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(REVIEW ARTICLE)

The bioactive ingredients and therapeutic effects of *Marrubium vulgare* - A review

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Abstract

Marrubium vulgare (Family: Lamiaceae) was used traditionally in the treatment of dyspeptic complains, pulmonary infections, cough, rheumatoid arthritis, night blindness, loss of appetite, as cholagogue, purgative, diuretic, bitter tonic, carminative and appetizer. The phytochemical analysis revealed that the plant contained alkaloids, sterols, steroids, terpenoids (diterpene), saponins, flavonoid, catecholic tannins, anthocyans, phenolic compounds and many other bioactive ingredients. The pharmacological investigations showed that the plant exerted anti-inflammatory, antiedematogenic, analgesic, antioxidant, antimicrobial, antidiabetic, cardiovascular hypolipidemic, antispasmodic and many other biological effects. This review discussed the bioactive contents and pharmacological activities of *Marrubium vulgare.*

Keywords: *Marrubium vulgare*; metabolites; constituents; traditional uses; pharmacology; therapeutic; toxicity

1. Introduction

Plants generally produce many secondary metabolites which are bio-synthetically derived from primary metabolites and constitute an important source of chemicals which are used as pharmaceuticals, agrochemicals, flavours, fragrances, colours, biopesticides and food additives. *Marrubium vulgare* (Family: Lamiaceae) was used traditionally in the treatment of dyspeptic complains, pulmonary infections, cough, rheumatoid arthritis, night blindness, loss of appetite, as cholagogue, purgative, diuretic, bitter tonic, carminative and appetizer. The phytochemical analysis revealed that the plant contained alkaloids, sterols, steroids, terpenoids (diterpene), saponins, flavonoid, catecholic tannins, anthocyans, phenolic compounds and many other bioactive ingredients. The pharmacological investigations showed that the plant exerted anti-inflammatory, antiedematogenic, analgesic, antioxidant, antimicrobial, antidiabetic, cardiovascular hypolipidemic, antispasmodic and many other biological effects. This review will highlight the bioactive contents and pharmacological activities of *Marrubium vulgare.* The current review will highlight the bioactive contents and pharmacological activities of *Marrubium vulgare.*

1.1. Synonyms

Marrubium apulum, Marrubium ballotoides, Marrubium germanicum, Marrubium hamatum, Marrubium uncinatum, Marrubium vaillantii, Marrubium vulgare subsp. apulum, Marrubium vulgare var. apulum, Marrubium vulgare var.

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caucasicum, Marrubium vulgare var. gossypinum, Marrubium vulgare var. lanatum, Marrubium vulgare var. microphyllum and *Marrubium vulgare var. oligodon*[1].

1.2. Taxonomic classification

Kingdom: Plantae, Subkingdom: Viridiplantae, Infrakingdom: Streptophyta, Superdivision: Embryophyta, Division: Tracheophyta, Subdivision: Spermatophytina, Class: Magnoliopsida, Superorder: Asteranae, Order: Lamiales, Family: Lamiaceae, Genus: Marrubium, Species: *Marrubium vulgare* [2].

1.3. Common names

Arabic: Hashishat Alkalib, Farasion Abiadh, Roubiya, Zekom, Ithn al-hemar, Ithn al-thawr; Chinese: ou xia zhi cao; English: Horehound, white horehound; French: marrube blanc, marrube vulgaire; German: Andorn; Hindi: Paharigandana; Portuguese: marroio; Persian: Faracion, Ghandnaye kohi, Shenar, Oftan-Sar, Korar; Russian: šandra obyknovennaja; Spanish: marrubio común; Swedish: kransborre [3-4].

1.4. Distribution

It is native to Africa (Algeria, Libya, Morocco and Tunisia), Asia (Armenia, Azerbaijan, Russian Federation- Ciscaucasia, China, Kazakhstan, Turkmenistan, Uzbekistan, Afghanistan, Iran, Iraq, Palestine, Jordan, Lebanon, Syria, Turkey and Pakistan), Europe (Belarus, Estonia, Latvia, Lithuania, Russian Federation-European part, Ukraine, Austria, Belgium, Czechoslovakia, Hungary, Netherlands, Poland, Switzerland, Denmark, Sweden, United Kingdom, Albania, Bulgaria, Former Yugoslavia, Greece, Italy, Romania, France, Portugal and Spain). It is naturalized in Africa (Cape Verde, Portugal – Azores, Lesotho and South Africa), Australasia (Australia and New Zealand), Northern America and Southern America and it is widely cultivated [3].

1.5. Description

Long-lived herbaceous plant usually growing 20-60 cm tall. Stems branched or unbranched. Leaves reduced upward; petiole 0.7-1.5 cm; leaf blade ovate to circular, 2-3.5 × 1.8-3 cm, adaxially polished, corrugate, and sparsely villous, abaxially densely scabrid strigose-villous, base broadly cuneate to rounded, margin dentate-serrate, apex obtuse to subrounded. Verticillasters axillary, many flowered, widely spaced basally, crowded upward, globose, 1.5-2.3 cm in diam.; bracts subulate, as long as to longer than calyx tube, reflexed. Calyx 10-veined, teeth 10, main 5 long, alternate with to 5 accessory teeth, 1-4 mm, subulate, hooked. Corolla white, ca. 9 mm; tube ca. 6 mm, densely pubescent outside, pilose annulate inside, upper lip as long as or slightly shorter than lower lip, straight or spreading, 2-lobed; middle lobe of lower lip reniform, undulate, 2-cleft. Nutlets triquetrous, ovoid, warty [5].

1.6. Traditional uses

Marrubium vulgare was used for dyspeptic complains and for loss of appetite. It also used as a choleretic or cholagogue herb [6].

Many Native American tribes extensively used the plant in formulations containing the leaves, or the leaves and flowers, for treatment of cough, mixed with honey because it is extremely bitter. Native Americans also used the root topically for rheumatoid arthritis [7]. In Mexico, the decoction of *Marrubium vulgare* was used in the traditional medicine for treatment of diabetes [8]. The herb was applied for the treatment of joint pain, inflammation, sore eyes, cough, bronchitis, pulmonary infections, cold and night blindness. The plant was also used to help in expulsion of fetus, as purgative, diuretic, bitter tonic, carminative and appetizer [9-10]. *Marrubium vulgare* raw material was recommended as a drink to accelerating the digestion processes [11].

1.7. Physicochemical characteristics

The petroleum ether, alcohol, and water extractive values of the whole herb were 2.77 ± 0.3, 8.66 ± 1.2% and 5.90 ± 0.8 % w/w respectively. The ash value was (10.7 ± 0.46%) and water soluble ash value was (8.9 ± 0.65%) [9].

1.8. Chemical constituents

The preliminary phytochemical screening showed that the plant contained alkaloids, sterols, steroids, terpenoids (diterpene), saponins, flavonoid, catecholic tannins, anthocyans and phenolic compounds [9, 12-13].

The dried herb of *Marrubium vulgare*, contained 0.05% essential oil [14]. The oil of *Marrubium vulgare* from Poland revealed the presence of 31 compounds. The main compounds identified in the essential oil of *Marrubium vulgare* were

E-caryophyllene (34.51–36.78%), germacrene D (22.45–27.18%), bicyclogermacrene (9.54–11.12%), δ-amorphene (6.15–8.18%), and carvacrol (4.71–6.64%) [15-16].

Thirty two compounds were determined in*Marrubium vulgare* essential oil cultivated in Egypt. The GC/MS analysis revealed that carvacrol (36.28%), followed by β-phellandrene (15.49%) and carvyl acetate (11.52%) were the main compounds of volatile oil extracted from *Marrubium vulgare* fresh herb. However, the isolated compounds were included (%):β- phellandrene 15.49, linalool 3.86, trans- sabinene hydrate 3.29, β-thujene 2.93, octen-3-ol 2.48, 1,8 - cineol 1.49, α-pinene 1.44, borneol 1.12, 1,8 -cineol 1.49, α-pinene 1.44, borneol 1.12, camphore 0.64, camphene 0.64, terpinen-4-ol 0.97, sabinene 0.25, α -terpineol 0.33, β-pinene 3.53, thymol 0.34, octanol 0.16, carvacrol 36.28,α-phellandrene 0.60 trans-caryophyllene 4.06, α-terpinene 3.83, α-humulene 0.19, carvyl acetate 11.52, (+)-carvomenthene 0.30, limonene 0.82, α-cubebene 0.47, 7-epi-sesquisabinene hydrate 0.27, caryophyllene oxide 0.67, cubenol 0.19, 2,6-dimethylheptadecane 0.18, α-copaene 0.22, cis- sabinene hydrate 0.59 and E-β-farnesene 0.24%[17]

The essential oils of *Marrubium vulgare* from Isfahan, Iran, contained 44 constituents, these included (%): trans-2-hexanal: 0.77, heptanal: 4.26, α-thujene: 0.22, α-pinene: 6.64, camphene: 0.36, benzaldeyde: 0.19, p-cymene: 4.76, - 1,8 cineole, 8.17, - ϒ- terpinene: 2.62, linalool: 0.43, ϒ- terpineol: 1.39, decanal: 0.95, carvone: 0.64, piperitone: 2.11, eugenol: 2.91, α-copaene: 0.51, β-cubebene: 1.08, β-caryophyllene: 32.19, geranyl linalool: 2.58, (E)-β-farnesene: 11.39, α-humulene: 1.59, alloaromadendrene: 0.97, germacrene D: 0.41, β-ionone: 1.16, ß-guaiene: 0.92, α-farnesene: 0.31, α-muurolene: 0.23, β-bisabolene: 0.81, trans–calamenene: 0.64, δ–cadinene: 0.36, α-calacorene: 0.37, spathulenol: 0.24, caryophyllene oxide: 4.06, viridiflorol: 0.28, - 1, 10 di-epi-cubenol: 0.17, epi-α-cadinol: 0.25, β-eudesmol: 0.19, α-cadinol: 0.27, β-cubebene: 0.18, citronellyl butanoate: 0.39, α-bisabolol: 0.19, geranyltiglate: 0.18, benzyl benzoate: 1.08 and cyclononasiloxane: 0.47%[18].

However, the hydrodistillation of the dried flowering aerial parts of *Marrubium vulgare* from the suburb of Nour, Mazandaran province, North of Iran, gave 0.4% (w/w) light yellowish oil. The oil contained 8 sesquiterpenoids (68.2%), 11 monoterpenoids (25.0%), and one non-terpenoid (0.3%). The compounds isolated from the oil of *Marrubium vulgare* were included (%): α-pinene 3.9, β-pinene: 4.8, δ-3-carene: 2.2, 1,8-cineole: 4.1, santolinyl acetate:0.7, nerol : 0.4, ascaridole: 0.4, cis-piperitone epoxide: 1.3, trans-piperitone epoxide: 2.7, isobornyl acetate: 4.2, isoascaridole: 0.3, α-copaene: 3.8, 1-tetradecene: 0.3, isocaryophyllene: 14.1, germacrene D: 3.1, bicyclogermacrene: 3.4, β-bisabolene: 20.4, α-bisabolene: 2.9, δ-amorphene: 1.4 and δ-cadinene: 19.1%[19].

Analysis of oils of the aerial parts of *Marrubium persicum* from Varzeghan in East Azarbaijan province, Iran, showed that the essential oil of *M. persicum* contained a mixture of non-terpenoids 51.5%, sesquiterpene hydrocarbons 27.9%, monoterpene hydrocarbons 9%, and oxygenated sesquiterpenes 4.8% and oxygenated monoterpenes 1.2%. The constituents isolated from the essential oil were included (%): n-nonane 1.6, benzaldehyde 0.9, α-pinene 4.6, 1-octen-3-ol 1.6, Sabinene 1.3, Myrcene 0.6, decane 0.2, α-tolualdehyde 2.4, limonene 2.5, acetophenone 14.6, m-tolualdehyde 19.1, o-tolualdehyde 3.5, nonanal 0.5, linalool 0.4, terpinen-4-ol 0.5, α-terpineol 0.3, α-cubebene 0.3, β-bourbonene 0.4, β-caryophyllene 7.4, β-farnesene 6.1, α-humulene 0.8, germacrene D 10.5, bicyclogermacrene 1.3, β-bisabolene 0.7, δ-cadinene 0.4, spathulenol 0.5, caryophyllene oxide 2.1, α-cadinol 0.5, α-bisabolol 1.7, hexahydrofarnesyl acetone 2.9, hexacosane 0.8, octacosane 1.1, nonacosane 2.3%[20].

While, the percent of essential oil obtained by hydrodistillation from aerial part of *Marrubium vulgare* collected from Zahedan and Kerman, south-eastern- Iran, was 0.34%. Thirty four compounds were identified in the oil, the main isolated compounds were γ-eudesmol (11%), germacrene (10%), D- citronelly formate (10%), β -citronellol (8%), geranyl tiglate (7.1%), geranyl formate (6.02%), lendene (5.15%), cyclononasiloxane- octadecamethyl (4.3), 1, 8-cineole (3.75%), geraniol (3.70%), neryl acetate (3.41), γ- cadinene (3.35) and β- cubebene (3.30)[21].

However, the components isolated from the essential oils of the leaves of *Marrubium vulgare* from Almus-Tokat-Turkey, were included (%): α-pinene (28.85%), β-pinene (18.31%), β-phellandrene (17.40%), 2- hexenal (14.80%), β- myrcene (5.07%), α-phellandrene (4.27%), caryophyllene (3.86%), α-limonen (3.88%), germacrene (1.05%), β-farnesene (0.88%) and sabinen (0.49%)[22].

Analysis of the essential oil from aerial parts of *Marrubium vulgare* from Algeria revealed that the total essential oils was 0.05 %, and the major constituents (%) included 4,8,12,16-Tetramethyl heptadecan-4-olid (16.97), germacrene D-4-ol (9.61), α- pinéne (9.37), phytol (4.87), dehydro-sabina ketone (4.12), piperitone (3.27), δ – cadinene (3.13), 1-octen-3-ol (2.35) and benzaldehyde (2.31)[23].

While, analysis of the essential oils of the dried aerial parts of Tunisian *Marrubium vulgare* showed that the oils contained thirty five components. The major isolated compounds were: sesquiterpenes (50.5 %), β-bisabolene (28.3 %), β- caryophyllene (7.8 %), (E)-β-farnesene (7.4 %) and 1,8-cineole (4.8 %)[24].

However, the amount of the essential oil obtained by hydrodistillation from aerial part of *Marrubium vulgare* from the village of Ouled Mnasser in Sidi Bouzid, Tunisia, was 0.34%. The oil composed of equal amounts of the oxygenated monoterpenes (40.02%) and sesquiterpenes hydrocarbons (42.7%). However, 34 compounds were isolated from the oil. ϒ-eudesmol (11.93%), ledene (5.35%), δ-cadinene (3.30%), transcaryophyllene (2.15%), β-bourbonene (1.96%), α-copaene (1.37%), 1,8 cineole (3.72%), geranial (2.74%) and α-thujone (2.29%) were the main isolated compounds[25].

Marruboside, 11-oxomarrubiin, marrubic acid, (+)(E)-caffeoyll-malic acid, acteoside, forsythoside B, arenarioside, ballotetroside, vulgarol, vulgarcoside A, β-sitosterol, lupeol, marrubiin, ladanein, vulgarin, apigenin -O-glucoside, luteolin, apigenin-7-lactates together with their 2''-O-glucuronides, 2''-O-O-glucosides, diosmetin, diosmetin-7-glucoside, vitexin, vicenin II, luteolin 7-glucoside, luteolin-7-rhamnoside, luteolin-7-O-β-glucopyranoside, acacetin, acacetin-7-rhamnoside, apigenin-7-O-β- glucopyranoside, apigenin-7-O-glucoside, apigenin-7-(6''-p-coumaroyl] glucoside, 3-hydroxyapigenin-4'-O-(6"-O-p-coumaroyl)-beta-D-glucopyranoside, chrysoeriol, quercetin 3-rhamnoglucoside and apigenin, stachydrine, ascorbic acid, caffeinic acid and tetrahydroisoquinoline alkaloids (emetine and cephaeline) were isolated from *Marrubium vulgare*[6,26-36].

The amount of marrubiin was calculated as 156 mg/g of the aerial parts of *Marrubium vulgare* extract [37]. The total phenolic contents of *Marrubium vulgare* leaves were 40.7-160 mg gallic acid equivalents/g and total flavonoids concentrations were 27.4- 66.3 mg catechin equivalents/g [38]. While, the total phenolic and flavonoids in the aerial parts of *Marrubium vulgare* were 625 mg gallic acid equivalent, and 1.62 g quercetin equivalent per 100 g of dried plant aerial parts respectively [39].

2. Pharmacological effects

2.1. Anti-inflammatory and analgesic effects

The anti-inflammatory effects of the methanolic extract of the aerial parts of *Marrubium vulgare* was evaluated in carrageenan- induced paw edema in rats. *Marrubium vulgare* methanolic extract (2.5, 5, 10 mg/kg) alleviated paw inflammation as determined by reduction of paw thickness ($P<0.001$) as well as, myeloperoxidase activity ($P<0.001$), which was associated with a marked decrease in tissue edema [39].

The anti-inflammatory effect of the methanolic extract (10, 20, and 40 mg/kg/12h) of *Marrubium vulgare*, was studied in isoproterenol-induced myocardial infarction (MI) in rat model. Isoproterenol injection increased inflammatory response, as shown by a significant increase in peripheral neutrophil count, myocardial myeloperoxidase (MPO) activity and serum levels of creatinine kinase-MB (CK-MB) and TNF-α ($p<0.001$). In the groups treated with 10, 20 and 40 mg/kg of *Marrubium vulgare* extract, serum CK-MB was subsided by 55.4%, 52.2% and 69%, respectively. 40 mg/kg of the extract was significantly reduced MPO activity, TNF-α level and peripheral neutrophil ($P<0.001$) in MI group. Interstitial fibrosis was significantly attenuated in extract treated groups compared with control MI group [40].

Comounds: 11-oxomarrubiin, vulgarcoside A and 3-hydroxyapigenin-4'-O-(6"-O-p-coumaroyl]-beta-D-glucopyranoside isolated from whole *Marrubium vulgare*, exhibited moderate to low level of inhibition on nitric oxide production. Vulgarcoside A also showed a moderate inhibition on pro-inflammatory cytokine TNF-α [30].

Marrubiin, exhibited significant analgesic effect against the writhing test in mice. Marrubiinic acid showed better activity in experimental models of pain in mice [41].

The antinociceptive profile of marrubiin, was studied in some models of nociception in mice. Marrubiin exhibited potent and dose-related antinociceptive effects. ID50 was: 2.2 micromol/kg, ip, in the writhing test, 6.6 micromol/kg, ip, (first phase) and 6.3 micromol/kg, ip, (second phase) in the formalin-induced pain test and 28.8 micromol/kg, ip, in the capsaicin test [42].

The analgesic effects of the hydroalcoholic extract of *Marrubium vulgare* were investigated in different models of pain in mice. The extract exhibited significant analgesic activity and antagonizing chemically- induced acute pain, the effects which could be attributed to the presence of steroids and terpenes [43].

2.2. Antioxidant effects

The Antioxidant activity of the methanolic extract of the aerial parts of *Marrubium vulgare* was evaluated using DPPH radical scavenging assay and nitric oxide radical inhibition assay. The extract RC50 values for DPPH and nitric oxide antioxidant activity of the methanolic extract were 177 µg/ml and 370.5 µg/ml respectively [39].

The alcoholic extract of *Marrubium vulgare* at concentrations of (8, 10 mg/ml), and some isolated flavonoids (acacetin, apigenin, and acacetin-7-rhamnoside) showed high antioxidant activity towards scavenging of DPPH radical. The activity was corresponding to 88.2 standard ascorbic acid at concentration of 100 µM [29].

The antioxidant potential of *Marrubium vulgare* essential oil was evaluated using scavenging of DPPH free radicals, β-carotene bleaching test and reducing power assay. The results showed that they possessed strong antioxidant activity [25].

The *in vitro* antioxidant assays was used to determine the ability of the compounds (luteolin-7-O-β-glucopyranoside, apigenin-7-O-β- glucopyranoside, oleanolic acid, β-sitosterol, luteolin-7-O-rutinoside, and rosmarinic acid) isolated from the dried leaves of *Marrubium vulgare*, using MTT and LPO assays. Luteolin-7-O-β-glucopyranoside, apigenin-7-O-β- glucopyranoside, and luteolin-7-O-rutinoside showed the highest antioxidant activity. Apigenin-7-O-β-glucopyranoside possessed antioxidant activity similar to that of vitamin C. Luteolin-7-O-rutinoside showed the highest LPO inhibition by 89% at 100 µg/ml concentration. Oleanolic acid and β-sitosterol inhibited LPO by 24% and 40%, respectively [28].

A strong radical scavenging activity was recorded for the methanol extract of *Marrubium vulgare* with RC_{50} value of 8.24µg/ml [37]. The methanolic extract of *Marrubium vulgare* (2.5–120 µg/ml) showed antioxidant activity (EC_{50} of 38.56 ± 0.10µg/ml) with the using DPPH assay [44].

2.3. Anticancer effects

The *in vitro* anticancer activity of *Marrubium vulgare* alcoholic extract and some isolated flavonoids (acacetin, apigenin, and acacetin-7-rhamnoside) were tested against Ehrlich tumor cell lines and human tumor cell lines U251 and MCF7 (brain tumor and breast carcinoma cell lines, respectively). The alcoholic extract, acacetin, apigenin and acacetin-7-rhamnoside possessed anticancer activity against against Ehrlich tumor cell lines and breast carcinom a MCF7 with ED_{50}< 20µg/ ml [29]. The cytotoxic activity of aerial parts of *Marrubium vulgare* essential oil was studied against HeLa cell lines. Essential oil inhibited the proliferation of HeLa cell lines with IC_{50} of 0.258 µg/ml[45].

Marrubium vulgare ethanolic extract dose-dependently reduced viability of melanoma (B16) and glioma (U251) cells, but not peripheral blood mononuclear cells. It arrested cell cycle in S + G2/M phase, associated with activation of MAP kinase p38 and up-regulation of antiproliferative genes p53, p21 and p27. *Marrubium vulgare* ethanolic extract also induced mitochondrial depolarization, activation of caspase-9 and -3, Parp cleavage, phosphatidylserine exposure and DNA fragmentation. The mitochondrial apoptotic pathway was associated with the up-regulation of proapoptotic genes Pten, Bak1, Apaf1, and Puma and down-regulation of antiapoptotic genes survivin and Xiap. It also stimulated the expression of autophagy-related genes Atg5, Atg7, Atg12, Beclin-1, Gabarab and Sqstm1, as well as LC3-I conversion to the autophagosome associated LC3-II. The most abundant phenolic components of *Marrubium vulgare* ethanolic extract (ferulic, p-hydroxybenzoic, caffeic and chlorogenic acids), did not exert a profound effect on viability of tumor cells, suggesting that other components were responsible for its cytotoxicity [46].

Ladanein, a methoxylated flavones isolated from *Marrubium vulgare*, displayed moderate (20-40 microM) activities against K562, K562R (imatinib-resistant), and 697 human leukemia cell lines [26].

2.4. Antimicrobial activities

The antimicrobial activity of *Marrubium vulgare* extracts was studied against *Pseudomonas aeruginosa*, Staphylococcus aureus, Escherichia coli and Proteus vulgaris. *Marrubium vulgare* showed activity against Escherichia coli on the ethyl acetate phase, and on the diethyl ether and butanol phases against *Pseudomonas aeruginosa*. However, the three phases of the extracts showed no activity against *Proteus vulgaris* [13].

The antibacterial activity of *Marrubium vulgare* leave extracts were investigated against *Enterobacter sp, S. aureus, E. coli, Acinetobacter baumanii, P. aeruginosa* and *S. epidermidi*. The results revealed that the antibacterial activity possessed by the extract was varied according to the extract concentration and the species of pathogen. The MICs were 125-250 mg/ml [47].

The antimicrobial activity of *Marrubium vulgare* flavonoids was evaluated against four bacterial strains pathogenic (*Klebsiella pneumonia, Pseudomonas aeruginosa* 7244, *Escherichia coli* 1429 and *Staphylococcus aureus*). The diameters of growth inhibition were 4 and 12 mm for *Pseudomonas aeruginosa* 7244, 0 to 14 mm for *Staphylococcus aureus*, on Mueller-Hinton medium, and 8-38 mm for *Pseudomonas aeruginosa* 7244 and between 0 to 6 mm for Staphylococcus aureus on Sabouraud medium[12].

Marrubium vulgare essential oil and ethanol extract were tested against 17 strains of *S. aureus* isolated from nose and throat sample from 160 healthy subjects. The ethanol extract and essential oil of *Marrubium vulgare* possessed growth inhibitory effect against most isolates. The least MIC value of ethanol extract of *Marrubium vulgare* was 2.5 mg/ml and the highest MBC values were 5 and 10 mg/ml [21].

The antimicrobial activity of aerial parts essential oil was studied against bacterial isolates (*Staphylococcus aureus* 1327, *Staphylococcus epidermidis*, *Micrococcus luteus*, *Enterococcus faecalis*, *Enterobacter cloacae*, *Staphylococcus aureus* 25923, *Bacillus subtilis*, *Bacillus cereus*, *Pseudomonas aeruginosa* 27853, *Klebsielle pneumoniae* WHO24 and *Escherchia coli* 25922), and fungal isolates (*Botrytis cinerea, Fusarium solan, Penicillium digitatum* and *Aspergillus niger*). *Marrubium vulgare* essential oil possessed significant activity against microorganisms especially Gram positive bacteria with inhibition zones and minimal inhibitory concentration values in the range of 6.6-25.2 mm and 1120-2600 µg/ml, respectively, whereas Gram negative bacteria exhibited high resistance. In antifungal study, *Botrytis cinerea* exhibited the highest sensitivity with inhibition zones of 12.6 mm, while, *Fusarium solani*, *Penicillium digitatum* and *Aspergillus niger* were less sensitive to *Marrubium vulgare* essential oil [45].

The antibacterial activity of the methanolic extract of *Marrubium vulgare* whole plant (50, 100, 200, 400 and 600 mg/ml) was tested by disc diffusion method against *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, S. epidermidis, Pseudomonos aeruginosa and Proteus vulgaris*. The result revealed that the extract was very much effective against *B. subtilis, S. epidermidis* and *S. aureus* (zones of inhibition of 24, 21 and 20mm for 600 mg/ml, respectively) and moderately effective against *P. vulgaris* and *E. coli* (zones of inhibition of 15 and 16mm for 600 mg/ml, respectively), while the extract was ineffective against *P. aeruginosa* [48].

Antimicrobial activity of methanol, acetone and ethyl acetate extracts from whole *Marrubium peregrinum* plant was investigated against 22 microorganisms including 15 bacterial strains (standard strains: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* ATCC 6633, *Bacillus pumilus* NCTC 8241 and clinical strains: *Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Pseudomonas aeruginosa, Proteus mirabilis, Sarcina lutea, Salmonella enterica, Bacillus subtilis* and *Bacillus cereus*) and 7 fungal species [*Aspergillus niger* ATCC 16404, *Penicillium italicum* PMFKG-F29, *Trichothecium roseum* PMFKG-F32, *Botrytis cinerea* PMFKG-F33; *Candida albicans* (clinical isolate); *Rhodotorula sp*. PMFKG-F27 and *Saccharomyces boulardii* PMFKG-P34]. The strongest antimicrobial activity was detected against Gram positive bacteria while the activities on other species were moderate. The activity of tested extracts varied depending on the species and type and concentration of the extract. The comparative analyses showed that the most active was methanol extract (MIC from 0.3125 mg/ml to 40 mg/ml) followed by ethyl acetate (MIC from 0.0781 mg/ml to 40 mg/ml) and acetone extract (MIC from 0.1563 mg/ml to 40 mg/ml). The most sensitive bacteria were *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853 and *Bacillus subtilis* (MIC 0.0781 mg/ml). The best antifungal extract was the methanol extract against *Aspergillus niger* ATCC 16404 (MIC: 0.625 mg/ml) [49].

2.5. Antiparasitic and molluscicidal effects

The anthelmintic activity of *Marrubium vulgare* aqueous and ethanolic leaves extracts (0.78, 1.55, 3.1, 6.2, 12.5, 25, and 50 mg/ml) was evaluated against digestive strongyles in naturally infected bovine using the egg hatch assay and larval mortality assay. The high effects were observed with 50 mg/ml, but the lowest reduction on parasite eggs hatchability was observed in cultures exposed to 0.78 mg/ml of both extracts. Both aqueous and ethanolic extracts of *Marrubium vulgare* (at 50 mg/ml) exhibited 45.8±1.99% and 51±2.53% larval mortality rate, respectively, at 24h [50].

Mortality of the 4th instar larvae of the mosquito *Culex pipiens* exposed to different doses of methanolic extract of *Marrubium vulgare* was varied with exposure time. The maximum mortalities (31, 40, 59%) were recorded for the concentration of 200, 500 and 900 mg/ml respectively, after 72 h of exposure [51].

The molluscicidal activity of *Marrubium vulgare* essential oils was investigated in adult and eggs *of Biomphalaria alexandrina*. The LC_{50} and LC_{90} of *Marrubium vulgare* essential oil against adult snails was 50 and 100 ppm/3hrs, respectively. Moreover, *Marrubium vulgare* showed LC_{100} ovicidal activity at 200 ppm/24 hrs [52].

2.6. Antidiabetic effects

The antidiabetic and antidyslipidemic effects of the methanolic extract of the aerial part of *Marrubium vulgare* were studied in streptozotocin- induced diabetic rats. *Marrubium vulgare* extract significantly reduced the blood glucose level from the second week. It also significantly increased plasma insulin and tissue glycogencontents. Furthermore, it also significant reduced plasma total cholesterol, triglycerides, and low density lipoprotein-cholesterol, and increased high density lipoprotein-cholesterol in diabetic rats [53].

A series *in vivo* experiments were carried out on albinos rats to determine the antidiabetic effects of the aqueous aerial part extract of *Marrubium vulgare* (100, 200 and 300 mg/kg bw, twice a day, orally). The aqueous extract of the *Marrubium vulgare* [200 and 300 mg/kg bw] induced significant antidiabetic effect (decreased blood glucose by 50% for the dose 100 mg/kg and more than 60% for 200 and 300 mg/kg) and antihyperlipidemic (dose-dependent effect). It significantly lowered the total lipids, triglycerides, and total cholesterol levels in treated animals, compared with diabetic controls group ($p<0.001$) [54].

The hypoglycemic effects of the acute administration of various ethanolic extracts (root, leaf and stem) of *Marrubium vulgare* were investigated in normoglycemic rats. Both extracts (root and stem) caused significant reductions of glycemia in healthy rat after intragastric administration of 100 mg/kg. Furthermore, the orally administered ethanolic root extract suppressed the elevation in the serum glucose in oral glucose tolerance test [55].

The effects of *Marrubium vulgare* infusion in renal and liver function were studied in alloxan diabetic rats. Diabetic rats showed elevation of plasma creatinine, oxidative stress markers, in addition to renal and hepatic histological alteration. The treatment with *Marrubium vulgare infusion* attenuated blood glucose and creatinine, reduced oxidative stress and improved histopathological alterations [56].

The antidiabetic effect of the aqueous extract of *Marrubium vulgare* was studied in type 2 non-controlled diabetic patients. Patients received a prepared infusion of the dry leaves of the plant treatment for 21 days. *Marrubium vulgare*treatment decreased the plasma glucose level by 0.64% and cholesterol and triglycerides by 4.16% and 5.78%, respectively [57].

A randomized, double-blind, and controlled clinical trial was conducted to evaluate the clinical effect of the aqueous extract on type 2 non-controlled diabetes mellitus. The product consisted of fresh *Marrubium vulgare* leaves that were dried under environmental temperatures and protected from direct light and then milled. Patients had to prepare the treatment immediately before administration. *Marrubium vulgare* extract was administered three times a day, before every meal. The study was carried out for 21 days. Prior to infusion administration, every seven days and after the clinical trial, the fasting glucose, cholesterol, triglycerides, urea, creatinine, and uric acid in blood were determined. The effectiveness was considered as a decrease in the basal concentration of glucose, cholesterol, or triglycerides by at least 25%. *Marrubium vulgare* caused that effect in only two of the 21 patients (9.52%). The mean of plasma glucose level was reduced by 0.64%, and that of cholesterol and triglycerides by 4.16% and 5.78%, respectively [58].

2.7. Hepatoprotective effect

The hepatoprotective effect of the methanol extract of whole *Marrubium vulgare* was evaluated on paracetamol induced hepatotoxicity in rats. The hepatotoxic effects of paracetamol were significantly inhibited by the extract manifested by the restoration of serum biochemical parameters to near normal levels [59].

The antihepatotoxic activities of the active compounds of *Marrubium vulgare* were studied using CCl_4- induced hepatic toxicity in rats. Vulgarin exhibited a significant antihepatotoxic activity by reducing the elevated levels of serum enzymes such as serum glutamate oxaloacetate transaminase serum glutamate pyruvate oxaloacetate transaminase and alkaline phosphatase, while the total protein levels were increased compared with standard drug silymarin. These biochemical observations were also confirmed by recovery of damaged liver cells in histopathological examinations of the liver sections [60].

The hepatoprotective activity of aqueous extract of *Marrubium vulgare* (500 mg of dry leaves/kg/day, for 30 days) was studied in cyclophosphamide toxicity in rats. Elevation of alanine amino transferase, aspartate amino transferase, lactate dehydrogenase, and alkaline phosphatase and increased lipid peroxidation confirmed cyclophosphamide-induced liver toxicity. Cyclophosphamide also decreased the enzymatic defense system against oxidative stress. *Marrubium vulgare* extract attenuated cyclophosphamide-induced enzymes alteration and the associated liver damage. The protective effect of the plant was mainly attributed to the existence of phenolic acids and flavonoids and their antioxidant properties [61].

Marrubic acid (p-menthane-5,6-dihydroxy-3-carboxylic acid) isolated from the *Marrubium vulgare*, showed protective effects against hepatotoxicity induced by CCl_4 in rats, by reducing the elevated levels of serum enzymes such as serum glutamate oxaloacetate transaminase by 40.16%, serum glutamate pyruvate oxaloacetate transaminase by 35.06%, and alkaline phosphatase by 30.51%[27].

The antihepatotoxic and antioxidant activities of *Marrubium vulgare* were studied against CCl_4- induced hepatic damage in rats. The extract was given orally in a dose of 500 mg/kg/day for 4 weeks along with CCl_4 started at the 7th week of induction of hepatotoxicity. The extract showed a significant antihepatotoxic effect by reducing the levels of AST, ALT and LDH significantly. However, ALP levels were decreased non-significantly. Furthermore, it exhibited significant antioxidant effects by increasing the GPx, GR and GST activities with increased GSH tissue contents and decreased production of MDA level. Furthermore, it alleviated histopathological changes in rats' liver treated with CCl_4[62].

2.8. Cardiovascular effects

The cardioprotective effects of aqueous fraction of hydroalcoholic extract were studied in ischaemic- reperfused isolated rat hearts. *Marrubium vulgare* aqueous fraction (40 µg/ml) significantly decreased infarction size compared with control. All doses reduced the total ventricular ectopic beats during 30 min of ischaemia. The extract (40 µg/ml) decreased the arrhythmias during the first 30 min of reperfusion. The aqueous fraction scavenged DPPH radical with RC_{50} value of 47 µg/ml. The total phenolic and flavonoids content of the fraction was 6.05 g gallic acid equivalent and 36.13 mg quercetin equivalent per 100 g of dry plant material [63].

Severe myocardial necrosis with a sharp decline in the arterial blood pressure, left ventricular contractility, with marked increase in the left ventricular end-diastolic pressure were seen in the isoproterenol group. All pathological changes induced by isoproterenol were significantly improved by the *Marrubium vulgare* extract treatment. The authors concluded that the therapeutic effects of *Marrubium vulgare* attributed to its antioxidant activities [64].

Subcutaneous injection of rats with isoproterenol (100 mg/kg/day,for 2 consecutive days) caused ST-segment elevation in ECG, left ventricular dysfunction, intensive myocardial fibrosis with a profound increase in myocardial myeloperoxidase (MPO) activity and serum levels of TNF- α. All doses of the *Marrubium vulgare* extract significantly amended the ECG pattern and improved the left ventricular systolic pressure, contractility and relaxation ($P<0.001$). Interstitial fibrosis was significantly attenuated in treated groups compared with control MI group. Treatment with the extract also reduced serum levels of TNF- α (at least 40.35%) and myocardial MPO activity (at least 30.47%) [65].

The crude extracts of the aerial parts of *Marrubium vulgare* were strongly inhibited the *in vitro* KCl-induced contraction of rat aorta. It appeared that furanic labdane diterpenes, marrubenol and marrubiin were the most active compounds [66].

The relaxant activity of marrubenol (a diterpenoid extracted from *Marrubium vulgare*), and the underling mechanism were studied in rat aorta. Marrubenol inhibited the contraction evoked by 100 mM KCl (IC_{50}: 11.8±0.3 microM, maximum relaxation: 93±0.6%) than of the contraction evoked by noradrenaline (maximum relaxation: 30±1.5%) in rat aorta. It also simultaneously inhibited the Ca2+ signal and the contraction evoked by 100 mM KCl, and decreased the quenching rate of fura-2 fluorescence by Mn^{2+}. Marrubenol inhibited Ba^{2+} inward current in a voltage-dependent manner (KD: 8±2 and 40±6 microM at holding potentials of -50 and -100 mV, respectively). The results revealed that Marrubenol inhibited smooth muscle contraction by blocking L-type calcium channels [67].

The hypotensive effect of the water extract of *Marrubium vulgare* was investigated in spontaneously hypertensive and in normotensive rats. Oral administration of *Marrubium vulgare* extract lowered the systolic blood pressure of spontaneously hypertensive rats but not in normotensive rats. *Marrubium vulgare* extract inhibited the contractile responses of rat aorta to noradrenaline and to KCl (100 mM). Inhibition was greater in aorta from spontaneously hypertensive rats compared to normotensive rats and was not affected by the NO synthase inhibitor, N-nitro-L-arginine [68].

The effects of 10 week- treatment with amlodipine or *Marrubium vulgare* water extract on systolic blood pressure, cardiovascular remodeling and vascular relaxation were studied in spontaneously hypertensive rats. Both treatments produced similar decrease in systolic blood pressure. Amlodipine treatment reduced left ventricle, aortic and mesenteric artery weight, while, marrubium treatment had a significant antihypertrophic effect in aorta only. Relaxation to acetylcholine (Ach) of mesenteric artery was improved by *Marrubium vulgare* but not by amlodipine treatment [69].

2.9. Hypolipidemic effects

The hypocholesterolemic and hypotriglyceridemic activities of four *Marrubium vulgare* herb extracts were evaluated using Triton WR-1339-induced hyperlipidemia in mice. After, 7 and 24 h intragastric administration of extracts, caused a significant decrease of plasma total cholesterol. Triglyceride levels were also significantly lowered by all extracts while petroleum ether extract produced the lowest decreasing level. Similar results were observed for LDL-cholesterol concentrations. Furthermore, the more polar extracts (methanol and ethyl acetate) showed a significant ameliorative action on elevated atherogenic index and LDL/HDL-C ratios, while these atherogenic markers were not statistically suppressed by the chloroform and petroleum ether extracts [70].

The aqueous extracts of *Marrubium vulgare* inhibited LDL oxidation and enhanced reverse cholesterol transport and can prevent cardiovascular diseases development. Incubation of LDL with the aqueous extracts of *Marrubium vulgare* significantly prolonged the lag phase (P=0.014), lowered the progression rate of lipid peroxidation (P=0.004), reduced the disappearance of electrophoretic mobility in a dose-dependent manner. Furthermore, incubation of HDL with the aqueous extracts significantly increased HDL-mediated cholesterol efflux from THP-1 macrophages implicating an independent ATP binding cassette A1 (ABCA1) pathways [71].

2.10. Antiedematogenic effects

The antiedematogenic profile of marrubiin, the main constituent of *Marrubium vulgare*, was studied in a model of microvascular leakage in mice ears. The results show that it exhibited significant and dose-related antiedematogenic effects. The ID_{50} values (mg/kg, ip) and maximal inhibition (%) for the different phlogistic agents were: histamine (13.84 mg/kg and 73.7%); bradykinin(18.82 mg/kg and 70.0%); carrageenan (13.61 mg/kg and 63.0%). In other phlogistic agonists, such as prostaglandin E2, it caused inhibition of less than 50%. Marrubiin (100 mg/kg) also significantly inhibited the Ovo-induced allergic edema in actively sensitized animals (maximal inhibition 67.6±4%)[72].

2.11. Gastroprotective effect

The gastroprotective effect of the methanol extract and marrubiin obtained from the leaves of *Marrubium vulgare* was studied using ethanol- and indomethacin- induced ulcers in mice. In ethanol-induced ulcers, the curative ratios were 49.31±0.57, 74.31±0.91 and 79.86±0.59% for the groups treated with 50 and 100mg/kg of the extract and omeprazole, respectively. In indomethacin-induced ulcers, the percentages of ulcer inhibition were 50.32±5.60, 66.24±4.30, 82.17±04.09 and 67.52±4.38, for the groups treated with 25, 50 and 100mg/kg *Marrubium vulgare* extract and cimetidine, respectively. In both models, the marrubiin (25mg/kg) significantly reduced all the parameters when compared with the control group (P<0.01). pH and mucus production were significantly increased in the groups treated with *Marrubium vulgare* extract and marubiin[73].

2.12. Antispasmodic effects

The antispasmodic effects of hydroalcoholic extract of the roots and aerial parts of *Marrubium vulgare* were evaluated in several smooth muscle preparations *in vitro*. The results showed that the extract possessed a significant antispasmodic activity, it inhibited the action of acetylcholine, bradykinin, prostaglandin E2, histamine and oxytocin, with putative selectivity for cholinergic contractions [74].

3. Toxicity and side effects

The rats given *Marrubium vulgare* at doses of 100, 250, 500, and 1000 mg/kg daily, for a period of 3 weeks and observed continuously for 3 weeks, showed no physical signs of toxicity such as writhing, gasping, palpitation and respiratory rate, or mortality[53].

The effects of ethanol-water (80:20) extract of *Marrubium vulgare* on the hematological parameters, macroscopic and histological aspects of the uterus and fetus in non-pregnant and pregnant rats were investigated. The results showed, a significant decrease on hematological parameters in normal rats and pregnant rats treated with the ethanol-water. Furthermore, the extract of *Marrubium vulgare* caused a significant decrease on the mean implantations of fetuses (82.5%, P< 0.001) and their size (47.2%, P< 0.01). However, the extract possessed no uterine histological change in non-pregnant treated rats, while, in pregnant rats, it induced severe histological change characterized by the existence of location of stopped gestation with lyses placental and embryo tissue. The results supported the abortifacient effect of *Marrubium vulgare* [75]. Extended use may lead to hypertension. While large doses can cause vomiting [76].

4. Conclusion

The current review discussed the chemical constituents, pharmacological effects and therapeutic importance of *Marrubium vulgare* as a promising medicinal plant with wide range of pharmacological activities which could be utilized in several medical applications because of its effectiveness and safety.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors confirm that this paper's content has no conflict of interests.

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