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REVIEW PAPER

Ballota nigra L. – an overview of pharmacological effects and traditional uses

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Summary

Ballota nigra, also known as black horehound is a common medical herb used in folk medicine around the world. First reported mentions of its medical properties and use goes as far as the 13th century. The use of black horehound depends on regions and countries. It is used mostly to treat e.g. mild sleep disorders, nervousness, upset stomach, wound healing. It can be used as an anti-inflammatory, antibacterial, antiprotozoal, antifungal drug. Moreover, it has been reported as a potential cancer drug. This extensive usage is particularly interesting for us. The aim of this review is to present available data on *B. nigra* pharmacological effects and known traditional uses gathered from a wide range of scientific articles published in 1997–2020.

Key words: ***Ballota nigra L., black horehound, pharmacology, medical herb***

Słowa kluczowe: ***Ballota nigra L., mierznica czarna, farmakologia, roślina lecznicza***

INTRODUCTION

Nowadays, the black horehound is an object of interest mainly in the countries of the Middle East, which is reflected in the amount of articles and research conducted in that region. Due to the number of its potential applications and common occurrence in Europe this article is to raise interest in this special herb. *Ballota nigra* is a plant from the *Lamiaceae* family, it is a herbaceous perennial, occurring in Europe, North Africa, Western Asia and North America. The whole plant is pubescent and fresh green. It has herbaceous ascending stems, wooden and branched at bottom, covered by down folded hairs. The plant has a taproot system. The leaves are 0.5 to 1 cm long and the lower leaves are a little bit longer. It has purplish flowers, that are organized in verticillasters, subspherical to about one-sided, with 15 to 30 flowers. The whole plant gives off an unpleasant odour [1].

Other names of black horehound are as follows: Ballota, Ballote Fétide, Ballote Noire, Ballote Puante, Ballote Vulgaire, Black Stinking Horehound, Marrube Fétide, Marrube Noir, Marrubio Negro.

The black horehound has been of interest to the medical world for many years, and to this day it is used in traditional medicine in many regions of the world. Its versability translates into a large number of 'traditional' uses, ranging from wound care to psychiatric problems. Most often it is used in the form of leaf tea, it can also be used in the form of essential oils or tinctures [2].

It is mainly used as an *ad hoc* sedative agent, but it can also be used to relieve discomfort in neurotic disorders and depression. The 5-phenylpropanoids compounds from the plant are mainly responsible for these properties [3]. An essential oil from aboveground parts has at least 70 chemical compounds [4]. The chemical compounds responsible for the pharmacological effects of black horehound are mainly substances from phenylpropanoid glycosides, diterpenes and carboxylic acids. One of the most important groups is the phenylpropanoid glycosides such as lavanduli folioside, which has a negative chronotropic effect and it lowers the blood pressure.[5] Other important compound in this group is forsythioside B, which has neuroprotective and cardioprotective properties [6, 7]. From the group of diterpenes, the substance ballonigrin is also interesting because it can potentially inhibit the development of cervix cancer [8].

In many researches that provided evidence for the pharmacological effects of *B. nigra*, researchers used an extract of black horehound, which makes it impossible to indicate exactly which compound was responsible for a given effect. This could be an idea for further work on this herb.

The aim of this article is to provide an overview of information about the black horehound, with particular emphasis on the possible clinical applications. The range of its traditional uses was also taken into account. In the chemotaxonomy part, the chemical composition of the plant is included, as well as the composition of essential oils, depending on the part from which they were obtained. The text also includes information on the dosage, forms of use, intake and the toxicity of *B. nigra*.

CHEMOTAXONOMY

B. nigra has a wide variety of phytochemical compounds. Many studies have proven that black horehound contains terpenoids, flavonoids, steroids, carboxylic acids, phenylpropanoids. Essential oils obtained from various parts of the plant also contain a wide range of chemicals.

Phytochemicals compaunds extracted from B. nigra:

- diterpenes: 7α-acetoxymarrubiin, ballonigrin [9], ballotenol [10], dehydrohispanolone (hispanone) [3], marrubiin [12], preleosibirin [13], 7α-acetoxyroyleanone [14];

- flavones: ladanein [14], apigenin, luteolin, chrysoeriol [15], tangeretin [16];

- flavonols: 5-hydroxy-3,7,4'-trimethoxyflavone, kumatakenin, retusin [17];

- flavone glycosides: apigenin-7-O-β-D-glucopyranoside [18];

- acyl flavonoid glycosides: luteolin-7-lactate, luteolin-7-O-[2-O-β-D-glucopyranosyl-lactate] [19];

- C-glycosyl flavonoids: vicenin-2 [18];

- triterpenoids: oleanolic acid, ursolic acid [20];

- steroids: β-sitosterol [14];

- carboxylic acids: caffeic acid, E-caffeoyl-L-malic acid, chlorogenic acid, fumaric acid, quinic acid, shikimic acid [21];

- nitrogen-containing compounds: choline (*foetida)* [22], 4-hydroxyproline betaine, stachydrine [23];

- phenylpropanoids: alyssonoside, angoroside A, arenarioside, ballotetroside, forsythoside B, lavandulifolioside [21], martynoside [14], verbascoside (acteoside) [24] and phytol [25].

Main compounds: (>3% of the essential oil):

B. nigra

- aerial parts: caryophyllene oxide, epi-α-muurolol, δ-cadinene, α-cadinol, γ-amorphene, β-bourbonene, 6,10,14-trimethyl-2-pentadecanone, (E)-caryophyllene, germacrene D, aromadendrene, γ-muurolene, germacrene D-4-ol, α-bisabolol, α-amorphene [26] β-pinene, α-pinene, sabinene, α-phellandrene [27];

- corollas: palmitic acid, 2,2,6-trimethyl-4-methylene-3,4-dihydro-2H-pyran, hexahydrofarnesylacetone, myristic acid, caryophyllene oxide, pentadecanoic acid, palmitoleic acid, germacrene D [29];

- calyx: palmitic acid, dodecanal, palmitoleic acid, myristic acid, pentadecanoic acid, lauric acid, trans-isoelemicin, hexahydrofarnesylacetone, pentadecene, methyleugenol;

- leaves: β-caryophyllene, germacrene D, α-humulene, (E)-phytol [28], palmitic acid, palmitoleic acid, myristic acid, pentadecanoic acid, farnesyl acetone, dihydroactinidiolide [29];

- stems: β-caryophyllene, germacrene D, α-humulene, δ-cadinene, (E)-phytol [28], methyl salicylate, palmitic acid, 2,2,6-trimethyl-4-methylene-3,4-dihydro-2H-pyran, myristic acid [29];

- root: *p*-vinylguaiacol, borneol, myrtenol, trans-pinocarveol, 1-octen-3-ol, pinocarvone, 2-methyl-3-phenylpropanal, *p*-cymen-8-ol, *trans*-carveol, β-pinene, α-pinene , sabinene, α-phellandrene [28]

B. nigra subsp. *anatolica*

- *aerial parts:* germacrene D, nerolidol sclareol oxide, linalyl acetate, β-caryophyllene, spathulenol, linalool, longipinene epoxide [30], hexadecanoic acid, β-bisabolene, hexahydrofarnesyl acetone, 1-isobutyl-4-isopropyl-2,2-dimethyl succinate, β-eudesmol [31], 1-hexacosanol, caryophyllene oxide, germacrene-D, α-selinene, Z-8-octadecen-1-ol acetate, 2,5-di-tert-butyl-p-benzoquinone, arachidic acid, tetracosane, heneicosane, heptacosane, 2-methyl-1-hexadecanol, octadecane, butyl phthalate [32], β-caryophyllene, germacrene D, 1-octen-3-ol, (E)-2-hexenal, α-humulene, caryophyllene oxide [33], β-caryophyllene, germacrene D, caryophyllene oxide, 1-octen-3-ol, (E)-2-hexenal, α-humulene, β-bourbonene [34], (E)-phytol, germacrene D, β-caryophyllene, caryophyllene oxide, (E)-β-ionone [35], germacrene D, β-caryophyllene, caryophyllene oxide, caryophylladienol I, (E)-2-hexenal, hexadecanoic acid, α-humulene [4];

- fruiting aerial parts: β-caryophyllene, caryophyllene oxide, germacrene D, (E)-2-hexenal, β-pinene, limonene, 1-octen-3-ol, linalool [36];

- aerial parts flowering: β-caryophyllene, caryophyllene oxide, germacrene D, (E)-2-hexenal, 1-octen-3-ol [36];

- flowers: hexenal, (E)-β-caryophyllene, germacrene D, cis-3-hexene-1-ol, pentanal, limonene, (E)-2-hexenal [37].

Ballota nigra subsp. *curdica*

- aerial parts: caryophyllene oxide, β-caryophyllene , germacrene D, 1-undecene, isoaromadendrene epoxide [38].

Ballota nigra subsp. *uncinata*

- aerial parts: caryophyllene oxide, hexadecanoic acid, β-caryophyllene, germacrene D, hexahydrofarnesyl acetone, spathulenol, caryophyllene II; bicyclogermacrene [31].

PHARMACOLOGICAL EFFECTS

Various biologically active compounds that are present in *B. nigra* have an impact on the human body. Many of those effects can be useful in pharmacotherapy. The variety of those compounds is reflected in numerous pharmacological effects such as: anticholinesterase activities, antidepressant activity, antiproliferative and cytotoxic activities, sedative activity, antiprotozoal activity, hypoglycaemic activity, hypolipidemic activity and tyrosinase inhibitory activity.

Antidepressant and sedative activity

Studies from 1996 showed that water extract of *B. nigra*, subsp. *anatolica* had antidepressant activity. In this study authors compared it with other substances such as amitriptyline and *Passiflora* extract. In the forced swimming test when compared to amitriptyline (379.0 s), *Passiflora* extract (388.9 s), *B. nigra* had an slightly longer time of immobility (496.4 s), however, the result compared to the control group (570.0 s) was better [39].

In a clinical study where patients with anxiety disorder and symptoms of depression and sleep

disorders, were administered liquid extract of *B. nigra* for 90 days. After 60 days, 65% of patients showed improvement, and after 90 days 73% of patients reported a relief in disease symptoms. Patients with sleep disorders experienced particularly remarkable improvement [40].

B. nigra is traditionally used and well-known for its sedative activity [40]. It is proven that 5 phenylpropanoids (feoyl-L-malic acid, verbascoside, forsythoside B, arenarioside, and ballotetroside) from *B. nigra* showed a binding affinity to morphinic, dopaminergic and benzodiazepine receptors [3].

Antiproliferative and cytotoxic activities

Ballotinone and ballonigrin, natural compounds of *B. nigra* have a docking activity against the active site of the protein E6, a HPV virus protein, that plays a major role in molecular genesis of cervix cancer. The E6 protein binds and deactivates to the human tumour suppressor protein p53, creating a p53-HPV oncoprotein complex. The binding of ballotine and ballonigrin to E6 protein can play an important role in prevention of cervix cancer [8]. Another compound that can be found in *B. nigra*, 7*α*-acetoxyroyleanone, was proven to have *in vitro* cytotoxic activity against MIA PaCa-2 and melanoma (MV-3) cancer cell lines [41].

Additionally, it seems that 7*α*-acetoxyroyleanone, through inhibition of DNA synthesis, causes cytotoxic activity against other cancer cell lines: murine skin (B16), breast (MCF-7), colon cancer (HCT-8), and leukaemia (CEM and HL-60) [42].

Anticholinesterase activities

The cholinesterase enzymes are known for hydrolysis of choline esters, most importantly ACh, which finishes the neural transmission in central neural system (CNS), neuromuscular junction and in autonomic nervous system. In comparison with BChE the AChE, concentration is much higher in the brain, however, the BChE concentration is much higher in the circulatory system. The BChE is also known as the nonspecific cholinesterase that can hydrolyse more substrates. On the other hand, the AChE is known to be a specific cholinesterase and it is 20-fold more efficient in ACh hydrolysis. The inhibition of those enzymes leads to the elevation of ACh, thus, it is useful in myasthenia gravis, glaucoma, Alzheimer's disease [43].

Studies have shown that an acetone extract from *B. nigra* subsp. *anatolica* (whole plant) have been proven to be superior over ether, water, methanol and the petroleum extracts in matter of inhibiting acetylcholinesterase and butyrylcholinesterase. The BChE inhibition was stronger (71.58%) than AChE inhibition (44.71%). Comparing the acetone extract of *B. nigra* with galanthamine (standard drug), the extract had higher inhibitory effect against BChE [32]. However, the inhibition of BChE has not found any usage in medicine [44].

Hypoglycaemic and hypolipidemic activity

The plant extract of *B. nigra* caused a reduction in blood glucose level in albino rats. It is suspected to have an insulinotropic effect, but further studies are necessary to prove it [45]. Nonetheless, this can be a very useful for treating diabetes mellitus in which control of the blood glucose level is the most important treatment target [46]. A study has also shown that *B. nigra* can decrease the total serum cholesterol with no changes in triglycerides levels [47]. LDL cholesterol increases the risk of cardiovascular disease, so its lowering will be a positive factor for human health [48]. Both of those effects can be also helpful in treatment of patients with metabolic syndrome, thus it can reduce the risk of coronary heart disease and stroke *etc*. [49].

Tyrosinase inhibitory activity

A study from 2014 proved that an extract from *B. nigra* subsp. *curdica* from Iran has a considerable tyrosinase inhibitory activity [50].

Considering the fact that tyrosinase is an enzyme that plays a key role in melanin biosynthesis, this extract can be helpful in the treatment of melasma, solar lentigo and other anomalous skin pigmentation [51].

Antibacterial activity

An EtOH extract from *B. nigra* stems can lower the production of *δ*-hemolysin, which is an virulence factor of *Staphylococcus aureus* and due to this fact it is safe to say that *B. nigra* has anti-quorum sensing activity. Hence, it might be useful as an alternative to antibiotics [52].

In the study it was reported that the water extract of *B. nigra* exhibited a dose-dependent biofilm

formation inhibition for methicillin-resistant *S. aureus* [53].

Other research indicating *B. nigra* antibacterial activity:
- *Escherichia coli, Staphylococcus aureus, Proteus mirabilis, Klebsiella pneumoniae, Enterococcus faecalis, Salmonella typhi* [54];
- *Escherichia coli, Staphylococcus aureus, Bacillus mycoides, B. subtilis, Micrococcus lysodeikticus, Klebsiella pneumoniae, Candida albicans* [28];
- *Staphylococcus aureus, S. aureus MRSA, Proteus mirabilis* [55];
- *B. nigra* subsp. *anatolica →Bacillus subtilis, B. cereus, Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Salmonella typhimurium, Pseudomonas aeruginosa* [56];
- *B. nigra* subsp. *anatolica → Bacillus cereus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus capitis, S. aureus, S. epidermidis, Propionibacterium acnes, Moraxella nonliquefaciens* [57];
- *B. nigra* subsp. *foetida → Escherichia coli, Enterobacter cloacae, Pseudomonas aerouginosa, P. fluorescens, Staphylococcus aureus, S. epidermidis* [33].

Antifungal activity

- *Aspergillus niger, A. flavus, A. fumigatus, Fusarium solani* [54];
- *B. nigra* subsp. *anatolica → Candida albicans, Debaryomyces hansenii, Kluyveromyces fragilis, Rosemonas rubra* [53];
- *B. nigra* subsp. *foetida → Candida albicans, C. glabrata, C. tropicalis* [34].

Antiprotozoal activity

In a 2014 study, extracts of *B. nigra*'s stems, leaves, and roots in EtOH were partitioned between water and several organic solvents and an antileishmanial activity was observed [54].

Antioxidant activity

Oxidative stress occurs when an overwhelming amount of free radicals are produced in human body. It can lead to the development of many diseases, such as cancer [58], Alzheimer's disease, Parkinson's disease, multiple sclerosis, amyotrophic lateral sclerosis (ALS), memory loss, depression [59], asthma and chronic obstructive pulmonary disease (COPD) [60], rheumatoid arthritis [61], cataracts[62], foetal growth restriction [63] and pre-eclampsia [64], hypertension [65], atherosclerosis, ischaemia, cardiomyopathy, congestive heart failure and cardiac hypertrophy [66].

The antioxidant activity of *B. nigra* was proven in many tests, such as: FRAP [67], SAFQ [68], DPPH [69, 21], and free radicals such as O_2, H_2O_2, OH [3], ABTS (*B. nigra* subsp. *anatolica)* [32].

Dosage

The single adult dose of *B. nigra* contains from 1.5 to 5 g of the drug, taken as a tea infusion or as an extracts prepared with ethanol (max. 45% *v/v*) or water. The same dosage is for elder people. For children (aged 3–12) preparations of *B. nigra* should be given only under medical supervision, it should be a non-alcoholic preparation and the dosage must be correlated with body weight [70].

No studies about the usage of black horehound during pregnancy and lactation is available, thus it should not be used during this period without medical advice [40].

Toxicity and adverse effects

Black horehound, when used in proper therapeutic dosage, seems to be a safe drug with no health hazards or side effects [1]. Studies available present little proofs of toxic activity of *B. nigra*. Mongold *et al.* tested the safety on mice, giving them 2 g/kg of their body mass. No death was stated, even after 15 days of administration there were no signs of toxic activity [71].

Studies on 28 patients, in which the black horehound was administered 3 times a day as 5 ml of liquid preparation for 90 days. Only 2 minor adverse effects were reported: nausea (alleviated by taking the drug after meals) and fatigue, which abated as the study progressed [70].

No cases of overdose are reported. There are no contraindications for *B. nigra*. However, due to its sedative effects, driving ability and machine operating might be affected [40].

TRADITIONAL USES

Black horehound is known in traditional medicine. For ages, it has been used for the treatment of many

symptoms and diseases. Some of pharmacological effects of *B. nigra*, as presented in this article, have been proven, however, many of them still lack research-based evidence [1].

The use of *B. nigra* differs among countries. As it was mentioned in 13th century Ibn al-Baytar's Compendium of Simple Medicaments, in Morocco and eastern Andalusia the black horehound herb was used for the treatment of animal bites, wounds, ulcers, hemorrhoids, menstruation disorders, toothaches, sores and nervousness [2]. In Turkish traditional medicine, *B. nigra*, called there "Yalanci Isirgan", was used for a variety of clinical situations as a sedative, diuretic, digestive, anti-inflammatory, antiseptic, and also as an antiparasitic drug. Otherwise it was used for the treatment of wounds, hemorrhoids, and dysmenorrhea [72]. *B. nigra* subsp. *uncinata* in the regional tradition of Taurus mountains (Turkey) is used to treat stomach upset and flatulence [73]. Another subspecies of *B. nigra* subsp. *anatolica*, also found use in the Turkish traditional medicine. It was used externally, as an antiseptic and for treatment of inflamed sore in foot or armpit [74]. In France, it is used for the management of nervous disorders (e.g. mild sleep disorders) and cough [1]. In Italy, in the upper Lucca Province, the folk tradition was to use it in the form of poultice for wounds and sprains [75] and in Dolomiti Lucane, a decoction of the black horehound was used in form of washes for its haemostatic properties [76]. In the south Italian pharmacopoeia, a rinse of *B. nigra* served as treatment of skin rashes and it was also drunk to promote circulation.

B. nigra is used not only in medicine. In the tradition of Rotonda (Italy), the whole plant of black horehound is used in repellent fumigation against insects, mostly due to containing diterpenes [77]. In Serbia, a tea from dried root and an alcohol extract of the black horehound have been used to treat nervous disorders and cancer [28]. Moldavians noted the sedative, antispasmodic, stimulant and vermifuge effect of the *B. nigra*. A plant infusion was used to treat flatulence, vomiting, convulsive cough, esophageal spasms, abdominal colic, rheumatism and mental disorders, such as hysteria, anxiety, psychic asthenia and palpitations [78]. *B. nigra* is used in repellent fumigation against insects. B. nigra from *Lamiaceae* was already reported for its content in diterpenes, compounds with well-known insecticide and antifeedant activities [77].

CONCLUSIONS

Taking into account the growing public interest in medicinal plants, as well as the above-mentioned variety of pharmacological effects of *B. nigra*, the black horehound can be a very useful herb in everyday life. The compounds contained in the *B. nigra* may prove helpful even in more complicated medical cases, however, the amount of clinical trials so far does not allow for unequivocal determination of its usefulness. Therefore, it is suggested to work further on this plant.

At the moment, *B. nigra* has a well-documented use as a sedative agent. Laboratory studies have also demonstrated other properties of substances of black horehound, such as anticholinesterase, antidepressant, antiproliferative and cytotoxic, antiprotozoal, hypoglycaemic, hypolipidemic and tyrosinase inhibitory activities.

Most compounds showing biological activity are found in the aboveground parts of the plant, among which the dominant groups are phenylpropanoids, flavonoids, diterpenes or derivatives of ortho-dihydroxycinnamic acid.

There is an evidence of the use of the black horehound even in the 13th century. The country where it has gained popularity as a traditional medicinal herb is Turkey. There is also evidence of its use in other countries such as Morocco, Italy, France, Serbia and Moldova. Use varied by region, but was most commonly used as a sedative and wound sanitizer.

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