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by

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Phytochemical and Biological Studies of Plants: *Scrophularia tenuipes* Coss & Durieu. and *Oenanthe virgata* Poiret.

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Dedication

To my parents

To my husband Hassen and my children: Iyado and Linouna

To my brothers and sisters

To everyone who believes in me

Zeynouba

Abstract:

This work aims to study the phytochemical composition of the two endemic plants *Scrophularia tenuipes* Coss & Durieu and *Oenanthe virgata* Poiret and to investigate their health-benefit properties.

UHPLC-ESI-DAD-MS^n analysis of *S. tenuipes* revealed that the main compound in EA is the phenylethanoid «acetyl martynoside» and the iridoid «harpagoside» was dominant in Bu. While the prominent compound for *O. virgata* is «dicaffeyolquinic acid» for EA and the flavonoid «rutin» for Bu. Regarding biological activities, *S. tenuipes* and *O. virgata* exhibited a considerable antioxidant potential. Moreover, the antibacterial activity of *S. tenuipes* depended on the type of pathogenic bacteria and EA of *O. virgata* has an antibacterial activity against three out of four tested bacteria. In addition, EA extract has the high potent with regard to *in vivo*/*vitro* anti-inflammatory effect for both plants. Furthermore, *S. tenuipes* exhibited a strong effect against α-amylase enzyme but weak α-glucosidase inhibitory effects. *O. virgata* showed a strong α-amylase inhibitory activity and EA has high α-glucosidase inhibitory effect. *S. tenuipes* and *O. virgata* showed a weak urease inhibitory. *S. tenuipes* has a weak tyrosinase inhibitory capacity and EA of *O. virgata* has a moderate effect. Furthermore, both plants have no anticholinesterase activity but have a high sun protection effect.

Keywords: *Scrophularia tenuipes*, *Oenanthe virgata*, ethyl acetate, *n*-butanol, UHPLC-ESI-DAD-MS^n and biological activities

الملخص:

يهدف هذا العمل إلى دراسة التركيب الكيميائي النباتي للنبتتين المتوطنتين *Scrophularia tenuipes* Coss & Durieu و*Oenanthe virgata* Poiret و البحث عن خصائصها الطبية.

كشف تحليل UHPLC-ESI-DAD-MS^n لنبتة *S. tenuipes* أن المركب الرئيسي في مستخلص أسيتات الإيثيل هو "acetyl martynoside" بينما "harpagoside" في مستخلص البوتانول. أما بالنسبة لنبتة *O. virgata* فالمركب السائد في مستخلص أسيتات الإيثيل هو "dicaffeyolquinic acid" اما "rutin" في مستخلص البوتانول. فيما يتعلق بالأنشطة البيولوجية، تظهر كل من النبتتين *O. virgata* و *S. tenuipes* نشاطا مضادا للأكسدة معتبرا. علاوة على هذا، يعتمد النشاط المضاد للبكتيريا لـ *S. tenuipes* على نوع البكتيريا المرضية بينما مستخلص أسيتات الإيثيل ل *O. virgata* له نشاط مضاد للبكتيريا ضد ثلاثة من أربعة بكتيريا تم اختبارها. بالإضافة إلى ذلك ، فإن مستخلص أسيتات الإيثيل لكلتا النبتتين يبدي نشاطا قويا فيما يتعلق بالتأثير المضاد للالتهابات في الاختبارين الوسط الحي / المختبر. إلى جانب ذلك، أظهرت *S. tenuipes* نشاطا قويًا ضد إنزيم α-amylase ولكن له تأثير مثبط ضعيف لإنزيم α-glucosidase. نبات *O. virgata* له نشاط مثبطً قوي ضد إنزيم α-amylase ومستخلصها أسيتات الإيثيل له تأثير مثبط

عالي لإنزيم α-glucosidase. أظهرت كل من النبتتين *O. virgata* و *S. tenuipes* تـأثيرًا مثبطًا ضـعيفا ضـد انـزيم urease. نبتة *S. tenuipes* لديها قدرة مثبطة لـtyrosinase ضـعيفة بينمـا *O. virgata* لهـا تـأثير معتـدل عليـه. علاوة على ذلك، كلتا النبتتين ليس لهما نشاط مضاد لـcholinesterase ولكن لهما فعالية كبيرة للحماية من أشعة الشمس.

الكلمـات المفتاحيـة: *Scrophularia tenuipes*، *Oenanthe virgata*، أسـيتات الإيثيـل ،بوتـانول، UHPLC-ESI-DAD-MS^n والنشاطات البيولوجية.

Résumé:

Le but de ce travail est d'étudier la composition phytochimique des deux plantes endémiques *Scrophularia tenuipes* Coss & Durieu et *Oenanthe virgata* Poiret et leurs propriétés bénéfiques pour la santé.

L'analyse UHPLC-ESI-DAD-MSn de *S. tenuipes* a révélé que le composé principal dans EA est le phényléthanoïde « acétyl martynoside» et l'iridoïde « harpagoside »est dominant dans Bu. Alors que les principaux composés d'*O. virgata* sont «l'acide dicaffeyolquinique» pour EA et le flavonoïde «rutine» pour Bu. Concernant les activités biologiques, *S. tenuipes* et *O. virgata* ont un pouvoir antioxydant considérable. De plus, l'activité antibactérienne de *S. tenuipes* dépend du type de bactéries pathogènes et EA d'*O. virgata* a une activité antibactérienne contre trois de quatre bactéries testées. On outre, l'extrait d'EA des deux plantes est le plus puissant en ce qui concerne l'effet anti-inflammatoire *in vivo* / *in vitro*. D'autre part, *S. tenuipes* a montré un fort effet contre l'enzyme α-amylase et un faible effet inhibiteur sur l'α-glucosidase. *O. virgata* a montré une forte activité inhibitrice de l'α-amylase et EA a un effet inhibiteur élevé sur l'α-glucosidase. *S. tenuipes* et *O. virgata* ont présenté un effet inhibiteur de l'uréase faible. *S. tenuipes* a une faible capacité inhibitrice de la tyrosinase et *O. virgata* a un effet moyen. De plus, les deux plantes n'ont pas d'activité anti-cholinestérase mais ont une activité de protection solaire élevé.

Mots clés: *Scrophularia tenuipes*, *Oenanthe virgata*, acetate d'ethyl, *n*-butanol, UHPLC-ESI-DAD-MS^n et activités biologiques.

List of Abbreviations

$A_{0.50}$: Concentration in which the absorbance is 0.50

ABTS: Acide 2,2'-azino-bis (3-ethylbenzothiazolin-6-sulfonic)

AChE: Acethylcholinesterase

BChE: Butyrylcholinesterase

BHA: Butylated hydroxyanisole

BHT: Butylated Hydroxytoluene

BSA: Bovine serum albumin

Bu: n-butanol extract

CUPRAC: Cupric Reducing Antioxidant Capacity

DAD: Diode array

DPPH: 2,2 diphenyl-1-picrylhydrasyl

DTNB: 5, 5'-dithiobis (2-nitrobenzoïc)acid

EA: ethyl acetate extract

GAE: Equivalent of galic acid

QE: Equivalent of quercetin

ESI–MS: electrospray ionization–mass spectrometry

GC-MS: Gas chromatography–mass spectrometry

IC_{50}: The half maximal inhibitory concentration

LC: Liquid chromatography

L-DOPA: L-3,4-dihydroxyphenylalanin

MS: Mass spectrometry

MS^n: Tandem mass spectrometry

NMR: Nuclear magnetic resonance

TCC: Total carotenoid content

TFC: Total flavonoid content

TPC: Total phenolic content

TR: Time of retention

UHPLC: Ultra high-performance liquid chromatography

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GENERAL INTRODUCTION

GENERAL INTRODUCTION

General Introduction

Throughout the ages, Man has been able to rely on nature to provide for his basic needs such as food, shelter, clothing and also for his medical needs. Plants have extraordinary therapeutic properties. Their uses for the treatment of several diseases in living beings and in particular in humans are very old and have always been made empirically (Svoboda *et al*., 2000; Atanasov *et al*., 2015).

Archaeological evidence indicates that the use of medicinal plants dates back to the Paleolithic age, approximately 60,000 years ago. Written evidence of herbal remedies dates back over 5,000 years to the Sumerians, who compiled lists of plants. Some ancient cultures wrote about plants and their medical uses in books called herbals. In ancient Egypt, herbs are mentioned in Egyptian medical papyri, depicted in tomb illustrations, or on rare occasions found in medical jars containing trace amounts of herbs (Nunn, 2002).

In fact, there are around 500 000 species of plants on Earth, 80 000 have medicinal properties (Quyou, 2003). According to the World Health Organization, approximately 25% of modern drugs used in the United States have been derived from plants. At least 7 000 medical compounds in the modern pharmacopoeia are derived from plants. Among the 120 active compounds currently isolated from the higher plants and widely used in modern medicine today, 80% show a positive correlation between their modern therapeutic use and the traditional use of the plants from which they are derived. Up to 80% of the population in Africa uses traditional medicine as primary health care (Fabricant and Farnsworth, 2001).

The Mediterranean region has an exceptional biological diversity, its flora richness estimated at 25,000 species of vascular plants, which corresponds to 9.2% of the world flora, on a territory representing only 1.5% of the Earth's surface (Quézel, 1997).

Algeria with its 237 639 100 ha, its different bioclimatic, ecological regions, as well as its remarkable specific diversity, ranks moderately with the countries known for their taxonomic, ecosystem, landscape and cultural diversity. Its biogeographical position privileged between the Mediterranean and sub-Saharan Africa, enriched by a floristic potential made up of Mediterranean, Palearctic, Ethiopian and endemic species. This mixing of species constitutes for our country a real floristic richness and estimated at around 3 139 species of wild plants, of which more than 600 species have medicinal properties (Mokkadem, 1999; Benkiki, 2006).

GENERAL INTRODUCTION

The main objects of this work are to study the two endemic plant *Scrophularia tenuipes* Coss & Durieu and *Oenanthe virgata* Poiret according to the following plan:

- The first part “literature review” comprises three chapters: The first chapter will discuss the botanical aspect of the two plants. The second chapter will be devoted to a reminder on the different classes of secondary metabolites as well as some therapeutic interests of these metabolites. The third chapter will give an insight about the different studied biological activities on the two plants.
- The second part "experimental study" contains two chapters: In the first chapter, we will discuss the materials and analytical methods used for extraction, colorimetric assays, Column Chromatography, NMR, GCMS analysis of essential oils and LCMS-MS analysis of extracts from the two plants, and the different biological activities studied. The second chapter will be devoted to the results obtained in this study and to their discussion.

FIRST PART: LITERATURE REVIEW

CHAPTER I: BOTANICAL ASPECT

I. Medicinal Plants

The term “medicinal plants” is usually used for plants that possess therapeutic properties or exert beneficial pharmacological effects on the human body. Medicinal plants naturally synthesize and accumulate some secondary metabolites, for example: terpenes, flavonoids, alkaloids, volatile oils, sterols etc. Since ancient times, the medicinal plants have been used for the treatment of illnesses and diseases. Actually, people in different parts of the world tend to use the same plants for treating the same illnesses (Abdul motaleb, 2011). Medicinal plants have many characteristics when used as a treatment, as following:

Synergic medicine: all the components of the plants interact simultaneously, so their uses can complement or damage others or neutralize their possible negative effects.

Support of official medicine: in the treatment of complex cases like cancer diseases the ingredients of the plants proved to be very effective.

Preventive medicine: the ingredients of the plants are characterized by their ability to prevent the appearance of some diseases (Rasool Hassan, 2012).

I.1. Monograph of the Plant *Scrophularia tenuipes* Coss. & Durieu.

I.1.1. Scrophualariaceae Family

The Scrophulariaceae family includes 220 genera and about 3000 species. The name was derived from European species of Scrophularia, the common figwort الجنازيرية. It is also known as the figwort family.The plants of this family were used to treat haemorrhoids, which were known as "figs".

The genera that can be finding in this family are: *Verbascum, Calceolaria*, *Linaria, Antirrhinum*, *Scrophularia*, *Penstemon*, *Mimulus, Gratiola*, *Veronica*, *Isoplexis, Bartsia, Euphrasia*, *Pedicularis, Digitalis, Melapyrum, Odontitis, Chaenorrhium* and *Bacopa***.**

Scrophualariaceae is a family of flowering plants which can be annual, perennial herbs or under shrubs with bilateral or rarely radial symmetry. It is characterized by bisexual flowers with tubular corollas which are bilaterally symmetrical and have four stamens in most, two of which are shorter than the other two. The pistil is superior and generally two celled. The geographical distribution is cosmopolitan majority being found in the temperate areas including tropical mountains. The main secondary metabolites are steroidal and triterpenoid saponins, cyanogenetic glycosides, aucubin glycosides, napthoquinones and anthraquinones, aurones and iridoid alkaloids (Cherotich, 2011)

I.1.2. *Scrophularia* Genus

Scrophularia consists of about 300 species, is one of the most important genera belonging to the Scrophulariaceae. Many species of this genus grow wild in nature and have not been cultivated yet. However, some species are in danger of extinction. Distribution of this genus occurs mainly through mountainous regions (e.g. *Scrophularia farinosa* Boiss. and *Scrophularia amplexicaulis* Benth.) to rarely in deserts (e.g., *Scrophularia deserti* Delile) (Pasdaran, 2017). Anatomical studies of *Scrophularia* are mainly focused on leaf features (Makbul, 2006). Most species of this genus are herb with woody base *(S. variegata, S. frigida, S. deserti* and etc.) and rarely suffruticose (*S. shulabadensis and S. papyracea* etc.). Also, plant can be spinose (just in *S. alhagioides*) or tortuse (in *S. tortuisissima*). Height of some taxa of *Scrophularia* can be more than 100 cm (in *S. mesopotamica*) and some of them up to 20 cm (*S. crassiuscula*) (Attar, 2006).The genus *Scrophularia* is represented in Algeria by eleven species: *S. hypericifolia* Wudl., *S. canina* L., *S. sambucifolia* L., *S. tenuipes* Coss. et Dur., *S. arguta* Solander, *S. Iaevigata* Vahl., *S. hispida* Desf., *S. aquatica* L. (Quezel and Santa, 1963).

I.1.3. Applications in Herbal Medicine

Scrophularia species have been used since ancient times as folk remedies for some medical treatments (scrophulas, scabies, tumours, eczema, psoriasis, inflammatory affections, etc.) (De Santos Galíndez, 2002). **Table 1** shows the traditional use of different *Scrophularia* species.

Table 1: The traditional use of *Scrophularia* species.

Name	Plant Medicinal Part	Traditional Uses	References
S. ningopoensis	Roots	Anti-inflammatory	(Wang *et al*., 2005)
S. aquatica	Roots and aerial parts	Laxative, heart stimulant, circulatory Stimulant	(Marty, 1999)
S. buergeriana	Roots	Treatment of fever, swelling, constipation, pharyngitis, neuritis and laryngitis	(Pinkas *et al*., 1994)
S. dentata	Aerial parts	Treatment of smallpox, measles, high-heat plague and poisoning	(Zhang *et al*., 2014)
S. nodosa	Roots and aerial parts	Treatment of fever, swelling, constipation, and laryngitis	(Zhu, 1998)
S. lucida L	Roots and aerial parts	Heart stimulant, circulatory stimulant, diuretic	(Goodyer, 1968)
S. chryasanthemifolia L.	Roots and aerial parts	Heart stimulant, circulatory stimulant and diuretic	(Goodyer, 1968)
S. canina	Roots	Treatment of dermatitis and rheumatoid arthritis	(Berdini *et al*., 1991)
S. tenuipes	Leaves	treatment of inflammation and haemorrhage	(Hamel *et al*. ,2018)

I.1.4. Main Secondary Metabolites from *Scrophualaria* and their Biological Activities

According to the literature, many *Scrophularia* species have been studied and found to contain many classes of secondary metabolites including iridoids, phenylpropanoids, phenolic acids, flavonoids and saponins. Some of these compounds were shown to have anti-inflammatory, antioxidant, antibacterial, hepatoprotective, immuno-modulator, cardiovascular, diuretic, fungicidal, cytotoxic, cytostatic and antitumour activities (De Santos Galíndez, 2002). **Table 2** summarises the biological activities and the bioactive substance of some *Scrophularia* species, while the **table 3** gives informations about the compounds isolated from different plants of the genus *Scrophularia*.

Table 2: Biological activities of some *Scrophularia* species.

Species	Pharmacological Activities	Responsible Compound or Extract	References
S. deserti	inhibiting an enzyme or enzymes of Type II fatty acid synthesis (FAS)	Unsaturated fatty acids, ethanolic extract	(Ahmed *et al*., 2003)
	Anti-inflammatory	Scropolioside-D2 and harpagoside B	(Stavri *et al*., 2006)
	Antidiabetic	Scropolioside-D2 and harpagoside B	(Bahmani *et al*., 2013)
S. dentata	Anti-inflammatory activity significantly inhibited CoA-induced splenocyte proliferation	Iridoids & Scrodentoids A–E, scropoliosides	(Zhang *et al*., 2014)
S. amplexicaulis	Antibacterial (against *S. aureus*)	Essential oil	(Pasdaran *et al*., 2016)
	Antimalaria	Methanolic extract and fractions	
	Free radical scavengering activities and general toxicity	Methanolic extract and fractions	
S. cryptophila	Antiprotozoal and antimycobacterial activities	Crypthophilic acid A, C and buddlejasaponin III, acetylharpagide	Tasdemir *et al*. (2008)

S. canina	Insecticidal activity	phenolic acids	Germinara *et al.* (2011)
S. lepidota	Anti-protozoal & Antiplasmodial	Ningpogenin, sinuatol	Tasdemir *et al.* (2005)
S. nodosa	Wound healing activity	Scopolioside A, scrophuloside A4 and scrovalentinoside	Stevenson *et al.* (2002)
S. takesimensis	Strong aldose reductase (AR) inhibitory Activity	Acacetin	(Kim *et al.*, 2012)
S. auriculata	Antibacterial	Phenolic acids	(Cuéllar *et al.*, 1998)
	Anti-inflammatory	Iridoids and saponins, Hydroalcoholic extract	(Giner *et al.*, 2000)
S. koelzii	Hepatoprotective & immunostimulant	Scropolioside-A, koelzioside, harpagoside, 6-O-(300-O-p-Methoxy-cinnamoyl)-a-L-rhmanopyranosyl catalpol, chloroform fraction of the aerial parts	(Garg *et al.*, 1994)

Table 3: Compounds isolated from the genus *Scrophularia.*

Plant's Names	Compounds	References
S. kotscyhana	8-O-acetyl-4′-O-(E)-(p-coumaroyl)-harpagide 8-O-acetyl-4′-O-(Z)-(p-coumaroyl)-harpagide β-sitosterol 3-O-β-glucopyranoside Apigenin 7-Oβ-glucopyranoside Apigenin 7-O-rutinoside Luteolin 7-Oβ-glucopyranoside Luteolin 7-O-rutinoside (7)	(Renda *et al.*, 2017)
S. auriculata	Scrovalentinoside	(Giner *et al.*,

	Verbascosaponin A Scropolioside A Ilwensisaponin A Verbascoside	2000; Giner *et al*., 1998)
S. amplexicaulis	Scropolioside D Scrophuloside B_4 Salidroside Verbascoside Eugenol Eugenol acetate 1-Octen-3-ol	(Pasdaran *et al*., 2016)
S. buergeriana	Buergerinin F Buergerinin E Ningpogenin Buergerinin D Buergerinin C Buergerinin B 8-O-E-p-methoxycinnamoyl harpagide 8-O-Z-p-methoxycinnamoyl harpagide 6'-O-E-p-methoxycinnamoyl harpagide 6'-O-Z-p-methoxycinnamoyl harpagide Trans-cinnamic acid (E)-p-methoxycinnamic acid (E)-p-methoxycinnamic acid methyl ester (E)-o-methoxycinnamic acid (E)-p-coumaric acid (E)-caffeic acid (E)-ferulic acid Homovanilline alcohol Buergeriside A1 Buergeriside B1 Buergeriside B2 Buergeriside C1	(Kim and Kim 2000; Lin *et al*., 2000; Kim, *et al.* 2002; Kim *et al*., 2003; Jeong *et al.*, 2008; Yan and Xie, 2011)

	Harpagoside	
S. canina	7,8-Didehydro-6b,10-dihydroxy-11-noriridomyrmecin 8-epi-Loganic acid Verbascoside (E)-Phytol	(Berdini *et al.*, 1991; Venditti *et al.*, 2015)
S. cryptophila	Crypthophilic acid A Crypthophilic acid B Crypthophilic acid C Buddlejasaponin III 8-O-Acetyl harpagide Harpagide	(Tasdemir, *et al*. 2008)
S. dentata	Scrodentoside A Scrodentoside B Scrodentoside C Scrodentoside D Scrodentoside E Scrodentoside F Scrodentoside G Scrodentoside H Scropolioside G Scropolioside H Saccatoside 6-O-Methyl catalpol Catalpol 6'-O-E-p-feruloyl harpagide Scropolioside D Cis-harpagoside Harpagoside Laterioside Scorodioside 6-O-α-L-(4"-O-trans-cinnamoyl)-rhamnopyranosylcatalpol 6-O-α-L-(4"-O-trans-p-coumaroyl)-rhamnopyranosylcatalpol	(Zhang, *et al*. 2015; Zhang, *et al*. 2014)

	(Scropolioside F) lagotisoside D 8-O-Acetyl harpagide 7-Deoxygardoside Ajugoside 8-epi-deoxyloganic acid 6'-O-p-Coumaroyl harpagide 10-Deoxygeniposidic acid Geniposidic acid Ajugol Harpagide Scrodentoid A Scrodentoid B Scrodentoid C Scrodentoid D Scrodentoid E Lipedosides A-I Osmanthuside B Martynoside Diacetylmartynoside Verbascoside Isoverbascoside 3-O-trans-Feruloylrhamnopyranose 2-O-trans-Feruloylrhamnopyranose	
S. deserti	3-(R)-1-Octan-3-yl-3-O-b-D-glucopyranoside 3(f)-Hydroxy-octadeca-4(E), 6(Z)-dienoic acid 6-O-a-L-rhamnopyranosylcatalpol Buddlejoside A8 Harpagoside B 9 8-O-Acetyl harpagide Koelzioside Scropolioside D	(Ahmed, *et al.* 2003, Stavri, *et al.* 2006)

	Scropolioside D2 Scropolioside B Scrospioside A Laterioside	
S. frutescens	(Z)-p-Coumaric acid (Z)-Caffeic acid (Z)-Isoferulic acid (Z)-p-Methoxycinnamic acid (E)-p-coumaric acid (E) 3, 4-Dimethoxy cinnamic acid (Z) Ferulic acid (Z)-Methoxycinnamic acid methyl ester Syringic acid Gentisic acid Protocatechuic acid Isovanillic acid Catalpinic acid Vanillic acid	(Fernandez, *et al*. 1998, Garcia, *et al*. 1998)
S. ilwensis	Ilwensisaponin A (Mimengoside A) Ilwensisaponin B Ilwensisaponin C Ilwensisaponin D Karsoside Scropolioside D Aucubin Harpagide 8-O-Acetylharpagide Ajugol Angoroside C Quercetin-3-O-rutinoside Kaempferol-3-O-rutinoside	(Calis *et al*. 1993. Calis and Zor 1993)
S. kakudensis	Songarosaponin A Saksisaponin A	(Yamamoto *et al*., 1993)

	Buddlejasaponin I Buddlejasaponin II Buddlejasaponin III Scrophulasaponin II Scrophulasaponin III Scrophulasaponin IV	
S. koelzii	Koelzioside Scropolioside B 6-O-(3”-O-p-Methoxy-cinnamoyl)-a-L-rhmanopyranosylcatalpol Scrokoelziside A Scrokoelziside B	(Bhandri *et al*. 1992, Garg, *et al*. 1994, Bhandari, *et al*. 1996, Bhandari, *et al*. 1997)
S. lepidota	Ajugoside Ajugol Sinuatol 6-O-b-D-Xylopyranosylaucubin Catalpol 6-O-Methyl catalpol 3,4-Dihydro-methyl catalpol 1-Dehydroxy-3,4-dihydro aucubigenin Scrolepidoside Aucubin Angoroside C Ningpogenin	(Tasdemir, *et al*. 2005)
S. ningpoensis	Haemoplantaginin 8-Hydroxycoumarin 6-Hydroxyindan-1-one 4-Methylcatechol trans-Cinnamic acid 3-Methylphenyl-O-b-xylopyranosyl-(1'6)-O-b-glucopyranoside 4-Hydroxybenzaldehyde 30-Hydroxyacetophenone	(Kajimoto, *et al*. 1989; Qian, *et al*. 1991; Qian *et al*., 1992; Li *et al*., 2000; Nguyen *et al*., 2005; Chen *et al*., 2007; Chen *et al*., 2008; Li, et al. 2009; Niu *et al*., 2009; Zhang *et al*., 2012; Zhu *et al*., 2013; Zhang *et al*.,

	Scrokoelziside A Buergeriside A1 Sibirioside A Cistanoside F Cistanoide D 6'-O-Caffeoyl harpagide 6'-O-E-p-Feruloyl harpagide 6"-O-b-Glucopyranosylharpagoside 8-O-Acetyl harpagide b-Sitosterol b-Sitosterol glucoside Angoroside C Nepitrin Buergerinin A Aucubin Ningpogenin Ningpogoside A Ningpogoside B 4'-hydroxyacetophenone 3',5'-Dimethoxy-4'-hydroxyacetophenone 3'-Methoxy-4'-hydroxyacetophenone (Z)-4-Hydroxycinnamic acid methyl ester (E)-*p*-Methoxycinnamic acid trans-Caffeic acid methyl ester Scropolioside B Scrophularianine A Scrophularianine B Scrophularianine C 2,6-Dimethoxy-4-methoxymethylphenol Homovanillic alcohol Scrophuloside B4 Scrophuloside A4 6-O-Feruloylb-fructofuranosyl-(2-1)-O- α -	2015)

	glucopyranosyl-(6-1)-O-α-glucopyranoside Scrokoelziside B 6-O-cinnamoyl b-fructofuranosyl-(2-1)-O-α-glucopyranosyl -(6-1)-O-α-glucopyranoside Ningposide A Ningposide B Homoplantaginin Eurostoside 2-(3-Hydroxy-4-methoxyphenyl)ethyl-O-α-arabinopyranosyl- (1!6)-O-α-rhamnopyranosyl-(1-3)-O-b-Glucopyranoside PhenylO-b-xylopyranosyl-(1-6)-O-b-glucopyranoside Ningpoensines B/C Vanillin 6-O-Methyl catalpol 8- O-Feruloylharpagide 8-O-(2-Hydroxycinnamoyl) harpagide 6-O-a-D-Galactopyranosylharpagoside Harpagoside Harpagide Ningposide C Ningposide D Buergeriside C1 Buergeriside B2 Buergeriside B1 Ningpoensine A	
S. oxysepala	Scrokoelziside A Scrokoelziside B Verbascosaponin Harpagoside B Scropolioside D	(Orangi *et al*., 2013; Orangi *et al*., 2016; Valiyari *et al*., 2012)

	2-(4-chlorobenzyl amino) ethanol Eugenol Dehydroeugenol Methyl benzyl alcohol 1-Octen-3-ol	
S. nodosa	Jionoside D Angoroside C Scrophuloside A2 Scrophuloside A4 Scrophuloside A5 Scrophuloside A6 Scrophuloside A7 Scrophuloside A8 Scrophuloside A1 Buddlejoside A5 Buddlejoside A3 Buddlejoside A4 Pulverulentoside II Scrophuloside A3 Verbascoside A Scrophuloside B1 Scrophuloside B2 Purpureaside C Verbascoside Angoroside A cis-Verbascoside	(Miyase and Mimatsu 1999; Stevenson *et al.*, 2002; Swiatek and Dombrowicz, 1972)
S. scopolii	Angoroside A Angoroside B Angoroside C Angoroside D Verbascoside Isoverbascoside Scropolioside A	(Calis *et al.*, 1988)

	ScropoliosideB	
S. striata	Quercetin trans-cinnamic acid Isorhamnetin-3-O-rutinoside Nepitrin Verbascoside 1-Octen-3-ol	(Monsef-Esfahani *et al*., 2010)
S. scorodonia	8-O-Acetyl harpagide Saikosapoinin I (Buddlejasaponin IV) Saikosapoinin II (Sandrosaponin I) Isoangoroside C Buddlejasaponin I	(Emam *et al*. 1997; De Santo *et al*. 2000; Bermejo *et al*. 2002; Diaz *et al*., 2004)
S. takesimensis	Isorhamnetin-3-O-rutinoside Nepitrin b-Sitosterol a-Spinasterol 3-O-b-D-glucopyranoside 5-Hydroxypyrrolidin-2-one trans-Cinnamic acid (E)-p-Methoxycinnamic acid (E)-o-Methoxycinnamic acid Acacetin	(Kim *et al*., 2012)
S. trifoliata	Catalpol Aucubin	(Ramunno *et al*., 2006)

I.1.5. Species of *Scrophularia tenuipes* Coss. & Durieu.

I.1.5.1. Botanical Aspect

It is perennial, glandular and a glabrescent plant. Length of stems varies between 10-15 decimetre. The leaves are all petiolate, cordate-ovoid, finely serrated or toothed. The cymes far exceed the leaves, long pedunculated, branching, with slender, elongated axes. The corolla is five millimetres, yellowish. The sepals are lanceolate, non-margines. The globular capsule is small, fragile and briefly acuminates (Quezel and Santa, 1963; Battandier, 1888). The **figure 1** is a picture taken from mountain in Jijel (Algeria).

Figure 1: *Scrophularia tenuipes* during floraison (picture taken by Sebti Mohamed 2015, June).

I.1.5.2. Distribution / Habitat

It's an Algerian-Tunisia endemic /protected species. It occur at four sites in Tunisia (Ain Draham, Kroumirie Babouch, valley Mellegue, and valley of the Medjerda Ghardimaou) and in Algeria, where it have widespread to Great Kabylie, mainly Bouira and Tizi-Ouzou (in the mountains of Djurdjura), and Little Kabylie, mostly Bejaia, Jijel, and Setif (in the Babors mountains), besides other cities, such as Skikda, Annaba, and El-Taref.. It lives in wet or marshy places at the edge of springs and streams (Quezel and Santa, 1963; Battandier, 1888).

I.1.5.3. Systematic

Scrophularia tenuipes Coss. & Dur. is classified in the plant kingdom as following:

Kingdom: Plant
Division: Angiospermae
Class: Dicotyledonae
Sub class: Sympetalae
Order: Tubiflorae
Sub order: Solanineae
Family: Scrophulariaceae
Genus: *Scrophularia*

Species: *Scrophularia tenuipes*

I.2. Monograph of the Plant *Oenanthe virgata* Poiret

I.2.1. Apiaceae Family

This family is commonly called carrot family. It was also named as Umbelliferae. It comprises 300–455 genera and some 3000– 3750 species. This family is nearly cosmopolitan, being particularly abundant in the northern hemisphere. One remarkable feature of umbellifers is the wide range of uses of different species, ranging from food and fodder to spices, poisons and perfumery. The carrot (*Daucus carota*) is a major vegetable crop, with a world production of 23.3 megatons. Herbs used for flavoring include fennel (*Foeniculum vulgare*), parsley (*Petroselinum crispum*) etc. Some of these are used as flavorings for alcoholic beverages, especially anise. Many umbellifers have medicinal uses, for gastrointestinal complaints, cardiovascular ailments, and as stimulants, sedatives, etc. the plants of this family are herbs, annual or perennial, rarely trees. The inflorescences are usually involucrate compound umbels (sometimes simple or condensed into a head). The flowers are epigynous, small, bisexual or staminate (unisexual male), regular, in simple or compound umbels. Leaves are alternate, rarely oppo-site or basal; petiole usually sheathing at base; stipules absent. The styles are basally swollen to form a nectar-secreting structure (stylopodium) atop the ovary. The fruit is a schizocarp, the two dry segments (mericarps) attached to an entire to deeply forked central stalk (carpophores). Seeds contain oil glands. The plants of this family are aromatic with ethereal oils, terpenoids, saponins and other compounds (San, 2005).

I.2.2. *Oenanthe* Genus

The genus *Oenanthe* L. (“water dropwort”, Apiaceae) is represented by numerous species, it includes about 30 species, mainly distributed in humid habitats and on riverbanks that are widely distributed in Eurasia, North America, and Africa. The genus Oenanthe is represented in Algeria by six species: *O. globulosa* L., *O. fistulosa* L., *virgata* Poiret, *O. peucedanifolia* Poll., *O. silaiifolia* MB., *O. Lachenalii* Gimel (Quezel et Santa, 1963). Aquatic plants can be found in flooded places, leaves are usually heteromorphic, with very dense umbellules and white flowers. *Oenanthe* species have long been used as vegetables and as traditional medicines. The fresh leaves and petioles of *Oenanthe* plants are rich on proteins, amino acids, calcium, Vitamin C, iron, and flavonoids (Fu, 2016).

I.2.3. Applications in Herbal Medicine

Asiatic people used some species of *Oenanthe* as food, and others are used for their therapeutic properties (Park and Kim, 1996). **Table 4** summarises the traditional uses of some species of *Oenanthe*.

Table 4: The traditional use of *Oenanthe* species

Name	Plant Medicinal Part	Traditional Uses	References
Oenanthe fistulosa	Aerial part	Treatment of rheumatism	(Allen, 2004)
Oenanthe crocata	Aerial part	applied in Ireland to scrofulous swellings in the neck sound	(Allen, 2004)
O. javanica	shoot	indigestion remedies	(Kala, 2005)
O. pimpinelloides	Aerial plant	treatment of burned, antihypertensive and analgesic	(Mumcu, 2018)
O. palustris	Root	use for Attracting buyers and customers to their shop by Crushing, drying and licking twice a day while in shop	(Ssegawa, 2007)
O. aquatic	Aerial part	Use for sedative and expectorant properties	(Benigni *et al*., 1964)
O. stolonifera	Aerial part	used for various cooked vegetable dishes and kimchi, a fermented vegetable dish	(Park and Kim, 1996)
O. javanica	Leaves	used as a seasoning in soups	(Facciola, 1990)

I.2.4. Main secondary Metabolites from *Oenanthe* and their Biological Activities

Table 5 summarises the biological activities and the bioactive substance of some *Scrophularia* Species, while the **table 6** gives informations about the compounds isolated from different plants of the genus *Oenanthe*.

Table 5: Biological activities of some *Oenanthe* species.

Species	Pharmacological Activities	Responsible Compound or Extract	References
O. javanica	Antioxidant activity	Aqueous and methanolic extracts	(Bhaigyabati, 2017)
	Anti-inflammatory effect	Isorhamnetin, hyperoside, and persicarin	(Lu, 2019)
	Antiviral effect	Total phenolics, flavones, and ethyl acetate extracts	(Wang, 2005)
	Neuroprotective activity	Ethanol extract	(Park, 2015)
	Ethanol elimination/Alcohol detoxication	Aqueous extract	(Kim, 2009)
	Hepatoprotective effect	Total phenolic acid	(Lee, 2015)
	Enhancing immunity	Total flavone	(Liu,2016)
	Anticancer activity	total phenolics acid extract	(Zhang, 2013)
	Anticoagulant/Antithrombotic activities	Persicarin, isorhamnetin, hyperoside, and isorhamnetin-3-O-galactoside	(Ku, 2013)
	Hypoglycemic effect	Aqueous extract	(Su, 2011)
	Cardiovascular protection	Persicarin and isorhamnetin-3-O-galactoside	(Ji, 1990)
O. stolonifera	Protective effects on myocardial injury induced by ischemia and reperfusion	Methanolic extract	(Zhang, 1995)
O. pimpinelloides	larvicidal effect	Essential oil	(Evergetis, 2009)
O. crocata	Antifungal, antioxidant and anti-inflammatory activities	Essential oil	(Valente, 2013)

O. virgata	Antimicrobial and antioxidant activities	Essential oil	(Zellagui, 2012)

Table 6: Compounds isolated from the genus *Oenanthe*.

Plant's Names	Compounds	References
O. javanica	Apigenin Isorhamnetin-3-*o*-*β*-*d*-glucopyranoside Quercetin Isorhamnetin-3-*o*-galactoside Afzelin Persicarin Isorhamnetin Hyperoside Luteolin Kaempferol Rutin Nictoflorin Quercetin-3-l-rhamnoside Xanthotoxin Bergapten Isopimpinellin Sioimperatorin Imperatorin Columbianadin 5-hydroxy-8-methoxypsoralen,6,7-dihydroxycoumarin, Scopoletin Neochlorogenic acid Chlorogenic acid Caffeic acid Gallic acid *α*-tocopherol Lunularin	(Ai *et al*., 2016; Zhang *et al*., 2012; Hou, 2017; Lu and Li, 2019)

	p-hydroxyphenylethanol ferulate 5-*p*-trans-coumaroylquinic acid Carvacrol Ferullic acid Catechin	
O. crocata	Oenanthotoxin	(Clarke *et al.*, 1949)
O. aquatica	2-*trans*-9-*cis*-2,9-pentadecadiene-4,6-diyne 2-*trans*-8-*cis*-10-*trans*-2,8,10-pentadecatriene-4,6-diyne 2-*trans*-8-*trans*10-*trans*-2,8,10-pentadecatriene-4,6-diyne, 2,8,10-Pentadecatriene-4,6-diyne	(Vincieri *et al.*, 1981)
O. fistulosa	Oenanthotoxin Dihydrooenanthotoxin 9-Epoxyfalcarindiol	(Appendino *et al.*, 2009).

I.2.5. Species *Oenanthe virgata* Poiret

I.2.5.1. Botanical Aspect

It is a perennial plant, 0.3-0.8m, with elongated-thick root tubers, sessile; fistulous stem, fluted, branched from the base. It has polymorphic leaves, the basal 2-3 entrenched, with petiole ≤ limb, triangular contour, cuneiform segments, divided-laciniate, acute-mucronate at the apex; higher flowers with longer segments, linear, finely petiolate, with apparent secondary nerves network. Umbels are 8-15 cm, pedunculated, with 6-12 long rays (3-8m) and thickened to fruition, 0-1 (2) fast deciduous bracts; umbellules with numerous lanceolate, fruiting subglobose; white flowers, persistent sepals with acute triangular teeth (1.2-1.5), external petal clearly radiating. Subcylindrical fruit, 5-6×1.5mm, erect style (4.5-5mm) almost as long as the fruit; mericarpes with little apparent ribs, thin airy tissue (thick on commisurales) (Lamarck, 1798; Jahandiez and Maire, 1932; Quézel and Santa,1963). **Figure 2** below is a picture taken from Meadows in Jijel (Algeria).

Figure 2: *Oenanthe virgata* during floraison (picture taken by Zeyneb Chaibeddra, 2015).

I.2.5.2. Distribution/ Habitat

O. virgata is endemic for the Maghreb (Morocco, Algeria, and Tunisia). It lives in streams, soggy lawns, swamp, plains and well watered medium mountains (Lamarck, 1798; Jahandiez and Maire, 1932; Quézel and Santa, 1963).

I.2.5.3. Systematic

O. virgata is classified in the plant kingdom as following:

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Sub class: Rosidae
Order: Apiales
Family: Apiaceae
Genus: *Oenanthe*
Species: *Oenanthe virgata*

CHAPTER II: SECONDARY METABOLITES

II.1.Definitions

II.1.1.Metabolites

A substance essential to the metabolism of a particular organism or to a particular metabolic process is called metabolite. Metabolites are the intermediate products of metabolism. Metabolites have various functions, including energy, structure, signalling, stimulant and inhibitory effects on enzymes (Altaf-Ul-Amin, 2019).

In plants, there are two major classes of metabolites:

II.1.2. Primary Metabolites

Primary metabolites are organic molecules that are found in all cells of a plant to ensure its survival. They are classified in four broad categories: carbohydrates, lipids, amino acids and nucleic acids (Raghuveer, 2015).

II.1.3. Secondary Metabolites

Secondary metabolites, also known as phytochemicals, natural products or plant constituents are responsible for medicinal properties of plants to which they belong. They are compounds biosynthetically derived from primary metabolites, not required for normal growth and development, and are not made through metabolic pathways common to all plants. They are accumulated by plant cells in smaller qualities than primary metabolites. These secondary metabolites are synthesized in specialized cells at particular developmental stages making extraction and purification difficult (Kabera, 2014).

II.2. Classification of Secondary Metabolites and their Pharmacological Actions

The classification is based on chemical structure, composition, their solubility in various solvents, or the pathway by which they are synthesized. The main classification system includes three major groups: terpenoids, phenolics and alkaloids. Glycosides, tannins and saponins are part of them according to their specific structure (Verpoorte, 1998).

II.2.1. Terpenoids

The terpenes, or isoprenoids, are one of the most diverse classes of metabolites. The Dictionary of Natural Products lists over 30000, mainly of plant origin, encompassing flavours and fragrances, antibiotics, plant and animal hormones, membrane lipids, insect

attractants and mediators of the essential electron-transport processes which are the energy-generating stages of respiration and photosynthesis (Buckingham, 2004).

Terpenoids are the largest and most diverse family of natural products, ranging in structure from linear to polycyclic molecules and in size from the five-carbon hemiterpenes to natural rubber, comprising thousands of isoprene units. All terpenoids are synthesized through the condensation of isoprene units (C5) and are classified by the number of five-carbon units present in the core structure (Mahmoud, 2009).

Many flavour and aromatic molecules, such as menthol, linalool, geraniol and caryophyllene are formed by monoterpenes (C10), with two isoprene units, and sesquiterpenes (C15), with three isoprene units. Other bioactive compounds, such as diterpenes (C20), triterpenes (C30) and tetraterpenes (C40) show very special properties (Raghuveer, 2015).

II.2.1.1. Monoterpenes and Sesquiterpenes (Plant Volatiles)

Plant volatiles are typically lipophilic liquids with high vapour pressures. Non-conjugated plant volatiles can cross membranes freely and evaporate into the atmosphere when there are no barriers to diffusion. The number of identified volatile chemicals synthesized by various plants exceeds 1000 and is likely to grow as more plants are examined with new methods for detecting and analyzing quantities of volatiles that are often minute (Dudareva, 2004; Pichersky, 2006). Studying the volatile fraction requires analytical methods and technologies that not only evaluate its composition exhaustively but also monitor variations in its profile and detect trace components characterizing the plant being investigated (Bicchi, 2011). The gas chromatography (GC and GC-MS) is a very powerful analytical tool for the identification of essential oil components. However, GC-MS has its limitations. Isomers usually give very similar mass spectra. This is particularly true for terpenes and even more for sesquiterpenes. Therefore, a favourable match factor between mass spectra is not sufficient for identification (Zellner, 2010). Retention indices have been used, together with mass spectrometry, for the proper identification of essential oils composition. Misidentification is not rare, however, either if a non-authentic mass spectra library is used, which means a database built with data from the literature, not from the analysis of real standards, or by misuse of retention indices (Joulain, 1998). Column chromatography (CC) has been applied to solve this problem. After isolation, a NMR analysis can be performed for the unknown or suspect compound, so that the correct mass spectrum and retention index can be recorded. Despite the advances in

analytical methods to evaluate the composition of essential oil, CC remains a powerful technology for separation and characterization of specific compounds of interest. Column chromatography has been largely used and reported for searching and identification of new molecules, sometimes associated with their antimicrobial, antibacterial and antifungal activities. Also, this technique has been successfully used to obtain sufficient amounts of a substance for the investigation of its biological properties and allowing the detection of its olfactory properties. Isolation is also applied to and, very important for volatiles, to evaluate its odor (Salamci, 2007). Monoterpenes are further classified into unsaturated hydrocarbons (e.g., limonene), alcohols (e.g., linalool), alcohol esters (e.g., linalyl acetate), aldehydes (e.g., citronellal) and ketones (e.g., Carvone). Monoterpenes and other volatile terpenes have a number of widespread medicinal uses. Compounds such as camphor and menthol are used as counterirritants analgesics and anti-itching agents. Many monoterpenes have been used as antihelmintics. A series of monoterpene glycosides appear to have vasodilation effect on coronary vessels and the femoral vascular bed (Yi, 2004). A number of sesquiterpene lactones show antibacterial, antifungal and antiprotozoan activities. Sesquiterpenes from *Vernonia colorata* inhibit *Entamoeba histolytica* at concentrations comparable to metronidazole, an antiamoebic drug. Helenalin and a series of related compounds are responsible for the cardiotonic properties of *Arnica montana* flowers. Atractylodis rhizoma, from *Atractylodis macrocephala* (Asteraceae), is clinically used as diuretic, analgesic and anti-inflammatory. The activity is related to the presence of active compounds including eudesma-4(14)-7(1 l)-dien-8-one and atractylenolide I. Several related medicinal plants are also used for the same purposes due to the presence of sesquiterpenes (Hikino, 1985; Seigler, 1995).

II.2.1.2. Diterpenes and Sesterterpenes

The diterpene compounds arise from geranyl diphosphate, and present 20 carbon units in their basic skeletal type. One of the simplest and most important of the diterpenes is phytol, a reduced form of geranylgeraniol, which forms the lipophilic side-chain of the chlorophylls (Vetter, 2011).

Cyclization reactions of geranyl diphosphate led to many structural types of diterpenoids, presenting a large range of polarity nature, from apolar hydrocarbons such as cembrene, (Villanueva, 2010) a 14-membered ring, to fully oxidized skeleton of virescenoside, isolated from marine fungus *Acremonium striatisporum* (Ebel, 2010).

Sesterterpenes (C25) may be the least common group of terpenoids. This class of compounds arises from geranylfarnesyl diphosphate, which by cyclization can give rise to various skeletal types, presenting different oxidation levels and several biological activities. Although many examples of these natural terpenoids are known, they are primarily isolated from fungi and marine organisms. Considering the large range of polarity nature presented by both diterpene and sesterterpene, the isolation and purification techniques vary and can be classic thin-layer chromatography (TLC), preparative thin-layer chromatography (PTLC), CC, flash chromatography (FC), or modern high performance liquid chromatography (HPLC), multiflash chromatography, vacuum liquid chromatography (VLC), solid-phase extraction and others (Lancas, 2008). Diterpenoids constitute the active constituents of a number of medicinal plants. Vitamin K1, an antihemorrhagic compound, first discovered in plants in 1929, is a diterpene. Vitamin A, a diterpenoid, is referred to, together with the related compounds, as "carotenes." The bitter principles of *Jateorhiza palmata* (calumba root) belong to furanoditerpenes. *Teucrium chamaedrys* (wall germander) and *T. scorodonia* (wood sage) family Labiatae, both produce diterpenes of the neoclerodane type. They are used in herbal medicine as diaphoretics and antirheumatics. Like all groups of terpenes, diterpenes have demonstrated a range of pharmacological properties including: analgesic, antibacterial, antifungal, anti-inflammatory, antineoplastic and antiprotozoal activities. Some diterpenes from *Kalmia latifolia* (Ericaceae) have antifeedant properties with respect to the gypsy moth. The gibberellins, first obtained from fungi of the genus *Gibberella* but also found in higher plants, are diterpenoid acids, which have a marked effect on growth of seedlings (Evans, 2009). An example of a sesterterpenoid is geranyl farnesol isolated from seed oils of *Camellia sasanqua* (sasanqua) and *Camellia japonica* (camellia), family Theaceae. Geranyl farnesol showed cytotoxic activity in mouse leukemic M1 cells (Ishikura *et al*., 1985).

II.2.1.3. Triterpenes

They consist of six isoprene units and have the molecular formula $C_{30}H_{48}$. The linear triterpene squalene, the major constituent of shark liver oil, is derived from the reductive coupling of two molecules of farnesyl pyrophosphate. Triterpenes constitute a significant portion of the lipid substances of all plants; more than 4000 triterpenoids have been isolated. These compounds are precursors to steroids in both plants and animals. Both triterpenes and steroids occur free, as glycosides or in other combined forms. The structures of triterpenes and steroids may be subdivided into about 40 major types. β-Boswellic acids (ursane-type

triterpene) and α-boswellic acids (oleanane-type triterpene) that are isolated from the oleo-gum-resin of *Boswellia carterii* are known for their anti-inflammatory and anti-rheumatic activities. One group of compounds showing a range of interesting biological activity is the quassinoids isolated from *Quassia amara*. These are degradation and rearrangement products of triterpenes. Quassia is used as a bitter tonic, as an insecticide and as an enema for the expulsion of thread worms (Culioli *et al.*, 2003).

II.2.1.4.Tetraterpenes (Carotenoids)

More than 650 carotenoids (C40) are found in nature, constituting the largest group of natural dyes. The carotenoids are substances with very special properties possessed by no other group of substances; these form the basis of their many varied functions and actions in all kinds of living organisms. Carotenoids are biosynthesized by plants, algae, fungi, yeasts and bacteria. The carotenoids are isoprenoid compounds, biosynthesized by tail-to-tail linkage of two geranyl diphosphate molecules. This produces the parent C40 carbon skeleton from which all the individual variations are derived. This skeleton can be modified:

a. by cyclization at one end or both ends of the molecule to give the seven different ends groups

b. by changes in hydrogenation level, and

c. by addition of oxygen-containing functional groups.

Carotenoids that contain one or more oxygen functions are known as xanthophylls, the parent hydrocarbons as carotenes (Britton, 1995). After being absorbed through human diet, some carotenes, among them beta-carotene are pro-vitamin A; other, such as lycopene are important due to their antioxidant properties. Carotenoid extracts have been screened by TLC and separated by CC, involving liquid-solid chromatography (adsorption). Various adsorbents have been applied in carotenoid analysis, including Al_2O_3, silica, magnesium oxide (MgO), calcium hydroxide [$Ca(OH)_2$], calcium carbonate [$CaCO_3$], siliceous earth as hyflosupercell and others. In normal phase CC, the adsorption affinity depends on the number of conjugated double bonds, cyclization and the presence of oxygen substituents (Rodriguez-Amaya, 1999). CC has been used for separations of mixtures of carotenes and xanthophylls, aiming for mainly analytical determinations, standard purifications, biological evaluations of carotenoids and the purification of synthesized carotenoids, especially by flash chromatography. Separations on basic adsorbents such as MgO and Ca $(OH)_2$ are mainly determined by the number and type of double bonds in the carotenoid molecules (Bernhard, 1995). The

procedure for isolating and purifying carotenoid standards was established because of the difficulty in obtaining standards commercially. The procedure consists of carotenoid extraction with cold acetone, partition to petroleum ether in a separatory funnel with addition of water, concentration in a rotatory evaporator and chromatographic separation of carotenoids on CC developed with petroleum ether containing increasing percentages of ethyl ether and acetone (Rodriguez-Amaya, 1999).

A variety of carotenoid standards have been isolated and purified using MgO: Hyflosupercel (1:1) CC developed with 2-8% ethyl ether in petroleum ether and 2-95% acetone in petroleum ether: 98% β-carotene (isolated from carrot), 94% lycopene, 99% β-cryptoxanthin, 91% γ-carotene and 91% rubixanthin (from pitanga) (Porcu, 2008). Several carotenoids have been reported to decrease the risk of diseases and disorders by reducing oxidative stress in target tissues, since they can be present in sufficient amounts and the correct location to have antioxidant function. For example, lutein and zeaxanthin have been studied widely and proven to show diverse beneficial effects on human health, particularly in optimizing eye health. The biological mechanisms for the protective effects of both these carotenoids may include their powerful blue-light filtering activities and antioxidant properties. There have been several studies into the relationship between astaxanthin antioxidant activity and the prevention of (cardiovascular disease) CVD. The prevention of atherosclerosis by astaxanthin intake can be explained by its protective effects on low-density lipoprotein (LDL) and vein endothelial cells against oxidative injury and dysfunction (Miyashita and Hosokawa, 2014). The **figure 3** below gives informations about the biosynthesis of terpenoids:

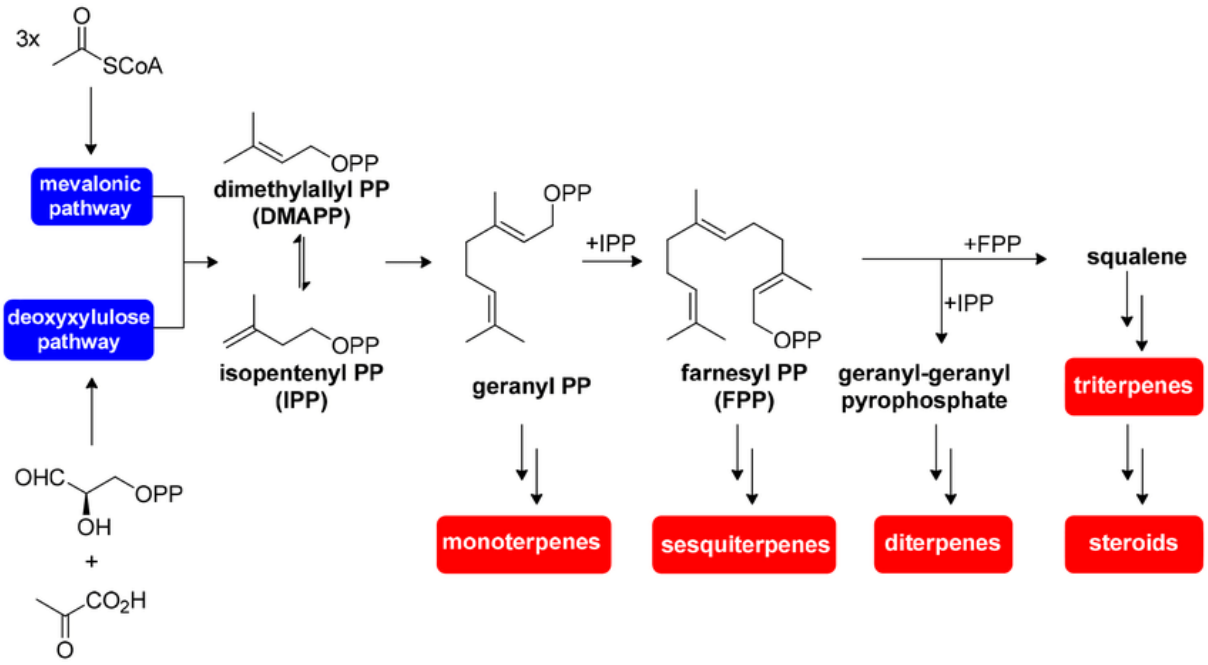


Figure 3: General scheme for the biosynthesis of terpenoids (Brocksom, 2017).

II.2.2. Phenolics

Phenolic compounds from plants are one of largest group of secondary plants constituents synthesized by fruits, vegetables, teas, cocoa and other plants that possess certain health benefits. They are characterized by the antioxidant, anti-inflammatory, anti-carcinogenic and other biological properties, and may protect from oxidative stress and some diseases (Park, 2001). Simple phenolics are bactericidal, antiseptic and anthlemintic. Phenol itself is a standard for other antimicrobial agents (Pengelly, 2004). They are distributed in almost all plants and subject to a great number of chemical, biological, agricultural, and medical studies (Herrmann, 1989; Dai, 2010). They are diverse in structure, and present in common the hydroxylated aromatic rings (e.g., flavan-3-ols). Most of phenolic compounds are polymerized into larger molecules such as the PA (proanthocyanidins; condensed tannins) and lignans. Furthermore, phenolic acids may occur in food plants as esters or glycosides conjugated with other natural compounds such as flavonoids, alcohols, hydroxyfatty acids, sterols, and glucosides (Dai, 2010). Hydroxybenzoic and hydroxycinnamic acids present two main phenolic compounds found in plants. In tea, coffee, berries and fruits, the total phenolic compounds could reach up to 103 mg/100 g fresh weigh (Manach, 2004).

The approach to classifying plant phenolics are based on:

(1) A number of hydroxylic groups. So, they may be divided into 1-, 2- and polyatomic phenols. Phenolic compounds containing more than one OH-group in aromatic ring are polyphenols;

(2) Chemical composition: mono-, di, oligo- and polyphenols;

(3) Substitutes in carbon skeleton, a number of aromatic rings and carbon atoms in the side chain. According to the latter principle, phenolic compounds are divided into four main groups:

Phenolics with one aromatic ring, with two aromatic rings, quinones and polymers.

Phenolic compounds with one aromatic ring: a large number of compounds, among them are simple phenols (C_6), phenol with attached one (C_6-C_1), two (C_6-C_2) and three (C_6-C_3) carbon atoms.

Phenolic compounds with two aromatic rings: this group includes benzoquinones and xanthones (C_6-C_1-C_6) containing two aromatic rings which are linked by one carbon atom; stylbenes (C_6-C_2-C_6) which are linked by two carbon atoms; and flavonoids, containing three carbon atoms (C_6-C_3-C_6). Flavonoids, depending on the structure of propane unit and an attaching place of side chain B, are divided into flavonoids in strict sense, which are derived from chromane or chromone, isoflavonoids and neoflavonoids.

Polyphenolics are more than 8,000 different compounds identified to date. That is why the terminology and classification of polyphenols is complex and confusing. Although all polyphenols have similar chemical structures, there are some distinctive differences. Based on these differences, polyphenols can be subdivided into two classes: flavonoids and non-flavonoids, like tannins (Somasegaran, 1994). **Figures 4**, **5**, **6**, **7**, **8** and **9** show some exemples of phenolic compounds.

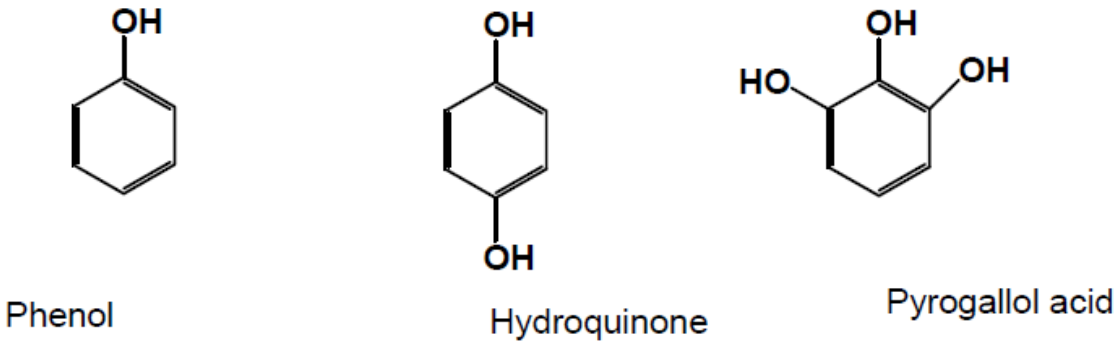
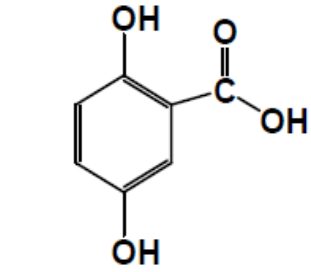
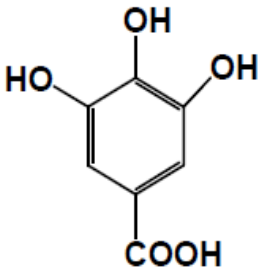


Figure 4: Examples of simple phenolics, C_6 (phenol, hydroquinone and pyrogallol acid) (Kabera, 2014).

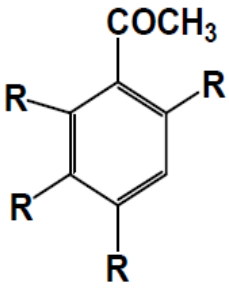


Salycilic acid

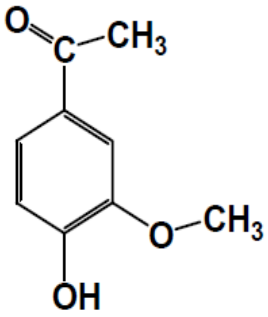


Gallic acid

Figure 5: Examples of C_6-C_1 phenolics: gallic acid and salicylic acid (Kabera, 2014).

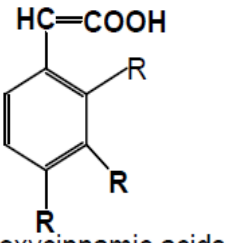


Acetophenones

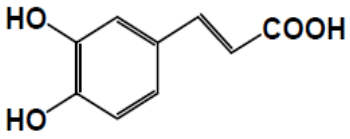


Apocynin

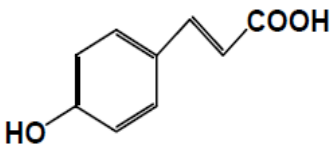
Figure 6: Examples of C_6-C_2: acetophenones, apocynin (Kabera, 2014).



Hyroxycinnamic acids



Caffeic acid



P-coumaric acid

Figure 7: Phenolics with C_6-C_3 (phenylpropanoids): hydroxycinnamic acid, caffeic acid and *p*-coumaric acid (Kabera, 2014).

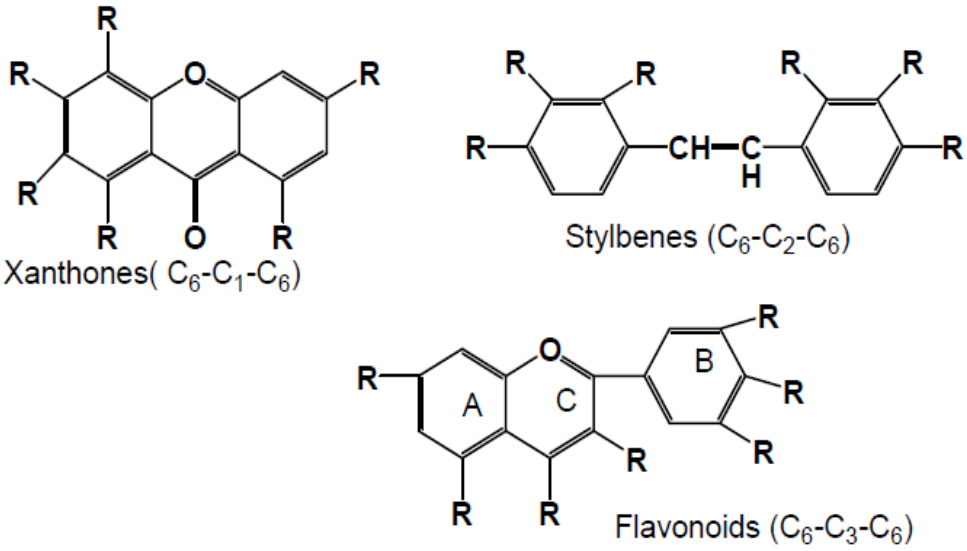


Figure 8: Some phenolics with two aromatic rings (xanthones: C_6-C_1-C_6; stylbenes: C_6-C_2-C_6 and flavonoids: C_6-C_3-C_6) (Kabera, 2014).

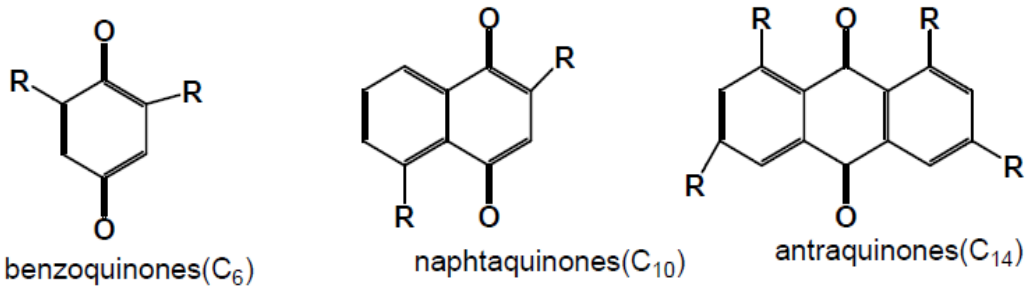


Figure 9: Main groups of quinines: benzoquinones, naphtoquinones, and antraquinones (flavonoids, tanins, glycosides, saponins) (Kabera, 2014).

II.2.2.1. Flavonoids

Flavonoids stand as the first class of polyphenols. They are water-soluble pigments found in the vacuoles of plant cells. They can also be divided into three groups: anthocyanins, flavones and flavonols. They are widely distributed in plants, fulfilling many functions such as flower coloration, producing yellow, red or blue pigmentation in petals designed to attract pollinator animals. In higher plant, flavonoids are involved in UV filtration, symbiotic nitrogen fixation and floral pigmentation. They may also act as chemical messengers, physiological regulators, and cell cycle inhibitors. Flavonoids are secreted by the root of their host plant *Rhizobia* help in the infection stage of their symbiotic relationship with legumes like peas, beans, clover, and soy (Galeotti, 2008). *Rhizobia* living in soil are able to sense the flavonoids and this triggers the secretion of nod factors, which in turn are recognized by the

host plant and can lead to root hair deformation and several cellular responses such as ion fluxes and the formation of a root nodule. Some flavonoids have inhibitory activity against organisms that cause plant disease, for example, *Fusarium oxysporum* (Middleton, 2000).

They have become very popular because of their health benefits. Some of the activities attributed to them include: anti-allergic, anti-cancer, antioxidant, anti-inflammatory and anti-viral (Hertog, 1995; Guardia, 2001). The flavonoids quercetin is known for its ability to relieve high fever, eczema, asthma and sinusitis. Epidemiological studies have illustrated that heart diseases are inversely related to flavonoid intake. Studies have shown that flavonoids prevent the oxidation of low-density lipoprotein thereby reducing the risk for the development of atherosclerosis (Lippi, 2010; McCullough, 2012). The contribution of flavonoids to the total antioxidant activity of components in food can be very high; for instance red wine contains high levels of flavonoids, mainly quercetin and rutin. The high intake of it by the French might explain why they suffer less from coronary heart disease than other Europeans, although their consumption of cholesterol rich foods is higher (French paradox) (Wu, 2001). Many studies have confirmed that one or two glasses of red wine daily can protect against heart disease (Halliwell, 2007). Their antioxidant activities in chemical and biological assays are undisputed, and many are associated with the health-promoting effects of fruits and vegetables. However, extending these effects to entire organisms and clinical outcomes in human disease in particular, remains a controversially discussed topic in nutrition science and disease prevention (Habauzit, 2012). Recently, it has been mentioned that growing consensus for the hypothesis that the specific intake of food and drink containing relatively high concentrations of flavonoids may play a meaningful role in reducing the risk of CVD (cardiovascular disease). The reviewers stated that research to date has been of poor quality. Large and rigorous trials are needed to better investigate the possible adverse effects associated with excessive polyphenol intake. Currently, a lack of knowledge about safety suggests that polyphenol levels should not exceed that which occurs in a normal diet (Loeb, 1989). **Figure 10** represents the basic structures of flavonoids.

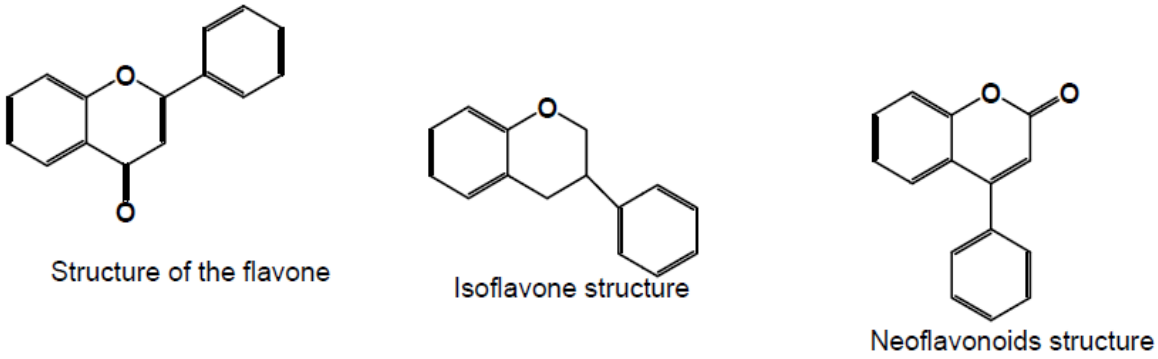


Figure 10: Basic structures of flavonoids (Kabera, 2014).

II.2.2.2.Tannins

Tannin is the name derived from French "Tanin" (tanning substance) and used for a range of natural polyphenols. The tannins are the phenolic compounds that precipitate proteins. They are composed by a very diverse group of oligomers and polymers. They can form a complex with proteins, starch, cellulose and minerals. They are synthesized via shikimic acid pathway, also known as the phenylpropanoid pathway. The same pathway leads to the formation of other phenolics such as isoflavones, coumarins, lignins and aromatic aminoacids. Tannins are water soluble compounds with exception of some high molecular weight structures. They are usually subdivided in two groups: HT (hydrolysable tannins) that include gallotannins, elligatannins, complex tannins, and PA, also known as condensed tannins (Lancini, 1993).

The tannins also constitute the active principles of plant-based medicines. According to the literature, the tannins containing plants are used as astringents against diarrhea (Fujiki, 2012), adiuretic against stomach and duodenal tumours (Trouillas, 2003), and anti-inflammatory (Brito Arias, 2007). **Figure 11** represents a type of tannins.

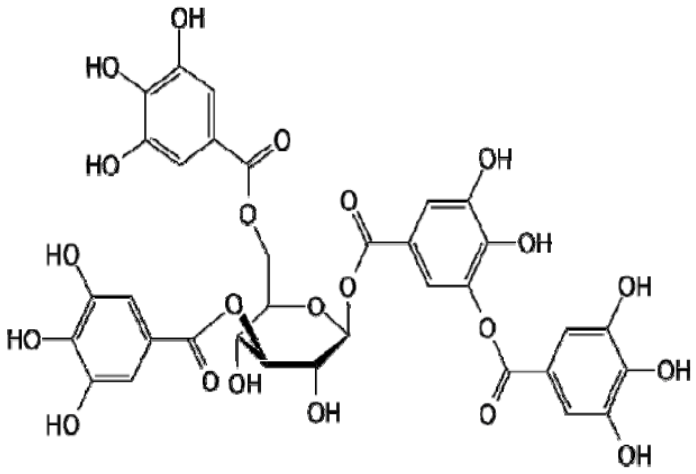


Figure 11: Tannic acid, type of tannins (Kabera, 2014).

II.2.2.3. Glycosides

Glycosides may be phenol, alcohol or sulfur compounds. They are characterized by a sugar portion or moiety attached by a special bond to one or non-sugar portions. Many plants store chemicals in the form of inactive glycosides, which can be activated by enzyme hydrolysis (Polt. 1995). For this reason, most glycosides can be classified as prodrugs since they remain inactive until they are hydrolysed in the large bowel leading to the release of the aglycone, the right active constituent.

The classification of glycosides is based on the nature of aglycone, which can be any of a wide range of molecular types including phenols, quinines, terpenes and steroids. They are heterogene heterogeneous in structure; therefore, they are not easy to learn as specific group and are described here in this review for the convenience. Glycosidic bonds are of great significance, since they link monosaccarides together to form oligosaccharides and polysaccharides (Levy, 1995; Newman, 2008). Concerning the therapeutic actions in different studies, it has been shown that glycosides have anticancer (Zhou, 2013), expectorant (Fernández, 2006), sedative and digestives properties (Galvano, 2004; Güçlü-Üstündağ, 2007). **Figure 12** represents an exemple of glycosides.

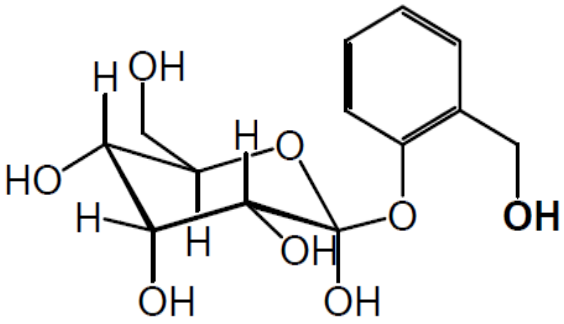


Figure 12: Salicin, a glycoside type related to aspirin (Kabera, 2014).

II.2.2.4. Saponins

Saponins are compounds whose active portions form colloidal solutions in water, which produce lather on shaking and precipitate cholesterol. They occur as glycosides whose aglycone is tripenoid or steroidal structures. The combination of lipophilic sugars at the end gives them the ability to lower surface tension, producing the detergent characteristic or soap-like effect on membranes and skin (Kabera, 2014).

They are largely distributed in plant kingdom, which have many physicochemical (foaming, emulsification, solubilisation, sweetness and bitterness) and biological properties (haemolytic, antimicrobial, antioxidant, moluscacide, insecticide and ichthyocide), exploited in many applications in food, cosmetics, pharmaceutical industries and soil bioremediation. Among the saponins properties, CMC (critical micellar concentration), maximum surface density and aggregation number (number of monomers in a micelle) are of great importance for application as surfactants and foaming agents. These are influenced by variables such as temperature, salt concentration, aqueous phase pH solvent concentration and type, such as ethanol or methanol.

Saponins have demonstrated numerous pharmacological properties. Some saponins have antitumor, piscicidal, molluscicidal, spermicidal, sedative, expectorant and analgesic properties. Glycyrrhizin from *Glycyrrhiza glabra* (Fabaceae) is useful as expectorant and antitussive agent. It is also used to treat chronic hepatitis and cirrhosis. Some saponins have anti-inflammatory properties as the saponins from *Bupleurum falcatum* (Apiaceae). *Phytolacca americana* roots are reputed to possess anti-inflammatory properties in Korean medicine. Similar properties have been demonstrated for a number of other saponins, for example aescin, from horse chestnut (*Aesculus hippocastanum*), has been shown to be 600

times more effective than rutin in reducing rat paw edema (Güçlü-Üstündağ and Mazza,2007). In **figure 13** a type of saponin is presented.

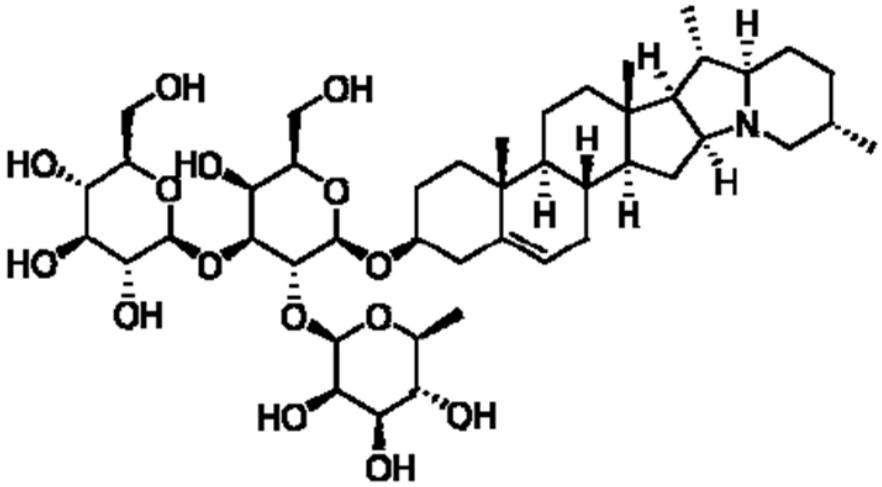


Figure 13: Type of saponin: solanine chemical structure.

II.2.3. Alkaloids

The alkaloids present the group of secondary metabolites that contain basic nitrogen atoms. Some related compounds with neutral and weakly acid properties are also included in the alkaloids. In addition to carbone, hydrogen and nitrogen, this group may also contain oxygen, sulfur and rarely other element such as chlorine, bromine and phosphorus (Nicolaou, 2011). Alkaloids are produced by a large variety of organisms, such as bacteria, fungi, animals but mostly by plants as secondary metabolites. Most of them are toxic to other organisms and can be extracted by acid-base. They have diverse pharmacological effects (Aniszewski, 2007), and have a long history in medication (Clarke, 1970).

The boundary between alkaloids and other nitrogen-containing natural compounds is not clear-cut (Giweli, 2013). Compounds like amino acids, proteins, peptides, nucleotides, nucleic acid, and amines are not usually called alkaloids.

Compared with most other classes of secondary metabolites, alkaloids are characterized by a great structural diversity and there is no uniform classification of them (Verpoorte, 1998). First classification was based on the common source because no information about chemical structure was yet available. Some classification is based on similarity of the carbon skeleton (Savithramma, 2011). Alkaloids are biosynthesized from amino acids such as

tyrosine (Evans, 1982). The typical example is the biosynthesis of morphine that includes a phenol coupling reaction involving a benzylisoquinoline alkaloid.

Alkaloids demonstrate a diverse array of pharmacological actions including analgesia, local anesthesia, cardiac stimulation, respiratory stimulation and relaxation, vasoconstriction, muscle relaxation and toxicity, as well as antineoplastic, hypertensive and hypotensive properties. The activity of alkaloids against herbivores, toxicity in vertebrates, cytotoxic activity, the molecular targets of alkaloids, mutagenic or carcinogenic activity, antibacterial, antifungal, antiviral and allelopathic properties have been reported in literature. Many alkaloids are sufficiently toxic to animals to cause death if eaten. Several (e.g., nicotine and anabasine) are used as insecticides (Seigler, 1995; Hoffmann, 2003).

CHAPTER III: INSIGHTS ON THE STUDIED BIOLOGICAL ACTIVITIES

III.1.Anti-oxidant Activity

In recent years, the world of biological and medical sciences is invaded by a new concept, that of "oxidative stress". Oxidative stress is involved in the pathogenesis of lifestyle-related diseases, including atherosclerosis, hypertension, diabetes mellitus, ischemic diseases, and malignancies (Yoshikawa and Naito, 2002).

III.1.1. Definition of Oxidative Stress

Oxidative stress is defined by an imbalance between increased levels of reactive oxygen species (ROS) and a low activity of antioxidant mechanisms. An increased oxidative stress can induce damage to the cellular structure and potentially destroy tissues. However, ROS are needed for adequate cell function, including the production of energy by the mitochondria. Increased oxidative stress has been incriminated in physiological conditions, such as aging and exercise, and in several pathological conditions, including cancer, neurodegenerative diseases, cardiovascular diseases, diabetes, inflammatory diseases, and intoxications. From a clinical standpoint, if biomarkers that reflect the extent of oxidative stress were available, such markers would be useful for physicians to gain an insight into the pathological features of various diseases and assess the efficacy of drugs (Preiser, 2012).

III.1.2. Free Radicals, Active Oxygen Species, and Oxidative Stress

Usually, an atom is composed of a central nucleus with pairs of electrons orbiting around it. However, some atoms and molecules have unpaired electrons and these are called free radicals. Free radicals are usually unstable and highly reactive because the unpaired electrons tend to form pairs with other electrons. An oxygen molecule (O_2) undergoes four-electron reduction when it is metabolized *in vivo*. During this process, reactive oxygen metabolites are generated by the excitation of electrons secondary to addition of energy or interaction with transition elements. The reactive oxygen metabolites thus produced are more highly reactive than the original oxygen molecule and are called active oxygen species. Superoxide, hydrogen peroxide, hydroxyl radicals, and singlet oxygen are active oxygen species in the narrow sense. Active oxygen species in a broad sense are listed in **table 7**. Only active oxygen species having an unpaired electron, indicated with a dot above and to the right of the chemical formula in the table, are free radicals. For aerobic organisms, a mechanism to remove these highly reactive active oxygen species is essential to sustain life. Therefore, various antioxidant defence mechanisms have been developed in the process of evolution. Also, the

high reactivity of these oxygen metabolites is utilized to control various biological phenomena. From a biological viewpoint, various oxygen-derived free radicals have been attracting attention for the following reasons: Various active oxygen species are generated in the body during the process of utilizing oxygen. Because the body is furnished with elaborate mechanisms to remove active oxygen species and free radicals, these by-products of oxygen metabolism are not necessarily a threat to the body under physiological conditions. However, if active oxygen species or free radicals are generated excessively or at abnormal sites, the balance between formation and removal is lost, resulting in oxidative stress. Consequently, active oxygen species and free radicals can attack molecules in biological membranes and tissues, thus inducing various diseases. In other words, oxidative stress is defined as a "state harmful to the body, which arises when oxidative reactions exceed antioxidant reactions because the balance between them has been lost." However, oxidative stress is actually useful in some instances. For example, oxidative stress induces apoptosis to prepare the birth canal for delivery. Also, biological defence mechanisms are strengthened by oxidative stress during appropriate physical exercise and ischemia. Therefore, a more useful definition of oxidative stress may be a "state where oxidation exceeds the antioxidant systems because the balance between them has been lost" (Yoshikawa and Naito, 2002; Halliwell and Whiteman, 2004; Preiser, 2012).

Table 7: Major active oxygen species

Active Oxygen Species	Abbreviation
Superoxide radical	$O^{2\cdot -}$
Hydrogen peroxide	H_2O_2
Hydroxyl radical	$HO^{\cdot}$
Singlet oxygen	$^1O_2$
Hydroperoxyl radical	$HOO^{\cdot}$
Alkylhydroperoxide	LOOH
Alkylperoxyl radical	$LOO^{\cdot}$
Alkoxyl radical	$LO^{\cdot}$
Hypochlorite ion	CIO^-
Ferryl ion	$Fe^{4+}O$
Periferryl ion	$Fe^{5+}O$
Nitric oxide	$NO^{\cdot}$

III.1.3. Biomarkers of Oxidative Stress

The biomarkers that can be used to assess oxidative stress have been attracting interest because the accurate assessment of such stress is necessary for investigation of various pathological conditions, as well as to evaluate the efficacy of drugs.

III.1.3.1. Measurement of Biomarkers of Oxidative Stress

III.1.3.1.1. Direct Measurement of ROS

The ideal method would be to measure directly the ROS, but these are labile compounds, and their direct detection and quantification are difficult. Free radicals are particularly short-living species; they can be measured only by electron paramagnetic resonance (EPR), most often coupled to spin trapping to increase their lifetime and the sensibility of the detection method. EPR has been successfully applied in animal models but is very difficult to use in vivo in humans (Lemineur *et al*., 2006).

III.1.3.1.2. Stimulated Production of ROS

The global production of oxidant species produced by stimulated phagocytes can be measured by chemiluminescence on freshly isolated cells, stimulated *ex vivo*. Unfortunately, this method does not allow an identification of ROS that have been produced. Moreover, to increase the sensitivity, chemiluminescence enhancers are used, which are highly sensitive to incidental (non-ROS-dependent) oxidation. The same criticism can be addressed to the fluorescence techniques developed for ROS identification on isolated cells (fluorescein, rhodamine) (Yoshikawa and Naito, 2000; Lemineur *et al*., 2006).

III.1.3.1.3. Assessment of Oxidative Damage

Many indirect methods have been developed to measure stable by-products of ROS activity on biomolecules: isoprostanes, hydroxyl-nonenal, nitrated proteins, chlorinated lipids, lipid peroxides, conjugated dienes, oxidized glutathione, malondialdehyde (MDA, detected as thiobarbituric acid reactants), and other breakdown products of cell membranes. However, these techniques have limitations. These are not specific of an oxidative stress and are at risk of artefacts. For example, isoprostanes can be produced by platelets independently from oxidant stress, and the chemical reaction of MDA detection is influenced by the iron present in the sample. Moreover, the intensity of the oxidative stress measured indirectly is also dependent on the type of ROS released. For instance, nitrated proteins will be much more influenced by the presence of peroxynitrite than by any other ROS (Halliwell and Whiteman, 2004; Lemineur *et al*., 2006).

III.1.3.1.4. Antioxidant Status

Additional approaches use the assessment of the "antioxidant status" and the "oxidant capacity". The antioxidant status evaluates the consumption of antioxidants or the changes in activity or expression of the antioxidant enzymes. It claims to evaluate the capacity of healthy humans to fight a potential oxidative stress, regardless of the type of antioxidant involved. The "total antioxidant capacity" measures the capacity of a biological sample (plasma, tissue extract) to inhibit the transformation of a selected substrate by an *in vitro* generated free radical. However, the total antioxidant capacity measurement also raises questions: the radical used in the technique does not represent the ROS that are produced *in vivo*, and the capacity of a biological sample to inhibit it can be unrelated to the *in vivo* situation. But the most important problem is the signification of this kind of technique when applied to plasma samples, in which the concentration of albumin, an excellent barrier against ROS, is high.

And finally, as there are no reference values for antioxidant status in humans, how should we interpret the changes? (Lemineur *et al*., 2006; Preiser, 2012)

III.1.3.1.5. Circulating Antioxidant Levels

The easily measurable plasma or serum levels of some antioxidant molecules have been used as biomarkers. However, the relation between these circulating levels and the magnitude of intracellular oxidative stress is not established. In addition, the level of a single molecule is unlikely to reflect the complex cascade of mechanisms implying numerous players (Lemineur *et al*., 2006)

III.1.3.1.6. Practical Issues

In addition to the limitations of each of these methods, the origin of samples and the timing are unsolved issues. As ROS react rapidly, they react *in situ*, at the site of production. For instance, if oxidative stress is suspected in the lungs, biomarkers should thus be measured ideally in bronchoalveolar lavage but not in blood. If ROS are produced in membranes, lipid derivatives should be searched (Yoshikawa and Naito, 2002; Halliwell and Whiteman, 2004; Lemineur *et al*., 2006; Preiser, 2012).

III.1.4. Antioxidant

An antioxidant can be defined as any substance that when present in low concentrations compared to that of an oxidisable substrate, significantly delays or inhibits the oxidation of that substrate. The physiological role of antioxidants, as this definition suggests, is to prevent damage to cellular components arising as a consequence of chemical reactions involving free radicals (Halliwell and Gutteridge, 1995).

III.1.4.1. Classification of Antioxidants

Antioxidants are grouped into two classes, namely,

III.1.4.1.1. Primary Antioxidants

They are the chain breaking antioxidants which react with lipid radicals and convert them into more stable products. Antioxidants of this group are mainly phenolic in structures and include the following (Hurrell, 2003):

(1) Antioxidant minerals: These are co factor of antioxidants enzymes. Their absence will definitely affect metabolism of many macromolecules such as carbohydrates. Examples include selenium, copper, iron, zinc and manganese.

(2) Antioxidant vitamins: They are needed for most body metabolic functions. They include vitamin C, vitamin E, vitamin B.

(3) Phytochemicals: These are phenolic compounds that are neither vitamins nor minerals. These include:

Flavonoids: These are phenolic compounds that give vegetables fruits, grains, seeds leaves, flowers and bark their colours. Catechins are the most active antioxidants in green and black tea and sesamol. Carotenoids are fat soluble colour in fruits and vegetables. Beta carotene, which is found in carrot, is converted to vitamin A when the body lacks it. Lycopene, high in tomatoes and zeaxantin is high in spinach and other dark greens. Herbs and spices-source include diterpene, rosmariquinone, thyme, nutmeg, clove, black pepper, ginger, garlic and curcumin and derivatives.

III.1.4.1.2. Secondary Antioxidants

These are phenolic compounds that perform the function of capturing free radicals and stopping the chain reactions, the compounds include (Hurrell, 2003):

1. Butylated hydroxyl anisole (BHA).
2. Butylated hydroxyrotoluene (BHT).
3. Propyl gallate (PG) and metal chelating agent (EDTA).
4. Tertiary butyl hydroquinone (TBHQ).
5. Nordihydro guaretic acid (NDGA)

III.2. Antimicrobial Activity

Antimicrobial activity refers to the process of killing or inhibiting the disease-causing microbes. Various antimicrobial agents are used for this purpose. Antimicrobial may be anti-bacterial, anti-fungal or antiviral. They all have different modes of action by which they act to suppress the infection. Currently, antibiotics are the invaluable weapons to fight against infectious diseases (Cheng *et al*., 2016).

III.2.1. Definition of Antibiotics

Infections caused by bacteria can be prevented, managed and treated through anti-bacterial group of compounds known as antibiotics. Antibiotics are natural, semi-synthetic or synthetic compounds that kill or inhibit the growth of bacteria (Bobbarala, 2012).

III.2.2. Classification and Mechanisms of Action

There are many ways to classify antibiotic drugs. Historically, the most used and recovered classification is based on both the chemical structures and the mechanisms of action that are linked to them. It classify on:

1- molecules that inhibit the synthesis of the bacterial cell wall, which include penicillins and cephalosporins;
2- molecules that act directly on the bacterial membrane by disturbing the permeability and that lead to the leakage of intracellular components, such as polymyxins;
3- molecules that bind to the 30S and 50S subunits of the bacterial ribosome and are responsible for reversible inhibition of protein synthesis; these bacteriostatic antibiotics include chloramphenicol, tetracyclines, macrolides;
4- antibiotic inhibitors of nucleic acids synthesis such as rifamycins or quinolones;
5- inhibitors of folate synthesis such as sulfonamides and trimethoprim (Casamajor and Descroix, 2009).

III.2.3. Natural Source for Antibiotics

III.2.3.1. Antimicrobials from Terrestrial Source (Higher Plants, Herbs, Herbal Extracts and Essential Oils)

For a long period of time, herbs and their preparations including the essential oils have been acknowledged as valuable source of natural products (lead compounds) for maintaining human health, both for prophylaxis and treatment. Most extensive studies relating to antibiotics from herbs have been reported in Indian Ayurvedic system and in Traditional Chinese System of Medicine. Yet, the higher plants, still largely seems to be unexplored for the identification of potent compounds active against microorganisms. Very few active compounds so far have been isolated and tested out of huge number of plant species (250,000 to 500,000) reported to be present in the globe, while the overall percentage of the plants so far been explored out of these huge numbers, the antimicrobials lies between 1 to 10%. Even with this low percentage, some very useful plants have been identified showing potential to

explore the possibility to use as drugs. Classical examples are present where plant extracts or plant based products have been used for some specific treatment when other resources were not available or remain unable to deliver the desired effect. For example, *Andrographis paniculata* (Kalmegh or Indian chiretta) was very successfully used to control influenza epidemic of Bengal (India) in 1919 using a tincture of *Andrographis paniculata*. During the same period it was also used to control cholera and dysentery in the Phhillippines, while China is reported to use this plant to treat fever and as anti-infective for centuries (Borris, 1996; Osman *et al.*, 2012).

III.2.3.2. Antimicrobials from Marine Organisms (Seaweeds, Sponges and Humic Acid Sediments)

Marine algae, sponge and humic acid sediments have been evaluated in different regions of the globe where coastal area is reported with rich source of some novel algal and other marine species responsible to act against wide variety of microorganisms. It is really very difficult to predict the actual number of marine species, but most researchers believe it to be around 800,000 to several millions or even. Though use of various algal species for therapeutic effect has a long history and is documented in Chinese herbal and Ayurvedic system of medicine, however no such application example (as a drug) is available in modern pharmaceutical system. At present, the algal derived products, e.g., agar, carrageenan and alginates are mainly used in the food industries and as excipients in pharmaceuticals. Some other products derived from microalgae, such as carotenoids (e.g., β-carotene and astaxanthin), and long-chain polyunsaturated fatty acid (LC-PUFAs), docosahexaenoic acid (DHA) have been commercially produced and used as nutritional supplements and nutraceuticals (Kenchington, 2002; Bouchet, 2006).

III.2.3.3. Antimicrobial Enzymes and Peptides

One of emerging strategy to overcome the resistance issue with the common pathogens is the introduction of antimicrobial enzymes and antimicrobial peptides, now brought into intense investigation with the aim to disrupt the bacterial cell and biofilm formation. The incidence relating to multiple resistance against various pathogens due to unsystematic use of antibiotics during treatment has significantly increased and the situation has perhaps, now become a global concern and challenge for the researchers to find a reasonable solution. A series of antimicrobial enzymes, such as proteolytic enzymes (e.g., subtilisins, lysostaphin,

bacteriophage lysins), polysaccharide-degrading enzymes (e.g., lysozymes, alginate lysases, dispersin B, amylases), oxidative enzymes (e.g., hydrogen peroxide–producing enzymes, hydrogen peroxide-responsive enzymes), anti-quorum sensing enzymes (e.g., acyl homoserine lactones) have now been studied and reported (Thallinger, 2013).

III.3. Anti-inflammatory Activity

III.3.1. Definition

Inflammation usually occurs when infectious microorganisms such as bacteria, viruses or fungi invade the body, reside in particular tissues and/or circulate in the blood. Inflammation may also happen in response to processes such as tissue injury, cell death, cancer, ischemia and degeneration. Mostly, both the innate immune response as well as the adaptive immune response is involved in the formation of inflammation. So, anti-inflammatory is the property of a substance or treatment that reduces inflammation or swelling (Artis and Spits, 2015).

III.3.2. Nonsteroidal Anti-inflammatory Drugs (NSAIDs)

Nonsteroidal anti-inflammatory drugs (NSAIDs) generally work by blocking the production of prostaglandins (PGs are key factors in inflammation; creating inflammation) through the inhibition of two cyclooxygenase (COX) enzymes (COX enzyme synthesizes prostaglandins) (Gunaydin and Bilge, 2018). In whole, the NSAIDs prevent the prostaglandins from ever being synthesized, reducing or eliminating the pain. Some common examples of NSAIDs are aspirin, ibuprofen, and naproxen. The newer specific COX-inhibitors are not classified together with the traditional NSAIDs even though they presumably share the same mode of action. On the other hand, there are analgesics that are commonly associated with anti-inflammatory drugs but that have no anti-inflammatory effects. An example is paracetamol (known as acetaminophen or Tylenol in the U.S). As opposed to NSAIDs, which reduce pain and inflammation by inhibiting COX enzymes, paracetamol has - as early as 2006 - been shown to block the reuptake of endocannabinoids, which only reduces pain, likely explaining why it has minimal effect on inflammation (Ottani *et al*., 2006; Dani et al.,2007).

III.3.3. Anti-inflammatory Foods

Suggested diets to reduce inflammation include those rich in vegetables and low in simple carbohydrates, and fats such as saturated fats and trans fats. Anti-inflammatory foods include most colourful fruits and vegetables, oily fish (which contain higher levels of omega-

3 fatty acids), nuts, seeds, and certain spices, such as ginger, garlic and cayenne. Extra-virgin olive oil contains the chemical oleocanthal that acts similarly to ibuprofen. Those following an anti-inflammatory diet will avoid refined oils and sugars, and show a preference for anti-inflammatory foods in their meal choices. Omega-3 fatty acids have been shown to disrupt inflammation cell signalling pathways by binding to the GPR120 receptor. This benefit however can be inhibited or even reversed if the ratio of Omega-6/Omega-3 is too high as Omega-6 serves as a precursor to inflammatory chemicals prostaglandin and leukotriene eicosanoids) in the body. A high proportion of omega-6 to omega-3 fat in the diet shifts the physiological state in the tissues toward the pathogenesis of many diseases: prothrombotic, proinflammatory and proconstrictive. Omega-6 competes with omega-3 for the same rate limiting factor which is required for the health-benefits of omega-3, directly reducing the action of omega-3 in addition to pharmacologically counteracting omega-3 benefits through its own action as a pro-inflammatory agent (Simopoulos, 2003; Wada *et al*., 2007; Willyard, 2012).

III.3.4. Secondary Metabolites as Anti-inflammatory Agents

Plant secondary metabolites have provided an important source of drugs since ancient times and now around half of the practical drugs used are derived from natural sources. Many of these herbal constituents are being prescribed widely for the treatment of inflammatory conditions. Phenolic compounds are of important pharmacological value, some having anti-inflammatory properties. Different types of phenolic compounds such as flavonoids, condensed tannins, and gallotannins are known to inhibit some molecular targets of pro-inflammatory mediators in inflammatory responses (Fawole *et al*., 2009). Coumarins represent a vast family of compounds which were naturally found in plants. It has been already reported that several coumarin derivatives have significantly antiinflammatory and antioxidant activities. Thus, coumarin derivatives could be particularly effective in the treatment of high protein oedemas. It was reported that some coumarins possessed the antioxidant capacity scavenging superoxide anion radicals and some coumarins could inhibit both the lipoxygenase and cyclooxygenase pathways of arachidonic acid metabolism (Kang *et al*., 2009). Some alkaloids such as isoquinoline, indole and diterpene are known to have good anti-inflammatory activity (Fawole *et al*., 2009). Many Plant-derived compounds are tested in humans in recent years; Fürst and Zündorf (2014) cited six very prominent plant-derived anti-inflammatory compounds: curcumin, colchicine, resveratrol, capsaicin, epigallocatechin-3-gallate (EGCG), and quercetin.

III.4. Enzyme Inhibitory Activity

An enzyme inhibitor is a molecule that binds to an enzyme and decreases its activity. Since blocking an enzyme's activity can kill a pathogen or correct a metabolic imbalance, many drugs are enzyme inhibitors (Martinek, 1969).

III.4.1. Anti-diabetic Activity

III.4.1.1. Definition

Diabetes mellitus is a chronic endocrine disorder that affects the metabolism of carbohydrates, proteins, fat, electrolytes and water. It includes a group of metabolic diseases characterized by hyperglycemia, in which blood sugar levels are elevated either because the pancreas do not produce enough insulin or cells do not respond to the produced insulin (West, 2000). Therefore a therapeutic approach to treat diabetes is to decrease postprandial hyperglycemia (Chakrabarti and Rajagopalan, 2002). This can be achieved by the inhibition of carbohydrate hydrolyzing enzymes like alpha amylase and alpha glucosidase. Alpha glucosidase and alpha amylase are the important enzymes involved in the digestion of carbohydrates. Alpha Amylase is involved in the breakdown of long chain carbohydrates and alpha glucosidase breaks down starch and disaccharides to glucose. They serve as the major digestive enzymes and help in intestinal absorption. Alpha amylase and glucosidase inhibitors are the potential targets in the development of lead compounds for the treatment of diabetes (Subramanian, 2008).

III.4.1.2. Natural Product as Anti-diabetic Agent

For a long time natural products from plants have been used for the treatment of diabetes, mainly in developing countries where the resources are limited and affordability and access to modern treatment is a problem. Extensive research has been carried out to screen the bioactivity of these inhibitors because of their significant importance in health care and medicine (Nickavar and Yousefian, 2009). Plant food rich in polyphenols have been reported to cause effects similar to insulin in the utilisation of glucose and act as good inhibitors of key enzymes like alpha amylase and alpha glucosidase associated with type 2 diabetes and lipid peroxidation in tissues. Studies have also shown that the bioactivity of polyphenols in plants is linked to their antioxidant activity and many of these plants also possess hypoglycaemic properties. Higher plants, animals and microorganisms are found to produce a large number of different protein inhibitors of alpha amylases and alpha glucosidases in order to regulate the activity of these enzymes (Choudhury, 1996). Some of these enzyme inhibitors act by directly

blocking the active centre of the enzyme at various local sites. In animals, alpha amylase inhibitors decrease the high glucose levels that can occur after a meal by slowing the speed with which alpha amylase can convert starch to simple sugars. This is of importance in diabetic people where low insulin levels prevent the fast clearing of extracellular glucose from the blood. Hence diabetics tend to have low alpha amylase levels in order to keep their glucose levels under control. Plants also use alpha amylase inhibitors as a defence mechanism as a protection from insects. These inhibitors alter the digestive action of alpha amylases and proteinases in the gut of insects and inhibit their normal feeding behaviour. Therefore alpha amylase inhibitors have potential roles in controlling blood sugar levels and crop protection. Therefore natural alpha amylase and glucosidase inhibitors from the dietary plants can be used as an effective therapy for treating post prandial hyperglycemia with minimal side effects (Mohamed *et al*., 2009).

III.4.2. Urease Inhibitory Activity

III.4.2.1. Definition

Ureases are found in numerous bacteria, fungi, algae, plants, and some invertebrates, as well as in soils, as a soil enzyme. Bacterial enzyme is of special importance since it has been demonstrated as a potent virulence factor for some species. Urease, the enzyme responsible for the rapid hydrolysis of urea to ammonia, is a key enzyme benefiting bacteria *Helicobacter pylori* through making its persistence possible in the acidic environment of the stomach (Urease activity increases the of its environment as ammonia is produced, which is basic) and as a result, cause gastrointestinal diseases, in particular gastritis, duodenal, peptic ulcer, and gastric cancer (Howson et al., 1986; Devesa *et al*., 1998). **Figure 14** explains the urease inhibitory activity.

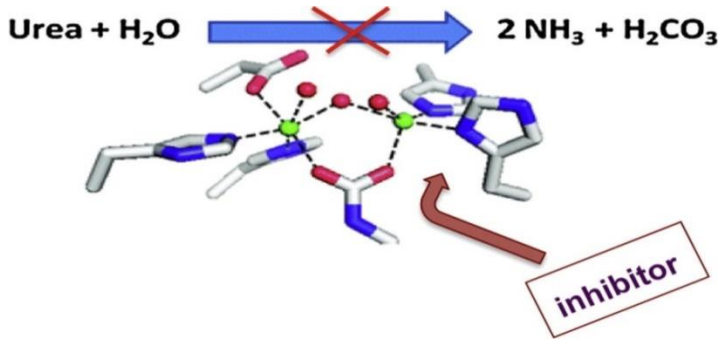


Figure 14: Graphical abstract of urease inhibitory activity (Kafarski and Talma, 2018)

It has already been proven that urease deficiency effectively risks the bacteria existence (Michetti, 1998). Additionally, urease activity leads to other disease like urinary stones and pyelonephritis. Also limiting nitrification procedure via restricting urease hydrolysis activity is also of great importance in the field of agriculture to control nitrogen leaching, greenhouse gas escape and ammonia volatilization from soil (Edmeades, 2004).

Although comprehensive studies have been performed on urease inhibition mechanisms and inhibitors until today, only a few of them are promising. According to the literature, most of the prescribed medicines and antibiotics for the mentioned disease treatment, not only evince adverse effects but also the bacteria grow resistance against. Furthermore, application of some compounds and especially synthetic ones for controlling urease function has been banned due to their toxicity and low chemical and physical stability in the natural environment. Medicinal plants have long been applied as remedies to cure diseases which nowadays are known as viral infections. Composites from *Euphorbia decipiens* (Ahmad *et al*., 2003) and sulfated polysaccharide found in different types of brown seaweed (fucoid an compounds) had been previously reported, are examples of natural substances with urease inhibition activities. While plants can be considered as the largest source of substances with pharmacological properties, their significant biological characteristics have not been investigated thoroughly. As a result, it is obvious that looking for efficient composites with natural origins to be used individually and/or as lead ones to design and develop new drugs with higher efficiency, stability and less toxicity is an important issue need to be more attention to (Limuro *et al*., 2003).

III.4.2.2. Inhibitors of Urease

III.4.2.2.1. Quinolones

Quinolone antibiotics constitute an important class of a large group of synthetic broad-spectrum antibacterial agents, which are nowadays the most successful clinically synthetic antibacterial drugs (Redgrave *et al*., 2014). They inhibit DNA synthesis. Nearly all quinolone antibiotics in modern use are fluoroquinolones. Their two popular representatives – Levofloxacin and Ciprofloxacin , as well as their analogues, appeared to be quite promising inhibitors of *Helicobacter pylori* and *Proteus mirabilis* enzymes (Abdullah *et al*., 2016).

III.4.2.2.2. Flavonoids

It is well known that structural diversity and complexity within natural products stimulates research on their use as lead compounds for various diseases. Extracts of various plants, including green tea and cranberries often have been used to treat gastritis or urinary tract infections. This effect is believed to result from the action of catechin and epigallocatechin gallate as urease inhibitors (Loes, 2013). Also flavonoids isolated from other plants: *Daphne retusa* (daphnretusic acid), *Pistacia atlantica* (transilitin and dihydro luteolin) and cotton (gossypol, gossypolone and apogossypol) appeared to be micromolar inhibitors of urease (Chen *et al*., 2015).

III.4.2.2.3. Other Natural Products

Natural products (mostly secondary metabolites) have been the most successful source of potential drug leads so far. Even if these efforts somewhat decline in interest they continue to provide unique structural diversity of potential enzyme inhibitors. This is also the case if considering research on urease. Representative examples of natural products of recently determined inhibitory action against urease are: boswellic acid a component of African medicinal plant *Boswellia arterii*, palmatine and epiberberine from *Coptis chinensis*, a plant traditionally used in China for the treatment of gastrointestinal diseases, andrographolide, the major diterpenoid lactone and the primary effective constituent of Chinese medicinal plant *Andrographis aniculata* and a popular antibiotic from garlic– allicin ((Kafarski and Talma, 2018).

III.4.3. Tyrosinase Inhibitory Activity

III.4.3.1. Definition

The increase in production and accumulation of melanin are the cause of a large number of skin ailments, e.g. acquired hyperpigmentation such as melisma, postinflammatory melanoderma, solar lentigo, etc, and it is an esthetic problem in humans (Briganti and Camera, 2003).Tyrosinase is the main enzyme recognized as responsible for this enzymatic browning and melanogenesis in mammals (Dembitsky and Kilimnik, 2016). Pharmacologically, melanogenesis can be controlled by inhibiting the activity of tyrosinase or other related melanogenic enzymes. Among melanogenic enzymes, tyrosinase is the rate-limiting enzyme for controlling the production of melanin. The use of tyrosinase inhibitors is

the most promising method for melanogenesis inhibition. Tyrosinase inhibitors specifically interact with melanogenic cells and do not lead to side effects compared with other melanogenesis inhibitors (Wang *et al*., 2017).

III.4.3.2. Tyrosinase Inhibitors

III.4.3.2.1.Tyrosinase Inhibitors Drugs

Since tyrosinase is a crucial enzyme in synthesizing melanin through melanogenesis, it becomes the most prominent and successful target for melanogenesis inhibitors that directly inhibit the tyrosinase catalytic activity. Most of cosmetics or skin lightening agents commercially available are tyrosinase inhibitors. The fact that the inhibitors target tyrosinase may specifically inhibit the melanogenesis in cells without side effects, as tyrosinase is exclusively produced only by melanocytes. Many tyrosinase inhibitors such as hydroquinone (HQ) arbutin, kojic acid, azelaic acid, *L*-ascorbic acid, ellagic acid and tranexamic acid have been used as skin-whitening agents, with certain drawbacks (Pillaiyar *et al*., 2017). **Figure 15** represents the structure of some well-known tyrosinase inhibitors.

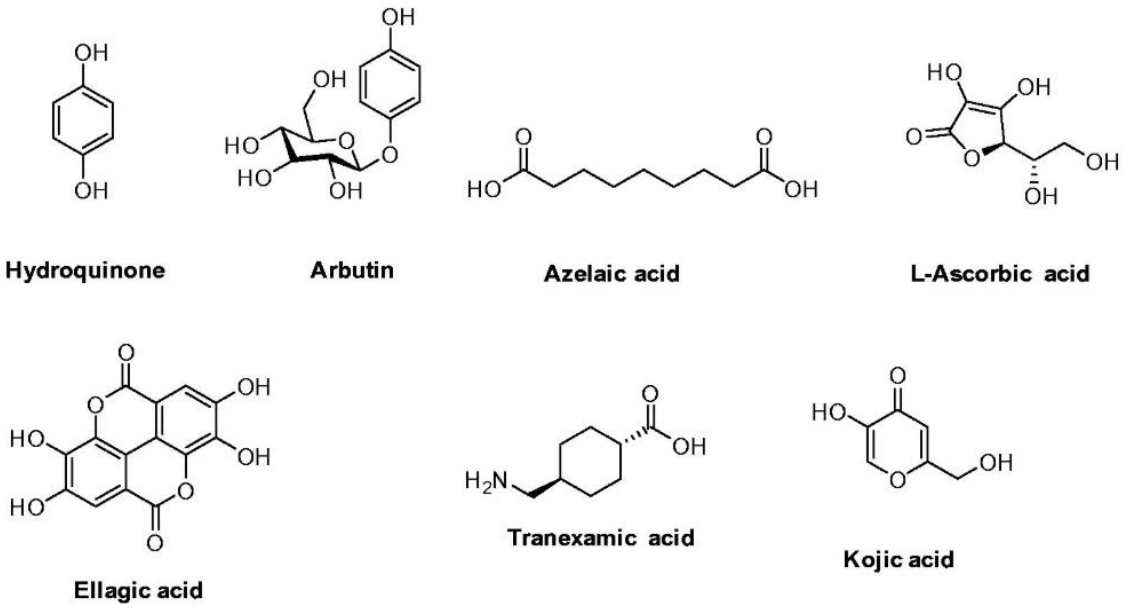


Figure 15: Chemical structure of well-known tyrosinase inhibitors as skin lightening agents

III.4.3.2.2. Natural Tyrosinase Inhibitor Sources

Natural sources; including plants, bacteria and fungi; have recently become of increasing interest for their anti-tyrosinase activity by producing bioactive compounds. A number of

researchers prefer to identify inhibitors from natural sources due to their lower toxicity and better bioavailability, especially for food, cosmetic and medicinal applications.

Plants

It is well known that phenolic compounds are the largest group of phytochemicals found in plants, which are mainly the factors responsible for the activities in plant extracts (Chang, 2009). Tyrosinase inhibitory activity of many plant extracts was investigated to find new sources of anti-tyrosinase compounds. For example, anti-tyrosinase activities of the following plants have been reported by various researchers: *Asphodelus microcarpus*, *Morus nigra* L, *Greyia radlkoferi Szyszyl*, *Limonium tetragonum*, *Arctostaphylos uva-ursi*, *Pleurotus ferulae*, *Agastache rugosa* Kuntze fermented with *Lactobacillus rhamnosus* and *Lactobacillus paracasei*, *Artemisia aucheri* Boiss, *Cassia tora*, *S. brevibracteata* subsp (Samaneh *et al.*, 2019).

Fungi and Bacteria

Fungi from different genera such *Aspergillus* sp., *Trichoderma* sp., *Paecilomyces* sp., *Phellinus linteus*, *Daedalea dickinsii*, *Dictyophora indusiata* along with a liquid culture of *Neolentinus lepideus* have been reported as a source of novel tyrosinase inhibitors by producing bioactive compounds. Also, there have been several reports on tyrosinase inhibitors from some marine fungi species such as *Myrothecium* sp. isolated from algae and *Pestalotiopsis* sp. Z233 (Wu et al., 2013; Li *et al.*, 2005; Ishihara *et al.*, 2018).

In addition, there are several reports on tyrosinase inhibition by bacterial species and their metabolites. Among them, *Streptomyces* sp., such as *S. hiroshimensis* TI-C3 isolated from soil, an actinobacterium named *Streptomyces swartbergensis* sp. and *Streptomyces roseolilacinus* NBRC 12815 are potential bacterial sources of tyrosinase inhibitors. Interestingly, some probiotics such as *Lactobacillus* sp. which are used in the fermentation process have been investigated as natural tyrosinase inhibitor sources (Chang *et al.*, 2008; Nakashima *et al.*, 2009; Ji *et al.*, 2018; le Roes-Hill *et al.*, 2018).

III.4.4. Anticholinesterase Activity

III.4.4.1. Definition

Alzheimer's disease (AD) is a chronic neurodegenerative disease that usually starts slowly and gradually worsens over time. It is the cause of 60–70% of cases of dementia. The most common early symptom is difficulty in remembering recent events. As the disease advances, symptoms can include problems with language, disorientation (including easily getting lost), mood swings, loss of motivation, not managing self-care, and behavioural issues (Burns and Iliffe, 2009).

According to the cholinergic hypothesis, the memory impairment in Alzheimer's disease results from the dysfunctions in the central cholinergic neurotransmission of acetylcholine. Cholinesterase inhibitors have been reported clinically to be effective in the first line of treatment for AD. The only present treatment of this disease is the use of acetyl- and butyryl-cholinesterase inhibitors (Mukherjee and Houghton, 2009).

III.4.4.2. Cholinesterase Inhibitors

III.4.4.2.1. Drugs

Cholinesterase (ChE) inhibitors are the mainstay of drug treatment for Alzheimer's disease (Cholinesterase inhibitors (for moderate disease) are: Donepezil 5-10 mg, Rivastigmine 6-12 mg and Galantamine 8-24 mg) (Burns and O'Brien, 2006). Cochrane reviews show that cholinesterase inhibitors have a moderate but worthwhile symptom modifying effect in a substantial minority of people with Alzheimer's disease and are generally well tolerated (Schneider, 2005).

III.4.4.2.2. Natural Sources

Several reviews on the newly discovered Acetylcholinesterase inhibitor (AChEi) obtained from plants, fungus and marine organisms have also been published over the last years. The majority of these AChEi belong to the alkaloid group, including indole, isoquinoline, quinolizidine, piperidine and steroidal alkaloids. On the other hand, several non-alkaloidal and potent AChEi have been obtained from natural sources, including terpenoids, flavonoids and other phenolic compounds (Murray *et al*., 2013).

Alkaloids with AChE Inhibitory Activity

Physostigmine (eserine) is an alkaloid with a pyrroloindole skeleton from *Physostigma venenosum* Balf. (*Leguminosae*) seeds that is a potent, short-acting and reversible inhibitor of AChE (Kamal *et al.*, 2000). Rutaecarpine and dehydroevodiamine, indole alkaloids from *Evodia rutaecarpa* (Juss.) Benth. (*Rutaceae*), have been used as templates to synthesise new AChE inhibitors, since the plant extract and dehydroevodiamine inhibit AChE *in vitro* and reverse scopolamine-induced memory impairment *in vivo* (Park *et al.*, 1996). Isoquinoline alkaloids from *Colchicum speciosum* Steven (Colchicaceae) corms are reversible inhibitors of both Acetyl- and butyrylcholinesterase enzymes (AChE and BChE) *in vitro*, and several benzylisoquinoline alkaloids from *Coptis* (Ranunculaceae) and *Corydalis* (Papaveraceae) species inhibit AChE (Kim *et al.*, 2002). Several quinoline and b-carboline alkaloids, including two new alkaloids (nigellastrines I and II) from the seeds of *Peganum nigellastrum* Bunge (*Zygophyllaceae*) showed AChE inhibitory activity in a TLC bioautographic assay, with results suggesting harmine, harmaline, harmol and harman showing similar activity to galantamine, although more quantitative data is needed. Although a number of AChE inhibitory alkaloids reported from *Lycopodium* species are structurally related to the quinolizidines, cryptadines A and B from *Cryptomerianum maxim* consist of a piperidine ring and two octahydroquinoline rings. These AChE inhibitors closely resemble lycoperine A, an AChE inhibitor from *Hamiltonii Spreng* (Hirasawa *et al.*, 2008). In addition, the following steroidal alkaloids: conessine, isoconessimine, conessimin, conarrhimin and conimin were isolated in a bioassay-guided fractionation from the seeds of *Holarrhena antidysenterica* (Apocynaceae) and were identified as active constituents against AChE (Yang *et al.*, 2012).

Non-Alkaloidal Compounds with AChE Inhibitory Activity

In spite of the fact that the majority of the most potent inhibitors known to date are alkaloids, several non-alkaloidal AChEi from the plant kingdom and with different structural characteristics (terpenoids, sterols, flavonoids and phenolic compounds, etc.) have been recognized as promising lead compounds as anti-Alzheimer's disease agents (Murray *et al.*, 2013).

Numerous essential oils (or oil absolutes) have shown inhibitory activity against cholinesterase enzyme ChE, including those from *Narcissus poeticus* L. (Amaryllidaceae), *Melaleuca* species (Myrtaceae), *Acorus calamus* L. (Acoraceae), *Eucalyptus camaldulensis*

Dehnh., *Marlierea racemosa* Kiaersk. (Myrtaceae), *Cymbopogon schoenanthus* Spreng. (Poaceae) and several oils from the Lamiaceae. Many constituents of these oils have been identified as AChE inhibitors including monoterpenoids (e.g. geranial, neral and linalool) and sesquiterpenoids (e.g. caryophyllene oxide, ar-tumerone) and some phenylpropanoids (e.g. eugenol, α- and ß-asarone) (Williams *et al.*, 2011).

From the methanolic extract of *Haloxylon recurvum* (Chenopodiaceae), a plant used in Pakistan for the treatment of several neuronal disorders, four new C-24 alkylated sterols and five known sterols were isolated, analyzed as AChEi and were found to inhibit AChE in a concentration-dependent manner acting as non-competitive inhibitors (Ahmed *et al*, 2006).

The flavonols present in *Sophora flavescens* (Fabaceae) were studied for several biological activities relevant for AD. Sophoflavescenol, icaritin, demethylanhydro-icaritin, 8-C-lavanduryl kaempferol and kaempferol were all found to be good AChE inhibitors and also significant BChE inhibition (Jung, 2011).

In several studies published during the period covered in the present review, various phenolic compounds with different structural characteristics were reported as AChEi. Some of them are structurally simple such as gallic acid and ellagic acid (Nag and De, 2011). Hopeahainol A, which was identified as a new compound isolated from *Hopea hainensis*, was observed to elicit a notable AChE inhibition with respect to huperzine A as a reversible mixed-type inhibitor (Ge *et al.*, 2008).

III.5. SPF and UV Protection Factor (UV-PF) Assessment

III.5.1. Definition

Light photons irradiating the earth consist of 56% of infrared light photons (wavelength 780–5000 nm) and 39% of visible light (400–780 nm). Ultraviolet radiations (UVR) are the 5% of the light photons irradiating the Earth. The UVR reaching the Earth's surface are UVB and UVA. UVB (290–320 nm) contributes for about 5% while UVA (320–400 nm) for about 95%. UVC (100–280 nm) are totally absorbed by atmospheric ozone. Sun is the main source of UVR, but artificial sources of UVR have been developed in the last decades. Skin is the organ most affected by environmental sunlight. Interaction between UVR and skin involves mutagenic lesions as well as indirect genotoxic effects mediated by oxidative stress. It is well known that UVR can damage many skin molecules and structures, including DNA (Cadet *et al.*, 2005). UVR can modify purines or pyrimidines, can disrupt the link between genes, or can even delete parts of the genome. All these damages are usually reversible, thanks to the

DNA-repair mechanisms. Unfortunately, sometimes the repair mechanism fails and inability to further read and transcribe can occur, leading to cell death or abnormal behavior like hyperproliferation or malignant transformation (Marrot and Meunier, 2008).

Photoprotection from UV damage is an essential prophylactic and therapeutic element, consisting of clothing and glasses and topical sunscreens or systemic agents. Topical sunscreens contain molecules or molecular complexes that can absorb, reflect, or scatter UV photons. Evaluation of the efficacy of sunscreen products has been made through the Sun Protection Factor (SPF), a mean of quantitatively assessing the degree of protection offered by sunscreen products against solar radiation. The SPF value offers no clear indication of the degree of protection against UVA1 (340–400 nm). It is based on an *in vivo* test that measures protection against sunburn or erythema, a biological response produced primarily by UVA2 (320–340 nm) and UVB (290–320 nm) (Colipa, 2006).

III.5.2. Sunscreen Products

III.5.2.1. Physical Sunblocks

There are two types of physical sunblocks that are mostly used: Zinc oxide and titanium dioxide. Both provide broad-spectrum UVA and UVB protection. They are gentle enough for everyday use, especially for individuals with sensitive skin and for children, because they rarely cause skin irritation. But, because of scattering effect, they often cause the so called "whitening" phenomenon when they are applied on the skin, which seriously affects the aesthetics and the efficacy of sunscreen products (Shao and Schlossman, 1999; Freund, 2010).

III.5.2.2.Chemical Sunblocks

Most chemicals only block narrow region of the UV spectrum. Therefore, most chemical sunblocks are composed of several chemicals with each one blocking a different region of UV light. Mostly, chemicals used in sunblocks are active in UVB region. Only a few chemicals block the UVA region. The best sunblock is the sunblock that combine both chemical and physical active ingredients. Dermatologists routinely recommend sunblocks that contain either a physical blocking agent or avobenzone (Parsol®1789) in combination with other chemicals. However, in the USA, combinations of avobenzone and physical sunscreens are not permitted. Avobenzone has been reported to be unstable when contained in formulations

with physical sunscreens. Surface coating of pigment has sometimes been shown to increase its stability (Nguyen and Schlossman, 2010).

III.5.2.3. Natural Sunblocks

The skin's natural sunblocks are proteins (the peptide bonds), absorbing lipids, and nucleotides. The high concentration of plant peptides protects the peptide bonds of the skin proteins. The high level of squalane (from olive oil) in some products protects the skin's sensitive lipids. Squalene is the skin's most important protective lipid. Allantoin is a nucleotide that naturally occurs in the body and absorbs the spectrum of UV radiation which damages the cell's fragile DNA. Allantoin is an extract of the comfrey plant and is used for its healing, soothing, and anti-irritating properties. This extract can be found in antiacne products, sun care products, and clarifying lotions because of its ability to help heal minor wounds and promote healthy skin. Some clinical studies confirm that allantoin enhance skin repair (Radava *et al*., 2011).

SECOND PART: EXPERIMENTAL STUDY

CHAPTER I: MATERIALS AND METHODS

I. Materials and Methods

I.1. Plant Material

The aerial parts of *Scrophularia tenuipes* Coss & Durieu. were collected during the flowering stage in June 2015 in Texanna/Jijel, Algeria. The plant was identified by the botanist Dr. Sebti from the Laboratory of Biotechnology Environment and Health, University of Jijel. While, the aerial parts of *Oenanthe virgata* Poiret. were collected during the flowering stage in March 2015 in Taher/Jijel, Algeria. This plant was identified by the botanist Dr. Bouldjedri, from the Laboratory of Biotechnology Environment and Health, University of Jijel.

The aerial parts of the two plants were dried at room temperature in the absence of direct sun light and then powdered by grinding.

I.2. Phytochemical Study

Table 8 represents the various phytochemical studies carried out on *S.tenuipes* and *O. virgata*:

Table 8: Phytochemical studies carried out on *S. tenuipes* and *O. virgata*.

Phytochemical Study	*S. tenuipes*	*O. virgata*
Extraction of Essential Oil	✓	X
Extraction of Flavonoids	✓	✓
Total Flavonoid Content	✓	✓
Total Polyphenol Content	✓	✓
Total Carotenoid Content	✓	✓
Polyamide Column Chromatography	✓	X
NMR	✓	X
LCMS-MS	✓	✓
GCMS	✓	X

I.2.1. Extraction of Active Substances

I.2.1.1. Extraction of Flavonoids

The powdered plant material (500 g for both plants) was macerated for 24 h with 80% methanol solution (500 mL) and the extraction procedure was repeated for two more times with solvent replacement. The hydro-alcoholic mixture from the extractions was combined, followed by concentration under reduced pressure to dryness. The obtained residue was dissolved in hot distilled water (500 mL). After filtration, the solution was partitioned with dichloromethane (1:1) for three times, ethyl acetate (1:1) for three times, and with *n*-butanol (1:1), also for three times (**figure 16**). The resulting ethyl acetate and *n*-butanol extracted solutions were evaporated to dryness, giving rise to the respective dried extracts (EA and Bu, respectively) (Akowuah *et al.* 2005).

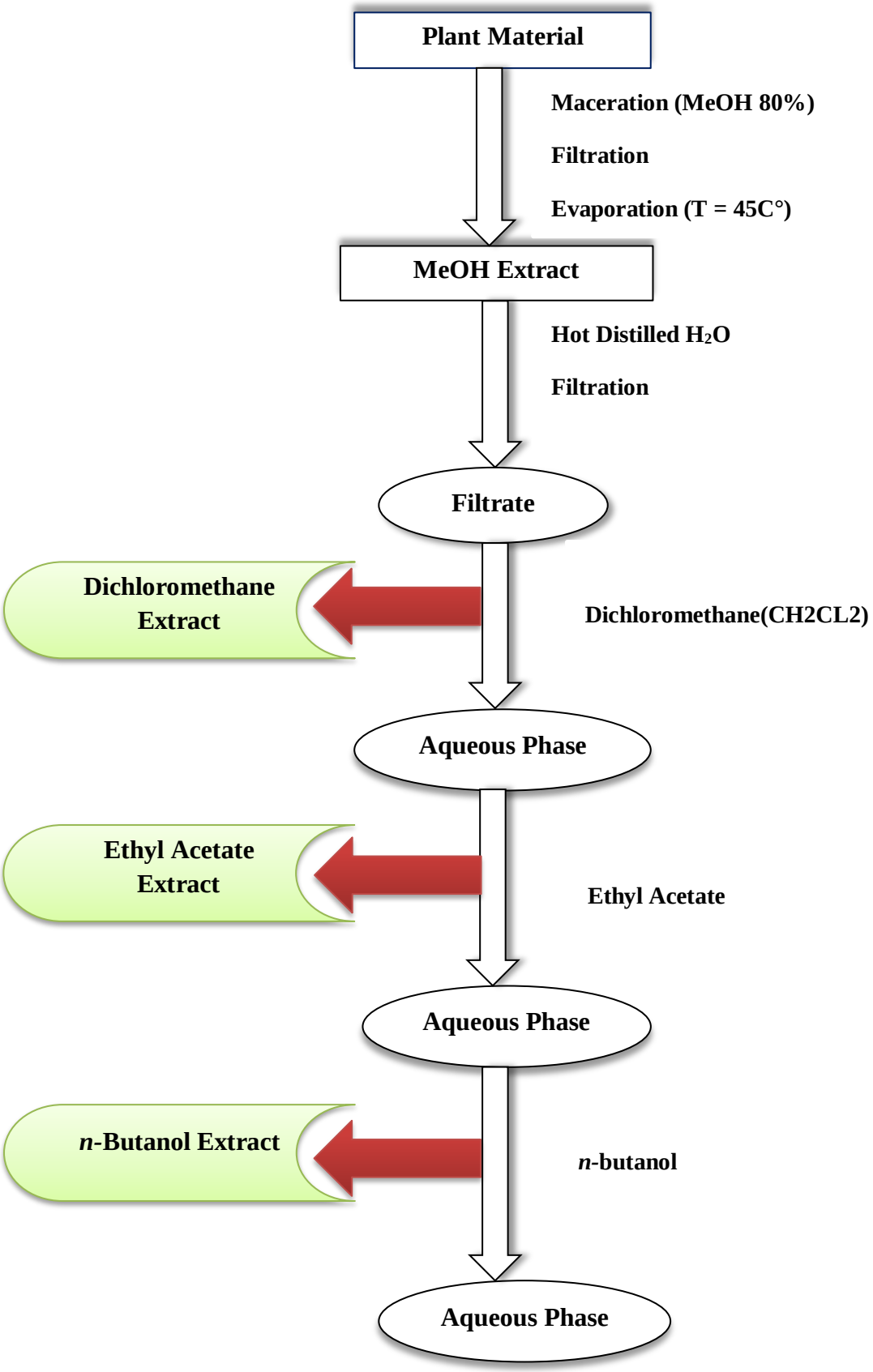


Figure 16: Protocol of Flavonoid's Extraction.

I.2.1.2. Extraction of Essential Oils

Extraction of the essential oils is carried out by hydrodistillation of the aerial part of *S.tenuipes*, where 100g of the powdered plant is introduced into a balloon with two necks, and impregnated with water; the whole is boiled for 2 hours. The water vapour loaded with essential oils, when crossing the refrigerant condense and fall into a separating funnel, water and oil separate by density difference (Ciulei, 1983).

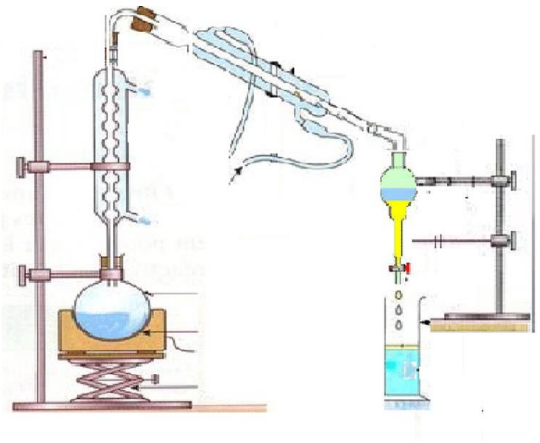


Figure 17: Hydrodistillation clevenger apparatus.

Calculation of essential oil yield: the ratio between the weight of the extracted essential oil and the weight of the plant to be treated. The percentage yield (Y) is calculated by the following formula:

Y = (WO x 100) / WP where

WO: weight of the essential **o**il extracted (g)

WP: weight of the plant treated (g)

I.2.2. Spectrophotometric Determination of some Secondary Metabolites

I.2.2.1. Total Phenolic Content

Total phenolic compounds of EA and Bu extracts of *S. tenuipes* and *O. virgata* were quantified by the method described by Müller *et al.* (2010) with slight modification. Briefly, 20 µL of the extract (or gallic acid), 100 µL of Folin-Ciocalteu reagent (FCR) (diluted 1:10 ratio with distilled water) and 75 µL of sodium carbonate were added to a 96-well microplate. The absorbance was measured at 765 nm after 2 h in a microplate reader (EnSpire Multimode Plate Reader perkin, USA). The total phenolic content was expressed as mg of gallic acid

equivalents (GAE) per g of extract. The amount was calculated from the regression equation of calibration.

I.2.2.2. Total Flavonoid Content

Estimation of total flavonoid concentration of EA and Bu extracts of *S.tenuipes* and *O.virgata* was based on aluminium nitrate method described by Park *et al.* (1997) with some modification. Briefly, 50 µL of plant extract (or quercetin), 130 µL MeOH, 10 µL of 1 M potassium acetate and 10 µl of 10% aluminium nitrate were mixed in each well of a microplate. Upon 40 min, the absorvance was read in a microplate reader. The total flavonoid content was expressed as mg of quercetin equivalents (QE) per g of extract. The amount was calculated from the regression equation of calibration curve.

I.2.2.3. Total Carotenoid Content

Carotenoids were extracted according to Sass-Kiss *et al* (2005) with slight modification. About 0.5 g of powdered plants (*S. tenuipes* and *O. virgata*) were weighted and extracted in dark with 20 ml n-hexane: acetone: ethanol (2:1:1) under agitating at 170 rpm for 30 min. The upper layer was filtered and collected by 10 ml of hexane for a second extraction. The two phases were brought together, and then the absorbance was measured at 450 nm for the determination of total carotenoids by microplate reader. The total carotenoids content were estimated by referring to regression equation of calibration curve using mother solution of β-carotene (0.1 g / mL) and the results are expressed as mg of β-carotene equivalents per g of extract.

I.2.3. Chromatographic Analyses

I.2.3.1. Column Chromatography (Separation and Purification)

The *n*-butanol extract (Bu) of *S.tenuipes* was fractionated firstly on a column of polyamide gel by a solid deposit. The elution was carried out using a mixture (toluene-methanol) with increasing polarities and a fractionation every 150 mL. The collected fractions were grouped according to the similarity of their thin layer chromatographic profile and the plates were visualized under a UV lamp (254 and 365 nm). The obtained results are grouped in **table 9**.

Table 9: Fractionation of *n*-butanol extract of *S. tenuipes*.

Fractions	Grouping of Fractions	System of Elution	
		% Toluene	% Methanol
1-3	1-50 →F1	100	0
4-12	51-60→F2	98	2
13-21	61 →F3	96	4
22-30	62-68→F4	94	6
31-39	69→ F5	92	8
40-48	70-75→F6	90	10
49-57	76→F7	85	15
58-66	77-81→F8	80	20
67-74	82→F9	70	30
75-78	83-85→F10	60	40
79-90	86-94→F11	50	50
91-93	95-99→F12	30	70
94-99		0	100

The fraction F5 (code in NMR: Z.F.69) seems to be pure, so they are subjected to NMR analysis.

I.2.3.2. Gas Chromatography/Mass Spectrometry (GC/MS) Analysis

The essential oil obtained from the aerial parts of *S. tenuipes* was analysed using a Perkin-Elmer GC-MS system. Gas chromatograph equipped with an automatic liquid sampler coupled to a mass selective detector in the electron impact mode (Ionization energy: 70 eV). GC analysis was performed on HP-5MS 5% phenylmethylsiloxane capillary column (30 m 0.25 mm, i.d., 0.25 μm film thickness) equipped with a flame ionization detector (FID). The oven temperature was set at 60°C for 2 min initially, followed by an increase of 4°C/min to 240°C in a 59 min total run and a solvent delay of 3 min was selected. Injector and detector temperatures were set at 210°C and 280°C, respectively. The sample was injected using a volume of 1 μl and used operating in the split mode injection (1:100). Helium was used as a carrier gas at a constant pressure mode (flow rate: 1 ml/min). All mass spectra were recorded in the scan mode at 70 eV (40–550 m/z). Repeatability was verified by analysing the sample three times and retention indices were calculated for all

components using a homologous series of n-alkanes injected in conditions equal to the sample one. The fragmentation patterns of mass spectra were also compared with those stored in the spectrometer database, spectrometric electronic libraries (WILEY, NIST)

I.2.3.4. UHPLC-ESI-DAD-MS^n Analysis

The analysis of EA and Bu extracts of *S. tenuipes* and *O. virgata* were performed in an Ultimate 3000 (Dionex Co., USA) apparatus equipped with an ultimate 3000 Diode Array Detector (Dionex Co., USA) and coupled to a mass spectrometer, following the general procedure previously described (Beder-Belkhiria *et al*., 2018). The chromatographic apparatus consisted of a quaternary pump, an autosampler, a photodiode-array detector and an automatic thermostatic column compartment. The column used was a 100 mm length, 2.1 mm i.d., 1.9 µm particle diameter, end-capped Hypersil Gold C18 column (Thermo Scientific, USA), and its temperature was adjusted to 30 ºC. The mobile phase was composed of (A) 0.1% (v/v) formic acid and (B) acetonitrile. Gradient elution was carried out with a mixture of 0.1% (v/v) of formic acid in water (solvent A). The solvent gradient consisted of a series of linear gradients, starting from 15-28% of solvent B over 5.6 min, increasing to 29% at 8.8 min, 100% of solvent B at 13.1 min and keeping up to 17 min, followed by the return to the initial conditions, with total running time of 20 min. The flow rate used was 0.2 mLmin^{-1} and UV–Vis spectral data for all peaks were accumulated in the range 200–600 nm. The mass spectrometer used was a Thermo LTQ XL (Thermo Scientific, USA) ion trap MS equipped with an ESI source. Control and data acquisition were carried out with the Thermo X calibur Qual Browser data system (Thermo Scientific, USA). Nitrogen above 99% purity was used and the gas pressure was 520 kPa (75 psi). The instrument was operated in the negative-ion mode with ESI needle voltage set at 5.00 kV and an ESI capillary temperature of 275 ºC. The full scan covered the mass range from m/z 100 to 2000. CID-MS/MS and MS^n experiments were simultaneously acquired for precursor ions using helium as the collision gas with collision energy of 25–35 arbitrary units.

I.3. Biological Activities

Many biological activities were carried out on EA and Bu extracts of *S. tenuipes* and *O. virgata* as shown in **table 10** below summarises it.

Table 10: Biological activities carried out on EA and Bu extracts of *S. tenuipes* and *O. virgate*.

Biological Activities	***Test Types***	***S. tenuipes***	***O. virgata***
Anti-oxidant Activity	DPPH free radical scavenging	✓	✓
	ABTS cation radical decolorization	✓	✓
	Cupric reducing antioxidant capacity	✓	✓
	Superoxide radical scavenging	✓	✓
	β-carotene bleaching	✓	✓
Antimicrobial Activity	Antibacterial	✓	✓
Anti-inflammatory Activity	Carrageenan-induced rat paw edema	✓	✓
	Xylene-induced ear edema	✓	✓
	Albumin denaturation	✓	✓
Anti-diabetic Activity	α-Amylase	✓	✓
	α-Glucosidase	✓	✓
Urease Inhibitory Activity	Urease inhibitory activity	✓	✓
Tyrosinase Inhibitory Activity	Tyrosinase inhibitory activity	✓	✓
Anticholinesterase Activity	Acetyl-cholinesterase inhibitory activity	✓	✓
	butyryl-cholinesterase inhibitory activity		
SPF and UV Protection Factor(UV-PF) Assessment	SPF and UV protection factor (UV-PF) assessment	✓	✓

I.3.1. Determination of Antioxidant Activity

I.3.1.1. DPPH Free Radical Scavenging Assay

The scavenging activity of EA and Bu extracts of *S. tenuipes* and *O. virgata* was determined spectrophotometrically by the 2, 2 diphenyl-1-picryhydrazyl radical ($DPPH^{\bullet}$) assay described by Blois (1958), with slight modification. Firstly, 6 mg of DPPH was dissolved in a volume of 100 ml of methanol and kept away from light. After that, 160 µL of prepared DPPH methanolic solution and 40 µL of sample solutions, dissolved in methanol, at different concentrations were mixed in a 96-well microplate. The plate was then incubated in dark at room temperature for 30 min and the absorbance of the reaction mixture was measured at 517 nm by a 96-well microplate reader. The scavenging capability of $DPPH^{\bullet}$ was

calculated using the following equation and the results were given as IC_{50} value (µg/mL) which is the concentration of 50% inhibition:

$$\text{DPPH radical scavenging (\%)} = A_{control} - A_{sample} / A_{control} \times 100$$

Where $A_{control}$ is the absorbance of reaction mixture in the presence of negative control (blank sample) and A_{sample} is the absorbance of reaction mixture in the presence of sample or standard (positive control). BHA and BHT were used as antioxidant standards.

I.3.1.2. ABTS Cation Radical Decolorization Assay

ABTS radical scavenging activity of EA and Bu extracts of *S.tenuipes* and *O.virgata* was determined according to the method described by Re *et al.* (1999) with slight modifications. The pre-formed radical monocation of 2,29-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) was produced by the reaction between 7 mM ABTS in H_2O and 2.45 mM potassium persulfate, kept away from light at room temperature for 12 h. Before usage, the absorbance of the solution obtained was adjusted by methanol to 0.700 ± 0.020 at 734 nm. After that, 160 µL of $ABTS^{\bullet+}$ solution was mixed with 40 µL of plant extract at different concentrations and the absorbance was measured at 734 nm after 10 min by a 96-well microplate reader. The ABTS radical scavenging activity was calculated using the following equation and the results were given as IC_{50} value (µg/mL), which is the concentration of 50% inhibition:

$$\text{ABTS radical scavenging (\%)} = A_{control} - A_{sample} / A_{control} \times 100$$

Where $A_{control}$ is the absorbance of reaction mixture in the presence of negative control (blank sample) and A_{sample} is the absorbance of reaction mixture in the presence of sample or standard (positive control). BHA and BHT were used as antioxidant standards.

I.3.1.3. Cupric Reducing Antioxidant Capacity (CUPRAC)

Cupric reducing antioxidant capacity of EA and Bu extracts of *S. tenuipes* and *O. virgata* was determined according to the method described by Apak *et al.* (2004) with slight modifications. 50 µL of 10 mM cupric chloride, 50 µL of 7.5 mM neocuprine, and 60 µL of ammonium acetate buffer (1 mol L-1, pH 7.0) solutions were added to each well (in a 96 well plate) containing 50 µL of extract at different concentrations. These mixtures were incubated for 1 hour at room temperature and measured against blank at 450 nm using a 96-well

microplate reader. Results were given as absorbance ($A_{0.5}$ µg/mL) comparing to those of BHA and BHT used as antioxidant standards.

I.3.1.4. Superoxide Radical Scavenging Activity

Superoxide radical of EA and Bu extracts of *S.tenuipes* and *O.virgata* was accessed by the alkaline DMSO method, using alkaline DMSO as the generating system (Elizabeth and Rao, 1990). Briefly, 130 µL of alkaline DMSO (20 mg NaOH dissolved in 100 mL of DMSO), 30 µl of NBT (1 mg/mL) and 40 µL of extract at different concentrations were added in a 96-well microplate. The absorbance was measured at 560 nm after 2 min in a 96-well microplate reader. The antioxidant activity was calculated using the following equation and the results were given as IC_{50} value (µg/mL):

$$\text{Superoxide scavenging (\%)} = A_{control} - A_{sample} / A_{control} \times 100$$

Where $A_{control}$ is the absorbance of reaction mixture in the presence of negative control (blank sample) and A_{sample} is the absorbance of reaction mixture in the presence of sample or standard (positive control). Tanic acid and α-tocopherol were used as antioxidant standards.

I.3.1.5. β-Carotene Bleaching Assay (BCBA)

The BCBA of EA and Bu extracts of *S. tenuipes* and *O. virgata* was performed based on the method of Marco (1968) with some modifications. Briefly, chloroform (1mL) was mixed with β-carotene (0.5 mg), Tween-40 (200µL), oxiginated water (50µL) and linoleic acid (25 µL). 160µL of the mixture was incubated with 40 µL of extract at different concentrations or standards (BHA and BHT) for 120 min at 50 C and the absorbance was read at 470 nm at time 0 and every 30 min thereafter in a 96-well microplate reader. The results were expressed as % β-carotene bleaching inhibition and calculated by the following equation:

$$\text{β-carotene bleaching inhibition (\%)} = [1 - (A_{sample\,(0)} - A_{sample\,(t)}) / (A_{control\,(0)} - A_{control\,(t)})] \times 100$$

Where $A_{sample\,(0)}$: value of the absorbance of β-carotene in the presence of the extract measured at t = 0;

$A_{control\,(0)}$: absorbance of reaction mixture in the presence of negative control measured at t = 0;

$A_{sample\,(t)}$: absorbance of reaction mixture in the presence of the sample measured at t = 120 min

$A_{control\ (t)}$: absorbance of reaction mixture in the presence of negative control measured at t = 120 min

I.3.2. Antibacterial Activity

The anti-bacterial test of EA and Bu extracts of *S. tenuipes* and *O. virgata* was carried out using the disk diffusion method against four human pathogenic bacteria, including Gram positive and Gram-negative bacteria. The bacterial strains were first grown on Muller Hinton medium (MHI) at 37 °C for 24 h prior to seeding onto the nutrient agar. A sterile 6-mm-diameter filter disk (Whatman 8) was placed on the infusion agar seeded with bacteria, and each extract (100 mg/mL) suspended in DMSO 10% was dropped onto each paper disk (40 µL per disk). Furthermore, a sterile filter disk was dropped by just DMSO 10%, used as negative control.The treated Petri dishes were kept at 4 °C for 1 h, and incubated at 37 °C for 24 h. The antibacterial activity was assessed by measuring the zone of growth inhibition surrounding the disks. Each experiment was carried out in triplicate (Singh *et al.*, 2015).

I.3.3. Assessment of Anti-inflammatory Activity

I.3.3.1. Carrageenan-induced Rat Paw Edema

The acute anti-inflammatory effect of EA and Bu extracts of *S. tenuipes* and *O. virgata* was evaluated by carrageenan induced rat paw edema. Four groups of 6 rats (female Wistar rats (180-200 g) each were treated by oral administration with EA and Bu extracts (200 mg/kg, po) (EA and Bu were dissolved in DMSO 10%), the standard diclofenac drug (Dic, 10 mg/kg, po), or vehicle for the control group (isotonic NaCl solution 0.9 %, po). One hour after, edema was induced to the rats by injecting carrageenan 0.1 mL 1% into the subplantar tissue of the right hind paw. Paw volume was measured using a plethysmometer before administering the inflammatory agent and 1, 2 and 3 h after induction of inflammation. Increase of paw volume was measured as the difference in paw volume at the beginning of the experiment, before inducing edema (0 hour) and paw volume at the respective hours. The percent inhibition of inflammation was calculated according to the following formula (Winter *et al.*, 1962):

$$\text{Edema inhibition (\%)} = 100(V_0 - V_t/V_0)$$

Where V_t is the volume of paw of rats given test extract or the standard drug at corresponding time and V_0 is the volume of paw of rats of control group at the same time.

I.3.3.2. Xylene-induced Edema

The xylene-induced ear edema test of EA and Bu extracts of *S. tenuipes* and *O. virgata* was performed as previously described by Tang *et al.* (1984). Animals were divided into four groups of six rats each. Edema was induced by applying 0.03 ml of xylene to the inner surface of the right ear 30 min after oral administration of saline (10 mL/kg, po), EA and Bu extracts (200 mg/kg, po) (EA and Bu were dissolved in DMSO 10%), or diclofenac (10 mg/kg, po). The left ear was considered as control. Two hours after the application of xylene, the rats were sacrificed using chloroform anesthesia and both ears were removed. Circular sections (6 mm diameter) of both the right (treated) and left (untreated) ears were sampled with a punch and weighted (Sowemimoa *et al.*, 2013). The edematous response was measured as the weight difference between the right and left ears, where the inhibition level was calculated according to the following equation:

$$\text{Edema inhibition (\%)} = 100\ (V_c - V_t / V_c)$$

Where V_c represents difference in weight of ear in control and V_t difference in weight of ear in group treated with standard/extracts.

I.3.3.3. Albumin Denaturation

The anti-inflammatory activity of EA and Bu extracts of *S. tenuipes* and *O. virgata* was also evaluated by using inhibition of albumin denaturation technique, which was carried out as previously described by Sakat *et al.* (2010) with slight modifications. A solution of 0.2% w/v of BSA was prepared in a Tris Buffer Saline and pH was adjusted to 6.6 using HCl. The extracts (2 mg/mL) or diclorofenac (1 mg/mL) were prepared by using DMSO (10%) as a solvent. Afterwards, 0.5 mL of extracts were added to 0.5 mL of BSA solution and heated at 72ºC for 5 minutes. After cooling, the turbidity of the samples was measured at 660 nm and the percent of inhibition of protein denaturation was calculated as follows:

$$\text{Denaturation inhibition (\%)} = A_{Control} - A_{Extract} / A_{Control} \times 100$$

Where A is the absorbance.

I.3.4. Enzyme Inhibitory Activity

I.3.4.1. α-Amylase

α-Amylase inhibitory activity of EA and Bu extracts of *S. tenuipes* and *O. virgata* was performed using the iodine/potassium iodide (IKI) method (Zengina *et al*., 2014) with slight modifications. The sample solution (25 µL) was mixed with α-amylase solution (50 µL) in phosphate buffer (pH 6.9 with 6 mM sodium chloride) and then incubated for 10 min at 37 ◦C. After that, a starch solution (50 µL, 0.1%) was added and incubated for 10 min. Finally, 25µl of HCl (1M) and 100 µl IKI were added to each well in the 96-well microplate. Similarly, a blank was prepared by adding to sample solution all reaction reagents except enzyme (alpha-amylase) solution. The absorbance of both sample and blank were read at 630 nm while blank absorbance was subtracted from that of the sample. The pharmacological inhibitor, acarbose, was used as standard and the α-amylase inhibitory activity was calculated as follows:

$$\text{Activity inhibition (\%)} = A_{control} - A_{sample} / A_{control} \times 100$$

Where $A_{control}$ is the absorbance of reaction mixture in the presence of negative control (blank sample) and A_{sample} is the absorbance of reaction mixture in the presence of sample or standard (positive control).

I.3.4.2. α-Glucosidase

α-glucosidase inhibitory activity of EA and Bu exracts of *S.tenuipes* and *O.virgata* was performed according to the methodology described by Sinéad Lordan *et al.* (2013). Sample solution (50 µL) was mixed with 50 µL of 5 mM p-nitrophenyl-α-D-glucopyranoside solution (in phosphate buffer) in a 96-well microplate. After 5 min of incubation at 37°C, phosphate buffer (100 µL) containing 0.1 U/ml α-glucosidase was added to each well. Blank readings (without enzyme) were subtracted from each well and the results were compared to the control. The absorbance of sample and blank were recorded at 405 nm and acarbose was used as standard. The activity of α-glucosidase was evaluated as follows:

$$\text{Activity inhibition (\%)} = A_{control} - A_{sample} / A_{control} \times 100$$

Where $A_{control}$ is the absorbance of reaction mixture in the presence of negative control (blank sample) and A_{sample} is the absorbance of reaction mixture in the presence of sample or standard (positive control).

I.3.4.3.Urease inhibitory Activity

Urease inhibitory activity of EA and Bu extracts of *S. tenuipes* and *O. virgata* was determined according to the protocol reported by Weatherburn (1967) with some modification. Exactly 25 µL of enzyme (Jack Bean Urease) solution and 10 µL of sample solution at different concentrations were incubated for 15 min at 30°C. The aliquot was taken after 15 min and again incubated with 50 µL of buffer containing 100 mM urea for 15 min at 30°C. Final volumes were maintained as 200 µL by adding 45 µL phenol reagent (1% w/v phenol and 0.005% w/v sodium nitroprusside) and 70 µL of alkali reagent (0.5% w/v NaOH and 0.1% active chloride NaOCl). The absorbance was measured at 630 nm after 50 min at pH=8.2. Thiourea was used as the standard inhibitor and percentage inhibitions were calculated as follows:

$$\text{Activity inhibition (\%)} = A_{control} - A_{sample}/A_{control} \times 100$$

Where $A_{control}$ is the absorbance of reaction mixture in the presence of negative control (blank sample) and A_{sample} is the absorbance of reaction mixture in the presence of sample or standard (positive control).

I.3.4.4.Tyrosinase Inhibitory Activity

Tyrosinase enzyme inhibitory activity of EA and Bu extracts of *S. tenuipes* and *O. virgata* was measured by the spectrophotometric method as described by Masuda *et al.* (2005) with slight modification. Mushroom tyrosinase was used, while L-DOPA was employed as substrates of the reaction. Briefly, 150 µL of phosphate buffer (0.1 M, pH 6.8), 20 µL of mushroom tyrosinase (250 U/mL) in the same phosphate buffer and 10 µL of sample at different concentrations. The resulting mixture was mixed and then incubated for 10 min at 37°C. After that, 20µL of L-DOPA was added to each well in a 96-well microplate plate, and the resulting mixture was incubated at room temperature for 7 min, and the absorbance at 475 nm was measured. Kojic acid was used as standard and percentage inhibitions were calculated as follows:

$$\text{Activity inhibition (\%)} = A_{control} - A_{sample}/A_{control} \times 100$$

Where $A_{control}$ is the absorbance of reaction mixture in the presence of negative control (blank sample) and A_{sample} is the absorbance of reaction mixture in the presence of sample or standard (positive control).

I.3.4.5. Anticholinesterase Activity

Acetyl- and butyrylcholinesterase inhibitory activities of EA and Bu extracts of *S. tenuipes* and *O. virgata* were measured, by slightly modifying the spectrophotometric method developed by Ellman *et al.* (1961). Acetyl- and butyrylcholinesterase enzyme (AChE and BChE) were used, while acetylthiocholine iodide and butyrylthiocholine chloride were employed as substrates of the reaction. DTNB (5,50-dithio-bis(2-nitrobenzoic) acid) was used for the measurement of the cholinesterase activity. Methanol was used as a solvent to dissolve test compounds and the controls. Briefly, 150 µL of 100 mM sodium phosphate buffer (pH 8.0), 10 µL of sample solution dissolved in methanol at different concentrations and 20 µL AChE (5.32×10^{-3} U) or BChE (6.85×10^{-3} U) solution were mixed and incubated for 15 min at 25°C, and 10 µL of 0.5 mM DTNB were added. The reaction was then initiated by the addition of 10 µL of acetylthiocholine iodide (0.71 mM) or butyrylthiocholine chloride (0.2 mM). The hydrolyses of these substrates were monitored spectrophotometrically by the formation of yellow 5-thio-2-nitrobenzoate anion, as the result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of acetylthiocholine iodide or butyrylthiocholine chloride, respectively, at a wavelength of 412 nm, utilising a 96-well microplate reader. Galantamine was used as the standard inhibitor and percentage inhibitions were calculated as follows:

$$\text{Activity inhibition (\%)} = A_{control} - A_{sample}/A_{control} \times 100$$

Where $A_{control}$ is the absorbance of reaction mixture in the presence of negative control (blank sample) and A_{sample} is the absorbance of reaction mixture in the presence of sample or standard (positive control).

I.3.4.6. SPF and UV Protection Factor (UV-PF) Assessment

The *in vitro* SPF was determined according to the methods described by Mansur (1986). 1gm of samples was weighed, transferred to a 100 ml volumetric flask, diluted to volume with ethanol, and then filtered through cotton. Rejecting the first 10 ml, a 5.0 ml aliquot was transferred to 50 ml volumetric flask and diluted to volume with ethanol. Then a 5.0 ml aliquot was transferred to a 25 ml volumetric flask and the volume completed with ethanol. The absorption spectra of each prepared aliquot were determined from 290-400 nm, taking ethanol as a blank. The absorption data were obtained in the range of 290 nm to 320 nm,

every 5 nm, and 2 determinations were made at each point, followed by the application of Mansur equation.

$$SPF_{spectrophotometric} = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

EE: erythemal effect spectrum

I: solar intensity spectrum

Abs: absorbance of sunscreen product

CF: correction factor (= 10)

The values of EE (λ) x I (λ) are constants as given by Sayre *et al.* (1979) are presented in **table 11** and the categories of protection in **table 12**.

Table 11: Normal function product used in the calculation of the SPF

Wave Length λ (nm)	EE (λ)x I(λ) (Norms)
290	0,0150
295	0,0817
300	0,2874
305	0,3278
310	0,1864
315	0,0837
320	0,0180
Total	1

Table 12: Categories of protection displayed on solar products based on protection factors measured, according to the recommendation of the European Commission 2006.

Category Indicated	Protection Factor Indicated	Sun Protection Factor Measured	Minimum UVA Protection Factor Recommended	Minimum Critical Wavelength Recommended
«Low Protection»	6	6 - 9,9	1/3 of sun protection factor indicated on the label	370 nm
	10	10 - 14,9		
«Medium Protection»	15	15 - 19,9		
	20	20 - 24,9		
	25	25 - 29,9		
«High protection»	30	30 - 49,9		
	50	50 - 59,9		
«Very High Protection»	50+	60 ≤		

I.4. Statistical Analysis

All data on all phytochemical study and biological activity tests are the means of analysis in triplicate. All the antioxidant, enzymatic tests were carried out at more than four concentrations, and the results are presented as IC_{50} and $A_{0.5}$ values and anti-inflammatory activities and SPF and UV protection factor test were tested just for one concentration. Values are means of three assays ± SD (Standard Deviation). The results of anti-inflammatory activity of S. tenuipes is analysed by one-way analysis of variance (ANOVA) followed by Tukey's test were used to detect any significant differences among different means. A p-value under 0.05 was assumed to indicate a significant difference. The results were analyzed using GraphPad Prism 6 (GraphPad Software, San Diego, CA, USA).

CHAPTER II: RESULTS AND DISCUSSION

II. Results and Discussion

II.1. Phytochemical Study

II.1.1. Yield of Extractions

The *n*-butanol (Bu) and ethyl acetate (EA) extracts obtained from the two plants *S. tenuipes* and *O. virgata* were screened for phytochemical study (spectrophotometric determination of some secondary metabolites and chromatographic analysis) and were investigated for their health-benefit properties, in particular with respect to *in vivo*/*in vitro* anti-inflammatory antimicrobial and antioxidant activities, their potential to inhibit some enzymes (anti-diabetic, urease, tyrosinase inhibitory activities and anticholinesterase Activity), as well as SPF and UV protection factor assessment.

The yields of the extracts (in %) in the two plants are presented in **table 13**.

Table 13: Yields of *S. tenuipes* and *O. virgata* extracts in percentage.

Plants	*S. tenuipes*	*O. virgata*
n-Butanol (Bu)	0.32%	0.31%
Ethyl Acetate (EA)	0.15%	0.14%
Essential Oils (EO)	0.24%	X

According to our results, the yield of the (Bu) and (EA) extracts from 500 g of the aerial part of the two plants *S. tenuipes* and *O. virgata* are almost the same; the yield of Bu is nearly the double of EA (≈30% and 15% for Bu and EA, respectively). While in the case of essential oils of *S .tenuipes*, in which 100 g of the aerial parts are used, the yield is 0.24%. According to previous studies, the yield of essential oils obtained from *S .tenuipes* is higher than that obtained from *S. chrysantha*, *S. kotschyana*, *S. olympica*, *S. cinerascens* and *S. zuvandica* with a yield of 0.10%, 0.15%, 0.11%, 0.16% and 0.11% (w/w), respectively (Gülin, 2017).

II.1.2. Determination of Total Phenolic Content

Total phenolic content of *S. tenuipes* and *O. virgata* extracts was performed according to the Folin–Ciocalteu method using gallic acid as standard. The amount was calculated from the

regression equation of calibration curve (y = 0.0309 x + 0.1708, R^2 = 0.9812). The results are shown in **table 14**. Total phenolic content (TPC) of *S. tenuipes* and *O. virgata* revealed that EA extract has a higher concentration than Bu extract for both plants (EA: 225.47and 713.71 mg GAE/g dry extract; Bu: 181.35 and 453.71mg GAE/g dry extract for *S. tenuipes* and *O. virgata*, respectively). In a study on *Scrophularia striata* done by Mahboubi *et al.* (2013), it was found that ethyl acetate extract has a high TPC in comparison to other fractions such as methanolic and aqueous extracts. According to Hwang *et al.* (2011), the TPC of ethyl acetate of *Oenanthe javanica* is higher than *n*-butanol extract.

Table 14: Total phenolic content of *S .tenuipes* and *O. virgata* extracts

Extracts	**Total Phenolic Content (TPC) (mg GAE/g Dry Extract)**	
	S. tenuipes	***O. virgata***
EA	225.47±0.9	713.71±0.2
Bu	181.35±0.5	453.71±0.7

II.1.3. Determination of Total Flavonoid Content

Total flavonoid content of *S. tenuipes* and *O. virgata* extracts was performed according to the aluminium nitrate method using quercetin as standard. The amount was calculated from the regression equation of calibration curve (y = 0.0083 *x* + 0.0475, R^2 = 0.977).The results are shown in **table 15**. Total flavonoid content (TFC) of *S. tenuipes* revealed that Bu extract has a higher concentration than EA extract (21.7 and 64.55 mg QE/g dry extract for EA and Bu, respectively). In contrast to *S. tenuipes* TFC, the amount of flavonoid content in Bu extract is lower than EA extract for *O. virgata* (272.22 and 173.12±1.44 mg QE/g dry extract for EA and Bu, respectively). In contrast to our results, the TFC of ethyl acetate of *S. striata* exhibits the highest concentration in comparaison to all other tested extracts (aquaeous, methanolic and ethanolic extract) (Mahboubi *et al*., 2013). Regarding *Oenanthe*, Hwang *et al.* (2011) found that the TFC of ethyl acetate of *Oenanthe javanica* is higher than that of *n*-butanol extract.

Table 15: Total flavonoid content of *S. tenuipes* and *O. virgata* extracts**.**

Extracts	Total Flavonoid Content (TFC) (mg QE/g Dry Extract)	
	S. tenuipes	***O. virgata***
EA	21.70±3.17	272.22±5.12
Bu	64.55±4.82	173.12±1.44

II.1.4. Determination of Total Carotenoid Content

Total carotenoid content of *S. tenuipes* and *O. virgata* extracts was screened using β-carotene as standard. The amount was calculated from the regression equation of calibration curve ($y = 185.15\ x + 0.0506$, $R^2 = 0.9988$). As shown in **table 16**, the total carotenoid content (TCC) of *S. tenuipes* is 14.3 µg β-carotene E /g dry extract and 3.9 µg β-carotene E /g dry extract for *O.virgata*. The total carotenoid content found in *Oenanthe javanica* (144 µg /g dry extract) (Othman *et al*., 2017) is much higher than that found in *Oenanthe virgata* (3.9 µg /g dry extract).

Table 16: Total Carotenoid content of *S. tenuipes* and *O. virgata* extracts

Plants	Total Carotenoid Content (µg β-carotene E /g Dry Extract)
S. tenuipes	14.3±1.2
O. virgata	3.9±0.6

II.2. Chromatographic Analysis

II.2.1. Column Chromatography (Separation and Purification)

Identification of compound (1) "Z.F.69"

Compound (1):

1H NMR (CD3OD, 600 MHz): phenylethyl moiety: δ 7.3 (1H, *d*, *J* = 1.8 Hz, H-2), δ 6.42 (1H, *d*, *J*= 8.0 Hz, H-5), δ 6.42 (1H, *dd*, *J* = 8.0/2.0 Hz, H-6), 3.70 (2H, *m*, H-α), 2.30 (2H, *m*, H-β); glucose moiety: δ 4.91 (1H, *d*, *J* = 6.6 Hz, H-1'), 3.23 (1H, *dd*, *J* = 7.3/8.5 Hz, H-2′), 3.36 (2H, overlapped, H-3′, H-4′), 3.52 (1H, *m*, H-5′), 4.51 (2H, H-6'); feruloyl moiety: δ 7.3 (1H, *d*, *J* = 1.8 Hz, H-2''), 6.80 (1H, *d*, *J* = 8.4 Hz, H-5''), 6.69 (1H, *dd*, *J* = 8.4/2.0 Hz, H-6''), 6.50 (1H, *d*, *J* = 16.2 Hz, H-*α'*), 7.64 (1H, *d*, *J* = 16.2 Hz, H-*β'*), 3.84 (3H, *s*, OMe); 13C NMR (CD3OD, 150 MHz): δ: 21.2(C-7), 44.8 (C-8), 54.21(O-CH3), 61.6 (C-6'), 70.3 (C-4'), 72.0(C-8) 73.1 (C-2'), 76.3 (C-5'), 85.7(C-3'), 105.4 (C-1'), 118.1 (C-8'), 115.2 (C-2′), 116.4 (C-5), 116.8 (C-5′), 117.2 (C-2), 121.3 (C-6), 123.6 (C-6), 127.5 (C-1′), 131.6 (C-1), 144.6 (C-4), 130.1 (C-2''), 134.3 (C-3''), 142.1 (C-7''), 144.6 (C-4''), 167.3 (C-9), positive ion ESIMS: *m*=*z* 493 $[M+H]^-$.

Positive ion ESIMS: *m*=*z* 493 $[M+H]^-$.

All the previous informations and the spectrums presented in the **figures 19**, **20** leads to the following structure (**figure 18**).

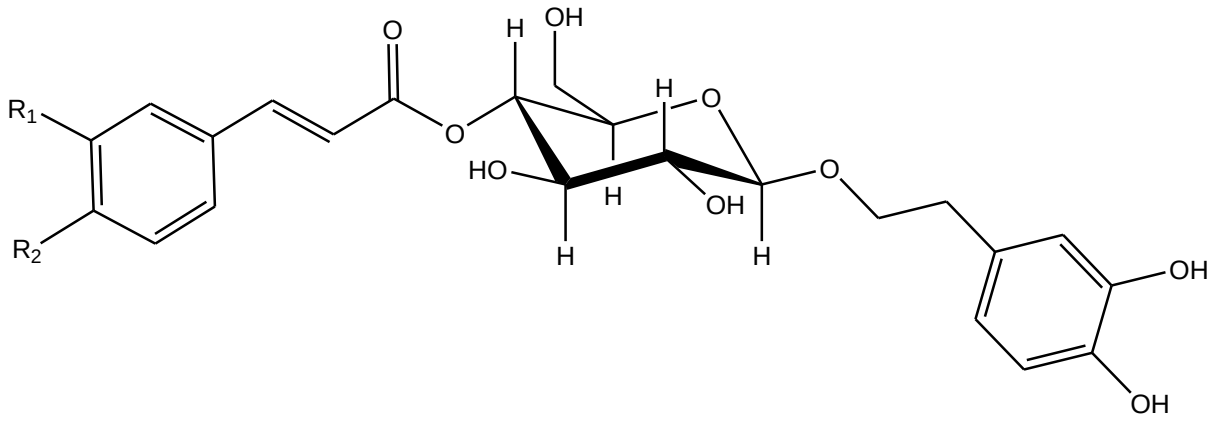


Figure18: Structure of compound (1).

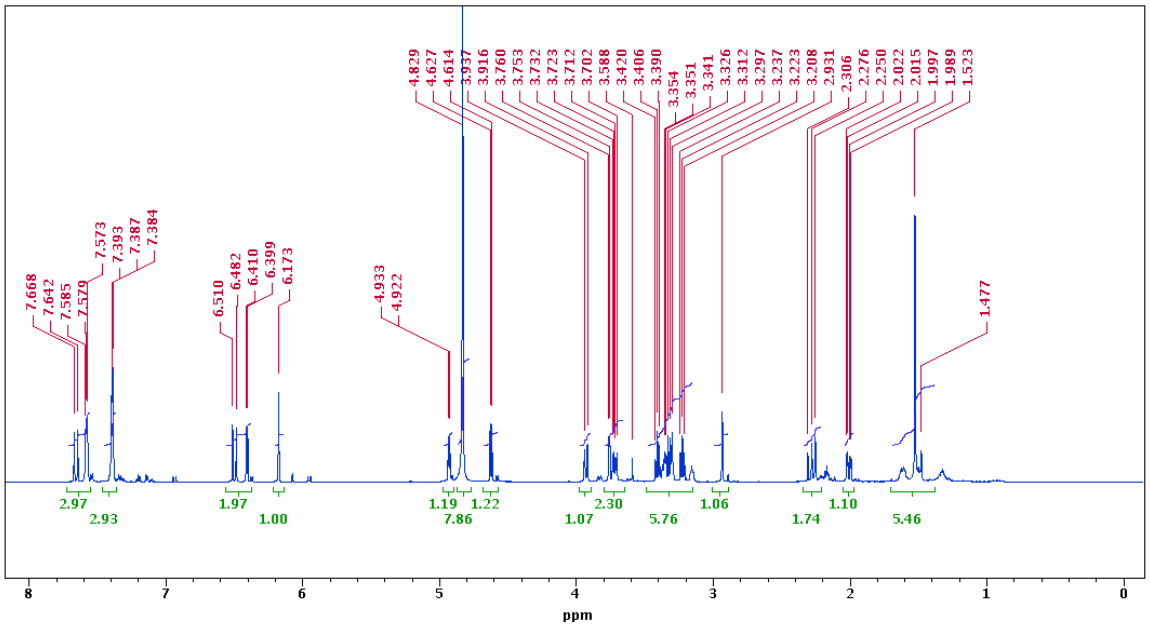


Figure 19: NMR proton **(600 MHz)** of compound (1).

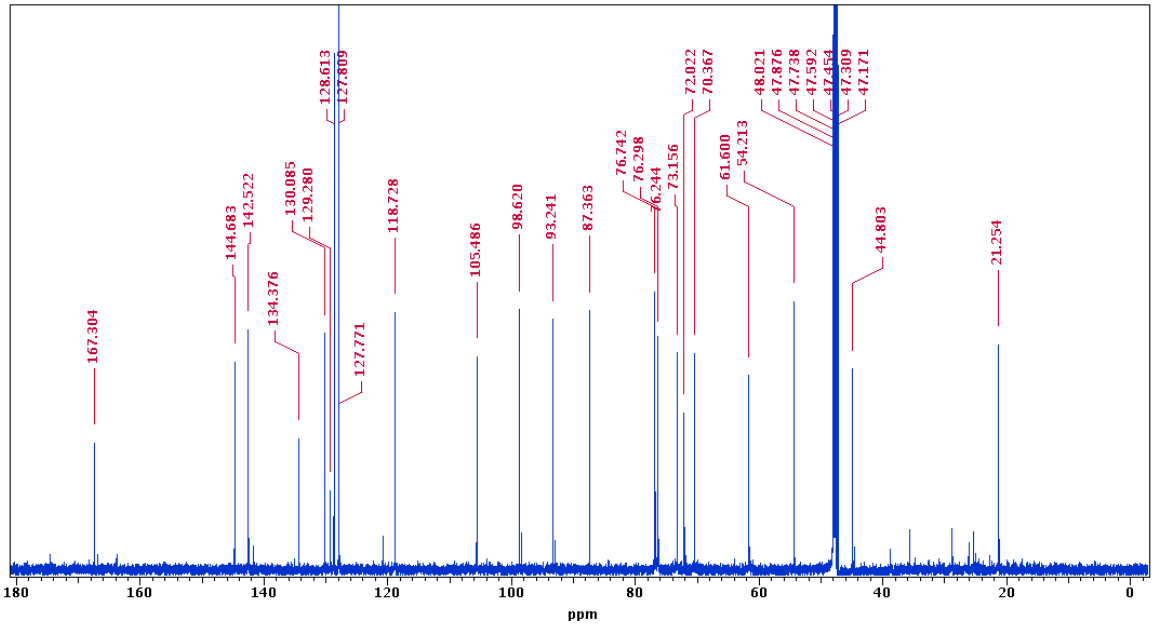


Figure 20: NMR- **^{13}C (150 MHz**) of compound (1).

II.2.2. Gas Chromatography/Mass Spectrometry (GC/MS) Analysis

The essential oils obtained from the aerial parts of *S. tenuipes* were analysed using a Perkin-Elmer GC-MS system. According to **table 17** the major constituents of the essential oils of *S. tenuipes* during flowering are: Hexahydrofarnesyl acetone (34.77%), Phthalic acid,

diisobutyl ester (19.36%), Dibutyl phthalate (14.94%). The major compound Hexahydrofarnesyl acetone, β-Damascenone and palmitic acid were also found in the essential oil of *S. subaphylla* and *S. khorassanica* (Asgharian *et al*., 2015; Barati and Sani, 2017).

Table 17: The major constituents of the essential oils of *S. tenuipes* during flowering.

N°	TR	%	Compounds
1	26,848	4,10	Damascenone
2	32,107	6,14	Formic acid, 3,7,11-trimethyl-1,6,10-dodecatrien-3-yl ester
3	33,194	3,29	Fluorene
4	35,128	2,92	Hexa-hydro-farnesol
5	38,727	5,73	Anthracene
6	39,63	**34,77**	Hexahydrofarnesyl acetone
7	40,396	**19,36**	Phthalic acid, diisobutyl ester
8	41,569	2,43	2,4,7,14-Tetramethyl-4-vinyl-tricyclo[5.4.3.0(1,8)]tetradecan-6-ol
9	42,515	6,32	Hexadecanoic acid (palmitic acid)
10	42,712	**14,94**	Dibutyl phthalate

II.2.3. UHPLC-ESI-DAD-MS^n Analysis

Chemical analysis of Ethyl Acetate (EA) and *n*-Butanol (Bu) Extracts of *S. tenuipes*

The EA and Bu extracts obtained from *S. tenuipes* represented 0.15% and 0.32% of the dried plant, respectively. Notably, the two extracts differed significantly regarding their contents in total phenolic compounds and total flavonoids, with EA presenting superior levels of TPC but less flavonoid than Bu (**table 14, 15**). Their distinct phenolic profile was also confirmed by UHPLC-DAD-ESI-MS^n analysis (**figure 21** and **table 18**). In fact, while the main chromatographic peak in EA corresponded to the phenylethanoid acetyl martynoside (peak 16, UV_{max} at 277 nm, $[M-H]^-$ at *m/z* 693→651), iridoids, namely two harpagoside isomers (peaks 14 and 15, UV_{max} at 280 nm, $[M-H]^-$ at *m/z* 493,) were dominant in Bu. Consistent with the results obtained from total flavonoids assay, a derivative of kaempferol, namely the kaempferol-*O*-dihexoside (peak 4, UV_{max} at 272 and 334 nm, $[M-H]^-$ at *m/z* 609→447), was prominent in Bu, while it was not detected in EA and in opposition, coumaric

acid (eluted in peak 5) was much more significant in EA compared to Bu. Regardless, the two extracts presented several coumaric acid derivatives, which appeared as minor components.

In general, the phenolic compounds herein found in the two *S. tenuipes* extracts are in line with previous reported data for *Scrophularia* plants, since phenylethanoid/phenylpropanoid glycosides and iridoids represent their major phenolic metabolites. In this regard, martynoside has been previously isolated from *S. dentata* (Pasdaran and Hamedi, 2017) *S. xanthoglossa* (Abbas *et al*., 2010), *S. koraiensis* (Pachaly *et al*., 1994), *S. umbrosa* (Han *et al*., 2018), while the iridoid harpogoside was reported for *S. ningpoensis* (Huang *et al*. 2011), *S. scorodonia* (Diaz *et al*., 1998; Fernández *et al*., 2005), *S. buergeriana* (Lee *et al*., 2007) and *S. nodosa* (Crisan *et al*., 2009). As well, caffeic acid, coumaric acid derivatives and kaempferol derivatives were also previously detected in *Scrophularia* plants. In more detail, coumaric acid and/or caffeic acid were reported in *S. frutescens*, *S. sambucifolia*, *S. buergeriana*, *S. nodosa* L. (Fernández *et al*., 1996; , Fernandez *et al*., 1998, Crisan *et al*., 2009), *S. kotscyhana*, *S. cinarescens*, *S. catariifolia*, *S. chrysantha* and *S. scopolii* (Gülin *et al*., 2018) and kaempferol and/or some of its glycosidic derivatives were described, among others, in *S. ilwensis*, *S. lucida*, *S. nodosa* L. and *S. scopolii* (Crisan *et al*., 2009; Calis *et al*., 1993; Lewenhofer *et al*., 2018).

Table18: Phenolic compounds of *S. tenuipes* extracts.

Peak	Rt (min)	λmax	$[M-H]^-$	ESI MS/MS Product Ions	Probable Compound	EA	Bu
1	8.9	294sh, 318	179	**135**	Caffeic acid	+	-
2	9.9	312	309	**187**,118,163	Coumaroyl-*O*-rhamnoside	+	+
3	10.5	277, 320sh	367	**349**,307,203,161,289,245,191,173	Feruloylquinic acid	+	-
4	10.7	272, 334	609	**447**, 285,489,429,255	Kaempferol-*O*-dihexoside	-	+
5	11.0	309	163	**119**	Coumaric acid	+	+
6	12.2	282, 334	447	**285**,284,327,255; MS^3 [285]: 267, 241, 185	Kampferol-*O*-hexoside	+	+
7	12.3	313	509	**307**, 265, 163, 235	Coumaric ac derivative	+	+
8	13.0	272, 331	447	**285**,284,327,255; MS^3[285]: 267, 241/239	Kaempferol-*O*-hexoside	+	+
9	13.6	313	351	**333**,229,187,273,163,119	Coumaroyl-*O*- acetyl-rhamnoside (isom 1)	+	+
10	13.8	313	351	**187**, 229, 333, 163, 119	Coumaroyl-*O*-acetyl-rhamnoside (isom 2)	+	+
11	14.9	314	679	**637**, 499, 351,619, 229, 333, 273	Coumaroyl-*O*-acetyl-rhamnoside derivative (isom 1)	+	+
12	15.0	314	679	**499**,351,637,619,333,229,273,517	Coumaroyl-*O*-acetyl-rhamnoside derivative (isom 2)	+	+
13	15.3	313	351	**163**, 187, 333, 119	Coumaroyl-*O*- acetyl-rhamnoside (isom 3)	+	-

14	15.7	281	493	**345**, 179,181	Harpagoside	+	+
15	16.4	280	493	**345**,179,201,147	Harpagoside	+	+
16	16.9	277	693	**651**,633 ,505,517,597	Acetyl martynoside	+	+

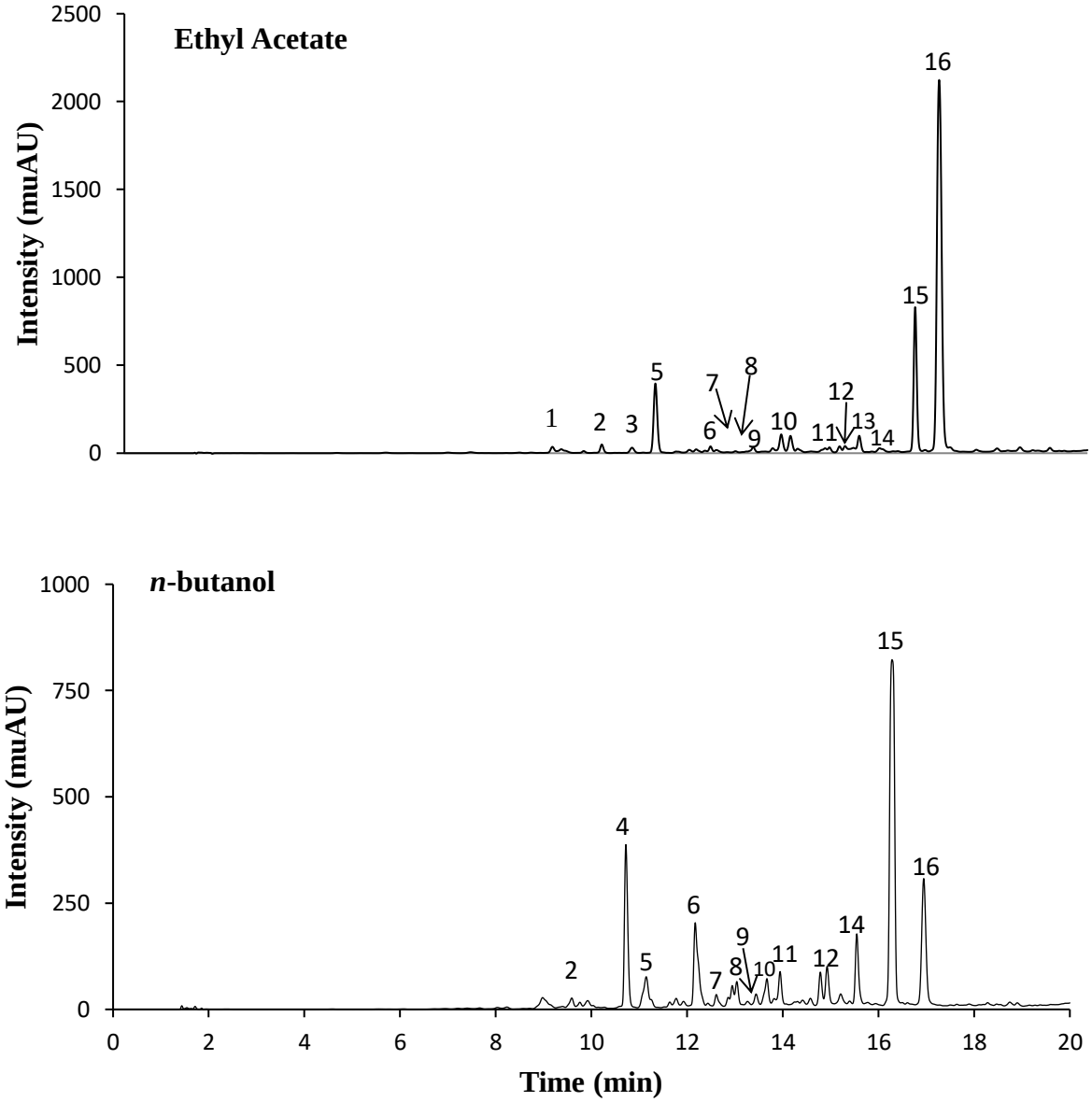


Figure 21: Chromatographic representation of *Scrophularia tenuipes* extracts at 280 nm. Numbers in the figure correspond to the UHPLC-DAD-ESI-MS^n peaks described in **table 18**.

Chemical Analysis of Ethyl Acetate (EA) and *n*-Butanol (Bu) Extracts of *O. virgata*

The EA and Bu extracts obtained from *O. virgata* gave 0.15% and 0.14% of the dried plant, respectively. Notably, the two extracts differed significantly regarding their contents in total phenolic compounds and total flavonoids, with EA presenting superior levels of TPC and TFC (**table 14, 15**). Their distinct phenolic profile was also confirmed by UHPLC-DAD-ESI-MS^n analysis (**figure 22** and **table 19**). In general, the phenolic profile of *O. virgata* is

represented by 27 compounds: 4 of them are not identified and the others are belonging to phenolic acids non-phenolic organic acid and flavonoids (flavanonols, flavonols and flavanones). Souilah *et al*. (2019) has carried out a study on *Oenanthe fistulosa* and has found the same phenolic profiles as reached in this work (9 phenolic acids, 8 flavonoids were and 3 non-phenolic organic acids were detected).

In fact, all the 27 compounds are presents in EA extract while the main chromatographic peak corresponded to the phenolic acid dicaffeyolquinic acid (peak 20, UV_{max} at 296sh, 326 nm, $[M-H]^-$ at *m/z* 515→353 and peak 26, UV_{max} at 295sh,326 nm, $[M-H]^-$ at *m/z* 515→353). In more details, phenolic profile of EA are represented by 4 non identified compounds which appears as minor components (eluted in peaks 4, 5, 7 and 9), non-phenolic organic acid (quinic acid), 12 phenolic acids (derivative of caffeic, ferulic and coumaric acid) and 10 flavonoids (flavanonol: taxifolin-*O*-hexoside and aromadendrin-c-hexoside; flavonol: derivative of kaempferol, derivative of quercetin and rutin; flavanone: Naringenin hexoside).

In contrast, Bu extract contains just 10 compounds which are (8) phenolic acids, (1) non-phenolic organic acids and (1) flavonoid (flavonol) namely rutin (peaks 15 UV_{max} at 256,265,354 nm, $[M-H]^-$at *m/z* 609) which is dominant.

In general, the phenolic compounds herein found in the two *O. virgata* extracts are in line with previous reported data for *Oenanthe* plants, since phenolic acids and flavonoids represent their major phenolic metabolites. In this regard, the phenolic acid dicaffeyolquinic acid is not previously isolated from any species of the genus *Oenanthe*, while the flavonoid rutin was reported for *O. javanica* (Chen *et al*., 2014), *O. linearis* (Seal, 2016) and *O. fistulosa*. As well, phenolic acids were also previously detected in *Oenanthe* plants. In more detail, ferulic acid was reported in *O. fistulosa* and *O. linearis*, coumaric acid in *O. fistulosa* and *O. linearis* (Seal, 2016; Souilah et al., 2018), caffeic in *O. javanica* and *O. linearis* (Chen *et al*., 2014; Seal, 2016), citric acid in *O. javanica* (Lee, 2001), protocatechuic acid *O.fistulosa* (Souilah *et al*., 2018). In addition, flavonoid kaempferol, quercetin were described in *O. javanica*, *O. fistulosa* and *O. linearis* (Chen *et al*., 2014; Seal, 2016; Souilah *et al*., 2018; Lu et al.,2019), naringenin in *O. fistulosa* (Souilah *et al*., 2018). The flavonoid taxifolin and aromadendrin were not described in the genus Oenanthe before that. Although, non-phenolic organic acids "quinic acid" was reported in *O. fistulosa* (Souilah *et al*., 2018).

Table 19: Phenolic compounds of *O. virgata* extracts.

Peak	Rt (min)	λmax	$[M-H]^-$	ESI MS/MS Product Ions	Probable Compound	EA	Bu
1	1.4	193,273	191	**127**, 85,173,93,171..	Quinic acid	+	+
2	1.9	205, 270	191	**111**,147,173,127,133,85,	Citric acid	+	+
3	4.3	260,294	153	109	Protocatechuic acid	+	-
4	6.7	279,309	183	139,137,153,165,,156	Not found	+	-
5	7.2	255	183	139,137,156,165,155,166	Not found.	+	-
6	8.8	298sh,325	353	191,179	Caffeoylquinic acid (isomere)	+	+
7	9.1	260,292	177	133	Not found	+	-
8	9.6	295sh,322	179	135	Caffeic acid	+	-
9	10.3	219, 272	159	97,115,141	Not found	+	-
10	12.7	289,330sh	465	303,285,177	Taxifolin-*O*-hexoside	+	-
11	12.83	243,325	367	191,173,193,247,261	Feruloyl-quinic acid	+	+
12	13	309	163	119	Coumaric acid	+	-
13	13.3	291,330sh	449	287,259,269,243,431	Aromadendrin-*C*-hexoside	+	-
14	14.5	295sh,322	367	191,179,135	5-Feruloylquinic acid	+	-
15	15.25	256,265,354	609	301,343	Rutin	+	+
17	15.76	256,265,353	463	301	Quercetin-*O*-hexoside	+	-
18	16.9	265,344	593	285	Kaempferol-*O*-rutinoside	+	+

19	17.2	295sh,331	515	353,335,173	3,4-Dicaffeyolquinic acid	+	+
20	17.5	296sh,326	515	353	3,5-Dicaffeyolquinic acid	+	+
21	17.9/18.1	281,330sh	287	259,269,243,201,125,	Dihydrokaempferol	+	+
24	18.6	291 : 330sh	433	271,	Naringenin hexoside		
25	18.9	219,243,325	447	285,284,327,	Kampferol-*O*-hexoside	+	
26	19	295sh,326	515	353, 299, 203,	1,4-Dicaffeoylquinic acid	+	+
31	20.8	315	499	353 146+353	Coumaroyl-caffeoylquinic acid	+	-
32	21.1	296sh,326	529	353,367,	feruloyl caffeoylquinic acid	+	-
33	21.7	255,369	301	179,151,257,273	Quercetin	+	-
34	23.5	266,364	285	257,243	Kaempferol	+	-

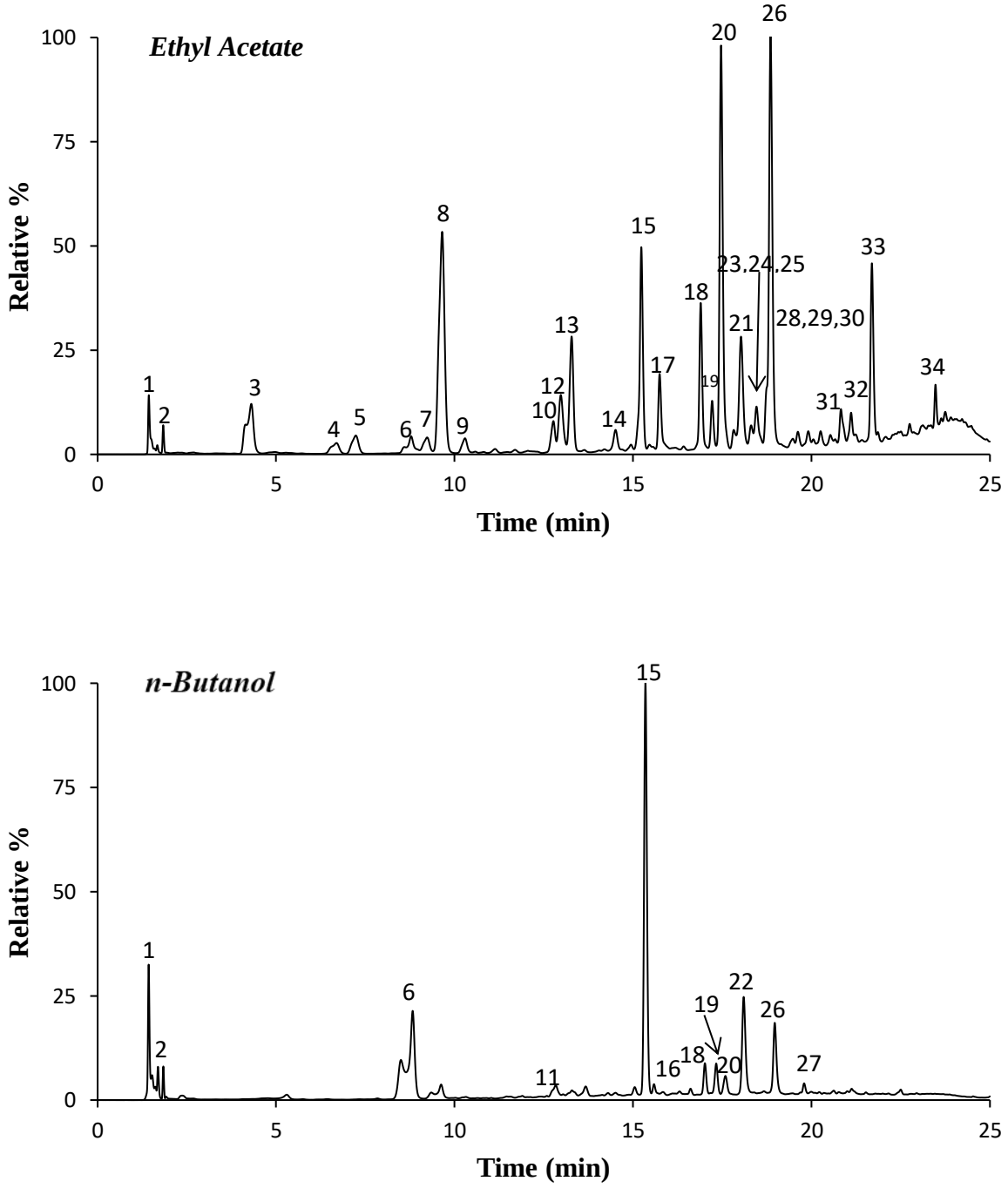


Figure 22: Chromatographic representation of *Oenanthe virgata* extracts at 280 nm. Numbers in the figure correspond to the UHPLC-DAD-ESI-MSn peaks described in **table 19**.

II.3. Biological Activities

II.3.1. Antioxidant Activity

Oxidative stress has been implicated in numerous pathologic conditions such as inflammation, diabetes, cardiovascular diseases, cancer and ageing (Rana *et al.*, 2010) while natural extracts are potential sources of bioactive compounds able to counteract such events. In this work, EA and Bu extracts of *S. tenuipes* and *O. virgata* were screened for their antioxidant abilities through ability to scavenge DPPH, ABTS, CUPRAC, superoxide radicals (DMSO Alcalin) and β-carotene. The results can be expressed as: percentage of the antiradical activity or using the parameter $IC_{50}/A_{0.50}$, which is defined as the concentration of the substrate which causes a 50% loss of the activity of the free radical. Evaluation of the antioxidant activity of the EA and Bu extracts of *S.tenuipes* and *O.virgata* was evaluated in comparison with the antioxidant standards BHT, BHA, tanic acid and α-tocopherol.

Antioxidant Activity for *S. tenuipes*

The results of antioxidant activity of *S. tenuipes* extracts tested against different free radicals are presented in **tables 20**, **21**, **22**, **23** and **24** as percentage of inhibition and as $IC_{50}/A_{0.5}$ in **figures 23**, **24**, **25**, **26** and **27**.

DPPH Free Radical Scavenging

Table 20: Antioxidant activity of *S. tenuipes* by the DPPH assay.

Extracts	% Inhibition in DPPH Assay						
	3.125 µg/mL	6.25 µg/mL	12.5 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL	200 µg/mL
EA	0.6±1.41	3.48±1.51	8.01±0.61	19.33±1.21	23.37±1.7	45.23±0.6	75.02±2.83
Bu	5.11±0.34	5.34±1.91	17.35±1.56	32.57±1.97	42.47±14.25	79.34±0.80	82.45±2.65
BHT	11.69±1.88	22.21±1.30	37.12±1.80	52.63±2.70	56,02±0.53	83.60±0.23	87.28±0.26
BHA	28.95±1.16	54.33±1.59	76.76±1.65	84.09±0.35	87.53±0.82	87.73±0.15	88.43±0.23

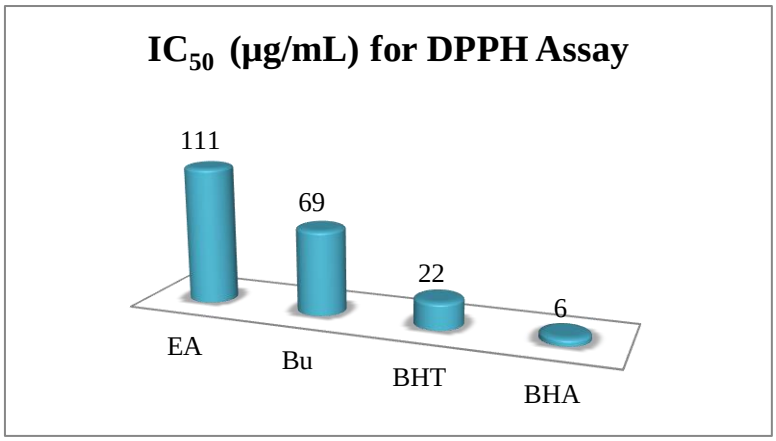


Figure 23: IC $_{50}$ of DPPH assay for standards and *S. tenuipes* extracts.

The highest DPPH radical scavenging activity (%) was recorded at 200µg/mL for both extracts, EA and Bu, with a percentage of 75.02% and 82.45%, respectively. Both extracts of *S. tenuipes* have a low activity compared to standards BHT and BHA.

ABTS Cation Radical Decolorization

Table 21: Antioxidant activity of *S. tenuipes* by the ABTS scavenging assay

Extracts	% Inhibition in ABTS Assay						
	3.125 µg/mL	6.25 µg/mL	12.5 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL	200 µg/mL
EA	19.08±2,45	31.45±1.66	44.57±1.4	57.47±1.72	66.34±0.86	80.16±2.83	87.63±1.01
Bu	14.99±1,31	24.39±1.21	38.51±0.86	57.47±1,09	75.26±0.86	89.44±0.56	91.83±0.36
BHT	59.22±0.59	78.55±3.43	90.36±0.00	92.18±1.27	93.37±0.86	94.87±0.87	96.68±0.39
BHA	83.42±4.09	93.52±0.09	93.58±0.09	93.63±0.16	93.63±0.95	94.20±0.90	95.39±2.62

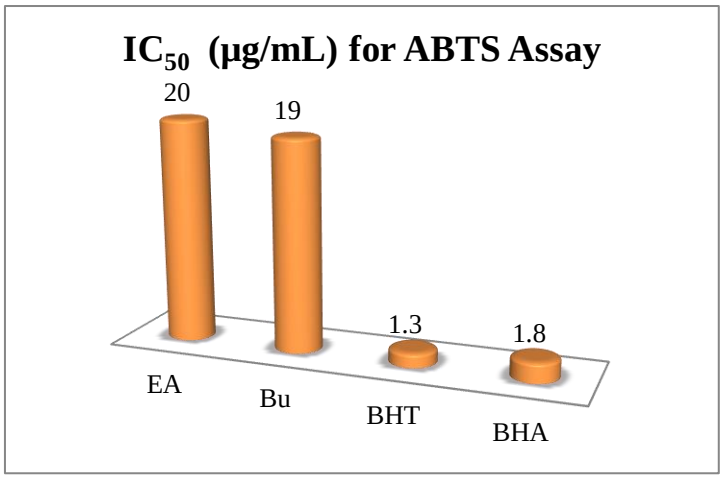


Figure 24: IC $_{50}$ of ABTS assay for standards and *S. tenuipes* extracts.

The highest ABTS radical scavenging activity (%) was noticed at 200µg/mL for both extracts, EA and Bu, with a percentage of 87.63% and 91.83%, respectively. The antioxidant activity of Bu is higher than that of EA (IC_{50}: 19g/mL). Both extracts of *S. tenuipes* have a low activity compared to standards BHT and BHA.

Cupric Reducing Antioxidant Capacity

Table 22: Antioxidant activity by the CUPRAC assay.

Extracts	Absorbance in CUPRAC Assay						
	3.125 µg/mL	6.25 µg/mL	12.5 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL	200 µg/mL
EA	0.16±0.02	0.18±0.01	0.21±0.02	0.30±0.01	0.45±0.03	0.79±0.08	1.10±0.06
Bu	0.15±0.01	0.20±0.03	0.24±0.01	0.35±0.03	0.55±0.04	0.97±0.10	1.77±0.06
BHT	0.19±0.01	0.33±0.04	0.66±0.07	1.03±0.07	1.48±0.09	2.04±0.14	2.32±0.28
BHA	0.46±0.00	0.78±0.01	1.34±0.08	2.36±0.17	3.45±0.02	3.76±0.03	3.93±0.01

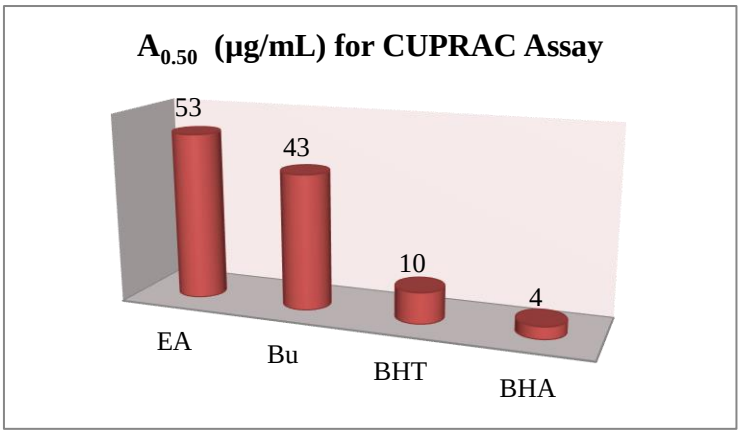


Figure 25: $A_{0.50}$ of CUPRAC assay for standards and *S. tenuipes* extracts.

The highest cupric reducing antioxidant capacity was recorded at 200µg/mL for both extracts, EA and Bu, with an absorbance of 1.1 and 1.77, respectively. Both extracts of *S.tenuipes* have a low activity compared to standards BHT and BHA.

Superoxide Radical Scavenging

Table 23: Antioxidant activity of *S. tenuipes* by the alkaline DMSO assay.

Extracts	% Inhibition in Alkaline DMSO Assay						
	3.125 µg/mL	6.25 µg/mL	12.5 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL	200 µg/mL
EA	11.48±0.0	24.31±3.53	42.64±3.10	58.92±1.72	71.41±0.54	73.28±1.52	75.77±0.00
Bu	3.43±0.00	17.73±2.25	44.03±2.28	54.51±3.43	67.78±2.64	72.51±2.09	76.26±3.07
Tanic Acid	83.58±1	88.35±0.61	92.66±0.42	94.98±0.85	96.53±0.23	96.95±0.81	97.54±0.68
α-tocopherol	70.09±1.84	79.07±2.54	85.1±1.36	89.48±0.88	94.76±0.28	96.62±0.29	96.86±1.53

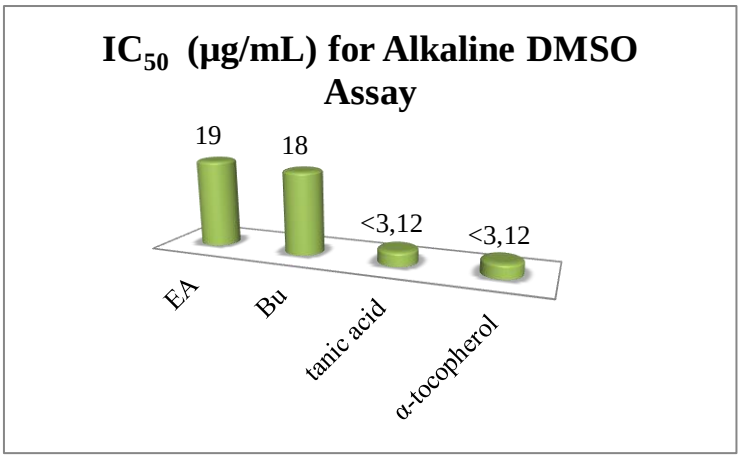


Figure 26: IC_{50} of alkaline DMSO assay for standards and *S. tenuipes* extracts.

The highest superoxide radical scavenging activity was found at 200µg/mL for both extracts, EA and Bu, with a percentage of 75.77% and 76.26%, respectively. Both extracts of *S. tenuipes* have a low activity compared to standards tanic acid and α-tocopherol.

β-Carotene Bleaching

Table 24: Antioxidant activity of *S. tenuipes* by the β-carotene assay

Extracts	% Inhibition in β-carotene Assay						
	3.125µg/mL	6.25 µg/mL	12.5µg/mL	25 µg/mL	50 µg/mL	100µg/mL	200 µg/mL
EA	7.17±1.95	5.34±1.68	15.24±1.35	15.52±3.24	18.41±0.32	21.77±1.65	36.27±1.59
Bu	3.42±4.78	8.77±2.96	6.63±1.82	16.19±3.42	26.09±1.39	37.41±2.28	45.52±0.76
BHT	81.14±0.84	86.0.9±1.0	87.52±4.24	91.67±0.52	94.11±0.42	94.41±0.32	95.28±3.25
BHA	84.23±1.14	90.11±0.68	94.59±0.77	96.09±0.02	97.35±1.08	99.59±0.14	99.76±

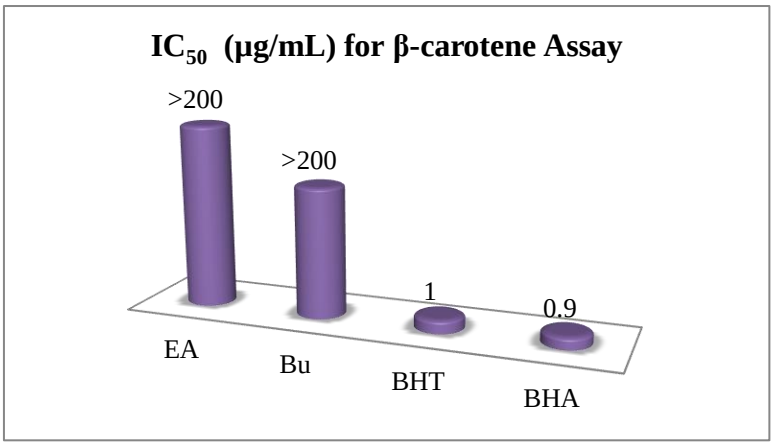


Figure 27: IC_{50} of β-carotene assay for standards and *S. tenuipes* extracts

The highest β-carotene scavenging activity was observed at 200µg/mL for both extracts, EA and Bu, with a percentage of 36.27% and 45.52%, respectively. IC_{50} for both extracts were not identified because it was higher than the area of measurement (IC_{50}> 200µg/mL). Both extracts of *S. tenuipes* have a low activity compared to standards BHT and BHA.

Figures 28 and **29** represent a comparison of $IC_{50}/A_{0.50}$ of of *S. tenuipes* extracts for all antioxidant assays.

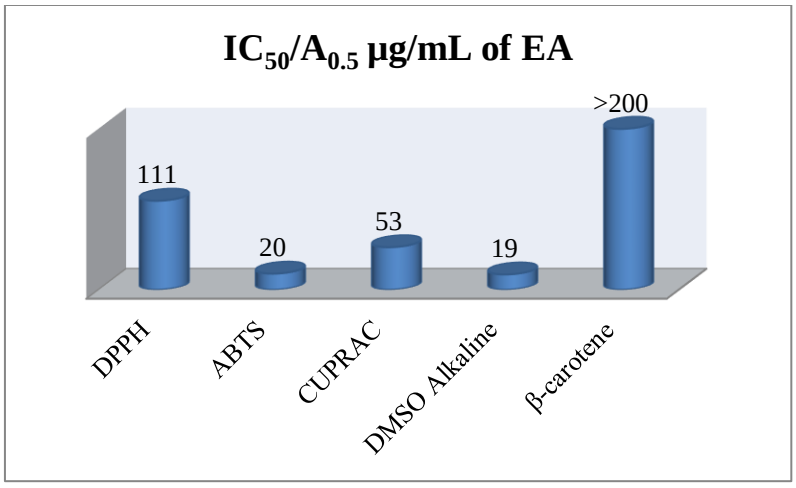


Figure 28: IC_{50} of *S. tenuipes* EA extract for all antioxidant assays.

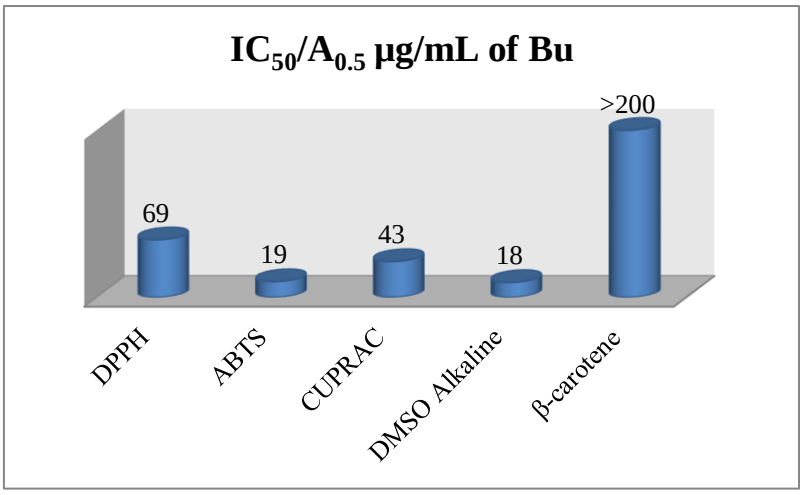


Figure 29: IC_{50} /$A_{0.5}$ of *S. tenuipes* Bu extract for all antioxidant assays.

S. tenuipes EA and Bu extracts were screened for their antioxidant abilities through ability to scavenge DPPH, ABTS, CUPRAC, superoxide radicals (DMSO Alcalin) and β-carotene. Both extracts exhibited considerable antioxidant potential which corresponds with many previous studies which reported that *Scrophularia* displayed an antioxidant potential such as *S. striata*, *S. ningopoensis*, *S. buergeri*, *S. oxysepala* and *S. amplexicaulis* (Mahboubi *et al*., 2013; Pasdaran and Hamedi, 2017). Regardless to the lower content of phenolic compounds, Bu was in all tests more effective than EA (except ß-carotene in which IC_{50} for both extract were not evaluated; >200 µg/mL), a fact that was particularly evident in DPPH$^{\bullet}$ (IC_{50} 69 and 111 µg/mL, respectively) and CUPRAC (43and 53 µg/mL, respectively) assays. Considering the distinct phenolic composition of the extracts, one may hypothesise that the superior antioxidant potential of Bu can in part be associated to its richness in flavonoids and/or the iridoid harpagoside, both previously pointed to be effective antioxidants. In fact, harpagoside isolated from *S. buergeriana* was previously shown to possess high antioxidant power in scopolamine-treated mice (Jeong *et al*., 2008).

Antioxidant Activity of *O. virgata*

The results of antioxidant activity of *O. virgata* extracts tested against different free radicals are presented in **tables 25**, **26**, **27**, **28** and **29** as percentage of inhibition and as IC_{50}/$A_{0.5}$ in **figures 30**, **31**, **32**, **33** and **34**.

Table 25: Antioxidant activity of *O. virgata* by the DPPH assay.

Extracts	% Inhibition in DPPH Assay						
	3.125 µg/mL	6.25 µg/mL	12.5 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL	200 µg/mL
EA	25.30±0.40	35.77±1.72	48.91±1.07	75.94±2.53	83.16±0.53	84.65±0.86	85.87±0.56
Bu	9.57±1.80	20.29±1.05	42.40±2.09	75.59±0.93	76. 30±2.12	78.21±1.41	80±0.39
BHT	11.69±1.88	22.21±1.30	37.12±1.80	52.63±2.70	56.02±0.53	83.60±0.23	87.28±0.26
BHA	28.95±1.16	54.33±1.59	76.76±1.65	84.09±0.35	87.53±0.82	87.73±0.15	88.43±0.23

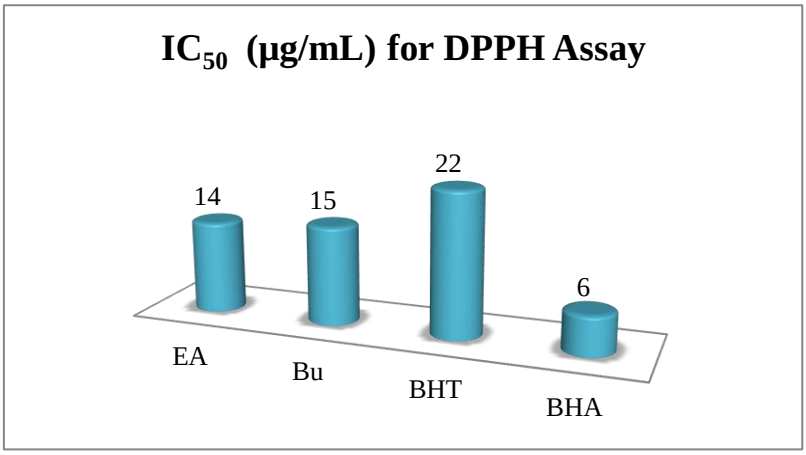


Figure 30: IC $_{50}$ of DPPH assay for standards and *O. virgata* extracts.

The highest DPPH radical scavenging activity (%) was recorded at 200µg/mL for both extracts, EA and Bu, with a percentage of 85.87% and 80%, respectively. Antioxidant capacity of EA is higher than Bu with IC_{50}: 14g/mL. Both extracts of *O.virgata* L. have a high activity compared to standards BHT and low capacity compared to BHA

ABTS Cation Radical Decolorization

Table 26: Antioxidant activity of *O. virgata* by the ABTS scavenging assay

Extracts	% Inhibition in ABTS Assay						
	3.125 µg/mL	6.25 µg/mL	12.5 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL	200 µg/mL
EA	29.40±2.63	48.95±1.89	73.34±2.55	90.90±0.20	91.95±0.35	91.31±1.59	92.36±0.27
Bu	21.82±4.34	41.31±2.75	73.69±4.75	91.60±0.18	92.30±0.18	92.24±0.27	92.18±0.10
BHT	59.22±0.59	78.55±3.43	90.36±0.40	92.18±1.27	93.37±0.86	94.87±0.87	96.68±0.39
BHA	83.42±4.09	93.52±0.09	93.58±0.09	93.63±0.16	93.63±0.95	94.20±0.90	95.39±2.62

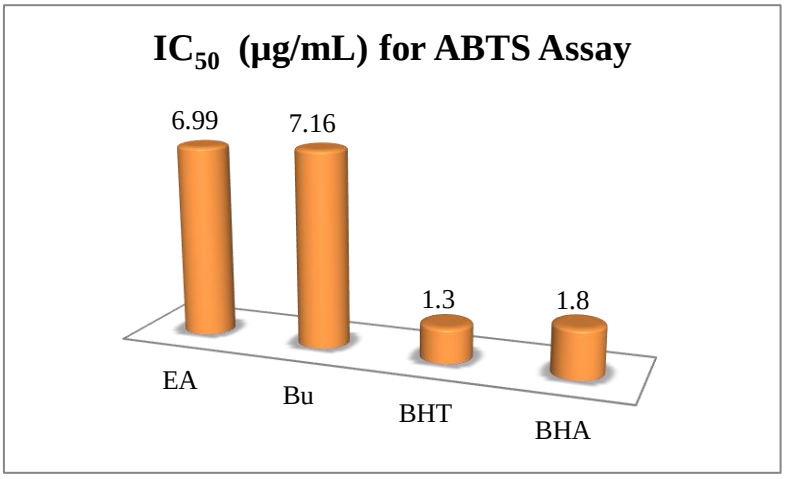


Figure 31: IC $_{50}$ of ABTS assay for standards and *O. virgata* extracts.

The highest ABTS radical scavenging activity (%) was noticed at 200µg/mL for EA extracts and at 50µg/mL for Bu, with a percentage of 92% for both. The antioxidant activity of EA is a slightty higher than Bu (IC_{50}: 6.99g/mL). Both extracts of *O. virgata*. have a low activity compared to standards BHT and BHA.

Cupric Reducing Antioxidant Capacity

Table 27: Antioxidant activity of *O. virgata* by the CUPRAC assay.

Extracts	Absorbance in CUPRAC Assay						
	3.125 µg/mL	6.25 µg/mL	12.5 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL	200 µg/mL
EA	0.26±0.01	0.36±0.0	0.59±0.06	0.98±0.07	1.75±0.19	2.96±0.17	4.23±0.15
Bu	0.25±0.02	0.36±0.03	0.55±0.03	0.95±0.02	1.58±0.14	2.57±0.10	4.01±0.02
BHT	0.19±0.01	0.33±0.04	0.66±0.07	1.03±0.07	1.48±0.09	2.04±0.14	2.32±0.28
BHA	0.46±0.00	0.78±0.01	1.34±0.08	2.36±0.17	3.45±0.02	3.76±0.03	3.93±0.01

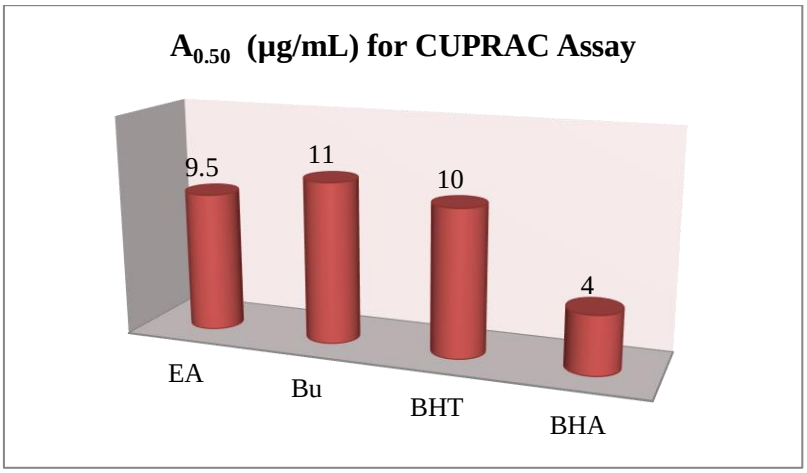


Figure 32: $A_{0.50}$ of CUPRAC Assay for standards and *O. virgata* extracts.

The highest cupric reducing antioxidant capacity was recorded at 200µg/mL for both extracts, EA and Bu, with an absorbance of 4.2 and 4, respectively. EA antioxidant capacity is higher than Bu and standard BHT with an $A_{0.50}$: 9.5µg/mL.

Superoxide Radical Scavenging

Table 28: Antioxidant activity of *O. virgata* by the alkaline DMSO assay.

Extracts	% Inhibition in DMSO Alcalin Assay						
	3.125 µg/mL	6.25 µg/mL	12.5 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL	200 µg/mL
EA	31.54±0.21	32.93±1.28	49.14±0.89	69.71±0.45	78.33±0.35	81.4±0.14	89. 31±1.6
Bu	22.46±0.11	41.34±0.19	57.64±0.61	76.02±0.13	79.80±0.45	89.83±1.11	92.43±2.11
Tanic acid	83.58±1	88.35±0.61	92.66±0.42	94.98±0.85	96.53±0.23	96.95±0.81	97.54±0.68
α-tocopherol	70.09±1.84	79.07±2.54	85.1±1.36	89.48±0.88	94.76±0.28	96.62±0.29	96.86±1.53

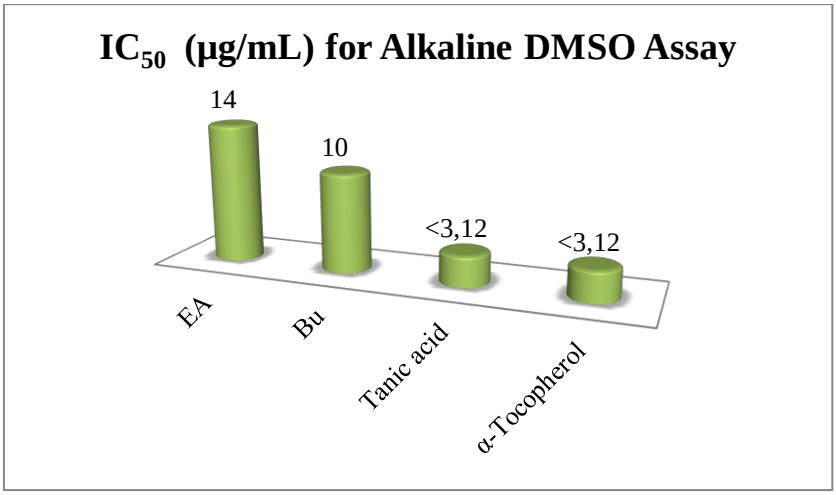


Figure 33: IC_{50} of DMSO alcalin assay for standards and *O. virgata* extracts

The highest superoxide radical scavenging activity was detected at 200µg/mL for both extracts, EA and Bu, with a percentage of 89.3 % and 92.4 %, respectively. The antioxidant activity of Bu is higher than EA (IC_{50}: 10µg/mL). Both extracts of *O.virgata* have a low activity compared to standards tanic acid and α-tocopherol.

β-carotene Bleaching

Table 29: Antioxidant activity of *O. virgata* by the β-carotene assay.

Extracts	% Inhibition in β-carotene Assay						
	3.125 µg/mL	6.25 µg/mL	12.5 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL	200 µg/mL
EA	8.75±2.24	5.17±1.71	12.33±0.63	21.70±1.94	40.88±1.84	60.48±1.15	71.76±2.93
Bu	16.18±0.96	25.18±2.13	33.79±1.97	45.72±1.44	57.72±3.01	71.47±1.64	81.41±1.87
BHT	81.14±0.84	86.0.9±1.04	87.52±4.24	91.67±0.52	94.11±0.42	94.41±0.32	95.28±3.25
BHA	84.23±1.14	90.11±0.68	94.59±0.77	96.09±0.02	97.35±1.08	99.59±0.14	99.76±0.19

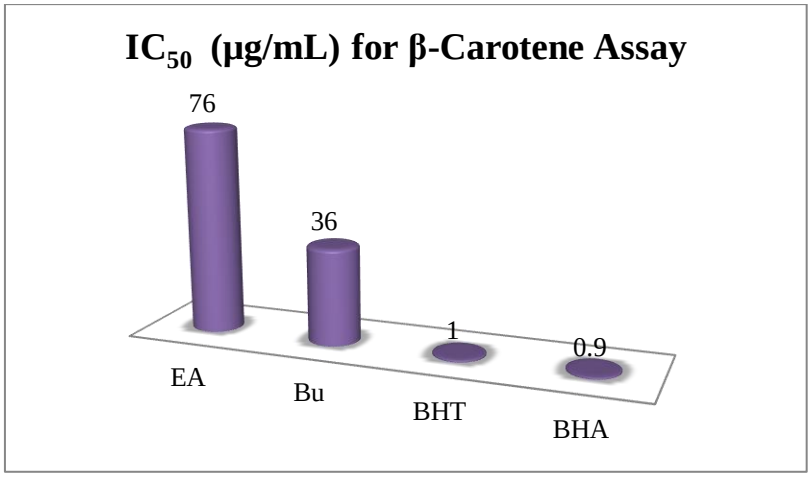


Figure 34: IC_{50} of β-carotene assay for standards and *O. virgata* extracts

The highest β-carotene scavenging activity was observed at 200µg/mL for both extracts, EA and Bu, with a percentage of 71.7% and 81.4%, respectively. Both extracts of *O. virgata* have a low activity compared to standards BHT and BHA.

Figures 35 and **36** represent a comparison of $IC_{50}/A_{0.50}$ of of *O. virgata* extracts for all antioxidant assays.

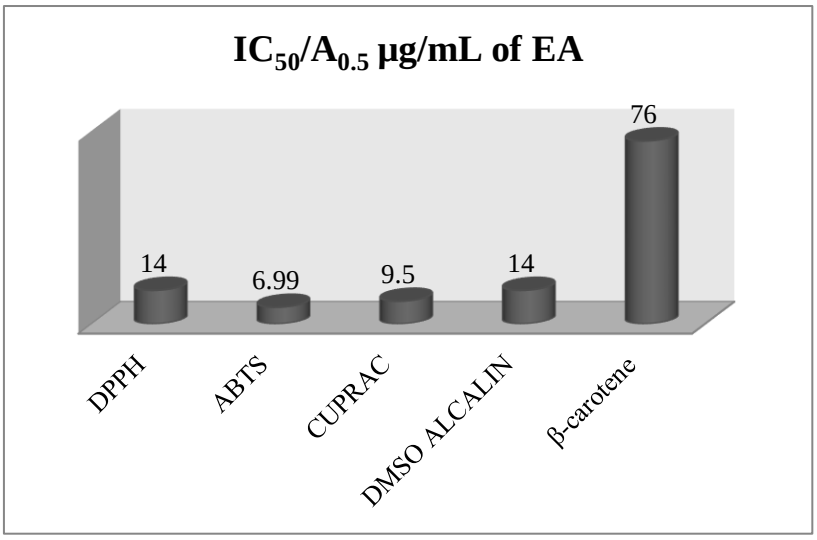


Figure 35: IC_{50} /$A_{0.5}$ of *O. virgata* EA extract for all antioxidant assay.

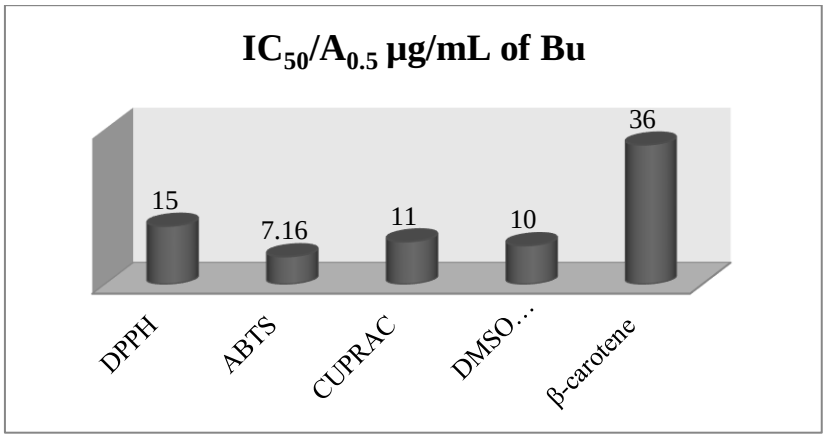


Figure 36: IC_{50} /$A_{0.5}$ of *O. virgata* Bu extract for all antioxidant assay.

In this work, *O. virgata* EA and Bu extracts were screened for their antioxidant abilities through ability to scavenge DPPH, ABTS, CUPRAC, superoxide radicals (Alcaline DMSO) and β-carotene. Both extracts exhibited a considerable antioxidant potential and curiously, EA was in 3 tests more effective than Bu. Considering the distinct phenolic composition of the extracts, one may hypothesise that the superior antioxidant potential of EA can in part be associated to its richness in flavonoids and phenolic compounds (Tungmunnithum *et al.*, 2018) or the phenolic acid dicaffeyolquinic acid which is the prominent compound in EA, Danino *et al.* reported in 2009 that the dicaffeyolquinic acids have higher DPPH radical scavenging activity than trolox and ascorbic acid, which are commonly used as antioxidants).

II.3.2. Antibacterial Activity

The evaluation of antibacterial activity of EA and Bu extracts of *S. tenuipes* and *O. virgata* were carried out using the disk diffusion method against four human pathogenic bacteria (two Gram-negative bacteria and two Gram-positive bacteria): *Escherichia coli* (-), *Pseudomonas aeruginosa* (-), *Staphylococcus aureus* (+) and *Bacillus subtilis* (+), then for each disk the diameters of growth inhibition zones of the bacteria were measured.

Antibacterial Activity of *S. tenuipes*

The results of antibacterial screening of *S. tenuipes* extracts are reported in **table 30**.

Table 30: Diameter of inhibition zone of *S. tenuipes* extracts

***S. tenuipes* Extracts**	**The Diameter of Inhibition Zone(mm)**			
	E. coli	*P. aeruginosa*	*S. aureus*	*B. subtilis*
EA	0	22±0.9	0	10±0.3
Bu	0	20±0.7	10±0.2	0

The results revealed that the antibacterial activity of EA exhibits a good inhibition against *P. aeruginosa*, moderate against *B. subtilis* and no effect was noticed against *E. coli* and *S. aureus* (at concentration=100mg/ml the diameter of inhibition zone: *P. aeruginosa*=22mm, *B. subtilis*=10mm). Like EA, Bu exhibited a good inhibition against *P. aeruginosa*, but moderate against *S. aureus* and no effect was seen against *E. coli* and *B. subtilis* (at concentration=100 mg/mL the diameter of inhibition zone: *P. aeruginosa*=20mm, *S. aureus* =10mm).

The results of this study have shown that the antibacterial activity of *S. tenuipes* depended on the type of pathogenic bacteria. These findings are consistent with what has been previously reached by Mansoor Ahmad (2012) on the two extracts of ethyl acetate and n-butanol of *Scrophularia nodosa* which was active against some bacteria like *B. subtilis*, *S. aureus* and *S. typhi* but inactive against others such as *E. coli*, *S. flexenari* and *P. aeruginosa*. In addition to that, a study done by Mahboubi (2013) has been shown that the antimbacterial activity of ethanolic, methanolic, ethyl acetate and aqueous extract of *S. striata* is dependent on the type of microorganism.

Antibacterial Activity for *O. virgata*

The results of antibacterial screening of *O. virgata* extracts are reported in **table 31**.

Table 31: Diameter of inhibition zone of *O. virgata* extracts

***O. virgata* Extracts**	**The Diameter of Inhibition Zone(mm)**			
	E. coli	*P. aeruginosa*	*S. aureus*	*B. subtilis*
EA	0	20±1.2	16±0.6	12±0.5
Bu	0	11±0.8	0	0

The results revealed that EA has antibacterial activity against three bacteria, while Bu has an activity against only one. In more details, EA exhibits a good inhibition against *P. aeruginosa*, and *S. aureus*, moderate against *B. subtilis* and no effect was shown against *E. coli* (at concentration=100mg/ml the diameter of inhibition zone: *P. aeruginosa*=20mm, *S. aureus*=16mm, *B. subtilis*=12mm). In contrast, Bu exhibits a moderate inhibition against *P. aeruginosa* (at concentration=100 mg/mL the diameter of inhibition zone : *P. aeruginosa*=11mm).

These results agree with the findings of Azouzi *et al*. (2011) which have shown that the antibacterial activity of ethyl acetate extract of *Oenanthe golobulosa* L showed good antibacterial activity against the microorganisms *Staphylococcus aureus* ATCC 29213 and *Staphylococcus aureus* ATCC 43300. In another study, done by Arbain (2005) on *Oenanthe javanica* D., the methanolic and ethanolic extract of this plant did not show any sign of bacterial inhibition to all tested bacteria.

II.3.3. Anti-inflammatory Activity

Inflammation is a complex physiological response of living tissues to injury and is known to involve a complex array of enzyme activation, mediators release, extravasations of fluid, cells migration, tissue breakdown and repair (Sowemimoa *et al*., 2013). In this respect, protein's denaturation is also pointed as a central cause of inflammation (Mizushima and Kobayashi, 1968). The potential anti-inflammatory activity of EA and Bu extracts of *S. tenuipes* and *O.virgata* were in two animal models, namely carrageenan-induced paw edema and xylene-induced ear edema models. Moreover, the ability to counteract protein denaturation was estimated as part of the investigation on the mechanism of the anti-inflammation activity

Anti-inflammatory Activity of *S. tenuipes*

The results of *in vivo*/*in vitro* anti-inflammatory activity of *S.tenuipes* extracts are presented in **table 32** and in **figures 37**, **38**, and **39**.

Table 32: Anti-inflammatory activities of *S. tenuipes* extracts.

Treatment	Paw-edema Volume (mL)			Ear-edema Weight (mg)	BSA Denaturation (% Inhibition) At 2mg/mL
	1h	2h	3h		
Control	2.40± 0.01[a]	1.68±0.04[a]	1.40± 0.03[a]	4.00±0.05[a]	-
EA	1.19±0.56[b]	0.91±0.15[b]	0.52±0.25[b]	1.9±0.09[b]	80.72±1.30[a]
Bu	1.96±0.36[c]	1.33±0.84[c]	1.08±0.93[c]	3.00±0.12[c]	NA
Diclofenac	0.54±0.08[d]	0.3±0.02[d]	0.25±0.09[d]	1.00±0.01[d]	94.04±0.84[b]

Values are means ± SEM. Means followed by different letters ([a,b,c,d]) in the same column are significantly different at $p < 0.05$ according to Tukey's test or T-test (BSA denaturation).

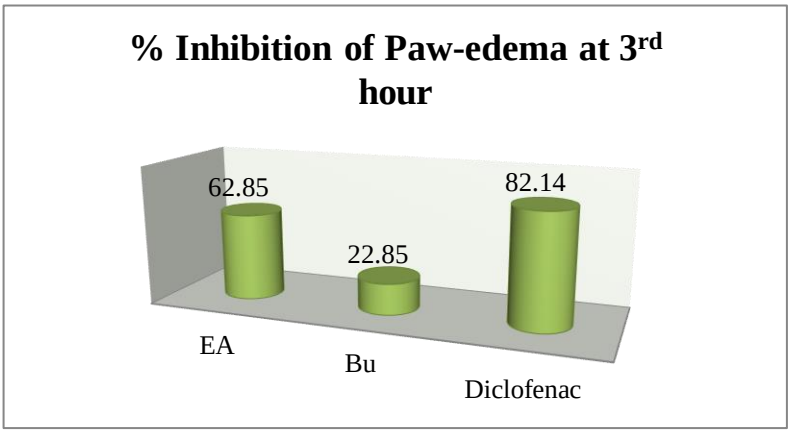


Figure 37: Effect of *S. tenuipes* extracts on carrageenan-induced rat paw edema at the 3rd hour.

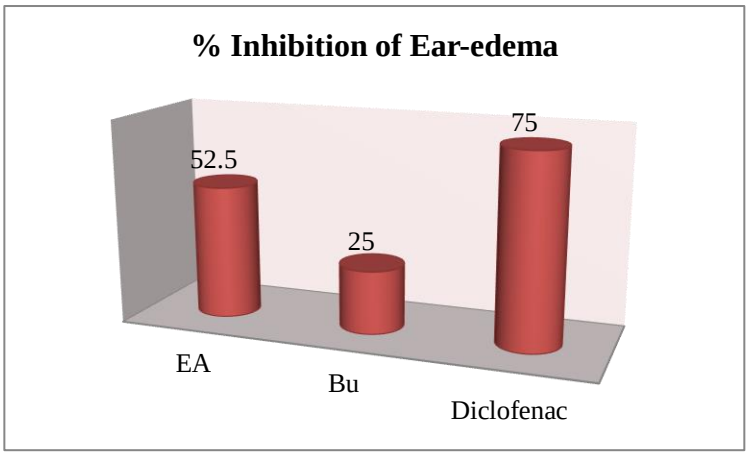


Figure 38: Effect of *S .tenuipes* extracts on xylene-induced rat paw edema.

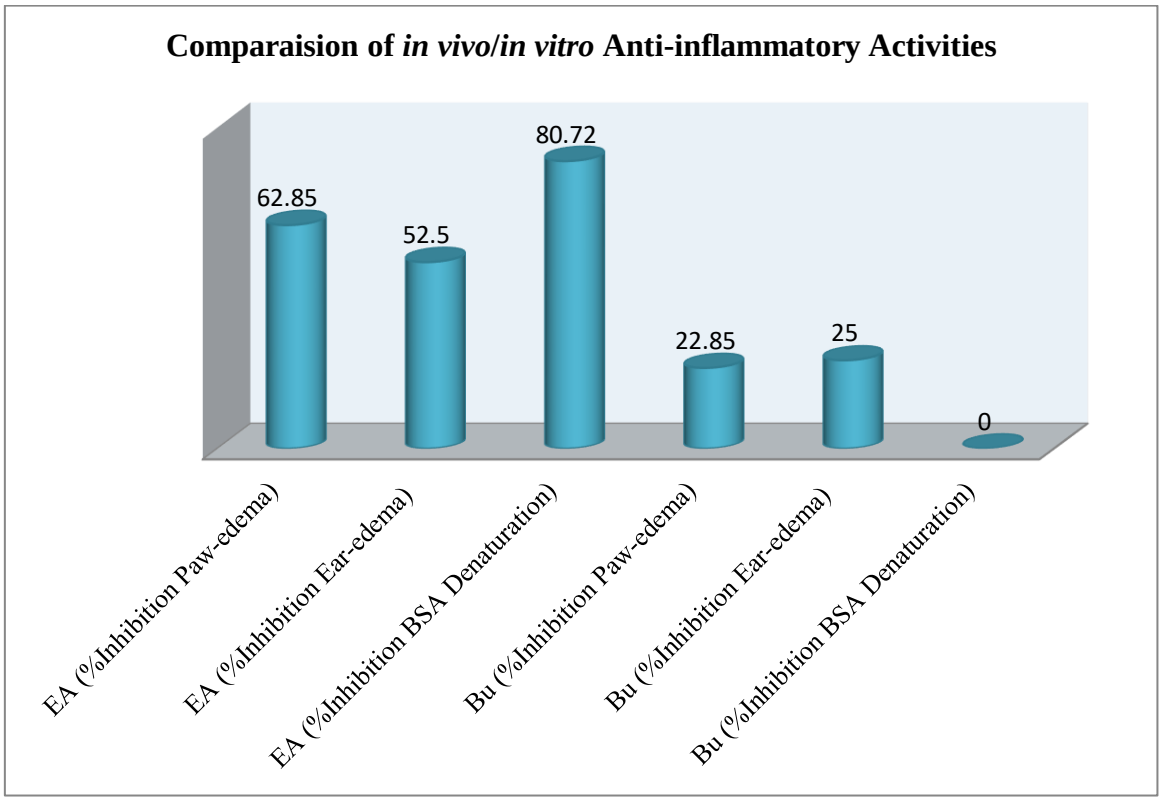


Figure 39: Comparison of the effect of *S. tenuipes* extracts on *in vivo*/*in vitro* anti-inflammatory activities.

As summarized in **table 32** the injection of the rats with carrageenan (1%) caused a paw edema, which was characterized by a volume of 2.4 mL upon 1 h and tended to decrease over time, reaching 1.4 mL at 3 h, in control conditions. The pre-supplementation of the animals' diet with *S. tenuipes* extracts, at a dose of 200 mg/Kg, considerably prevented inflammation. The major preventive effect was obtained with EA extract, for which paw-edema volume was

reduced by approximately half in the first 2 hours and by 62.85% at the third hour, as compared to control. Although less effective, Bu extract also prevented the paw edema of the rats over the experiment time (22.85% as compared with control at 3 h). It should be mentioned that edema formation is a result of complex interactions among various inflammatory mediators that contribute to the increase of vascular permeability and/or blood flow. In the case of the carrageenan-induced edema, this occurs as a biphasic event, in which the early phase, observed about 1 h after carrageenan injection, is due to the release of the modulators serotonin, histamine, while the late phase occurs due to to release of bradykinin, protease, prostaglandin and lysosome, meaning that suppression of early phase and late phases may be due to inhibition of release of early mediators and by inhibition of cyclo-oxygenase, respectively (Hafeez *et al*., 2013). Based on this, our results suggest that the inflammatory potential of *S. tenuipes* extracts occurs by both mechanisms.

Xylene-induced ear-edema is frequently used as an acute inflammation model. Ear-edema formation may involve a release of inflammatory mediators of promoting vasodilation and increasing vascular permeability and these mediators can induce ear edema (Zhao *et al*., 2018).

The anti-inflammatory capacity of *S. tenuipes* EA and Bu extracts and the superior potential of the first were also evidenced in the xylene-induced ear edema model. In fact, rats administered with EA and Bu at 200 mg/Kg showed an ear-edema suppression of 52.5% and 25% compared to the control group, respectively**.** This result further suggests that *S. tenuipes* extracts may produce anti-inflammatory effects through inhibiting the inflammatory mediators of the acute phase of inflammation. As regard to the ability to impair protein denaturation processes, our results showed that this event was also effectively inhibited by *S. tenuipes* EA (80.72%), while Bu was ineffective.

Hence, the overall gathered results support the anti-inflammatory claims of *Scrophularia* plants and in particular, of S. *tenuipes*. In addition, considering the richness of EA in total phenolic compounds and in particular of phenylethanoid acetyl martynoside, it is possible to suggest that this is a critical metabolite for the anti-inflammatory potential of *S. tenuipes*. Martynoside isolated from the Scrophulariaceae family was reported to have a remarkably anti-inflammatory activity: when isolated from the genus *Verbascum salviifolium* and tested on a carrageenan-induced hind paw edema in mice, it exerted a significant anti-inflammatory potential (60% inhibition) (Tatlia *et al*., 2008). In a study carried out on *Veronica anagallis-aquatica*, martynoside are combined with aquaticoside A, aquaticoside B, and tested on

carrageenan-induced hind paw edema in mice and inhibit the inflammation by 48.9% (Küpeli *et al*., 2005).

To our knowledge, distinct species of *Scrophularia* genus have an anti-inflammatory potential. In this context, the iridoids (scropolioside B and scropolioside D) isolated from *S. denata* were shown to exhibit anti-inflammatory effects in NF-$_k$B-mediated reporter gene luciferase assay (Zhang *et al*., 2014). Verbascosaponin isolated from *S. auriculata* strongly inhibited the carrageenan paw oedema and ear oedema induced by 12-*O*-tetradecanoylphorbol 13-acetate (TPA test) (Giner *et al*., 2000). According to Fernandez *et al*., several phenolic acids isolated from *S. frutescens*, including ferulic, gentisic, protocatechuic and syringic acids, were active in the TPA test (Fernandez *et al*. 1998). Moreover, the ethyl acetate extract of *S. striata*, in several anti-inflammatory models, inhibits IL-1b, TNF-a and prostaglandin E2 (PGE2) secretion in mouse peritoneal macrophages induced by lipopolysaccharide (LPS) (Azadmehr *et al*., 2013).

Anti-inflammatory Activity of *O. virgata*

The results of *in vivo*/*in vitro* anti-inflammatory activity of *O.virgata* extracts are presented in **table 33** and in **figures 40**, **41** and **42**.

Table 33: Anti-inflammatory activities of *O. virgata* extracts.

Treatment	Paw-edema Volume (mL)			Ear-edema Weight (mg)	BSA Denaturation (% Inhibition) At 4mg/mL
	1h	2h	3h		
Control	2.40± 0.01	1.68±0.04	1.40± 0.03	4.00±0.05	-
EA	1.44±0.04	1.17±0.02	0.80±0.09	2.1±0.01	81.58±2.4
Bu	1.79±0.06	1.51±0.03	1.18±0.07	3.2±0.05	NA
Diclofenac	0.54±0.08	0.3±0.02	0.25±0.09	1.00±0.01	94.04±0.84

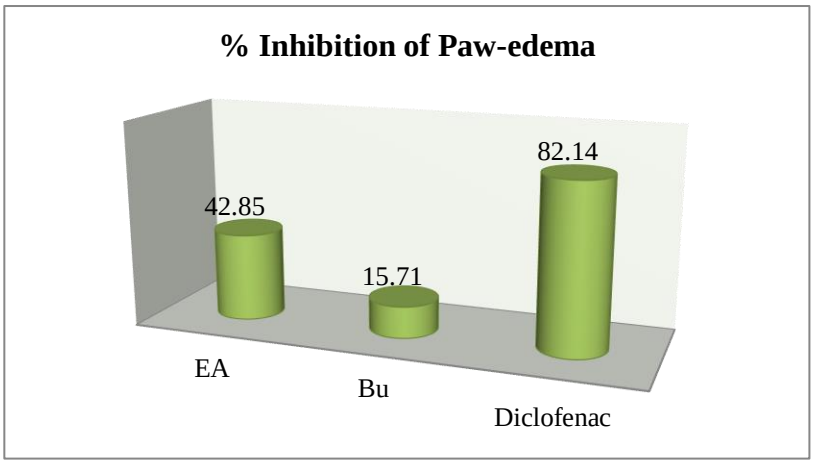


Figure 40: Effect of *O. virgata* extracts on carrageenan-induced rat paw edema at the 3rd hour.

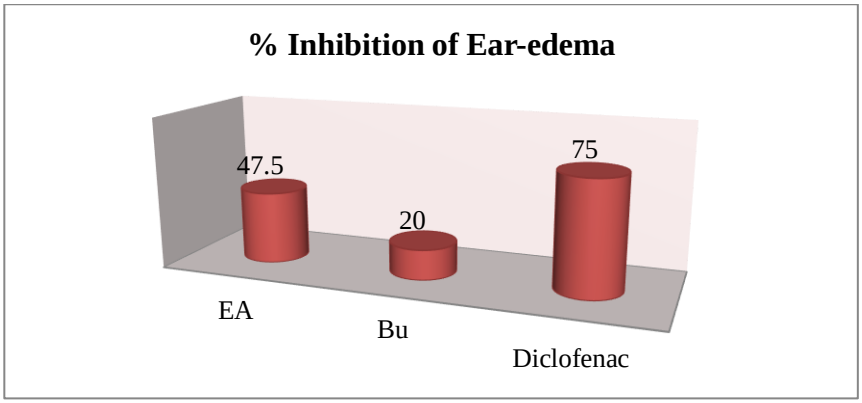


Figure 41: Effect of *O. virgata* extracts on xylene-induced rat paw edema

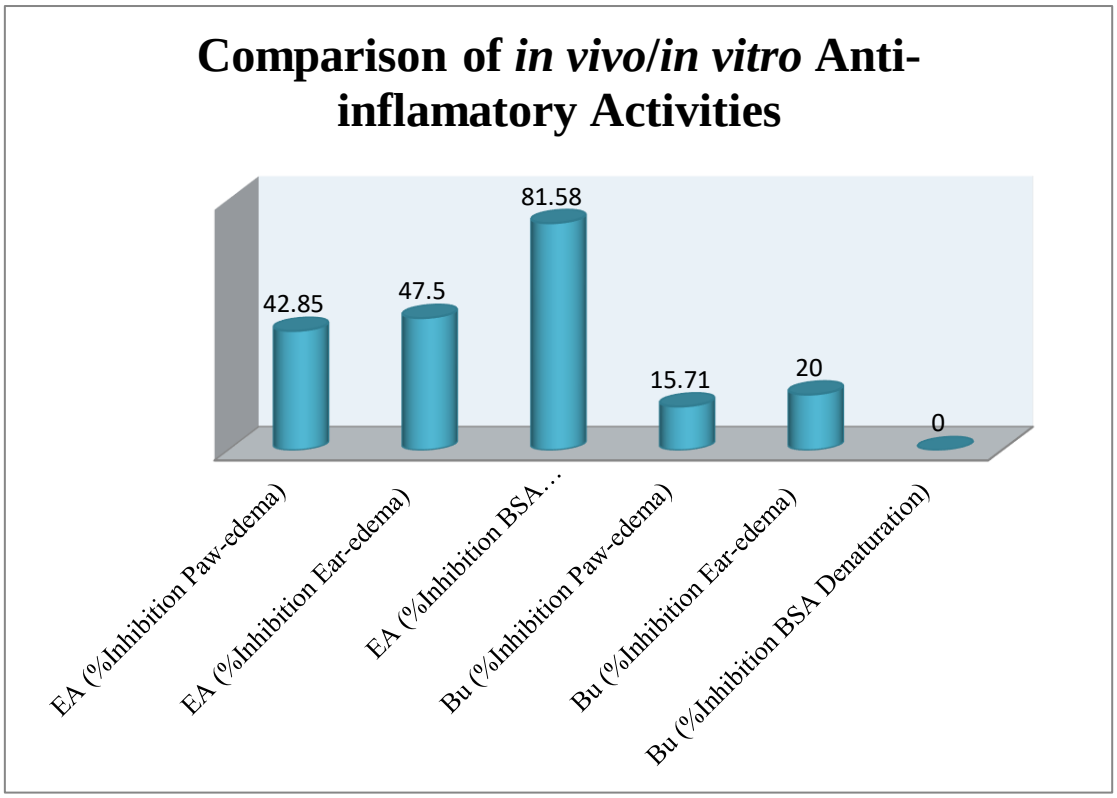


Figure 42: Comparison of effect of *O. virgata* extracts on *in vivo*/*in vitro* anti-inflammatory activities.

As summarized in **table 33**, the injection of the rats with carrageenan (1%) caused a paw edema, with a volume of 2.4 mL upon 1 h and tended to decrease over time, reaching 1.4 mL at 3 h, in control conditions. The pre-supplementation of the animals' diet with *O. virgata* extracts, at a dose of 200 mg/Kg, considerably prevented inflammation. The major preventive effect was obtained with EA extract, for which paw-edema volume was reduced by 42.85% at the third hour, as compared to control. Although less effective, Bu extract also prevented the paw edema of the rats over the- experiment time (15.71% as compared with control at 3 h).

The anti-inflammatory capacity of the *O. virgata* EA and Bu extracts and the superior potential of the first were also evidenced in the xylene-induced ear edema model. In fact, rats administered with EA and Bu at 200 mg/Kg showed an ear-edema suppression of 47.5% and 20% compared to the control group, respectively**.** This result further suggests that *O. virgata* extracts may produce anti-inflammatory effects through inhibiting the inflammatory

mediators of the acute phase of inflammation. As regard to the ability to impair protein denaturation processes, our results showed that this event was also effectively inhibited by *O. virgata* EA (81.58%), while Bu was ineffective.

As a consequence, the overall gathered results support the anti-inflammatory claims of *O. virgata*. In addition, considering the distinct phenolic composition of the extracts, one may hypothesise that the superior anti-inflammatory potential of EA can in part be associated to its richness in flavonoids and phenolic compounds (Tungmunnithum *et al.*, 2018) or the phenolic acid dicaffeyolquinic acid which is the prominent compound in EA (dicaffeyolquinic acids was reported as anti-inflammatory agent (Park, 2009).

Oenanthe javanica was reported as anti-inflammatory plant (Her *et al.*, 2019; Lu and Li, 2019).

II.3.4. Enzyme Inhibitory Activity

II.3.4.1. α-Glucosidase and α-Amylase Assay (Anti-diabetic Activity)

An unexpected increase of glucose levels in blood causes hyperglycemia in type-2 diabetes patients due to hydrolysis of starch by pancreatic α-amylase and the consequent absorption of glucose by intestinal α-glucosidase (Gray, 1995). Thus, the strong inhibition of α-glucosidase and mild inhibition of pancreatic α-amylase is believed to be an effective strategy for type-2 diabetes management (Krentz and Bailey, 2005). In this respect EA and Bu extracts of *S. tenuipes* and *O. virgate* were screened for their anti-diabetic activity.

Anti-diabetic Activity of *S. tenuipes*

The results of the inhibitory activity toward α-glucosidase and α-amylase assay of *S. tenuipes* extracts are presented in **tables 34**, **35** and in **figures 43** and **44**.

Table 34: Inhibitory activity of *S. tenuipes* extracts toward α-glucosidase assay.

Extracts	% Inhibition in α-glucosidase Assay						
	15.62µg/mL	31.25µg/mL	62.5µg/mL	125 µg/mL	250 µg/mL	500 µg/mL	1000µg/mL
EA	3.96±0.25	7.51±0.14	12.90±2.1	18.72±4.2	22.08±3.2	24.93±2.3	29.76±5.1
Bu	3.51±0.1	6.72±2.1	9.05±1.4	8.86±1.8	11.80±1.9	21.26±3.1	23.91±1.5
Acarbose	17.58±2.1	24.87±1.2	28.36±0.1	32.24±1.7	45.25±3.7	65.31±1.8	78.69±2.1

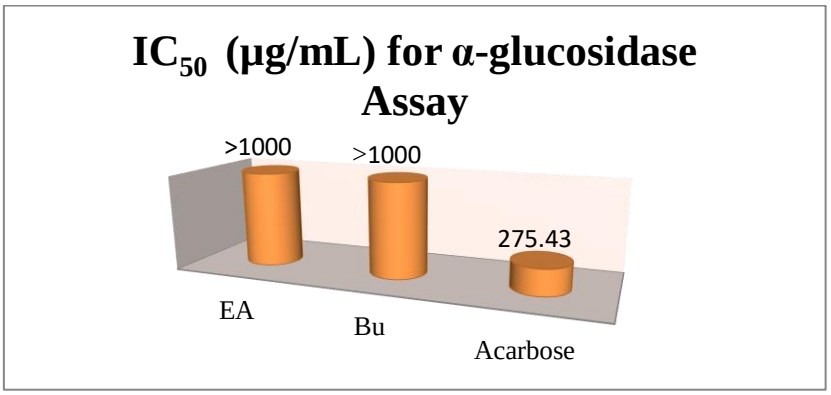


Figure 43: IC_{50} of α-glucosidase for standards and *S. tenuipes* extracts.

Table 35: Inhibitory activity of *S. tenuipes* extracts toward α-amylase assay.

Extracts	% Inhibition in α-Amylase Assay						
	6.25 µg/mL	12.5µg/mL	25 µg/mL	50 µg/mL	100µg/mL	200µg/mL	400µg/mL
EA	47.82±0.1	54.71±1.2	71.92±0.9	78.3±2.84	73.0±3.37	75.8±3.94	84.81±2.01
Bu	46.66±0.3	52.01±2.1	57.14±2.9	61.29±1.03	64.44±2.4	69.81±1.9	70.0±3.0
Acarbose	17.69±0.2	22.98±1.2	38.45±1.8	55.23±1.4	63.14±2.4	67.01±2.3	69.17±1.8

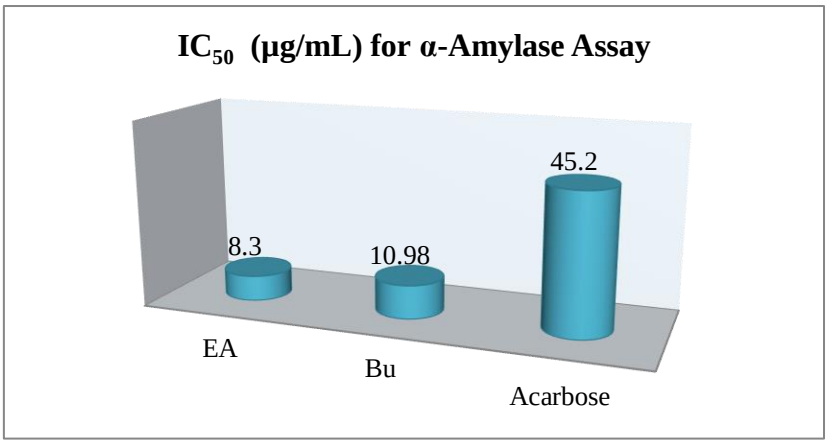


Figure 44: IC_{50} of α-amylase for standards and *S. tenuipes* extracts

Albeit the two extracts of *S. tenuipes* showed a weak α-glucosidase inhibitory effects (**tables 34**, **35**), but their potency to suppress α-amylase activity was stronger than that of the commercial drug acarbose (IC_{50} value 8.3 µg/mL, 10.98 µg/mL and 45.20 µg/mL for EA, Bu and acarbose, respectively). Among the two extracts, EA was the most promising one (about 5.5 fold the potency of acarbose), a fact that may also be associated to its phenolics richness and in particular to its major phenolic compound, i.e. acetyl martynoside, or even to phenolic synergies, as suggested by McCue and Shetty (2004). As far as we know, the *in vivo* antidiabetic ability of *Scrophularia* plants and the simultaneous association to α-amylase inhibitory activity was previously reported by Ahmed *et al*. (2003), for *S. deserti*. Consistent with our results, *S. frigida* methanolic and aqueous extracts showed weak inhibitory effects towards α-glucosidase (Gholamhoseinian *et al*., 2008).

Anti-diabetic Activity of *O. virgata*

The results of the inhibitory activity toward α-glucosidase and α-amylase assay of *O.virgata* extracts are presented in **tables 36**, **37** and in **figures 45** and **46**.

Table 36: Inhibitory activity of *O. virgata* extracts toward α-glucosidase assay.

Extracts	% Inhibition in α-Glucosidase Assay						
	15.62 µg/mL	31.25 µg/mL	62.5µg/mL	125 µg/mL	250 µg/mL	500 µg/mL	1000µg/mL
EA	13.08±0.2	15.76±0.8	33.56±0.9	56.03±1.9	73.93±1.4	84.67±2.8	86.05±3.1
Bu	7.80±0.1	10.56±0.4	15.39±1.7	21.98±1.6	24.74±2.1	31.80±2.3	50.06±2.1
Acarbose	17.58±0.4	24.87±1.2	28.36±1.5	32.24±1.7	45.25±2.1	65.31±1.8	78.69±3.7

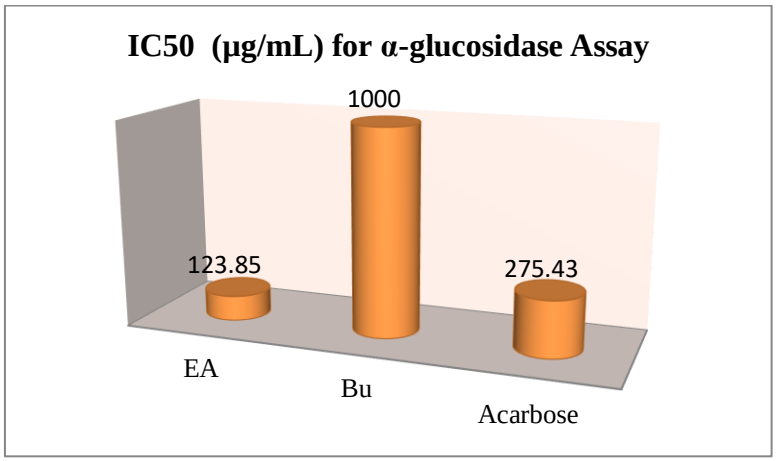


Figure 45: IC_{50} of α-glucosidase for standards and *O.virgata* extracts.

Table 37: Inhibitory activity of *O. virgata* extracts toward α-amylase assay.

Extracts	% Inhibition in α-Amylase Assay						
	6.25 µg/mL	12.5µg/mL	25 µg/mL	50 µg/mL	100µg/mL	200µg/mL	400µg/mL
EA	60.03±0.2	64.7±0.59	66.4±1.33	72.88±2.14	76.9±231	77.37±3.8	78.8±1.54
Bu	58.97±0.44	65.95±0.74	67.56±1.6	69.2±3.08	68.2±1.19	71.9±2.98	71.7±6.47
Acarbose	17.69±0.3	22.98±0.4	38.45±1.2	55.23±1.8	63.14±0.7	67.01±1.9	69.17±2.6

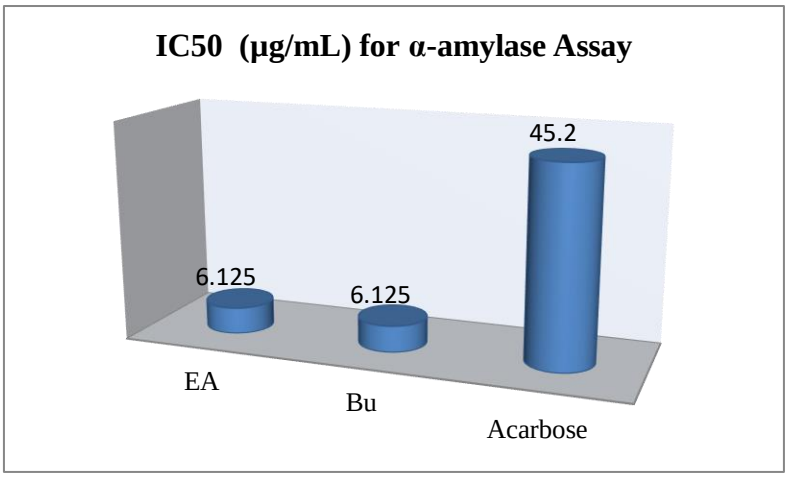


Figure 46: IC_{50} of α-amylase for standards and *O. virgata* extracts

EA extract of *O.virgata* showed a high α-glucosidase inhibitory effects (**table 36**, **37**), in which its IC_{50} is lower than the standard acarbose (123.85 µg/mL). In contrast to α-glucosidase inhibitory assay, the potency of both extracts (EA and Bu) to suppress α-amylase activity was stronger than that of the commercial drug acarbose (IC_{50} value 6.125 µg/mL, 45.20 µg/mL for both extract and acarbose, respectively). Among the two extracts, EA was the strongest and have a higher effect than standard acarbose in both assays, a fact that may also be associated to its richness in flavonoids and phenolic compounds (Ali Asgar, 2012; Saritha, 2017) or the phenolic acid dicaffeyolquinic acid which is the prominent compound in EA: Simeonova (2016) and Chen (2014) have reported the antidiabetic effect of dicaffeyolquinic acid). Consistent with our results, *O. javanica* showed antidiabetic potential (Yang *et al.*, 2000; Lee, 2017).

II.3.4.2. Urease Inhibitory Activity

Urease enzyme has a crucial role in the persistent habitation of *Helicobacter pylori* (*H. pylori*) that induces gastrointestinal diseases, in particular gastritis, duodenal, peptic ulcer, and gastric cancer. Plants have long been utilized as the biggest source of substances with medicinal properties from natural origin and therefore result in less toxicity and adverse side

effects upon usage (Mahernia, 2015). In this respect EA and Bu extracts of *S.tenuipes* and *O. virgata* were examined against Jack bean urease activity.

Urease Inhibitory Activity of *S. tenuipes*

The results of the inhibitory activity toward urease assay of *S. tenuipes* extracts are presented in **table 38** and in **figure 47**.

Table 38: Inhibitory activity of *S. tenuipes* extracts toward urease.

Extracts	% Inhibition in Urease Assay						
	3.125µg/mL	6.25µg/mL	12.5µg/mL	25 µg/mL	50 µg/mL	100µg/mL	200µg/mL
EA	9.47±0.2	10.69±0.8	12.38±1.2	11.36±0.8	21.68±0.7	37.09±1.8	56.12±1.4
Bu	4.15±0.1	8.23±0.7	10.87±0.45	11.04±1.4	20.39±2.5	27.49±4.1	33.79±2.7
Thiourea	4.49±0.78	19.85±2.74	55.64±4.24	94.17±0.15	98.42±0.19	98.49±0.41	98.90±0.05

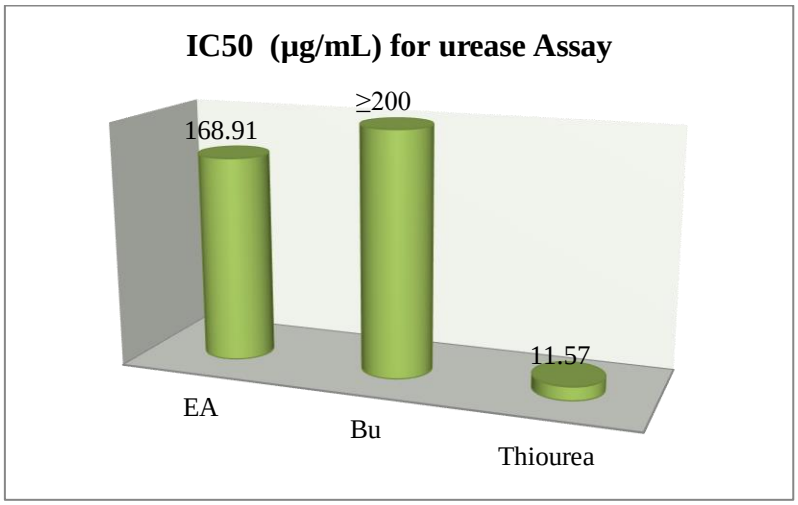


Figure 47: IC_{50} of urease for standards and *S. tenuipes* extracts.

The results of urease inhibition activity indicated that EA and Bu extracts of *S. tenuipes* showed a weak urease inhibitory effect (**table 38**), in which its IC_{50} of both extracts is much more than standard thiourea (IC_{50} value 168.91 µg/mL, ≥200µg/mL and 11.57 µg/mL for EA, Bu and thiourea, respectively). In contrast to our result, *S. nodosa* ethyl acetate and butanol

extracts showed a significant urease inhibitory effect and butanol had a higher effect than ethyl extract (Ahmad *et al*., 2012).

Urease Inhibitory Activity of *O. virgata*

The results of the inhibitory activity toward urease of *O. virgata* extracts are presented in **table 39** and in **figure 48.**

Table 39: Inhibitory activity of *O. virgata* extracts toward urease.

Extracts	% Inhibition in Urease Assay						
	3.125µg/mL	6.25µg/mL	12.5µg/mL	25 µg/mL	50 µg/mL	100µg/mL	200µg/mL
EA	7.29±1.2	15.11±1.7	19.23±2.0	34.63±1.4	49.90±2.6	75.41±3.4	89.41±2.8
Bu	5.14±0.8	7.46±0.9	13.56±1.8	16.98±2.3	30.46±2.2	49.32±2.4	75.86±3.6
Thiourea	4.49±0.78	19.85±2.74	55.64±4.2	94.17±0.15	98.42±0.1	98.49±0.4	98.90±0.05

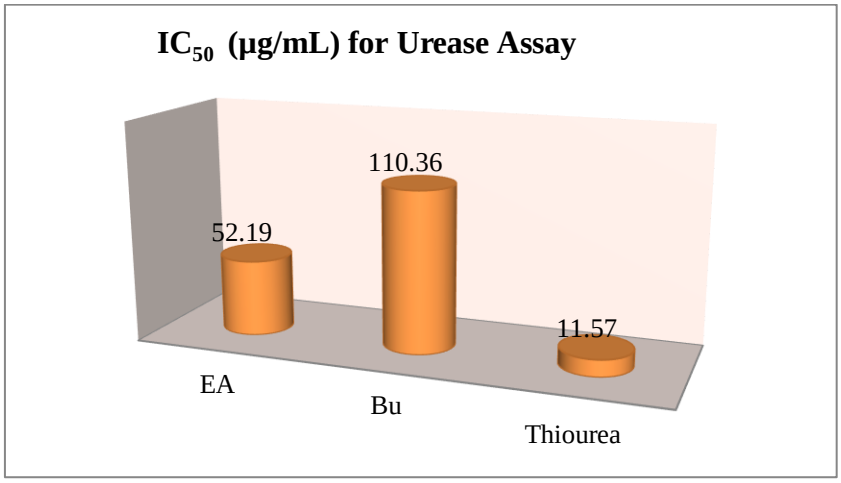


Figure 48: IC_{50} of urease for standards and *O. virgata* extracts

The results of urease inhibition activity revealed that both extracts of *O. virgata* have a weak urease inhibitory effect. On the other hand, EA urease inhibitory effect is higher than Bu with an IC_{50} equal to 52 µg/mL. There are no previous studies on *Oenanthe* genus regarding this assay but many plants from Apiaceae family were tested against urease at 10mg and showed moderate activity like: *Anethum graveolins, Apium graveolens, Diplotaenia damavendica, Echinophora platyloba* (Nabati *et al.*, 2012).

II.3.4.3. Tyrosinase Inhibitory Activity

Tyrosinase inhibition has become a strategy to prevent excess formation and accumulation of melanin in the skin, thereby preventing hyperpigmentation disorders such as melasma and agespots (Ullah *et al*., 2016). For that, tyrosinase inhibitory activity of EA and Bu extracts of *S. tenuipes* and *O. virgata* were screened.

Tyrosinase Inhibitory Activity of *S. tenuipes*

The results of the inhibitory activity toward tyrosinase assay of *S. tenuipes* extracts are presented in **table 40**.

Table 40: Inhibitory activity of *S. tenuipes* extracts toward tyrosinase.

Extracts	% Inhibition in Tyrosinase Assay						
	3.125µg/mL	6.25µg/mL	12.5µg/mL	25 µg/mL	50 µg/mL	100µg/mL	200µg/mL
EA	0.87±0.3	1.2±1.1	3.12±1.5	5.25±1.8	6.39±1.4	21.46±2.1	33.7±3.5
Bu	0.98±0.1	1.58±0.3	2.14±0.8	3.48±0.11	8.25±1.2	26.1±2.2	30.27±2.7
Kojic acid	14.2±0.6	25.12±1.1	30.5±2.1	51.26±1.9	72.36±1.6	85.12±2.8	99.7±2.1

The results of urease inhibition activity revealed that EA and Bu extracts of *S. tenuipes* has a weak tyrosinase inhibitory effect in comparison to the standard (IC_{50} >200µg/mL and 8.02 µg/mL for both extract and kojic acid, respectively). In contrast to our results, Zengin (2018) study revealed that *Scrophularia lucida* showed a significant tyrosinase inhibitory activity.

Tyrosinase Inhibitory Activity of *O. virgata*

The results of the inhibitory activity toward tyrosinase of *O. virgata* extracts are presented in **table 41.**

Table 41: Inhibitory activity of *O. virgata* extracts toward tyrosinase assay.

Extracts	% Inhibition in Tyrosinase Assay						
	3.125µg/mL	6.25µg/mL	12.5µg/mL	25 µg/mL	50 µg/mL	100µg/mL	200µg/mL
EA	3.12	5.74	11.25	16.15	29.55	40.57	61.17
Bu	1.04	2.48	6.47	11.25	18.58	26.52	33.33
Kojic acid	14.2	25.12	30.5	51.26	72.36	85.12	99.7

The results of tyrosinase inhibition activity revealed that EA of *O. virgata* has a moderate tyrosinase inhibitory effect (IC_{50}: 142µg/mL). On the other hand, Bu showed a weak tyrosinase inhibitory effect with an IC_{50} >200µg/mL. According to Souilah (2019), *O. fistulosa* has a good tyrosinase inhibitory activity.

II.3.4.4. Anticholinesterase Activity

EA and Bu extracts of *S. tenuipes* and *O. virgata* were screened for their Acetyl- and butyrylcholinesterase inhibitory potential. The result of Acetyl- and butyrylcholinesterase inhibitory activities of EA and Bu extracts of *S. tenuipes* and *O. virgata* revealed that both plants have no anticholinesterase activity. According to previous study, *S. lucida* have strong inhibitory activity against AChE Zengin (2018) and also *O. fistulosa* displayed very strong action against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes Souilah (2019).

II.3.5. Sun Protection Factor and UV Protection Factor (UV-PF) Assessment

Exposure to sun rays may lead to skin damages includes sunburns, skin cancer, oxidative damage to skin cells, and photo-aging. Sun protection factor is the abbreviation of SPF. This helps in grading the probable ability of a sunscreen to block UV B radiation. Chemical sunscreens, which are available in the market, are incorporated into moisturizers, lotions, creams, and hair tonics. Continuous usage of these chemical sunscreens are not safe from protection and unavoidable from side effects. In contrast, natural substances of plant origin are considered as potential sunscreen resources as they

absorb UV radiation. Therefore, *S. tenuipes* and *O. virgata* extracts are investigated for sun protection effect.

The results of UV protection factor of *S. tenuipes* and *O. virgata* extracts are presented in **table 42**.

Table 42: Categories of protection displayed on solar products of *S. tenuipes* and *O. virgata* extracts based on protection factors measured, according to the recommendation of the European commission 2006

Extracts	Sun Protection Factor Measured	Protection Factor Indicated	Category Indicated
EA *S. tenuipes*	37.07±1.09	30	High protection
Bu *S. tenuipes*	35.45±0.34	30	High protection
EA *O. virgata*	38.36±0.35	30	High protection
Bu *O. virgata*	36.25±0.17	30	High protection

The results of sun protection factor revealed that the extracts of both *S.tenuipes* and *O. virgata* have a high sun protection effect. Those extracts can be used as additives in other sunscreen formulations to enhance their SPF. No previous study has been achieved yet on *Scrophularia* and *Oenanthe* genera.

CONCLUSION AND PERSPECTIVES

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Conclusion and perspectives

Due to the increasing problem of drug resistance, new and improved medicines are required. Natural products and biotherapeutics offer a vast resource for new drugs. Natural products and traditional medicines are of great importance. From earlier decades medicinal plants existed on Earth. The world is decorated with medicinal herbs, which is a rich wealth of endurance. Every plant is identified by its own different therapeutic properties due to active bioactive molecules. In the modern system of medicine, natural drug substances are reported to be vital and have appreciable roles. Their therapeutic role was justified by the presence of their bioactive molecules. Due to disease-inhibiting capabilities, they are extremely useful as natural drugs, provide basic bioactive compounds that are less toxic and more effective, and bring biological and chemical means of modification and extraction of natural products into potent drug.

The purpose of this work was to study the phytochemical composition (spectrophotometric determination of some secondary metabolites and chromatographic analysis) of the two endemic plants *Scrophularia tenuipes* Coss & Durieu and *Oenanthe virgata* Poiret and to investigate their health-benefit properties, in particular with respect to *in vivo*/*in vitro* anti-inflammatory antimicrobial and antioxidant activities, their potential to inhibit some enzymes (anti-diabetic, urease, tyrosinase inhibitory activities and anticholinesterase activity), as well as SPF and UV protection factor assessment.

Total phenolic content was performed according to Folin–Ciocalteu method. Total phenolic content (TPC) of *S. tenuipes* and *O. virgata* revealed that EA extract has a higher concentration than Bu extract for both plants (EA: 225.47and 713.71 mg GAE/g dry extract; Bu: 181.35 and 453.71mg GAE/g dry extract for *S. tenuipes* and *O. virgata*, respectively).

Total flavonoid content was performed according to the aluminium nitrate method. Total flavonoid content (TFC) of *S. tenuipes* revealed that Bu extract has a higher concentration than EA extract (21.7 and 64.55 mg QE/g dry extract for EA and Bu, respectively). In contrast of *S. tenuipes* TFC, the amount of flavonoid content in Bu extract is lower than EA extract for *O. virgata* (272.22 and 173.12±1.44 mg QE/g dry extract for EA and Bu, respectively).

Total carotenoid content (TCC) was evaluated and the results revealed were 14.3 µg of β-carotene E /g dry extract for *S. tenuipes* and 3.9 µg of β-carotene E /g dry extract for *O. virgata*.

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Gas chromatography/mass spectrometry (GC/MS) analysis of *O. virgata* essential oil revealed that the major constituents of the essential oils of *S. tenuipes* during flowering are: hexahydrofarnesyl acetone (34.77%), phthalic acid, diisobutyl ester (19.36%) and dibutyl phthalate (14.94%).

UHPLC-ESI-DAD-MS^n analysis of EA and Bu extracts of *S. tenuipes* revealed that the main compound in EA corresponded to the phenylethanoid acetyl martynoside and the iridoid harpagoside was dominant in Bu. As regards UHPLC-ESI-DAD-MS^n analysis of *O. virgata* the phenolic acid dicaffeyolquinic acid is the main compound for EA and the flavonoid rutin is dominant for Bu.

EA and Bu extracts of *S. tenuipes* and *O. virgata* were screened for their antioxidant abilities through ability to scavenge DPPH, ABTS, CUPRAC, superoxide radicals (DMSO Alcaline) and β-carotene. *S. tenuipes* exhibited considerable antioxidant potential while *O.virgata* showed considerable potential. Bu was almost more effective than EA for *S. tenuipes* while for *O. virgata* was EA.

The evaluation of the anti-bacterial activity of EA and Bu extracts of *S. tenuipes* and *O.virgata* were carried out using the disk diffusion method against four human pathogenic bacteria (two Gram-*negative bacteria* and two Gram-*positive bacteria*: *Escherichia coli* (-), *Pseudomonas aeruginosa* (-), *Staphylococcus aureus* (+) and *Bacillus subtilis* (+).The results of this study have shown that the antibacterial activity of *S. tenuipes* depended on the type of pathogenic bacteria. For example, EA exhibited a good inhibition against *P. aeruginosa*, a moderate one against *B. subtilis* and no effect was noticed against *E. coli* and *S. aureus*. As well as EA, Bu exhibited a good inhibition against *P. aeruginosa*, a moderate one against *S. aureus* and no effect was seen against *E. coli* and *B.subtilis*. Regarding *O. virgata*, the results revealed that EA has antibacterial activity against three bacteria, while Bu has an activity against only one.

The *in vivo* anti-inflammatory activity of EA and Bu extracts of *S. tenuipes* and *O.virgata* were evaluated in two widely used rat models, namely carrageenan-induced paw edema and xylene-induced ear edema models. Moreover, the *in vitro* anti-inflammatory potential was estimated using inhibition of albumin denaturation technique. The *S. tenuipes* and *O. virgata* anti-inflammatory screening showed that EA extract was the stronger with regard to *in vivo*/*vitro* anti-inflammatory effect.

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The anti-diabetic activity of EA and Bu extracts of *S.tenuipes* and *O. virgata* were evaluated using α-glucosidase and α-amylase assay. Albeit *S.tenuipes* showed a weak α-glucosidase inhibitory effects, but their potency to suppress α-amylase activity was stronger than that of the commercial drug acarbose. EA extract of *O.virgata* showed a strong α-glucosidase inhibitory effect, in which its IC_{50} is lower than the standard acarbose. In contrast to α-glucosidase inhibitory assay, the potency of both extracts of *O. virgata* (EA and Bu) to suppress α-amylase activity was stronger than that of the commercial drug acarbose.

EA and Bu extracts of *S. tenuipes* and *O. virgata* were examined against Jack bean urease activity. *S. tenuipes* and *O. virgata* showed a weak urease inhibitory.

Tyrosinase inhibitory activity of EA and Bu extracts of *S.tenuipes* and *O.virgata* were screened. *S. tenuipes* has a weak tyrosinase inhibitory capacity and EA of *O. virgata* has a moderate effect.

EA and Bu extracts of *S. tenuipes* and *O. virgata* were screened for their Acetyl- and butyrylcholinesterase inhibitory potential. The result revealed that both plants have no anticholinesterase activity.

S. tenuipes and *O. virgata* extracts were investigated for sun protection potential using sun protection factor and UV protection factor (UV-PF) assessment. The results revealed that both *S. tenuipes* and *O. virgata* have a high sun protection effect.

These results remain preliminary; it would therefore be interesting to continue the investigations on these two plants *Scrophularia tenuipes* Coss & Durieu and *Oenanthe virgata* Poiret by extending the panel of species studied with other biological tests: anti-tumor, *in vivo* anti-oxidant… etc. In addition, further studies concerning the isolation of the active compounds responsible for biological activities will be necessary.

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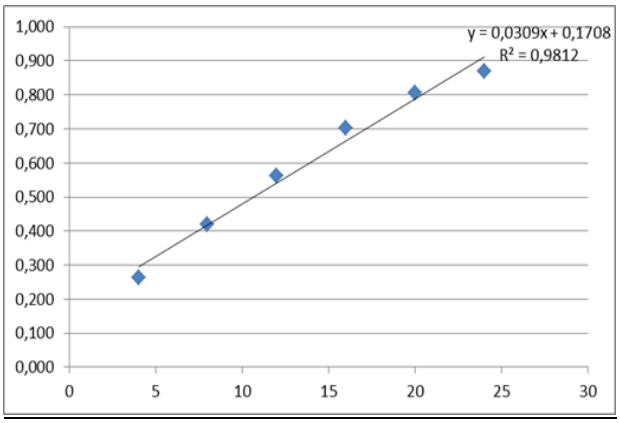
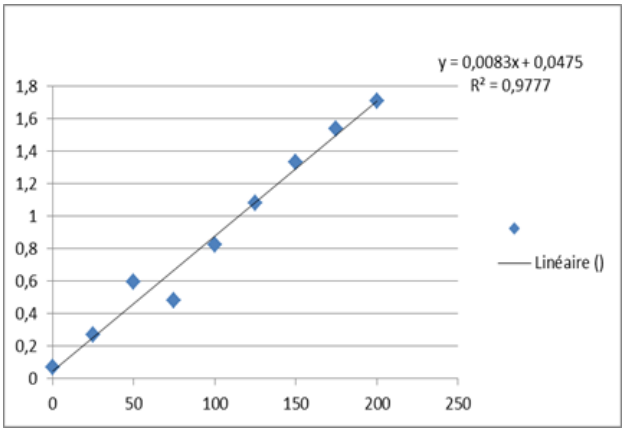
APPENDICES

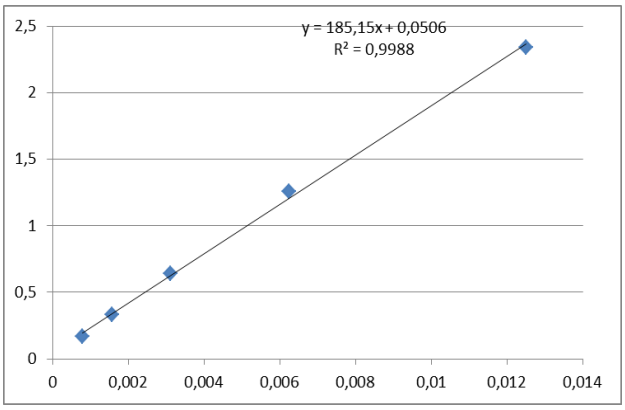
APPENDICES



Appendice 1: Calibration curve of standard gallic acid.



Appendice 2: Calibration curve of standard quercetin.



Appendice 3: Calibration curve of standard β-carotene.