Pharmacological Activities of *Ballota nigra* (L.) Benth: A Mini Review

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***Abstract*—The *Ballota* L. genera belonging to the Lamiaceae family found mainly in the Mediterranean area, Middle East, and North Africa. This genus has been largely explored from the ancient times for their biological properties. The phytochemical investigations revealed that diterpenoids are the main constituents of the genera. A large number of flavonoids and other metabolites were also identified. This mini review, covers the traditional and pharmacological properties of *Ballota nigra* L. species.**

***Index Terms*—*Ballota*, medicinal use, pharmacology, traditional use**

I. Introduction

From the ancient time several plants have been explored for their medicinal purposes. More recently, plant extracts have been developed and proposed for various biological process. In developing countries herbal medicine has been improved as a substitute solution to health problems and costs of pharmaceutical products. The development of drug resistance pathogens against antibiotics has required a search for new antimicrobial substances from other sources, including plants. Plants contain a wide range of substances that are used to treat chronic as well as infectious diseases [1].

The genus *Ballota* L. (Lamiaceae) consists of about 33 species growing mainly in the Mediterranean region. In Turkey, the genus *Ballota* is represented by 11 species, 6 subspecies, 10 of which are endemic [2]. *B. nigra* was described by Carl Von Linnaeus in 1753 [3] and the epithet "*nigra*" is derived from Latin word '*nigra*' which refers to means 'blackish' [4]. *B. nigra* contains a characteristic phyto-constituent, namely, betaines. Tradition¬ally it has been used to cure various diseases especially neuro-sedative [5]. Dioscorides, the Greek physician recommended its leaves plaster with salt in dog bites treatment. A balm prepared from its dried leaves and honey purified infected wounds and ulcer [6].

Manuscript received February 11, 2021; revised April 26, 2021.

II. Distribution

The native range of this plant is Macaronesia, Europe, Mediterranean to West Asia and introduced into Alabama, Argentina, Kirgizstan and New Zealand [7].

III. Botanical Description

Perennial herb, with hairy stems to c. 1 m tall. Petioles 1–3 cm long. Lamina of upper cauline lvs 2.5–5 × 2–3 cm, ovate or ovate-oblong, densely hairy on both sides, strongly crenate; base subcordate; apex acute. Verticels dense; bracts cuneate, serrate or crenate-serrate. Bracteoles <calyx, ± filiform. Calyx 6–9 mm long, with nerves densely hairy; teeth ovate, acuminate. Corolla 12–15 mm long, pinkish to purple or white, almost glabrous outside; middle lobe ovate and slightly reflexed. Filaments hairy at base. Nutlets not seen [8].

IV. Traditional Uses

B. nigra leaves were used as an antidote for rabid dog bites. In Balkanic area it was used as a sedative/tranquilizer in cases of hysteria and hypochondria [9]-[12]. It is also used for wound-healing properties in Italy [13], [14]. It used to treat various disease like whooping cough, to increase bile flow, sedative, nervousness, vomiting, upset stomach and nausea [12], [15]. In Moldova it is used against worm infestation in the form of enemas and suppositories [9] and as insecticide and repellent against fleas in northern Spain [16]. Various ethnopharmacological uses of *B. nigra* in different countries are provided in Table I.

V. Pharmacological Effects

A. Antioxidant Effect

Matkowski and coworkers studied the antioxidant activity of *B. nigra* aerial parts by using DPPH and HO scavenging and phosphomolybdenum reduction methods. The ethyl acetate and n-butanol fraction of *B. nigra* showed maximum inhibition of deoxyribose degradation (79.32 ± 1.62% and 82.04 ± 2.28%, respectively). They concluded that plant antioxidant activity is not only influenced by secondary metabolites such as carotenoids,

doi: 10.18178/ijpmbs.10.3.114-119

vitamins, volatile oils and diterpenes but also by phenolics (including flavonoids). Plant polyphenols possess an ideal structural chemistry for free radical scavenging activity which makes them important dietary antioxidants [19]. In Turkish folk medicine various *Ballota* species have been used to treat hemorrhoids, gastrointestinal disorders, wounds and burns, as diuretics and to prevent coughs. Citoğlu et al., investigated the antioxidant properties of 16 *Ballota* species on lipid peroxidation and superoxide anion formation. The *B. nigra* subsp. *anatolica* extract showed significant anti-superoxide anion formation [20]. In the south Italian pharmacopoeia *B. niger* aerial parts are drunk to promote circulation and used as a rinse for skin rashes. Various phytochemical studies [21]-[24] have been executed on *B. niger*, and numerous phenylpropanoid glycosides have been recognized and related to moderate growth inhibition in *Staphylococcus aureus* [25]. *B. niger* contains diterpenes (marubiin), ferulic acid derivatives and caffeic [26]. Quave and coworkers observed that aqueous extraction of the aerial parts of *B. niger* was the most effective at inhibiting both biofilm growth and adherence [27]. *B. nigra* has many neurosedative properties. Caffeoyl malic acid, verbascoside, ballotetraside, forsythoside, arenareoside are its active substances possess scavenging properties against hydroxyl radicals, hypochlorite, hydrogen peroxide and superoxide generated in cell-free systems and released from stimulated neutrophils. Their inhibitory concentrations were equivalent to mesna and N-acetyl cysteine [28]. Based on the information of traditional use and potent antioxidant activities, Rigano and coworkers studied the inhibitory effect of *B. undulata* (Sieber ex Fresen.) Benth, *B. saxatilis* (Sieber ex C.Presl) and *B. nigra* ssp. *foetida* (Vis.) Hayek on human breast cancer MCF-7 and human hepatoma HepG2 cell lines. They concluded the effectiveness in vitro of the *B. undulata*, *B. saxatilis*, *B. nigra* ssp. *foetida* EOs by inhibiting the human hepatoma HepG2 cell proliferation in a dose dependent way [29]. Makowczynska et al. [30], studied the antioxidant properties, total phenolic and flavonoid contents of *B. nigra* shoots in methanolic extracts initiated in vitro (from nodal explants) and in vivo (from seeds). They observed that antioxidant potential of the *B. nigra* extracts may be due to their scavenging of free radicals (DPPH assay) and metal reducing (FRAP test). The shoot extracts of in vitro derived exhibit the greatest antioxidant properties (EC50 - 56 μg/mL), characterized by the highest content of phenolic compounds. The cardiac tissues can absorb polyphenolic substances, which are responsible for radical scavenging and reducing activities [31].

TABLE I. ETHNOPHARMACOLOGICAL USES OF *B. NIGRA* L.

Country	Uses
Mt.,Macedonia	digestive [15].
Moldova	sedative, antispasmodic stimulant, vermifuge [9].
Northeast Bosnia-Herzegovina	nervous system disorders, sedation [10].
Lucca, Italy	wounds and sprains [13].
Mediterranean Area	skin disorders, sore throat in horses [17].
Albanians, North Basilicata, Italy	diuretic, hemostatic [14], [18].
Bosnia-Herzegovina	hysteria [11].
Jadovnik Mt., Serbia	remedy for upset stomach, nausea, and vomiting; symptomatic, treatment of nervous disorders, sleep disorders, coughs, inflammation, gout [12].
North Spain	insecticides and repellents against fleas [16].

B. Hypoglycemic Effect

Nusier *et al.*, studied hypoglycemic effect of *B. nigra* extract and observed significant reduction in glucose level in both healthy and diabetic Albino rats. The decrease in glucose level (32% and 22.3% in normoglycemic and alloxanized rats, respectively) was time-dependent and was observed 6 hours after administration of the *B. nigra* extract [32].

C. Neurosedative Effect

B. nigra contains various phenylpropanoid derivatives which showed remarkable neurosedative and antioxidant activities which are of curative interest [21], [33]. Phenylpropanoid glycosides might act as a neuroleptic drug increases sleeping time, reduce locomotor activity and produce a slowing of the electroencephalographic activity [34]. Some studied discover the antidepressant activities of *B. nigra* var. *anatolica* [35] and neurosedative activities of *B. nigra* [28]. Daels-Rakotoarison *et al.*, [28] studied the antioxidant properties of phenylpropanoid derivatives such as verbascoside, forsythoside B, arenarioside, ballotetroside, and caffeoyl malic acid isolated from *B. nigra* using cell-free experiments and cellular experiments including isolated polymorphonuclear neutrophils (Inhibitory concentrations at 50% were 0.4 - 4.7 mg/ml).

D. Antitumor Activity

In Italy the essential oils of B. undulate, *B. saxatilis*, and *B. nigra* was examined for their in vitro cytotoxicity toward the Hep-G2 hepatocarcinoma and MCF-7 breast carcinoma cell lines (IC50: 54.7, 65.4, and 69.9 μg/mL, respectively) [29].

E. Anti-Cancer Activity

B. nigra contains 7α-Acetoxyroyleanone (73) [36] which showed promising anticancer activities against MIAPaCa-2 and melanoma (MV-3) cancer cell lines (IC50 = 4.7 and 7.4 μg/mL) [38] and cytotoxic activities against breast (MCF-7), human leukemia (CEM and HL-60), murine skin (B16), and colon cancer (HCT-8) cell lines (IC50 = 0.9–7.6 μg/mL). It seemed that its cytotoxic activity may be related to inhibition of DNA synthesis [38].

F. Hepatoprotective Effects

The crude n-Hex extract of *B. nigra* subsp. kurdica was examined for tyrosinase inhibitory activity by the colorimetric tyrosinase inhibition assay (IC50 = 3.67 μg/mL); however, no individual active molecules were isolated [39].

G. Antifungal and Antimicrobial Activity

The antifungal activity *Aspergillus niger*, *A. flavus*, *A. fumigatus*, and *F. solani* and antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, and *Salmonella typhi* of root, stem, and leaves extract of *B. niger* were examined in Pakistan by using Agar tube dilution method and well assay method. The crude extract, ethyl acetate, and chloroform fractions were the most active fractions against all pahogens [40].

Fraternale and Ricci collected aerial parts of *B. nigra* ssp *foetida* at flowering and fruiting times to evaluate their antifungal activity against *Fusarium oxysporum*, *F. solani*, *F. coeruleum*, *F. sporotrichioides*, *F. tabacinum*, *F. verticillioides*, *Botrytis cinerea*, and *Alternaria solani* using the agar dilution method. The major compounds identified in the flowering and fruiting aerial parts oils respectively were β-caryophyllene (22.6% and 21.8%), caryophyllene oxide (18.0% and 20.5%) and germacrene-D (16.5 and 13.1%). The oils showed in vitro antifungal activity against some species of *Fusarium* (300-350 ppm), *B. cinereal* (600 ppm), and *A. solani* (750 ppm) [41].

TABLE II. VARIOUS PHYTOCHEMICALS ISOLATED FROM *B. NIGRA* L.

Names	Compounds
Labdane diterpenes	7α-acetoxymarrubiin [43].
	Ballonigrin [43].
	13-hydroxyballonigrolide [44], [45].
Diterpenes	7α-acetoxyroyleanone [36].
Flavones	ladanein [36].
	Luteolin [46].
	Chrysoeriol [46].
	tangeretin [47].
Acyl flavonoid glycosides	luteolin-7-O-[2-O-β-D-glucopiranosyl-lactate] [21].
Triterpenoids	ursolic acid [48].
Steroids	β-sitosterol [36], [49].
Carboxylic acids	caffeic acid [33], [46].
	E-caffeoyl-L-malic acid [25], [33], [50]-[52].
	chlorogenic acid [33], [46].
	ferulic acid [46].
	fumaric acid [33].
	laballenic acid [51].
	quinic acid [33].
	shikimic acid [33].
Nitrogen-containing compounds	4-hydroxyprolinebetaine [51].
	Stachydrine [51].
Penylpropanoids	alyssonoside [25], [33].
	angoroside A [25].
	arenarioside [25], [45], [53].
	ballotetroside [23], [25], [33], [45].
	forsythoside B [25], [33], [36], [45], [50]-[53].
	lavandulifolioside [25].
	martynoside [36].
Other metabolites	phytol [49].
Essential oils	caryophyllene oxide (7.9), epi-α-muurolol (6.6), δ-cadinene (6.5), α-cadinol (6.3), γ-amorphene (4.3), β-bourbonene (4.1), 6,10,14-trimethyl-2-pentadecanone (4.0), (E)-caryophyllene (4.0), germacrene D (3.8), aromadendrene (3.4), γ-muurolene (3.2), germacrene D-4-ol (3.2), α-bisabolol (3.2), α-amorphene (3.0) [54].
	β-caryophyllene (35.4), germacrene D (27.4), α-humulene (7.4), δ-cadinene (3.8), (E)-phytol (2.5) [55].
	β-caryophyllene (39.1), germacrene D (35.7), α-humulene (10.4), (E)-phytol (3.8) [55].
	p-vinylguiacol (9.2), borneol (7.5),myrtenol (7.1), trans-pinocarveol (5.2), 1-octen-3-ol (5.1), pinocarvone (4.4), 2-methyl-3-phenylpropanal (4.3), p-cymen-8-ol (4.3), trans-carveol (3.5) [55].
	β-pinene (39.0), α-pinene (34.5), sabinene (7.7), α-phellandrene (4.1) [56].
	palmitic acid (573)[a], 2,2,6-trimethyl-4-methylene-2H-pyran (172)[a], hexahydrofarnesylacetone (167)[a], miristic acid (100)[a], caryophyllene oxide (57)a, pentadecanoic acid (50)[a], palmitoliec acid (40)[a], germacrene D (40)[a] amg/kg [57].
	palmitic acid (1620)[a], dodecanal (519)[a], palmitoliec acid (306)[a], miristic acid (271)[a], pentadecanoic acid (182)[a], lauric acid (67)a, trans-isoelemicin (67)[a], hexahydrofarnesylacetone (60)[a], pentadecene (54)[a], methyleugenol (40)[a] amg/kg [57].
	palmitic acid (656)[a], palmitoliec acid (197)[a], miristic acid (187)[a], pentadecanoic acid (121)[a], farnesylacetone (69)a, dihydroactinidiolide (44)[a] amg/kg [57].
	methylsalicilate (313)[a], palmitic acid (130)[a], 2,2,6-trimethyl-4-methylene-2H-pyran (42)[a], miristic acid (42)[a] amg/kg [57].

H. Insecticide

The whole plant of *B. nigra* L. is used in repellent fumigation against insects and its diterpenes compounds are well known for insecticide and antifeedant activities [42].

VI. Phytochemical Contents

The phytochemical research of *B. nigra* and its subspecies led the isolation and identification of several groups of phytochemical constituents. We have briefly summarized various phytochemicals of *B. acetabulosa* in Table II and Table III.

TABLE III. Various Phytochemicals Isolated from Various Subspecies of *B. nigra*

Names	Compounds
B. nigra subsp. *foetida* Hayek	
Nitrogen-containing compounds	Choline [58].
	Stachydrine [58].
Other metabolites	Stachyose [59].
Labdane diterpenes	Ballonigrin [60]-[62].
	Ballotenol [63].
	Ballotinone [64]
	Marrubiin [62], [65].
	Preleosibirin [22].
Essential Oils	β-Caryophyllene (25.1), germacrene D (24.2), 1-octen-3-ol (7.3), (E)−2-hexenal (6.1), α-humulene (4.3), caryophyllene oxide (4.2) [66].
	β-Caryophyllene (20.0), germacrene D (18.0), caryophyllene oxide (15.0), 1-octen-3-ol (6.8), (E)−2-hexenal (6.1), α-humulene (4.5), β-bourbonene (3.2) [67].
	β-Caryophyllene (22.6), caryophyllene oxide (18.0), germacrene D (16.5), (E)−2-hexenal (6.5), 1-octen-3-ol (5.5) [41].
	(E)-Phytol (56.9), germacrene D (10.0), β-caryophyllene (4.7), caryophyllene oxide (3.6), (E)-β-ionone (3.4) [68].
	Germacrene D (23.1), β-caryophyllene (20.3), caryophyllene oxide (6.2), caryophylladienol I (3.3), (E)-2-hexenal (3.1), hexadecanoic acid (3.1), α-humulene (3.0) [29].
B. nigra subsp. *uncinata* (Fiori & Beg.) Patzak	
Labdane diterpenes	Dehydrohispanolone (hispanone) [60], [61].
Essential oils	Caryophyllene oxide (21.2), hexadecanoic acid (19.9), β-caryophyllene (18.9), germacrene D (4.6), hexahydrofarnesyl acetone (4.4), spathulenol (4.2), caryolphyllenol II (3.8), bicyclogermacrene (3.7) [69].
B. nigra subsp. *anatolica* P.H. Davis	
Essential Oils	Germacrene D (18.1), nerolidol epoxyacetate (15.4), sclareol oxide (12.1), linalyl acetate (11.5), β-caryophyllene (10.5), spathulenol (9.0), linalool (5.2), longipinene epoxide (4.7) [70].
	Hexadecanoic acid (40.9), β-bisabolene (13.4), hexahydrofarnesyl acetone (7.9), 1-isobutyl-4-isopropyl-2,2-diemethyl succinate (6.6), β-eudesmol (3.5) [1].
	1-Hexacosanol (26.7), caryophyllene oxide (9.3), germacrene-D (9.3), α-selinene (8.7), Z-8-octadecen-1-ol acetate (7.1), 2,5-di-tertoctyl-p-benzoquinone (7.3), arachidic acid (6.0), tetracosane (4.5), heneicosane (4.4), heptacosane (4.3), 2-methyl-1-hexadecanol (3.3), octadecane (3.0), butyl phthalate (3.0) [71].
Other metabolites	Phytol [72].
B. nigra subsp. *kurdica* P.H.Davis	
Essential Oils	Caryophyllene oxide (39.4), β-caryophyllene (24.9), germacrene D (7.6), 1-undecene (4.2), isoaromadendrene epoxide (3.2) [72].

VII. Conclusion

In this mini review we have briefly summarized the traditional uses, ethnobotanical description, ethnopharmacological properties and phytochemical constituents that have been isolated from *B. nigra* and their subspecies. Further research should be conducted to explore new potential therapeutic agents and their ethnopharmacological properties of *B. nigra* for the treatment of life-threatening diseases.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Sanjay Kumar and Reshma Kumari conducted the research, analyzed the data, wrote the paper and approved the final version.

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