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Presented by

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Theme

Bibliographic review of the *Ballota* genus and evaluation of the DPPH free radical scavenging capacity of *Ballota hirsuta*'s methanolic and aqueous extracts.

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Class : 2019-2020

بسم الله الرحمن الرحيم

Dedicated

TO

MY LOVELY MOTHER AND

DEAREST FATHER

MY BELOVED WIFE, CUTE SONS

AND MY SISTERS AND BROTHERS

TO

ALL THOSE WHO ALWAYS WISHED TO SEE ME GLITTERING HIGH ON THE SKIES OF SUCCESS

***HAMZA KHEMISSA ***

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Contents

Abstract

Medicinal plants are significant in today's drug source. Most current medicinal drugs are prescribed by their subsidiaries. *Ballota hirsuta* benth is perennial shrub plant belonging to the Lamiaceae family growing in Algerian centre, and are being utilized as a part of the traditional medicinal system of our country. The aims of the present study were to review pharmacology activities, phytochemistry, traditional use, and offer therapeutic knowledge of *Ballota* genus and to evaluate antioxidant properties of this plant in essential oil, methanolic and aqueous extract forms. The aerial parts of the plant were subjected to hydrodistillation to have the essential oil (yield: 0.42%). The methanolic maceration aqueous decoction extractions yielded in 11± 0.62% and 13.52± 0.28% respectively. The free radical scavenging effect was evaluated using the free radical DPPH. The IC_{50} were 0.120 ± 0.027mg/ml and 0.124 ± 0.012 mg/ml for the methanolic and aqueous extract respectively.

Key words - *Ballota hirsuta* benth, Lamiaceae, biological properties, traditional uses, aqueous extract, methanolic extract, antioxidant activity.

List of abbreviations

ATCC	**:**	American Type Culture Collection ;
AqE	**:**	Aqueous extract
BHT	**:**	Butylated hydroxytoluene;
MBC	**:**	Minimum bactericidal concentration
MIC	**:**	Minimum Inhibition Concentration
DNA	**:**	Deoxyribonucleic acid
DPPH	**:**	2,2'-diphenyl-1-picrylhydrazyl
EO	**:**	Essential oil
H_2O_2	**:**	Hydrogen peroxide
I%	**:**	Inhibition percentage.
IC_{50} %	**:**	Inhibitory concentration for 50% of activity
LOO°	**:**	Lipid peroxide radical
MeE	**:**	Methanolic extract
MH	**:**	Muller Hinton
NO^*	**:**	Radical nitroxid
O_2^*	**:**	Superoxid Radical
OH^*	**:**	Hydroxyl Radical
PDA	**:**	PtatosDextros Agar
RNS	**:**	Reactive nitrogen species
ROS	**:**	Reactive oxygen species
SD	**:**	Standard deviation

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INTRODUCTION

Introduction

Over the millennia, people have relied for essential needs such as food, clothes and shelter on various sections of plants (Phillipson, 2001). Plants were used since antiquity as medicine for treating different human diseases and illnesses, and also for other uses, such as fertilizer development, flavor, and scent development (Halberstein, 2005; Gurib-Fakim, 2011). Traditional forms of medicine, such as Chinese, Ayurvedic, Native American and African systems operate for centuries and continue to give different treatments to humans (Salim *et al.*, 2008). Medicines are also the dominant source of medication in developed countries and about 60-80% of citizens in the world eat plants to satisfy their health needs (Palombo, 2006)

The treatments of human disease were focused on natural products extracted from different sources, particularly plants (Morteza-Semnani *et al.* 2016). Many natural products have biological processes used in production of drugs and design (Benzie and Wachtel-Galor, 2011), and natural product fragments can be combined with other molecules essential to next generation drug exploration (Ghanbarimasir *et al.*, 2018). The use of herbal remedies played a major role in healing the human body in both ancient and present-day cultures (Benzie and Wachtel-Galor, 2011). Certain *Ballota* species are known to the medicinal use in many diseases (Dulger and Dulger, 2017).

Natural antioxidant sources, primarily phenolic, are often known as major factors to avoid oxidative stress for the human body, and several researchers have stated that plant's antioxidant activities could be associated with oxidative stress protection (Rice-Evans *et al.*, 1997).

Nowadays, polyphenols or extracts from different plants known to be effective antioxidants have been established and developed as a significant area of public health science, pharmaceutical research and medical study (Ramkissoon *et al.*, 2013). Nearly 80% people of developing countries, particularly in Africa, are dependent on herbal medicines, including wounds, metabolic and infectious diseases (Agyare *et al.*, 2009).

In addition, antimicrobial resistance to conventional antimicrobials has increased and become a significant public health concern. This has facilitated studies into better effectiveness combination therapies (Hübsch *et al.*, 2014).

Our study aims at providing a review on the botany, traditional use, phytochemical and pharmacological properties of the *Ballota* genus and focus on the antioxidant activity of the methanolic and aqueous extracts of *Ballota hirsuta* by use of the DPPH test.

This work was interrupted due to the consequences of the Covid-19 pandemic. Therefore possible planned work (complementary antioxidant activity tests, antibacterial and antifungal tests) was stopped.

CHAPTER I

LITERATURE REVIEW

1. Secondary metabolites

1.1. Polyphenols

The plant polyphenols are essential components of human and animal diets and constitute part of the secondary metabolites of separate plant parts (Madhan *et al.*, 2007). The term "phenolic compounds" covers a large variety of substances with an aromatic ring that has one or more hydroxyl substitutes (figure 1) (Bennick, 2002). Over 8,000 phenolic structures are known (Urquiaga and Leighton, 2000).

1.1.1. Structure and classification

Based on the number of the base skeleton carbon atoms natural polyphenols vary in structure from simple molecules, such as gallic acid, phenolic acids, caffeic acid, coumaric acid, chlorogenic acid, ferulic acid to very polymerized substances, such as quinones, flavonoids, stilbenes, coumarins, curcuminoids, condensed tannins and lignans (Crozier *et al.*, 2009). Basically, phenolic compounds may be classified as illustrated in Table 1 (Harborne, 1989; Baxter *et al.*, 1999). The major dietary phenolic compounds are flavonoids, phenolic acids and tannins (King and Young, 1999).

Class	Structure
benzoquinones, Simple phenolics	C6
Hydroxybenzoic acids	C6–C1
phenylacetic acids, Acethophenones	C6–C2
phenylpropanoids, Hydroxycinnamic acids, (chromones, chromenes, coumarins, isocoumarins,)	C6–C3
Napthoquinones	C6–C4
Xanthones	C6–C1–C6
Anthraquinones, Stilbenes,	C6–C2–C6
Flavonoids, isoflavonoids	C6–C3–C6
Lignans, neolignans	(C6–C3)2
Biflavonoids	(C6–C3–C6)2
Lignins	(C6–C3)n
Condensed tannins (flavolans or proanthocyanidins)	(C6–C3–C6)n

Table 1 Classes of phenolic compounds of plants.

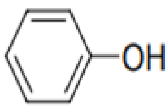

Figure 1 - Simple Phenol Structure (C6) (Garcia *et al.*, 2010).

1.1.2. Biological activities

Phenolics have strong capacity as antioxidant, anticancer, antimutagenic, antimicrobial and antidaibetic compounds. Their antioxidant properties are due to their capacity to neutralize free radicals. In general, the presence and number of hydroxyl groups on their ring are induced by antioxidant and radical scavenging processes of phenolic compounds. Furthermore, glycosylation of aglycons and other H-donor groups (-NH, -SH), etc. reduces their activity by flavonoid glycosylation (Cai *et al.*, 2004).

1.2. Flavonoids

Flavonoids are commonly found in plant tissue, and along with chlorophylls and carotenoids. They have brown, black, red, orange and purple colors (Khoddami *et al.*, 2013) and provide protection from UV radiations and other environmental stresses (Saleem *et al.*, 2010). Over 4000 complexes of flavonoids are identified and are abundant in the plants in their leaves and fruit skin. Significant locations of flavonoids include fruit, vegetables, spices, herbs, nuts, stems flowers, seeds, tea (Pham-Huy *et al.*, 2008).

1.2.1. Structure and classification

Flavonoids are the main set of polyphenolic compounds with a core pyrene (C6–C3–C6) of 15 carbons and are essential natural phenolics (Pracheta *et al.*, 2011). The molecular composition of these compounds is the 2-phenyl chromone, which consists of two benzene rings (A and B), paired with a pyrone ring (C ring) (fig 2). In addition, flavonoids are subdivided into different classes, including flavonones, flavanols, isoflavones, anthocyanae, proanthocyanidins, catechins(Khoddami *et al.*, 2013)

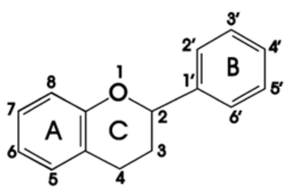

Figure 2- Basic structure of flavonoids (Redrejo *et al.*, 2004).

1.2.2. Biological activities

Flavonoids are significant secondary metabolites that are involved in anticancer, antioxidants, anticoagulants, antiallergic, antihistamines, anti-inflammatory, antimicrobials, antivirals and antiulcers (Agoreyo *et al.*, 2012; Hossain *et al.*, 2013). A significant flavonoid subgroup is known as flavanols includes anthcyanidins, proanthocyanidins, catechin, and tannins. These have very strong antioxidant, anticancer and cardioprotective effects (Rakesh *et al.*, 2010). Flavonoids demonstrate antioxidant behavior through a variety of processes such as free radical scavenging by contributing hydrogen atoms, chelating metals such as iron and copper, inhibiting lipid peroxidation and further defending against many diseases such as strokes, heart attacks (Kalim *et al.*, 2010). Quercetine is one of the flavones, having antiallergy, immunomodulatory activities and antitumor (Leela and Saraswathy, 2013).

1.3. Essential oils (EO)

Essential oils are secondary aromatic metabolites classified as volatile, raw, liquid, complex components. The biological activities and organoleptic properties biological activities of essential oils are distinguished by their respective compositions (Cherrat *et al.*, 2014). They can be found in various parts of the plant in glandular ducts such as seed, bulbs, buds, leaves, brindles, bark, herbs, timber, seeds, and roots. While oils, in particular for seeds, leaves, stem, bark or fruit in particular, are usually present and various parts of one plant species can have different chemical compositions (Hili *et al.*, 1997).

1.3.1. Chemical composition

Essential oils consist nearly entirely of three elements: carbon, hydrogen and oxygen. The most popular type of components is by far the terpenes. Terpenes consist of combinations of different units or isoprene, or 5-carbons-base (C5) (Gunther, 1952). In addition, various low molecular weight aliphatic hydrocarbons, alcohols, bases, acyclic esters or lactones, aldehydes, and sometimes N- and S-containing molecules, equivalents and coumarins of the phenylpropanoids can also occur as EOs, including monoterpenes (C10), sesquiterpenes (C15) and diterpenes (C20) (Benchaar *et al.*, 2008) .

1.3.2. Biological activities

The essential oils have anticancer, antibacterial, antinociceptive, antiphrogistic, antiviral and antioxidant properties in human medicine (Teixeira *et al.*, 2013). Moreover, the use of essential oils improves food properties such as shelf-life and customers are more conscious of the health issues caused by a variety of synthetic preservatives (Teixeira *et al.*, 2013), and because of their comparatively low toxicity, high volatility, biodegradability and transient nature (Hendel *et al.*,2019). Many plant essential oils have shown a wide range of activities against pest insects, including avoidance of ovipositories, regulatory growth, repellents, anti-feeding, antivector, plant fungi disease and insecticide activities (Arshad *et al.*, 2014).

1.4. Biological activities of plants

1.4.1. Antioxidant activity

For survival, oxygen is a critical factor. The cells are used to create energy by catabolism. However, free radicals or reactive oxygen species (ROS) are processed as by-products in this process (Ragavendran *et al.*, 2012). These free radicals act in small amounts as transducers of signals, regulators for development and as part of the immune system (Jindal *et al.*, 2012). At a lower degree, the natural protection mechanism in the body even neutralizes these reactive oxygen molecules. Excess ROS release is not regulated and contributes to oxidative stress by the body protection system. Bio-molecular (DNA, lipids and protein) and pathogenic disorders like aging mellitus, cardiovascular disease, lung diseases , carcinogenesis, cataracts, neurological abnormalities, cancer and also Alzheimer's disease are damaged due to oxidative stress (Battu *et al.*, 2012; Raghavendra *et al.*, 2013).

1.4.2. Oxidative stress and free radicals

Oxidative stress was defined as an imbalance in the equilibrium between antioxidants and oxidants (Lykkesfeldt and Sense, 2007). This imbalance occurs by increasing oxidant production, reducing antioxidant levels (Kirschvink *et al.*, 2008). Free radicals were defined in their outer orbits as atoms, molecules, or parts of molecules that contain one or even more unpaired electrons. They have a very short half-life and a large amount of reactivity (Valko *et al.*, 2006). ROS takes or converts the unpaired electrons via stable molecules. ROS is found in radical form such as hydroperoxylic ($HO_2^{\bullet}$), superoxide ($O_2^{\bullet -}$), lipid peroxyl ($LOO^{\bullet}$) and peroxylic (ROO), alkoxyl ($RO^{\bullet}$) and hydroxylic ($OH^{\bullet}$) and nonradical forms as singlet oxygen ($1O_2$), ozone (O_3), hypochlorous acid (HOCl), lipid peroxide (LOOH) and hydrogen peroxide (H_2O_2) (Pracheta *et al.*, 2011). In biological processes, ROS are considered to play a significant role because it can be detrimental to or benefit live systems. Beneficial effects of ROS include the physiological function and involvement of a variety of cellular signaling mechanisms in the defense against infection agents. ROS may be major mediators of disruption to cell structures, including nucleic acids, proteins and lipids, at elevated concentrations (Rahman, 2007).

1.4.3. Antioxidants

An antioxidant is identified as a molecule that is able to slow or prevent the oxidation of other molecules, or as a compound that greatly slows or prevents the oxidation of the substrate at low concentrations in comparison to the substrate oxidized (Aher *et al*, 2011). Antioxidant structures are categorized into two main classes, enzyme antioxidants and antioxidants non-enzymatic (Kunwar and Priyadarsini, 2011).

1.5. Antimicrobial activity

1.5.1. Antimicrobial agents

Antimicrobial agents seem to be the essential compounds commonly used by their disease prevention properties in current healthcare procedures by removing or destroying microorganisms (Atlas, *et al.* 1995). Infectious diseases are treated by two classes of antimicrobial agents:

- Antibiotics that are natural substances formed by certain microorganism groups.
- Chemotherapy agents synthesized chemically.

Selective toxicity of a specific antimicrobial agent should be evaluated owing to the fact that the antibiotic agent is more toxic for the infecting pathogen than for the host organism (Atlas, *et al.* 1995; Davidson and Harrison, 2002).

1.5.2. Resistance to antimicrobial agents

In the last 60 years, bacteria, including human pathogens, have increasingly developed antimicrobial drug resistance (Courvalin, 2005). Antimicrobial use is widely spread and often inadequate, as the main causes of the growth of antimicrobial bacterial resistant species (Lowy, *et al.* 2003) It is also important today to look for and find new and efficient antimicrobials by presenting them with new action mechanisms and new strategies for intervention (Cloutier, 1995). There are various explanations why the antimicrobial resistance appears. However, genetic mutation or transfers to housekeeping structural or control genes are the prevalent factors. Antimicrobial resistance can also be innate in bacteria (Courvalin, 2005). Genetic mutations that cause bacterial resistance include single point mutations and even several stage mutations, and plasmid transfers and transposons play the role of genetic transfers in the resistance (Patel *et al.*, 2005). These plasmids are the DNA fragments which can only contain a small number of intact genetic materials, but are significant stress that several copies of plasmids can be exchanged between various bacteria (Cloutier, 1995).

1.5.3. Mode of action of antimicrobial agents

The particular modes of action against bacterial cells identify the antimicrobial agents. These agents can interfere with the synthesis of nuclear acid and cell walls, interrupt the synthesis of proteins, or inhibit the metabolism pathway (figure 3).

- Interfering with the synthesis of DNA by blocking the DNA gyrase enzyme: Antibiotic agents can attach to the enzyme gyrase, which causes diagnosis and relaxation of DNA during replication, which leads to cell death and releases the broken DNA strand to the cell.

- Effect of protein synthesis by binding ribosomal sub-units: Antimicrobial substances can also bind to a 70S activation complex with ribosomal sub-units, including 30S and 50S. Protein chains break and protein synthesis is inhibited.
- Cytoplasmic membrane interference: these types of antimicrobial agents are necessary to interrupt and destabilize the membrane.
- Cell wall interference: Certain antimicrobial agents inhibit peptidoglycan base synthesis to block cell wall synthesis (Stephen, *et al.* 2005).

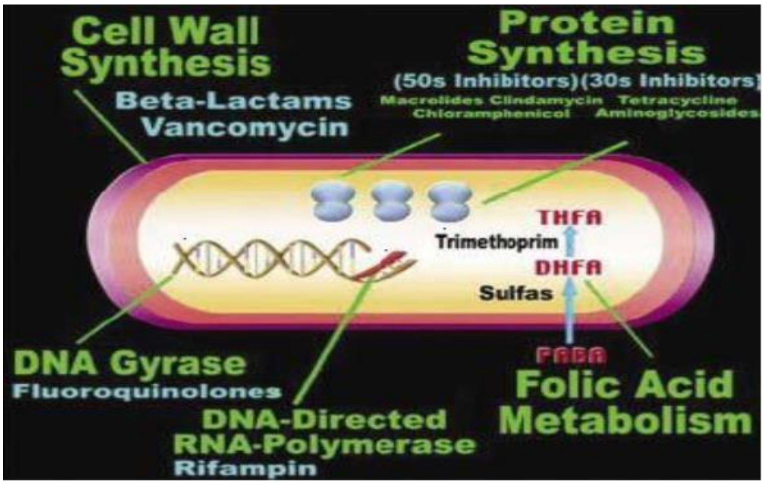

Figure 3- Specific sites for certain antimicrobials (Stephen, *et al.* 2005).

2. The genus *Ballota*

2.1. Botanical description

Perennial grasses or shrubs, very hispid with square stems. Leaves opposite and oval ± cordiform. Inflorescences in long spikes of interrupted leafy whorls. Chalice monophyllous, tubular, with ten ridges and five-pointed teeth, thin and very open at the top. Monopetal corolla labiate, with a cylindrical tube, having the upper right lip, a little concave and slightly crenate, the lower one larger, with three lobes, including the middle one, wider, is more or less indented; four unequal stamen, two by two; four upper ovaries, from which arises a filiform style terminated in a bifid stigma; four naked seeds, oval and attached to the bottom of the calyx (Tutin *et al.*, 1972).

2.2. Classification

A recent *Ballota* genus classification cites 31 species: *B. royleoides, B. wettsteinii , B. integrifolia, B. nigra, B. fruticosa, B. frutescens, B. andreuzziana, B. somala, B. undulata, B.*

pseudodictamnus, B. damascena, B. acetabulosa, B. hildebrandtii, B. hirsuta, B. bullata, B. africana, B. macrodonta, B. larendana, B. rotundifolia, B. rupestris, B. macedonica, B. kaiseri, B. aucheri, B. cristata, B. antilibanotica, B. labillardieri, B. saxatilis, B. semanica, B. philistea, B. stachydiformis, B. platyloma (Rosselli *et al.*, 2019).

2.3. Geographical distribution

Ballota (horehound) is really a genus with a highly Mediterranean region diverse with low-growing perennial herbs and sub-shrubs native to temperate regions in Asia, Africa and Europe (Đorđević *et al.*, 2016).

2.4. Traditional uses

In traditional medicine various plant species belonging to the genus *Ballota* have been used. Table 2 provides summaries of their traditional uses.

Table - 2 Traditional uses *Ballota* species.

Scientific name	region	Uses	Reference
B. africana (L.) Benth	South Africa	stomachache, headache, backache, wounds, pediatric, coughs and also bronchitis, burning of feet, mastitis, convulsions, earache, chest disease,	Nortje *et al.*, 2015
B. acetabulosa (L.) Benth.	Turkey	bowel disorder, stomach discomfort	Tuzlaca *et al.*, 2007
B. arabica Hochst. & Steud.	Pakistan	anthelmintic, astringent, abortifacient , hemostatic, diuretic, stimulant	Akhtar *et al.*, 2010
B. aucheri Boiss.	Pakistan	tonic of hair, cleaning of dental	Bano and Malik., 2014
	Iran	tonic of hair, strengthening the gums, dental brightness and cleaning, preventing hair damage	Sadeghi *et al.*, 2014
B. deserti (Noë) Jury, Rejdali & A. J. K.Griffiths	Tunisia	asthma, diuretic, diabete	Edziri *et al.*, 2012
	Sahara Central	colics, nausea, cough, respiratory trouble, fever, helminthiasis, colds, digestive diseases	Dendougui *et al.*, 2011
B. cinerea D. Don	Nepal, Kashmir	malaria, fever, scabs, skin trouble, insect repellent, jaundice	Bisht *et al.*, 2016
	India	analgesic, stomachache	Natarajan *et al.*, 2010
B. hirsuta Benth.	West Algeria	injuries, contusion, rheumatic trouble	Kechar *et al.*, 2016
	High Atlas, Morocco	gastrointestinal, pediatric, gynecological	Teixidor *et al.*, 2016
	Tibet	gynecological, intestinal, stomach trouble	Olennikov and Tankhaeva, 2011
B. nigra L	Moldova	sedative, vermifuge, antispasmodic excitant	Ciocîrlan, 2016
	Italy	safe of injury and also sprains	Pieroni, 2000
	Bosnia	sedation, Nervous system disturbances	Šarić *et al.*, 2011
	Macedonia	digestive trouble	Rexhepi *et al.*, 2014
	Albania, Italy	diuretic, hemostatic	Pieroni *et al.*, 2004
	North Spain	flea repellents and insecticides	González *et al.*, 2011
	Serbia	coughs, anxiety, sleep problems, a medication for gastrointestinal pain, inflammation, nausea.	Nićiforović *et al.*, 2010

	Mediterranean Area	horses' weakness of the flesh, disorders of skin	Mulas, 2006
	Bosnia	hysteria	Redžić, 2007

2.5. Phytochemicals

Ballota species comprise several distinct phytochemical classes. These are summarized in Table 3

Table - 3 Phytochemistry of the *Ballota* species.

Phytochemicals	Name	References
Flavonoids	ladanein, luteolin-7-glucosyl-lactate, luteolin-7- lactate, allopyranosy1-(1–2)-β-D-glucopyranoside,tangeretin,vicenin-2	Makowczyńska *et al.*, 2015
Diterpenoids	Ballonigrin, hispanolone, dehydrohispanolone, 18-Hydroxyballonigrin, ballonigrinone, ballotinone, 3β-hydroxyballotinone, siderol, 13-hydroxyballonigrolide, 7α-acetoxyroyleanone, 7α-Acetoxymarrubiin, ballotenol.	Hussein *et al.*, 2007; Tóth *et al.*, 2007
Triterpenoids	oleanolic acid, β-Amyrin.	Ahmad *et al.*, 2004b; Abdelshafeek *et al.*, 2010
Organic acids	shikimic acid, Aconitic acid, malic acid, ascorbic acid, fumaric acid, oxalic acid, citric acid,, quinic acid.	Vrchovská *et al.*, 2007
Phenolic acids	gallic acid, Caffeic acid, chlorogenic acid, rosmarinic acid, p-coumaric acid.	Askun *et al.*, 2013
Iridoids	Verminoside.	Siciliano *et al.*, 2005
Secoiridoids	Oleuropein.	Askun *et al.*, 2013
Phenylpropanoid glycosides	forsythoside B, verbascoside, Betonyoside, lysionotoside.	Siciliano *et al.*, 2005
Phytosterols	β-Sitosterol, stigmasterol, Cholesterol.	Abdelshafeek *et al.*, 2010
essential oils	epi-Bicyclosesquiphellandrene, Hexahydrofarnesyl acetone.	Erdogan *et al.*, 2014; Kaya *et al.*, 2017

2.6. Biological properties

2.6.1. Antibiofilm activity

Water extract of *B. nigra* has demonstrated an IC_{50} rate of 8 μg/ mL for *Staphylococcus aureus* biofilm inhibition resistant to methicillin (Quave *et al.*, 2008).

2.6.2. Anticholinesterase activity

Six forms of clerodane and tetracyclic diterpenes derived from the chloroform extract of the whole *B. Limbata* had potential inhibitory against butyrylcholinesterase (IC_{50} = 14.0 –51.0 μM) when contrasted with galantamine (IC_{50} = 8.5 μM) (Ahmad *et al.*, 2004a).

2.6.3. Anti-inflammatory activity

An inhibition of rat paw edema by carrageenan was induced by aerial extract *B. glandulosissima* (100 mg/kg) in 34.22%; indomethacin (3 mg/kg i.p) was used as a guide in the inflammatory agent that developed an inhibition of 95.70% (Özbek *et al.*, 2004).

2.6.4. Antidepressant activity

Antidepressants behavior in the forced swimming was recorded in aerial component excerpts of *B. nigra* subsp (240 mg/kg, i.p) relative to amitriptyline (5 mg/kg, i.p.) and passive flora extract (60 mg/kg). *B. nigra* subsp (496.4s), amitriptyline (379.0s), passive flora extract (388.9s) and rats, the cumulative time of immobility over the 15-minute evaluation cycle was measured and the control group (570.0s) (Vural *et al.*, 1996).

2.6.5. Antimicrobial activity

The evaluated antimicrobial studies of *Ballota* species showed antimicrobial activity of some extracts, and strong antifungal and antibacterial activities of essential oils (Çitoglu *et al.*, 1998). Although some tests of this genus have shown antimicrobial activity and reported minimal inhibitory concentration (MIC) values above 1000 µg/mL (Morteza and Ghanbarimasir, 2019). The values of MIC and MBC (minimal bactericidal concentration) can vary over a broad interval and are often less than those of the antibiotics selected as positive controls. For examples, ethanolic extract from *B. acetabulosa*, with MIC of 32 µg/mL and MBC of 64 µg/mL values equal to ampicillin, was found significantly active against *Escherichia coli* (Rosselli *et al.*, 2019). Methanolic extracts from the *B. acetabulosa* leaves were of high antimicrobial activity with inhibition zones 12.8 – 18.6 mm against bacteria, causing complicated urinary tract infections (Dulger and Dulger, 2012).

2.6.6. Antioxidant activity

The *B. nigra* infusion has been shown to have high antioxidant activity; DPPH scavenging ability IC_{25} = 4.81µg/mL; Hypochlorous acid (HOCl) scavenging activity was 80 % at 500 µg/mL; xanthine/xanthine oxidase (X/XO) =14.6µg/mL; Nitric oxide (*NO) IC_{25} =122µg/mL (Vrchovská *et al.*, 2007). The IC_{50} value obtained by the extracts of *B. glandulosissima* was 15mg/mL showed higher activity in lipid peroxidation than α-tocopherol (IC_{50} = 3 mg/mL) was seen. all the ethanol extracts of *Ballota* inhibited superoxide anion formation to different extent. The ethanol extracts of *Ballota antalyense*, *B. macrodonta*, *B. glandulosissima*, *B. larendana*, *B. pseudodictamnus*, *B. nigrasubsp. anatolica*, *B. saxatilis*, *B. rotundifoliaand*, *B. saxatilis* subsp. *brachyodonta* exhibited remarkable antisuperoxide anion formation. Their IC_{50} values ranged from 0.50 to 0.87 mg/ml. The extracts of *B. inaequidens*, *B. glandulosissima*, *B. saxatilis*, *B.*

macrodonta and *B. antalyense* inhibited lipid peroxidation. Their IC_{50}values ranged from 12 to 20 mg/ml (Çitoğlu *et al.*, 2004a).

2.6.7. Antinociceptive activity

The dose-related inhibition of the induced abdominal stretching of the acetic acid in mice (ED_{50} = 85.38 mg/kg) was caused by aqueous *B. inaequidens* extract (30, 65, and 100 mg/kg, i.p.) (Sever Yılmaz *et al.*, 2006).

2.6.8. Antimalarial activity

The antimalarial assay demonstrated 32,3% inhibition against *Plasmodium falciparum* strain FCR3 by a 1:200 aqueous extract of the aerial sections of *B. undulate* (Sathiyamoorthy *et al.*, 1999).

2.6.9. Antitumor activity

The most effective *B. undulata* essential oil at concentration 100 µg/mL was 81.36% inhibited (IC_{50}=54.75 µg/mL) for Hepg2 cells, while the most active oil was 100 µg/mL for the essential oil of *B. saxatilis*, at inhibition 24.18% for MCF-7 cells (Rigano *et al.*, 2017).

2.6.10. Hypoglycemic activity

High reduction in glucose concentration was recorded six hours after administration of the crude extract of *B. nigra* (400 mg/kg, orally) to healthy and diabetic rats (Nusier *et al.*, 2007b).

2.6.11. Antitussive activity

Methanol extracts of the *B. limbata* (200, 400 and 800 mg/kg, s.c.) demonstrated the highest rate of SO_2 mediated cough defence in this 800 mg/kg dosage fraction after 60 minutes of mice (Haq *et al.*, 2011).

2.6.12. Hepatoprotective activity

Aqueous extracts of *B. glandulosissima* (100 mg/kg i.p) and CCl4 extract (0.8 mL/kg i.p.) used during 7 days were shown to protect against hepatic damage caused by CCl4 (Özbek *et al.*, 2004).

2.6.13. Anxiolytic activity

Aqueous extract of *B. larendana* (240 mg/kg), in contrast with diazepam (4 mg/kg, i.p.) showed anxiolytic efficacy in a high-progressive plant maze test 5 minutes of test time (Vural *et al.*, 1996).

2.6.14. Lipoxygenase inhibitory activity

Two diterpenoids types, ballotenic acid, and ballodiolic acid derived from *B. limbata* demonstrated inhibitory activity against lipoxygenase enzyme (Ahmad *et al.*, 2004b).

2.6.15. Hypolipidemic activity

Ballota undulata ethanol extract with oral administration (1.2 g/kg body weight/day) reduced in serum cholesterol from 940.7 to 119.2 mg/dL (87.32 percent) before the end of the hyperlipidemic rabbit experiment (Qazan, 2008).

2.6.16. Hypnotic activity

The *B. africana* methanol extract (25–400 mg/kg) and the dosage based on pentobarbitone (40 mg/kg, i.p.) stimulate the duration sleep in mice (Amabeoku *et al.*, 2016).

2.6.17. Insecticidal activity

Aqueous extract of the *B. undulata* has acted as an anti-sweet potato parasite agent named *Bemisia tabaci* (Ateyyat *et al.*, 2009).

3. Plant studied

Lamiaceae family of plants is also known as Labiatae. It is generally consisting of trees, shrubs, subshrubs or perennial or annual herbs, rarely climbers, aromatic or not, roots rarely tuberous. It contains approximately 7.173 species spread through 236 genera (Harley *et al.*, 2004).It is also known as the Mint family and is the 6th largest family of angiosperms (Drew and Sytsma, 2012). The family Lamiaceae is known for the abundance of medicinal species used since early times, many of which are popular in the Mediterranean region (Ali *et al.*, 2000). In Algerian flora, 29 genera and 149 species present the Lamiaceae (Quezel and Santa, 1963). The genus *Ballota* is represented by only 2 plants perennials widespread in Algeria and which are illustrated in the Table 4. Our study plant, *B. hirsuta*, made part of this genus

Table 1 - Location and main characters of species of the genus *Ballota* located in Algeria (Quezel and Santa, 1963).

Species	Location	Main characters
B. nigra Linne.	Very common in all Algeria	Funnel-shaped chalice with 5 veryshort, a little spread out. Herbaceous stems. Leaves allpetiolate dentate.
B. hirsuta Benth.	Fairly common in: the Oran sector, sector of the Saharan Atlas, sector of Northern Sahara.	Calyx with broadly leafy blade, rotaceous reticulate multifid, with 5 teeth main. Upper leaves sessile with very obtuse teeth. Rosy corolla with a bifid upper lip.

3.1. Botanical characters of *B. hirsuta*

Stems woody and hairy 24-60 cm. Upper leaves sessile with very obtuse teeth, the lower and medium cauline leaves are 3-6 x 3-5 cm, roped or truncated at the base, ovate or suborbicular, crenellated; petiole of the lower leaves 5-40 mm long. Calyx is 10-12 mm with a leaf blade of 8-10 mm of diameter, 10 lobes or more, up to 2 mm, triangular-acuminate, sometimes toothed, extensively rotated 5-tooth multifid irregular main (Figure4). Sublet bracts, linear, membranous. Flowers are in whorls. Pink corolla with bifid upper lip (Patzak, 1958; Quézel and Santa 1963; Tóth, 2007). The main habitats for *B. hirsuta* are clear forests, rocks, scrub, plain and low and medium mountains (Bammi and Douira, 2004)

Figure 4- *B. hirsuta* Benth photo (by the author).

3.2. Systematics of *B. hirsuta*

The APG (Angiosperm Phylogeny Group) classification is the most recent scientific classification of Angiosperms established by the work of a group of researchers, the Angiosperm Phylogeny Group. It translates the efforts made into systematic so that classification systems best reflect the phylogeny of families highlighted by constant advances in genetics (Spichiger *et al.*, 2000). This classification, revised in 2009 (APG, 2009) and based largely on analyzes of chloroplast genes, even at the family level, notable changes with the classic classification.

According to APG IV (2016) the classification of *B. hirsuta* occupies in the systematics is the following:

- Domain Eukaryota
- Regnum Plantae
- Phylum: Spermatophyta
- Subphylum Angiospermae
- Class Dicotyledonae

- Order Lamiales
- Family Lamiaceae
- Genus *Ballota*
- Species *B. hirsuta*

3.3. Medicinal uses

B. hirsuta has been used traditionally in treatment of gastrointestinal, contusion, painful rheumatism, and heal wounds. It is also used to treat dental caries, antiseptic, pediatric and gynecological problems (Kechar *et al.*, 2016; Teixidor *et al.*, 2016).

3.4. Phytochemical contents

Phytochemical studies on *B. hirsuta* species isolated many classes of phytochemicals (Table 5).

Table 2- phytochemistry of the species *B. hirsuta*.

Phytochemicals	References
Flavonoids : Glucoside, nuchensin, , salvigenin, Apigenin, apigenin− 7-, genkwanin, apigenin−7-(p-coumaroyl)-glucoside, kumatakenin ,isokaempferide, ladanein, luteolin, luteolin−7-glucoside,luteolin−7-rutinoside, quercetin− 3-glucoside, vicenin	Ferreres *et al.*, 1986; Pieroni *et al.*, 2004 ; Redžić, 2007
Labdane diterpenes : hispanolone	Savona, 1978
Penylpropanoids : verbascoside Carboxylic acids : E-caffeoyl-L-malic acid	Tóth *et al.*, 2007
Anthraquinones, Anthocyanins ,Tannins, alkaloids, mucilage, saponoside, Sterols	Kechar *et al.*, 2015 a
Essential oils	Kechar *et al.*, 2015 b
Polyphénols, Gallic Acid	Kechar *et al.*, 2016

CHAPTER II

MATERIALS & METHODS

1. Plant material

The plant was harvested in March 2020 in the mount of Boutaleb (to the south of the Sétif district). This mount is 65 km north-east of M'sila town, and belongs to the Honda mountain range. The plant is washed in tap water then dried at room temperature in shady and ventilated place for 10 to 15 days. The collected aerial parts were stored in paper bags and placed in the shelter of the light and heat until use.

2. Extraction processes

2.1. Methanolic extraction by maceration

The plant was subjected to methanolic extraction by maceration: 500 ml of methanol were added to 50g of the ground plant aerial parts and the mixture was kept under stirring for 24 hours at room temperature (Figure 5a). The extract (MeE) was filtered through Whatman filter paper N°1, and then concentrated in vacuo at 45 °C using a rotary evaporator (Figure 5b). After drying in an oven (40 °C), the dried extract was collected, weighed and stored at 4 °C until use (Atere *et al.*, 2018). The extraction yield percentage is calculated as follows:

$$\textbf{Yield}_{ext}\,\textbf{(\%)} = \textbf{weight}_{ext}\,/\textbf{weight}_{sam} \times \textbf{100} \text{ ----------------- } \textbf{(1)}$$

Where,
Yield_{ext}: Extraction yield
weight_{ext}: weight of the extract after evaporation of the solvent in g.
weight_{sam}: weight of the plant sample in g.

2.2. Aqueous extraction by decoction

The aqueous extraction was carried out by decoction as follows: Fifty grams of the crushed plant (aerial parts) was directly immersed in 500 ml of distilled water and subjected to boiling for 20-30 min (figure 5c). The extract was filtered through Whatman filter paper N°1, and dried in an oven (40 °C). The dry extract (AqE) is collected and stored at 4 °C in the dark until use (Mbiantcha *et al.*, 2011). The extraction yield percentage is calculated as above (formula -1).

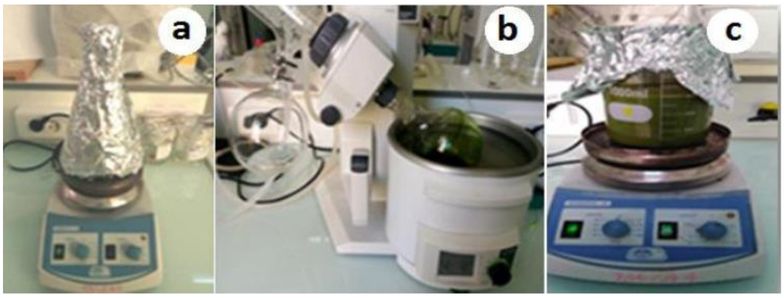

Figure 5- Extraction devices: a) Maceration, b) Vacuum evaporation, c) decoction.

2.3. Essential oil (EO) extraction

The procedure is carried out as follows: one hundred grams of the aerial parts (leaves and flower) of *B. hirsuta* plant were powdered then were hydro-distilled in distilled water (1000 mL) for 3 h using a Clevenger. The water, in which plant material was fully submerged, is heated to produce steam carrying the most volatile chemicals. The steam is then chilled (by a condenser) and the resulting distillate is collected. The essential oil will normally float on top of the aromatic water and then is separated off. The obtained EO was dehydrated over anhydrous sodium sulfate and stored at 4 °C (Kechar *et al.*, 2015).

$$\%\text{Yield}_{EO}\ (w/w) = W'/W \times 100$$

Where,
W': weight of the obtained EO in g.
W: weight of the plant sample in g.

3. Evaluation of the antioxidant activity

3.1. DPPH test

The free radical scavenging power of MeE and AqE of *B. hirsuta* was determined using 2,2diphenyl-1picrylehydrazile (DPPH) as the free radical. A volume of 0.5 ml methanolic solution of MeE or AqE (at different concentrations) was mixed with 0.5 ml of a methanolic solution of DPPH (0.004%). In the control, MeE or AqE was replaced by methanol. The reaction medium is vigorously stirred and then incubated at in the dark for 30 minutes at room temperature. The absorbance at 517nm was then measured against a blank using a spectrophotometer. The BHT was used as standard and the percentage inhibition of the free radical DPPH is calculated by the following formula:

$$\text{Inhibition (\%I)} = \frac{\text{Control Absorbance} - \text{Sample Absorbance}}{\text{Control Absorbance}} \times 100$$

The extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentage plotted against extract concentration (Atere *et al.*, 2018).

4. Statistical analysis

Analyzes of antioxidant activity were performed in three replicates and comparison of means was performed by Analysis of Variance (ANOVA). Statistical comparisons were made using Tukey's test ($P \leq 0.05$).

CHAPTER III

RESULTS & DISCUSSION

1. Extraction yield

1.1. Extraction yields of MeE and AqE

The methanolic extraction by maceration of *B. hirsuta* yielded in 11± 0.62%. The crude extract shows a more viscous appearance, a strong odor and a Blackish brown color. The aqueous extraction yielded in 13.52± 0.28%. It is clear that decoction (in water) gives higher yield than maceration (in methanol). This agrees with findings by Fertout *et al.* (2016). The difference in yielding may be due to the extraction techniques used and the nature of the extractant. The chemical composition differs thereafter from one extract to another.

The average yield of EO of *B. hirsuta* aerial parts was 0.42 ± 0.02%. It is relatively weaker compared to some aromatic plants from the same family.

2. Antioxidant activity

The stable DPPH radical scavenging model is a commonly used way of determining the free racial scavenging capacities of different samples (Ebrahimzadeh *et al.*, 2010). DPPH is a constant free radical based on nitrogen whose colour, either by a donation mechanism of hydrogen or of electron, transforms from violet to yellow. Substances that are capable of this reaction are known as antioxidants and radical scavengers (Moyo *et al.*, 2012). The results showed that the MeE and AqE will reduce the signal strength of DPPH and increase their radical splattering with increased concentration (Figure 6).

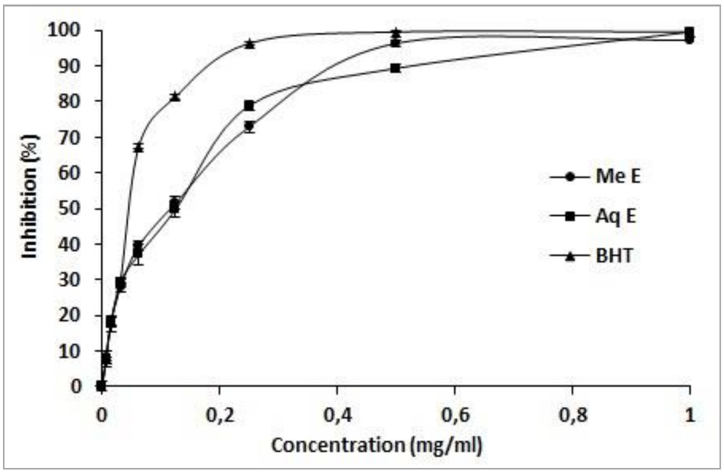

Figure 6- Antioxidant activity of the *B. hirsuta* extracts (methanol extract=MeE; aqueous extract= AqE) and BHT, as assessed by DPPH method. Data are given as mean ± SD (n = 3).

The IC_{50} values of the MeE, AqE extract and BHT were 0.120 ± 0.03; 0.124 ± 0.01 and 0.050 ± 0.01 mg/ml respectively. Therefore, they may be presented according to the order BHT> Met.E > AqE. Statistical analysis showed that *B. hirsuta* MeE and AqE extracts have a free radical scavenging power with IC_{50} values not statistically different and quite different when compared to that of BHT, which is approximately 2.5 times important (figure 9). The IC_{50} value is inversely related to the antioxidant capacity of a compound. It expresses the amount of antioxidant necessary to decrease the concentration of the free radical by 50%. The lower the IC_{50} value, the stronger the antioxidant activity of a compound (Ismaili *et al.*, 2017).

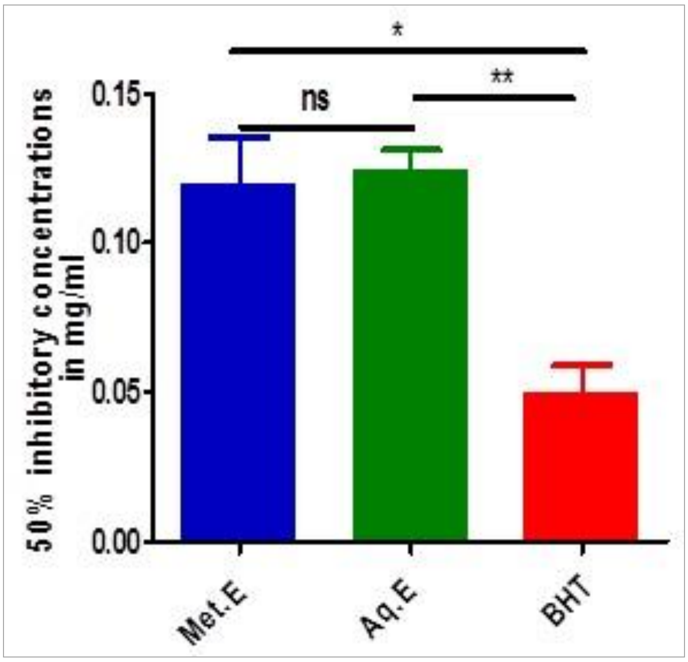

Figure 7- comparison between different plant extracts in DPPH free radical scavenging activity. Met.E: methanolic extract, AqE : aqueous extract. Data were presented as IC_{50} means ± SD (n = 3). (*p < 0.05, $^{ns}p > 0.05$, **p < 0.01).

The study of the antioxidant activity of AqE of *B. hirsuta* by Kechar *et al.* (2016) showed a lower anti-free radical capacity than that obtained in the present study with IC_{50} values of 0.35mg/ml for AqE of the aerial parts. However, the antioxidant activity of our methanolic extract is stronger than that obtained by Edziri *et al.* (2012). For their studies concerning a plant of the same genus *Ballota*; leaf extracts of *B. deserti* growing in Tunisia with IC_{50} equal to 0.150 mg/ml. However, it should be noted that this is neither the same solvent nor the same activity protocols, and the shelf life and temperature could explain the difference observed between our results and those in the bibliography (Sokol-Letowska *et al.*, 2007).The differences may generally be related to the polarity of the extraction solvents and/or the climatic conditions of the collection areas. In addition, the synergistic interactions between antioxidants in a mixture makes the antioxidant activity depends not only on the concentration, but also the structure and nature of antioxidants. Anti-free radical activity is also correlated with the content level of polyphenols and flavonoids in extracts from medicinal plants (Locatelli *et al*., 2010).

CONCLUSION & PERSPECTIVES

Conclusion and perspectives

Nowadays, a large number of medicinal plants have very important biological properties that find many applications in various fields, namely medicine, pharmacology, cosmetology and agriculture. This renewed interest comes partly from the fact that medicinal plants represent an inexhaustible source of bioactive substances, and on the other hand, drug-induced side effects are of concern to users who turn to less aggressive care for the body.

In the present review study, different pharmacological activities from some extracts, compounds and essential oils derived from *Ballota* species have been shown to display different pharmacological activities. Many effects include antitussive, antidepressant, sedative-hypnotic and anxiolytic activity, were beneficial in experimental animals.

The practical study treated only on the antioxidant activity of methanolic and aqueous *Ballota hirsuta* extracts by the DPPH free radical reduction test. The results showed that the plant exhibits important antioxidant activity with no difference between the 02 extracts tested.

This work was interrupted due to the consequences of the Covid-19 pandemic. These results obtained *in vitro* constitute only a first step in the research of biologically active substances of natural origin, an *in vivo* study is desirable, to get a more in-depth view of antioxidant activities and antimicrobial extracts from this plant.

We propose some perspectives to continue this work:

- expand the study spectrum by studying the plant from other regions for comparative purposes;
- perform a complete phytochemical screening for secondary metabolites (flavonoids, tannins, saponosides, waxes, etc.).
- carry out a toxicological study of this plant.

Industrial exploitation of natural sources of antioxidants depends on many requirements: high concentration of active molecules, sufficient, regular supply and if possible not seasonal, good conservation of the raw material, reasonable cost, ease of extraction, weakly aromatic character of preparations, absence of toxicity compounds present in the extract, legal authorization for use in industries food and pharmacology or cosmetics.

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ملخص

تعتبر النباتات الطبية مهمة في مصدر الأدوية اليوم. يتم وصف معظم الأدوية الطبية الحالية من قبل الشركات التابعة لها. *Ballota hirsuta* benth. شجيرة معمرة تنتمي إلى العائلة الشفوية و تنمو في الوسط الجزائري، ويتم استخدامها كجزء من النظام الطبي التقليدي لبلدنا. كانت أهداف هذه الدراسة هي مراجعة الأنشطة الصيدلانية ، والكيمياء النباتية ، والاستخدام التقليدي ، وتقديم المعرفة العلاجية لجنس *Ballota* وتقييم الخصائص المضادة للأكسدة لهذا النبات في شكل الزيت العطري والمستخلصين الميثانولي والمائي. عرضت الأجزاء الهوائية من النبات للتقطير المائي للحصول على الزيت العطري (المحصول: 0.42٪). نتج عن الاستخلاص الميثانولي و المائي بالنقع 11 ± 0.62٪ و 13.52 ± 0.28٪ على التوالي. تم تقييم تأثير إزالة الجذور الحرة باستخدام الجذر الحرDPPH . كانت قيم IC_{50} 0.120 ±0.027 مغ/مل و 0.124 ± 0.012 مغ/مل للمستخلص الميثانولي والمائي على التوالي.

كلمات مفتاحية: *Ballota hirsuta* benth. ، العائلة الشفوية ، الخصائص البيولوجية ، الاستخدامات التقليدية ، المستخلص المائي ، المستخلص الميثانولي ، نشاط مضادات للأكسدة.

Abstract

Medicinal plants are significant in today's drug source. Most current medicinal drugs are prescribed by their subsidiaries. *Ballota hirsuta* benth is perennial shrub plant belonging to the Lamiaceae family growing in Algerian centre, and are being utilized as a part of the traditional medicinal system of our country. The aims of the present study were to review pharmacology activities, phytochemistry, traditional use, and offer therapeutic knowledge of *Ballota* genus and to evaluate antioxidant properties of this plant in essential oil, methanolic and aqueous extract forms. The aerial parts of the plant were subjected to hydrodistillation to have the essential oil (yield: 0.42%). The methanolic maceration aqueous decoction extractions yielded in 11± 0.62% and 13.52± 0.28% respectively. The free radical scavenging effect was evaluated using the free radical DPPH. The IC_{50} were 0.120 ± 0.027mg/ml and 0.124 ± 0.012 mg/ml for the methanolic and aqueous extract respectively.

Key words - *Ballota hirsuta* benth, Lamiaceae, biological properties, traditional uses, aqueous extract, methanolic extract, antioxidant activity.

Résumé

Les plantes médicinales sont importantes quant à la source de médicaments d'aujourd'hui. La plupart des médicaments courants sont prescrits par leurs filiales. *Ballota hirsuta* benth est une plante arbuste vivace appartenant à la famille des Lamiaceae qui pousse dans le centre Algérien, et est utilisée dans le cadre du système médicinal traditionnel de notre pays. Les objectifs de la présente étude étaient de passer en revue les activités pharmacologiques, la phytochimie, l'utilisation traditionnelle, d'offrir des connaissances thérapeutiques du genre *Ballota* et d'évaluer les propriétés antioxydantes de cette plante sous forme d'huile essentielle, d'extrait méthanolique et aqueux. Les parties aériennes de la plante ont été soumises à une hydrodistillation pour avoir l'huile essentielle (rendement: 0,42%). Les extractions aqueuses par décoction et de macération méthanolique ont donné respectivement 11 ± 0,62% et 13,52 ± 0,28%. L'effet de piégeage des radicaux libres a été évalué en utilisant le radical DPPH. Les CI_{50} étaient de 0,120 ± 0,027 mg / ml et 0,124 ± 0,012 mg / ml pour l'extrait méthanolique et aqueux respectivement.

Mots clés - *Ballota hirsuta* benth, Lamiaceae, propriétés biologiques, usages traditionnels, extrait aqueux, extrait méthanolique, activité antioxydante.