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Review Article

Marrubium vulgare L.: A review on phytochemical and pharmacological aspects

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ABSTRACT

Marrubium vulgare L. (family: Lamiaceae), also known as the white horehound, is widely used as an herbal remedy for chronic coughs and colds. It is used in various disorders related to skin, liver, gastric, heart, and immune system. This review abridges phytochemical, pharmacological studies, and medicinal uses of *M. vulgare* and provides scientific proof for various ethnobotanical claims to identify gaps, which will give impulsion for novel research on *M. vulgare* based herbal medicines. This review summarizes selected scientific evidence on phytochemistry and pharmacological properties of *M. vulgare* over the past 48 years (1968-2016). Works related to *M. vulgare* was reviewed from various sources such as books, internet source, i.e., Google Search engine, PubMed, and Science Direct, and chemical abstract. The exhaustive literature was studied, and critical analysis was performed according to their phytochemical and pharmacological properties. Phytochemical investigations on different parts of *M. vulgare* have been reported the presence of flavonoids, steroids, terpenoids, tannins, saponins, and volatile oils (0.05%). The aerial parts contain marrubiin, together with ursolic acid and choline. Pharmacological activities such as antinociceptive, antispasmodic, antihypertensive, antidiabetic, gastroprotective, anti-inflammatory, antimicrobial, anticancer, antioxidant, and antihepatotoxic activity have been reported. *M. vulgare* has therapeutic potential in the treatment of inflammatory conditions, liver disorders, pain, cardiovascular, gastric, and diabetic conditions. Aerial parts of *M. vulgare* is a good source of labdane type diterpene especially marrubiin which is present in high concentrations. However, further scientific studies are needed to explore clinical efficacy, toxicity and to explore the therapeutic effect of major secondary metabolites such as diterpenes, phenylpropanoid, and phenylethanoid glycosides of *M. vulgare*.

KEY WORDS: Diterpenoids, marrubiin, *Marrubium vulgare*, marrubenol, phenylpropanoid

INTRODUCTION

Natural products originated from plant, animal, and minerals have been the basis of treatment of human disease. Herbal medicines are currently in demand and their popularity is increasing day by day. According to the WHO, about 70-80% of world population uses herbal medicines for their therapeutic effects [1]. Traditional system of medicine is based largely on plants species and animals for primary health care. Herbal medicines have an important value in the developing countries for their medicinal value, sociocultural and spiritual use in rural and tribal [2]. About 50,000-80,000 of flowering plants are uses for medicinal purposes by the peoples worldwide. Different indigenous systems such as Ayurveda, Siddha, Unani, and Allopathy use a number of plant species to treat different ailments [3,4] and becoming more popular due to toxicity and side effects of allopathic medicines. The practices continue today because of its biomedical benefits as well as place in cultural beliefs in many parts of the world and have made a great contribution toward maintaining human health [5].

A clear understanding of the herb's benefits and possible risks, as well as, a clearly defined patient diagnosis are essential for the practitioner to safely and effectively counsel patients as to safe and effective choices in the herb use [6]. In addition, the objective is to separate active constituents of medicinal plants in pure form, that can be possible to clarify its mode of action, and this study is major in phytotherapy. Thus, the subject of phytochemistry demonstrated characterization of number of chemical constituents and establishes their exact chemical formulae [7].

The Lamiaceae is most diverse plant family in terms of ethnomedicine. Due to high volatile content, it has great

medicinal value [8]. It contains about 236 genera and 6900-7200 species. Many species of this family are highly aromatic and produces volatile oil, due to the presence of external glandular structures [9]. Lamiaceae is also taxonomically known as mint family of flowering plants.

The genus *Marrubium* L. (Lamiaceae) has nearly 30 species indigenous to Asia and Europe [10]. Among them, *Marrubium vulgare* L. is a perennial herb which is commonly known as "white horehound" in Europe, and "Marrubia" in Tunisia. It grows naturally in North and South America and is extensively distributed in areas raising sheep, especially around bedding and watering areas. It attains approximately one-foot height, branched below, densely covered in young stage, with a thick, white, and cottony felt [11]. The plant grows in waste ground throughout Europe and Western Asia as far as India, especially in the Kashmir region at 5000-8000 ft [12]. *M. vulgare* is a hardy plant cultivated in many parts of USA. It thrives in almost any soil but does best in light calcareous, rather dry, soil and sunny situations. Leaves and tops are harvested just before full green color. The *M. vulgare* has musky odor which diminishes on drying and a pungent bitter, yet pleasant and aromatic in taste [11].

White horehound was included in the Pharmacopoeias and Merck's Index of Phytotherapy (1910). In 1927, researchers described that white horehound has been used in pulmonary disorders [13]. In 1941, it has been described that white horehound is the most popular herbal pectoral remedies and used as bitter tonic, expectorant, and diuretic [14]. It produces potential effects in coughs, colds, and pulmonary affections. In 1954, Belgian literature, the "Materia Medica Vegetabilis," illustrated the use of white horehound as a decoction with honey syrup against bronchitis and coughs [15]. It is also used against jaundice, piles, and diarrhea.

Recent pharmacological investigations shows that *M. vulgare* offers various *in vivo* and *in vitro* pharmacological activities including antihypertensive, antioxidant, anti-inflammatory, antidiabetic, effect on respiratory system, digestive stimulant, antiasthmatic, hypolipidemic, antibacterial, and antifungal effects [16]. Extensive phytochemical studies on *M. vulgare* were results over 54 secondary metabolites. These metabolites include diterpenes, sesquiterpenes, flavonoids, and phenylpropanoids were identified from different parts of *M. vulgare* [16-19]. Marrubiin, marrubiinic acid, and marrubenol are major diterpenes which exhibits analgesic and antiedematogenic activities. Arenarioside, acteoside, forsythoside B, and ballotetroside are phenylpropanoids possessing potent anticancer and anti-inflammatory activities.

Although the article [20] summarizes the present medicinal status of *M. vulgare* including phytochemical composition and selected pharmacological activities in brief. However, to date, there is no comprehensive review highlighting the ethnomedicinal values phytochemistry and pharmacological profile of this species. The present review aimed to discuss about the traditional use, phytochemistry and pharmacological studies as well as clinical studies of *M. vulgare*. In addition, the aim of the present review is to establish a relationship between traditional uses and reported systematic studies. This review will also answer the gaps between them and significant for the development of new drug from this species. In addition, the future perspectives of *M. vulgare* are also discussed in this review.

METHODOLOGY

The synonyms of *M. vulgare* were confirmed through plant data available on site (www.theplantlist.org). The published articles on *M. vulgare* were collected using popular search engine such as Google Scholar, PubMed, web of knowledge, and science direct. Other literature source was also used including books and journals available in library. About 180 literature articles were studied, and only 126 literature references were included in this review. The literature and databases were selected on the basis of topic covered. We did not included articles or literature related to other species, cultivation, physiological, and anatomical aspects of *M. vulgare*. The literature related to species distribution, taxonomy, morphological characters, ethnobotany, phytochemistry, clinical study, pharmacology, and toxicity of *M. vulgare* was included. These articles reviewed comprehensively, and data were critically analyzed and organized with accurate information. The phytochemical data were arranged according to category of constituents. The pharmacological data table consists of a plant part, extract, type of model, dose studied, and results of each study [Table 1].

BOTANICAL DESCRIPTION AND MEDICINAL USES

White horehound grows throughout most of Europe, especially in dry waste places and by roadsides, chiefly where it is warm and sunny. It is a tall robust herbaceous perennial, found in Kashmir and extending westward, at an altitude of 1500-2400 m. *M. vulgare* has fibrous roots and numerous stems, which are quadrangular, erect, very downy and from 12 to 18 inches high. The leaves are roundish ovate, dentate or deeply serrate, wrinkled, veined hoary on surface and supported in pairs. The flowers are white and in crowded axillary whorls. The calyx is tubular and divided into 10 narrow segments at the margin, which are hooked at the end. The corolla is also tubular, with a labiates margin, of which the upper lip is bifid, the under reflected and three cleft, with the middle segment slightly scalloped. Seeds are lying in the bottom of calyx [21].

M. vulgare is traditionally used in various parts of Europe, France, Pakistan, Brazil, Tunisia, and Morocco. The Physician's Desk Reference for Herbal Medicines (1998), has recommended the folk uses of white horehound for acute and chronic bronchitis, pulmonary catarrh, respiratory infections, tuberculosis, asthma, and jaundice and externally, for skin damage and ulcers. *M. vulgare* juice and infusion used internally as a gastric secretion stimulant due to the presence of bitter ingredients particularly marrubinic acid as a choleretic agent. In Germany, *M. vulgare* is traditionally used as a bitter remedy and is also used for respiratory disorders in Anglo-American and Mediterranean [22]. Leaves paste is applied for boils and rheumatism. Dried herb's infusion is used for debility and in

Table 1: Pharmacological activities of *Marrubium vulgare* L.

Activity reported	Plant parts	Type of extracts/compounds	Experimental models (*in vitro*/*in vivo*)	Control (positive/negative)	Doses	Results	References
Analgesic activity	Leaves, stems and roots	Hydroalcoholic extract	*In vivo*: Acetic acid-induced, formalin test and hot-plate test	+ve: Morphine (5 mg/kg, s.c.) −ve: 0.9% NaCl (10 ml/kg, i.p.) solution	22.2 and 272.2 mg/kg, i.p. and p.o., respectively	Significant ($P<0.05$) effect was observed after i.p. 30 min or p.o. 60 min and 60 mg/kg, i.p. or 600 mg/kg, p.o.	[71]
	Leaves	Methanol extract, marrubiin, marrubiinic acid and marrubenol	*In vivo*: Acetic acid-induced writhing, formalin test, hot-plate test, capsaicin-induced and glutamate-induced nociception	+ve: Morphine (10 mg/kg, s.c.) and aspirin (10 mg/kg, s.c.) −ve: Saline solution (10 ml/kg, i.p.)	10 mg/kg i.p. and 50 mg/kg p.o.	Marrubiinic acid, exhibited 80% inhibition of the abdominal constrictions, ID_{50} value, 12 μmol/kg	[32]
	Whole plant	Methanol extract	*In vivo*: p-benzoquinone-induced abdominal constriction test	+ve: Acetylsalicylic acid (100 mg/kg) −ve: Distilled water	200 mg/kg	Extract (200 mg/kg) significantly inhibited (35.3%) abdominal constriction	[72]
Antinociceptive activity	Aerial parts	Hydroalcoholic extract and marrubiinic acid	*In vivo*: Acetic acid-induced writhing, formalin test, hot-plate test, capsaicin-induced nociception	+ve: Aspirin and diclofenac− ve: 0.9% NaCl solution (10 ml/kg, i.p.)	3-90 μmol/kg by i.p. or 90-900 μmol/kg by p.o.	Potent antinociceptive effects with ID_{50} values 2.2, 6.6, 6.3, and 28.8 μmol/kg, i.p. in the writhing test, formalin-induced pain test (first phase), (second phase) and capsaicin test, respectively	[73]
Anti-inflammatory activity	Flowered aerial parts	(+) (E)-caffeoyl-L-malic acid, acteoside, forsythoside B, arenarioside and ballotetroside	*In vitro*: Cyclooxygenase catalyzed prostaglandin biosynthesis	+ve: Nimesulide (10^{-4} M) and indomethacin (10^{-5} M) −ve: DMSO 10% in Tris buffer	10^{-3}, 10^{-4}, and 10^{-5} M	Acteoside, forsythoside B, and arenarioside showed the strongest Cox-2 inhibition from 23.1% to 32.8% at a concentration of 10^{-4} M and IC_{50} varying from 0.49 to 0.69 mM, while ballotetroside exhibits a weaker activity (IC_{50} on Cox-2>1)	[19]
	Whole plant	Methanol extract	*In vivo*: Carrageenen and PGE-2 induced inflammation	+ve: Indomethacin (10 mg/kg) and acetylsalicylic acid (100 mg/kg) −ve: 0.9% NaCl solution (25 μL)	100 and 200 mg/kg	Extract showed significant inhibition (34.0%) at a dose of 200 mg/kg in the carrageenan-induced hind paw edema test Maximum inhibition (27.2%) in PGE-2 induced inflammation was observed after 45 min	[72]
	Aerial parts	Hydromethanolic extract	*In vitro* and *in vivo*: Rats pleural polymorphonuclear leukocytes stimulated by opsonized zymosan	−ve: 0.9% NaCl solution	For the *in vitro* study, 10-100 mg/ml and for *in vivo,* 100, 200, 300 and 400 mg/kg/day	*In vitro,* the plant extract exerted a significant ($P<0.05$) inhibition at 80 mg/ml, was 54%. *In vivo*, only high doses (300 and 400 mg/kg/day) exerted the significant ($P<0.05$) inhibitory effect on PMNs oxidative metabolism	[76]

(*Contd...*)

Table 1: (*Continued*)

Activity reported	Plant parts	Type of extracts/compounds	Experimental models (*in vitro*/*in vivo*)	Control (positive/negative)	Doses	Results	References
	Aerial parts	Methanol extract	*In vivo*: Isoproterenol-induced myocardial infarction	−ve: Normal saline solution (0.5 ml)	10, 20, and 40 mg/kg/12 h	All doses (10, 20 and 40 mg/kg), significantly decreased the serum TNF-α and myocardial MPO levels. However, at the dose of 40 mg/kg, extract elicited the highest significant effect on serum CK-MB activity	[77]
Antiedematogenic activity	Aerial parts	Marrubiin	*In vivo*: Microvascular leakage mice model and anaphylic oedema in mice	+ve: Sodium diclofenac (100 mg/kg, i.p.) −ve: Saline solution 0.9% (0.1 mL/10 g, i.p.)	100 mg/kg	ID_{50} values (mg/kg, i.p.) and maximal inhibition were as follows: Histamine (13.84% and 73.7%); bradykinin (18.82% and 70.0%); carrageenan (13.61% and 63.0%). Marrubiin (100 mg/kg) showed significant maximal inhibition 67.6±4%	[78]
Antispasmodic activity	Roots and aerial parts	Hydroalcoholic extract	*In vivo*: Acetylcholine and oxytocin-induced contractions	−ve: 0.9% NaCl solution	0.01-1 mg/ml	1 mg/rnl of extract concentration showed maximum effect	[79]
Gastroprotective activity	Leaves	Methanol extract and marrubiin	*In vivo*: Ethanol-induced and indomethacin-induced ulcers in mice	+ve: Omeprazole (30 mg/kg) and cimetidine (100 mg/kg) −ve: 1% Tween-80 aqueous solution	25, 50 and 100 mg/kg	Methanol extract at doses of 50 and 100 mg/kg, marrubiin at 25 mg/kg caused increased gastric pH and significantly decreased the H^+ions concentration in comparison to the control	[80]
Antihypertensive activity	Aerial parts	Aqueous extract	*In vivo*: Normotensive Wistar-Kyoto rats	+ve: Verapamil	80 mg/kg/day	SBP remained significantly lower for 2 days and then progressively increased	[81]
	Aerial parts	Aqueous and cyclohexane extract	*In vivo*: KCl-induced contraction of rat aorta	+ve: Verapamil −ve: Ethanol	0.032, 0.064, 0.18, 0.36, and 0.72 mg/ml	Marrubenol was slightly more potent than marrubiin (IC_{50} value, 7.7±1.9 μM and 24±2.3 μM, respectively), these significant ($P<0.05$) effect due to blocking L-type calcium channels	[82,83]
	Aerial parts	Aqueous extract	*In vivo*: Spontaneously hypertensive rats	-	12 μM and 80 mg/kg/day	Extract has significant antihypertrophic effect, and it inhibited intestinal smooth muscle az contraction half-maximally at a concentration of 12 μM	[84,85]

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Table 1: (*Continued*)

Activity reported	Plant parts	Type of extracts/compounds	Experimental models (*in vitro*/*in vivo*)	Control (positive/negative)	Doses	Results	References
	Stem, flower and root	Ethanol extract	*In vivo*: Norepinephrine induced contraction	+ve: Carbachol −ve: Distilled water	40, 72 and 120 μM g/ml	Root extract significantly ($P<0.05$) inhibited contraction in dose-dependent manner	[86]
	Aerial parts	Methanol extract	*In vivo*: ECG changes induced by Isoproterenol	−ve: Normal saline solution (0.5 ml)	10, 20 and 40 mg/kg	Extract prevented myocardial injury in a dose-dependent manner and, it could be related to its antioxidant activity	[87]
Antidiabetic and antihyperlipidemic activity	Aerial parts	Ethanol extract	*In vivo*: Alloxan-induced diabetes	+ve: Chlorpropamide (5 mg/kg) −ve: 0.9% NaCl solution	300 mg/kg	Extract produced moderate effects with inhibition rates of 30.3%	[89]
	Aerial parts	Methanol extract	*In vivo*: Streptozotocin-induced diabetes	+ve: Glibenclamide (5 mg/kg) −ve: 0.1 M sodium citrate buffer and 1% CMC-Na	500 mg/kg/day	Significant ($P<0.05$) reduction in the plasma glucose level starting at 14-28th day as reaching 42% reduction compared to the diabetic control	[88]
	Aerial parts	Aqueous extract	*In vivo*: Alloxan-induced diabetes	+ve: Glibenclamide (5 mg/kg) −ve: Distilled water	100, 200 and 300 mg/kg	A significant ($P<0.001$), decrease in the blood glucose level by 50% for the dose 100 mg/kg and more than 60% for doses 200 and 300 mg/kg were found	[90]
	Whole plant	Methanol, water and butanol extract	*In vivo*: Cyclosporine A and streptozotocin-induced diabetes	−ve: Normal saline solution	2, 2, and 1 mg/ml	All extracts significantly decreases the blood glucose level and decrease in the elevated level of TNF-α, IFN-γ and NO	[92]
Anti-hepatotoxic activity	Aerial parts	Methanol extract	*In vivo*: CCl_4-induced toxicity	+ve: Silymarin (150 mg/kg/day) −ve: 1% CMC-Na solution	500 mg/kg	Extract significantly ($P<0.05$) reduced the blood levels of the AST, ALT, and LDH	[93]
	Aerial parts	Methanol extract and vulgarin	*In vivo*: CCl_4-induced toxicity	+ve: Silymarin (10 mg/kg p.o.) −ve: Normal saline solution	500 mg/kg (methanol extract) and 50 mg/kg (vulgarin)	Extract had significant decrease in SGOT (92.33), SGPT (72.92), ALP (36.58 units/ml) and Vulgarin SGOT (59.12), SGPT (45.24) and ALP (42.54) units/ml	[94]
	Whole plant	Methanol extract	*In vivo*: Paracetamol induced liver toxicity	+ve: Silymarin (200 mg/kg) −ve'' 0.5% CMC suspension (1 ml/kg)	100 and 200 mg/kg/day	The extract showed significant ($P<0.01$) protection by restoring the levels of ALT, AST, and ALP in dose-dependent manner	[95]

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Table 1: (*Continued*)

Activity reported	Plant parts	Type of extracts/compounds	Experimental models (*in vitro*/*in vivo*)	Control (positive/negative)	Doses	Results	References
	Aerial parts	Methanol extract and 6-ODA	*In vitro*: HSC-T6, luciferase reporter assay	+ve: Tetrandrine (2 g/ml) −ve: DMSO	15 and 30 μg/ml	6-ODA stimulated lipid accumulation in 3T3-L1 cells in a PPARc-dependent manner	[91]
	Aerial parts	Petroleum ether and 70% ethanol extracts	*In vivo*: CCl_4 induced liver toxicity	+ve: Silymarin (25 mg/kg) −ve: Normal saline solution (0.5 ml/day/5 days)	250 mg/kg/day/5 days	Petroleum ether extract showed most potent protective effect against CCl_4 induced damage, it reduced significantly GOT and GPT activities	[96]
Immunomodulatory activity	Flowers	Aqueous extract	*In vitro*: MTT assay	+ve: Con-A−ve, Phosphate-buffered saline	100 μg/ml	High mitogenic activity against splenocytes and increase in cell proliferation more than Con-A (162.2%)	[97,98]
Antioxidant activity	Leaves	Acetone and water extracts	*In vitro*: Peroxide assay	-	200 μg/ml	Acetone extract showed higher activity than water extract	[51]
	Leaves	Aqueous extract	*In vitro*: Two-stage Trolox based assay	+ve: Trolox	27-972 μmol/g	Antioxidant capacity was 560 μmol Trolox equivalent/g	[101]
	Aerial parts	Aqueous extract	In vitro: DPPH assay	−ve: Ethanol	25, 50, 75 and 100 μg/ml	100 μ g/ml extract shows significant ($P<0.01$) scavenging activity (70%) after 30 min	[99]
	Aerial parts	Essential oil	*In vitro*, DPPH assay, β-carotene bleaching test and reducing power assay	+ve: BHT −ve: Ethanol	500 μg/ml	IC_{50} value in DPPH assay was 79.00±3.00% at 300 μg/ml; In β-carotene bleaching test IC_{50} value was 36.30 μg/ml at 70 μg/ml; In reducing power assay IC_{50} value was 0.45±0.032 at 70 μg/ml	[102]
	Aerial parts	Methanol (80%) – water (19%) - acetic acid (1%)	*In vitro*: DPPH assay, β-carotene bleaching test, ABTS+assay and DPPH assay	+ve: BHT and rosmarinic acid	-	The activities of verbascoside and forsythoside B are similar to the rosmarinic acid in ABTS+assay. The butanol fraction of methanol (80%)–water (19%) - acetic acid (1%) extract showed highest antioxidant activity	[67]
	Aerial parts	Essential oil	*In vitro*: DPPH assay	+ve: BHT −ve: Ethanol	25-1000 μg/ml	IC_{50} value was 153.84 μg/ml	[103]
	Leaves	Methanol extract	*In vitro*: DPPH and ABTS assay	+ve: BHT −ve: Methanol	25-100 μg/ml	DPPH (IC_{50}=35±0.01) and ABTS (IC_{50}=25±0.2) for radicals scavenging.	[99]

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Table 1: (*Continued*)

Activity reported	Plant parts	Type of extracts/compounds	Experimental models (*in vitro*/*in vivo*)	Control (positive/negative)	Doses	Results	References
Antimicrobial activity	Leaves and flowers	Ethanol extract	*In vitro*: Rapid XTT colorimetry and bacterial enumeration methods	+ve: Amikacin, ampicillin, cefuroxime, chloramphenicol, clindamycin and streptomycin (170 μg/ml) −ve: DMSO (8.5% v/v)	340 μg/ml	Extract exhibited antimicrobial activity against two of the tested organisms, *Bacillus subtilis* and *Staphylococcus aureus* with reduction in viable count 99.99%	[105]
	Roots	Ethanol extract	*In vitro*: Micro titer dilution method	−ve: Tween 80	8 and 32 μg/ml	Ethanol extract was the most effective for inhibiting both biofilm formation (IC_{50}=32 μg/ml) and adherence (IC_{50}=8 μg/ml)	[107]
	Aerial parts	Methanol extract	*In vitro*: Anti-*Helicobacter pylori* on broth microdilution	-	10 mg/ml	MIC was found 800 μg/ml for 50% inhibition	[109]
	Aerial parts	Essential oil	*In vitro*: Agar disc diffusion method	+ve: Ampicillin (10 μg/disc) and cycloheximide (10 mg/ml) −ve: Ethanol	1120-2600 μg/ml	Significant activities against Gram-positive bacteria with inhibition zones and MIC were 6.6-25.2 mm and 1120-2600 μg/ml, respectively, whereas the strongest antifungal activity exhibited for *Botrytis cinerea* with inhibition zones of 12.6 mm	[110]
	Leaves	Ethanol extract	*In vitro*: Agar disc diffusion assay	+ve: Gentamicin (10 μg/disc) and nystatin (50 μg/disc) −ve: DMSO	1000 μg/disc	Antimicrobial effect (zone diameters, 8-12 mm) of ethanol, acetone and ether extracts, were detected 48.0%, 40.1%, and 35.7%, respectively	[108]
	Aerial parts	Aqueous extract	*In vitro*: BACTEC, MGIT960 susceptibility test	−ve: Saline solution	1600 and 3200 μL	Aqueous extract showed significant, antimycobacterial activity (75%) at 3200 μL	[111]
	Aerial parts	Essential oils	*In vitro*: MHB dilution method	−ve: Ethanol (5%)	0.1-15 μl/ml	Essential oil inhibited the bacterial growth for Gram-positive and Gram-negative bacterium, with MIC values ranging from 0.1 to 15 μl/ml	[103]
	Leaves	Ethanol extract and essential oil	*In vitro*: Agar disc diffusion assay	+ve: Tetracycline, ampicillin, trimethoprim-sulfamethoxazol, erythromycin, ceftazidime, penicillin, amikacin and ceftriaxone −ve: Saline solution	2.5 mg/ml	Least MIC value of alcohol extract was 2.5 mg/ml, and highest value was 5 mg/ml, for essential oil least MIC value was 0.3 mg/ml, and the highest value was 2.5 mg/ml	[99]

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Table 1: (*Continued*)

Activity reported	Plant parts	Type of extracts/compounds	Experimental models (*in vitro*/ *in vivo*)	Control (positive/negative)	Doses	Results	References
	Leaves	Methanol extract	*In vitro*: Disc-diffusion and broth dilution method	+ve, Gentamicin (10 μg/disc) −ve: Sterile water	12.5-25 mg/ml	MIC and MBC values for tested microorganisms were found in the range of 12.5-25 mg/ml and 25-50 mg/ml, respectively	[100]
Anticancer activity	Aerial parts	Methanol extract and phenylpropa-noids	*In vitro*: LDL induced cytotoxicity in bovine aortic endothelial cells	+ve: BHT−ve: Normal LDL, phosphate-buffered saline	10 μM	Phenylpropanoids inhibit minimally oxidized-LDL induced cytotoxicity by reducing lipoprotein oxidation through endothelial cell mediation and by reducing hydroperoxide formation. Oxidized LDLs also increase ET-1 secretion in endothelial cells.	[113,114]
	Leaves	Methanol extract	*In vitro*: Human colorectal cancer cells (HCT-116 cell line)	−ve: Phosphate-buffered saline	100 and 250 μg/ml	Extract significantly ($P<0.05$) suppressed cell growth of HCT-116 at concentration of 250 μg/ml	[115]
	Aerial parts	Ladanein	*In vitro*: K562, K562R, and 697 human leukemia cell lines	+ve: Daunorubicin hydrochloride and camptothecin −ve: DMSO	20-40 μM	Ladanein showed moderate activities against K562, K562R, and 697 human leukemia cell lines with IC_{50} value of 25.1±1.0, 38.0±1.8 and 38.0±0.7 μM, respectively.	[66]
	Aerial parts	Essential oil	*In vitro*: MTT assay, HeLa cell lines	−ve: Phosphate-buffered saline	3.91-3000 μg/ml	Essential oil inhibited the proliferation of HeLa cell lines with IC_{50} value of 0.258 μg/ml, and at concentration higher than 500 μg/ml, all HeLa cells were destructed	[110]
	Aerial parts	Alcohol extract and flavonoids (acacetin, apigenin, and acacetin-7-rhamnoside)	*In vitro*: U251 (brain tumor cell line) and MCF7 cell lines (breast carcinoma)	-	25-100 μg/ml	Total alcoholic extract, acacetine, apigenin and acacetine-7-rhamnoside show high anticancer activity against brain carcinoma U251 with $ED_{50}<20$ μg/ml	[116]
	Aerial parts	Methanol, dichloromethane and n-hexane fraction	*In vitro*: LN229, SW620 and PC-3 cell lines	−ve: DMSO	50 μg/ml	Dichloromethane fraction showed a significant reduction in cell viability of LN229 cells with IC_{50} values, 30 μg/ml	[117]

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Table 1: (*Continued*)

Activity reported	Plant parts	Type of extracts/compounds	Experimental models (*in vitro*/ *in vivo*)	Control (positive/negative)	Doses	Results	References
Molluscicidal and mosquitocidal activity	Aerial parts	Essential oil	*In vitro*: Eggs of *Biomphalaria alexandrina* and *Culex pipiens*	−ve: Dechlorinated water	75-1000 ppm	LC_{50} and LC_{90} of essential oil were found 50 and 100 ppm/3 h, respectively. It shows LC_{100} ovicidal activity at 200 ppm/24 h	[52]
	Leaves	Methanol extract	*In vitro*: Larvae of the mosquito *Culex pipiens*	-	900 mg/L	High mortality rate (59%) after 72 h of exposure with the dose of 900 mg/L	[118]
Antiprotozoal activity	Leaves and stems	Acetone and methanol extracts	*In vitro*: Against *Entamoeba histolytica* and *Giardia lamblia*	+ve: Metronidazole	-	Acetone and methanol extracts were active against *E. histolytica* with IC_{50} =7 and 12 μg/mL, respectively	[119]
	Aerial parts	Methanol extract	*In vitro*: Epimastigote form of *Trypanosoma cruzi*	+ve: Nifurtimox (10 μg/ml) −ve: DMSO (1%)	4.68-150 μg/ml	Growth inhibition between 88 and 100% at 150 μg/ml (IC_{50} =22.66 μg/ml)	[120]

+ve: Positive control, −ve: Negative control, i.p.: Intraperitoneal, s.c.: Subcutaneous, p.o.: Per os, ED_{50}: Effective dose 50 (the concentration of test extracts which exhibits 50% of the maximum activity), IC_{50}: Inhibitory concentration 50 (the concentration at which cell proliferation is inhibited by 50% compared to that of untreated control), ID_{50}: Infectious dose 50, LC_{100}: Lethal concentration 100, LC_{50}: Lethal concentration 50, LC_{90}: Lethal concentration 90, MBC: Minimum bactericidal concentration, MFC: Minimum fungicidal concentration, MIC: Minimum inhibitory concentration, TNF-α: Tumor necrosis factor-α, COX-2: Cyclooxygenase-2, PPM: Parts per million, IFN-γ: Interferon gamma, NO: Nitric oxide, LDL: Low-density lipoprotein, VLDL: Very low-density lipoprotein, HDL: High-density lipoprotein, XTT: 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide, DMSO: Dimethyl sulfoxide, MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, $CuSO_4$: Copper sulfate, CCl_4: Carbon tetrachloride, DPPH: 2,2-diphenyl-1-picrylhydrazyl, ABTS: 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid), SGOT: Serum glutamic oxaloacetic transaminase, SGPT: Serum glutamic pyruvate transaminase, ALP: Alkaline phosphatase, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, GOT: Glutamic oxaloacetic transaminase, GPT: Glutamic pyruvic transaminase, 6-ODA: 6-octadecynoic acid, BHT: Butylated hydroxyanisole, ECG: Electrocardiogram, PGE-2: Prostaglandin E-2, PMNs: Polymorphonuclear

high blood pressure. Leaves, flowers, and stem infusion are used as a stomachic for diabetes and in cardiac problems [23].

White horehound has diuretic property and is used in respiratory disorders. It produces laxative effects in large doses and employed as a domestic remedy for colds, coughs and pulmonary affections in the form of infusion, syrup, or candy. Preparations and extracts of white horehound find their way into proprietary cough mixtures and lozenges, often in association with demulcent drugs, such as coltsfoot and licorice. An extract of fresh white horehound is useful in the treatment of cardiac extrasystoles. The herb has been considered useful in the treatment of cholera and prolonged fevers [11]. It is also used as carminatives, detergent, antipyretic, useful in joint pain, and liver disorders. Leaves have been used in inflammation, sore eyes, night blindness, strengthen the teeth, and facilitate the expulsion of fetus [24]. *M. vulgare* is also used for flavoring beverages and candies in USA [25,26]. The volatile oil present in *M. vulgare* has a folk reputation for claiming a nervous heart. The small amount of marrubiin has normalizing effect on irregular heartbeats. The hot infusion of white horehound produces sweat-inducing effect, and cold infusion is used as a bitter tonic for digestive system. *M. vulgare* has also been used to reduce fevers and used to treat malaria [27].

The dry flowering stem has irritant effects on mucosa. In herbalism, it is used for menstrual pain and menstrual irregularities. It is also used externally to treat painful and inflamed wounds. Due to the presence of volatile constituents, it is used as an infusion in one to two-ounce doses, as a stimulant, resolvent, anthelmintic, dyspepsia, amenorrhea, chronic rheumatism, and in hepatitis [28,29]. The appetite stimulant effect of *M. vulgare* is through activation of bitter receptors [30]. White horehound is traditionally used to prepare tea, candy, and ale in Norfolk and some other parts of England. Romans and Egyptians used it as an antidote for poison. An infusion of white horehound sprayed on fruiting trees that help to kill cankerworms. It was claimed to ease digestion, destroy intestinal worms, and heartburn.

At the first sign of a cold, some people chop nine small leaves and mix them with a tablespoon honey and then eat slowly to ease a sore throat. A candy that contains four ounces of fresh leaves of white horehound, three crushed cardamom seeds, one teaspoon crushed anise seed and 2.5 cups water, is used to treat cough in children [31]. In Brazil, white horehound has been used traditionally to treat inflammation, respiratory diseases, and gastrointestinal disorders [32]. A decoction of dried herb and seeds or the green herb juice of *M. vulgare* is taken with honey, which is a remedy for short winded cough. It is administered to expel out those who have ingested poison or bitten by venomous serpents. The leaves and its juice when mixed with honey purge foul ulcers, clean eyesight and with rose oil it also relieves ear pain. An ointment prepared from boiled green leaves has been used to heal dog bites [33]. Syrup containing leaves and stems has been used to cure chronic coughs in asthmatic or short winded patients. An infusion of leaves is given as an insecticide and against caterpillars [34].

PHYTOCHEMISTRY

More than 54 secondary metabolites have been isolated and identified from different parts of *M. vulgare*. Among them, diterpenes, sesquiterpenes, and flavonoids are considered to be major categories of compounds, some of which exhibit potential biological activities *in vitro* and *in vivo*.

Diterpenoids

Diterpenoids represent the major class of compounds presents in aerial parts of *M. vulgare*. Till date, nine different types of diterpenes including their alcohol derivatives have been isolated and identified from *M. vulgare* [Figure 1]. In 1998, a rapid, inexpensive, and efficient method was developed for the isolation of marrubiin (a major diterpenoid) using chitin, a natural biopolymer for chromatographic support [35]. Marrubiin is a diterpenoid unsaturated γ-lactone, related to podocarpic acid and isolated from aerial parts of *M. vulgare*. In another study, a low pressure based liquid chromatographic method for isolation of marrubiin as well as high-performance liquid chromatography method was developed for quantitative determination in crude drug [36]. A study is consisting X-ray crystallography for structural characterization and to confirm stereochemistry of marrubiin was reported [37]. It was observed that molecule is highly strained with Ring A (a distorted boat) and Ring B (a flattened chair). The dehydration of marrubiin in exocyclic alkene is also discussed.

Some diterpene alcohol, such as marrubenol, marrubiol, sclareol, peregrinin, and dihydroperegrinin has been isolated from leaves and flower tops [38-40]. Effect of light and irrigation with constant fertilization can be used to optimize productivity of chemical constituents in *M. vulgare*. The irrigation regime modified both phosphate and chemical constituent in tissue. Irregular nutrient supply could decrease premarrubin concentration under the high natural light levels received by this species [41].

Premarrubiin, premarrubenol, and vulgarol have been also reported in *M. vulgare* shoots [17,42,43]. In a study, labdane skeleton was served as a precursor for many diterpenes and in the biosynthesis of marrubiin which follows a non-mevalonate pathway in plantlets and shoot culture of *M. vulgare* [44]. Accumulation of furanic labdane diterpenes has been investigated in different parts of *M. vulgare* [45]. It has been proven that furanic labdane diterpenes were produced and accumulated only in the aerial parts mostly in leaves and flowers. The maximum accumulation occurs just before flowering and in matured leaves. By tracer techniques using radiolabeled [3H]-geranylgeranyl diphosphate, confirm that the marrubiin biosynthesis proceeds through the 1-deoxy-D-xylulose-5-phosphate pathway, which was also characterized by gas chromatography with electron impact mass spectrometry technique [46]. Other metabolites and transcription process in *M. vulgare* for different diterpene synthases (diTPSs), i.e., ent-kaurene synthase, (+)-copalyl diphosphate synthase, and functional diTPS were also identified in leaves [47]. A new secondary metabolites, 11-oxomarrubiin was reported from methanolic extract of whole parts of *M. vulgare* [48]. Two new labdane diterpenoids, 12(S)-hydroxymarrubiin, and 3-deoxo-15-methoxyvelutine C were isolated from methanol extract of whole plant of *M. vulgare* collected from Srinagar, Kashmir, and India [49].

Essential Oil Including Monoterpenes and Sesquiterpenes

From different areas of the world, the chemical compositions of essential oil obtained from *M. vulgare* were reported by different researchers. Saleh and Glombitza [50] reported essential oil components such as tricyclene, bisabolol, β-pinene, β-elemone, and isomenthon-8-thiol as the major compounds of *M. vulgare*. In Lithuania, the volatile components of *M. vulgare* were hydrodistilled and analyzed by gas chromatography (GC) and GC mass spectrometry (MS). The major constituents of the essential oil were reported as β-bisabolene, δ-cadinene, and isocaryophyllene [51]. In Egypt, Salama *et al*. [52] reported that thymol and γ-cadinene as were the major constituents of *M. vulgare* oil. From Libya, EL-Hawary *et al*. [53] investigated the main components of volatile oil of *M. vulgare* were carvacrol, E-β-farnesene, and thymol. In Tunisian, Hamdaoui *et al*. [54] reported the major components such as β-bisabolene (28.3%), (E)-β-farnesene (7.4%), and β-caryophyllene (7.8%) containing essential oil of *M. vulgare*. In Algeria, Abadi and Hassani [55] reported the main components of the oil of *M. vulgare* were 4,8,12,16-tetramethyl heptadecan-4-olid (16.97%), germacrene D-4-ol (9.61%), α-pinene (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%). In Iran, Golparvar *et al*. [56] were identified about 44 compounds in the essential oil from aerial parts of *M. vulgare* by GC/MS. The major components were as β-caryophyllene (32.19%), (E)-β-farnesene (11.39%), 1,8-cineole (8.17%), and α-pinene (6.64%). Study on chemical composition of essential oil from different area can be concluded that variation in chemical composition may be due to extraction methods as well as a change in environmental and climatic conditions.

The content of sesquiterpenoids up to 20% of total content was observed in the flowering aerial parts of *M. vulgare* [57]. In Iran, the aerial parts of *M. vulgare* containing essential oil were analyzed by GC-MS, and about 47 different constituents were identified. The major constituents were β-caryophyllene, (Z)-β-farnesene, germacrene D, and α-humulene [58,59]. A new monoterpene, from whole plant of *M. vulgare* L., has been characterized as p-menthane-5,6-dihydroxy-3-carboxylic acid also known as marrubic acid [60]. 34 components were identified in oil, representing 95.1% of the total oil. Essential oil was characterized by high amount of sesquiterpenes (82.5%) with β-bisabolene (25.4%), β-caryophyllene (11.6%), and E-β-farnesene (8.3%) as the major components. Vulgarin, a sesquiterpene lactone has been isolated from aerial parts of *M. vulgare*. Some other terpenes studied in essential oil as α-pinene, sabinene, limonene, camphene, p-cymol, α-terpinolene, camphene, and *p*-fenchene [61,62] from leaves and flower tops of *M. vulgare* [Figure 2]. The study of essential

oil composition in the seeds of *M. vulgare* showed the presence of 34 compounds. The total essential oil content found as 0.05%. Major components were found as E-caryophyllene (25.91-32.06%), germacrene D (20.23-31.14%), and δ-amorphene (8.38-10.22%) [63].

Flavonoids and their Glycosides

Flavonoids are an important class of compounds and widely distributed in a variety of plants. More than 10 flavonoid constituents as both aglycone and glycoside are reported from different parts of *M. vulgare* [Figure 3]. Leaves of *M. vulgare* contains maximum total phenolics amount (293.34 mg gallic acid equivalent/g dry weight) were obtained with 60% aqueous methanol at 25°C; total flavonoids (79.52 mg catechin equivalent/g dry weight) with 80% aqueous methanol at 20°C, and condensed tannins (28.15 mg catechin equivalent/g dry weight) with 60% aqueous acetone at 50°C [64]. A total of 11 flavonoids, including some glycosides, have been isolated from leaves of *M. vulgare* as vitexin, chrysoeriol, quercetin 3-*O*-α-l-rhamnosyl-glucoside, isoquercitrin, luteolin, apigenin, apigenin 7-*O*-glucoside, apigenin 7-lactate, apigenin 7-(6"-p-coumaroyl)-glucoside, luteolin 7-*O*-β-D-glucoside, and luteolin 7-lactate [18,39,65]. A flavone derivative 3-hydroxyapigenin-4'-O-(6"-*O*-p-coumaroyl)-beta-D-glucopyranoside isolated from methanolic extract of whole plant of *M. vulgare* [48]. Ladanein was isolated at the first time from dichloromethane extract of aerial parts of *M. vulgare* [66]. In another study, aerial parts of *M. vulgare* extracted with a solvent mixture of methanol–water–acetic acid (79:20:1). 7-O-β-glucuronyl luteolin was reported first time from *M. vulgare* along with other known compounds, i.e., 5,6-dihydroxy-7,40-dimethoxyflavone (ladanein) and 7-O-β-glucopyranosyl luteolin [67].

Phenylpropanoid and Phenylethanoid Glycosides

In 2002, some phenylpropanoid, e.g., (+) (E)-caffeoyl-L-malic acid, forsythoside B, acteoside, ballotetroside, and arenarioside isolated from flowering aerial parts of *M. vulgare* [19,43]. Verbascoside and forsythoside B were isolated from aerial parts of *M. vulgare* [67] with a solvent mixture of methanol–water–acetic acid (79:20:1). Vulgarcoside A [Figure 4] is diglycosides of diterpene peregrinol isolated from methanol extract of whole plant of *M. vulgare* [48]. Some new phenylethanoid glycosides as marruboside and acethyl marruboside isolated from aerial parts of *M. vulgare* [68].

Miscellaneous Compounds

In addition to above-mentioned compounds, two phenolic *acids*, *two* phytosterols, and traces of alkaloids have been studied from *M. vulgare*. A pentacyclic triterpene named ursolic acid and steroids including β-sitosterol and stigmasterol, additionally two phenolic acids, gallic acid, and caffeic acid have been reported from aerial part of *M. vulgare* [18,69]. Trace amount of alkaloid named pyrrolidine betonicine and its isomer turicine have been isolated from leaves and flower tops [40,62,70]. In 2010, few normal alkanes and four types of branched alkanes, i.e., 2-(omega-1)-dimethylalkanes, 2-methylalkanes, 3-methylalkanes, and 3-(omega-9)-dimethylalkanes were isolated from aerial parts of *M. vulgare* [16].

PHARMACOLOGICAL ACTIVITIES

Various pharmacological activities of *M. vulgare* are attributed to the presence of diterpenoids, flavonoids, phenylpropanoids, and other phenolic compounds. Table 1 summarizes the pharmacological activity of different extracts, and isolated compounds reported.

Analgesic and Antinociceptive Activity

M. vulgare is used in folk medicine against various ailments such as intestinal infections, gastrointestinal disorders, and inflammatory processes. The hydroalcoholic extract of *M. vulgare* (aerial parts) showed significant analgesic activity in chemically-induced acute pain animal model in a dose-dependent manner when given at 60 mg/kg, i.p. or 600 mg/kg, p.o. [71]. The inhibitory effect of hydroalcoholic extract was found in acetic acid-induced writhing responses in mice. The results showed its analgesic potency with inhibitory dose 50% (ID_{50}) values at 22.2 and 272.2 mg/kg for the i.p. and p.o., respectively. These effects may be due to the occurrence of steroids and terpenes components. In the formalin test, edema was inhibited only by i.p. dose (maximal inhibition 62.9%). In another study, aerial parts of *M. vulgare* have been recorded analgesic activity due to the presence of marrubiin, a furane labdane type diterpene. Meyre-Silva *et al*. [32] observed its potent biological activity and high yield of marrubiin. He studied on structural modifications to obtain appropriate biological active compounds. Marrubiinic acid and its two ester derivatives exhibited significant antinociceptive effect against acetic acid-induced abdominal writhing model in mice (10 mg/kg i.p. and 50 mg/kg orally). Recently, methanolic extract (200 mg/kg) of *M. vulgare* has been reported as an analgesic similar to acetylsalicylic acid, in p-benzoquinone-induced abdominal constriction test [72]. De Jesus *et al*. reported antinociceptive effect in nociception mice models was attributed due to the presence of marrubiin [73]. Marrubiinic acid exhibited dose-dependent antinociceptive effects with 3-90 μmol/kg by i.p. route or 90-900 μmol/kg by p.o. against the writhing test. Marrubiinic acid has ID_{50} value of 12 μmol/kg and is about 11-fold more active than the standard drugs and less active than marrubiin.

A study comprising hot-plate induced pain model showed that marrubiin did not increase the latency period. The exact mechanism of action of marrubiin is unknown but it does not interact with the opioid receptor. The results showed that marrubiin is highly effective in inhibiting acetic acid-induced writhing responses in mice with an ID_{50} value of 2.2-90 μmol/kg, i.p. Marrubiin practically abolished the non-specific pain of the writhing test but the mechanism of antinociceptive activity remains unclear. These results suggest that, like the hydroalcoholic extract of *M. vulgare*, it does not involve the inhibition of cyclooxygenase products derived from

the arachidonic acid pathway or the participation of the opioid system. It is well known that the opening of ring structure in any compound also affects the biological activity of that particular compound [74]. In conclusion, the opening of the chain in marrubiinic acid led to a decrease of activity and ring structure is essential for greater biological activity. The presence of carboxylic group in marrubiinic acid is responsible for acidic character and could have a major role in its antinociceptive activity.

Anti-inflammatory Activity

M. vulgare is commonly distributed in Europe. Aqueous and hydroalcoholic extracts from flowering aerial parts are widely used for the treatment of cough and biliary complaints [75]. It is used in traditional medicine for the treatment of neurosedative and inflammatory disorders [19]. The five major phenylpropanoid esters (+) (E)-caffeoyl-

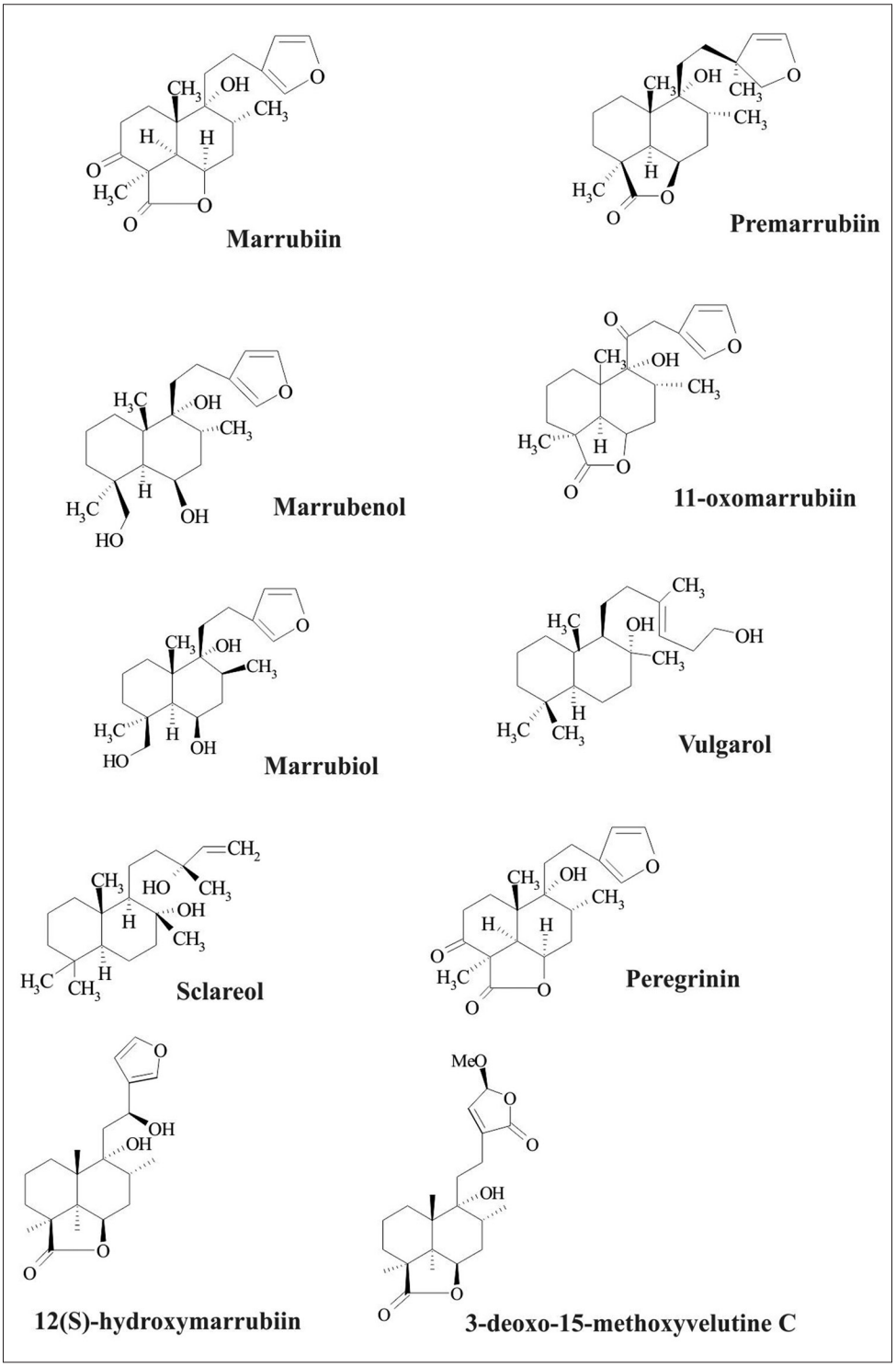

Figure 1: Chemical structure of diterpenoids from *M. vulgare*

L-malic acid, arenarioside, acteoside, forsythoside B, and ballotetroside were identified from the aerial parts of *M. vulgare* and their anti-inflammatory activity was reported on cyclooxygenase catalyzed prostaglandin biosynthesis. Results showed that the glycosidic phenylpropanoid esters showed cyclo-oxygenase-2 (Cox-2) inhibitory activity. Remaining acteoside, forsythoside B, and arenarioside are also produces some inhibitory effect on Cox-1.

Anti-inflammatory activity of *M. vulgare* against carrageenan and prostaglandin E2 induced inflammation in Swiss mice was reported [72]. Methanolic extract reduced inflammation significantly at a dose of 200 mg/kg. In another study, hydromethanolic extract of aerial part of *M. vulgare* has been selected for anti-inflammatory activity [76]. The oxygen consumption, production of superoxide anions and rat pleural polymorphonuclear (PMNs) leukocytes stimulation was

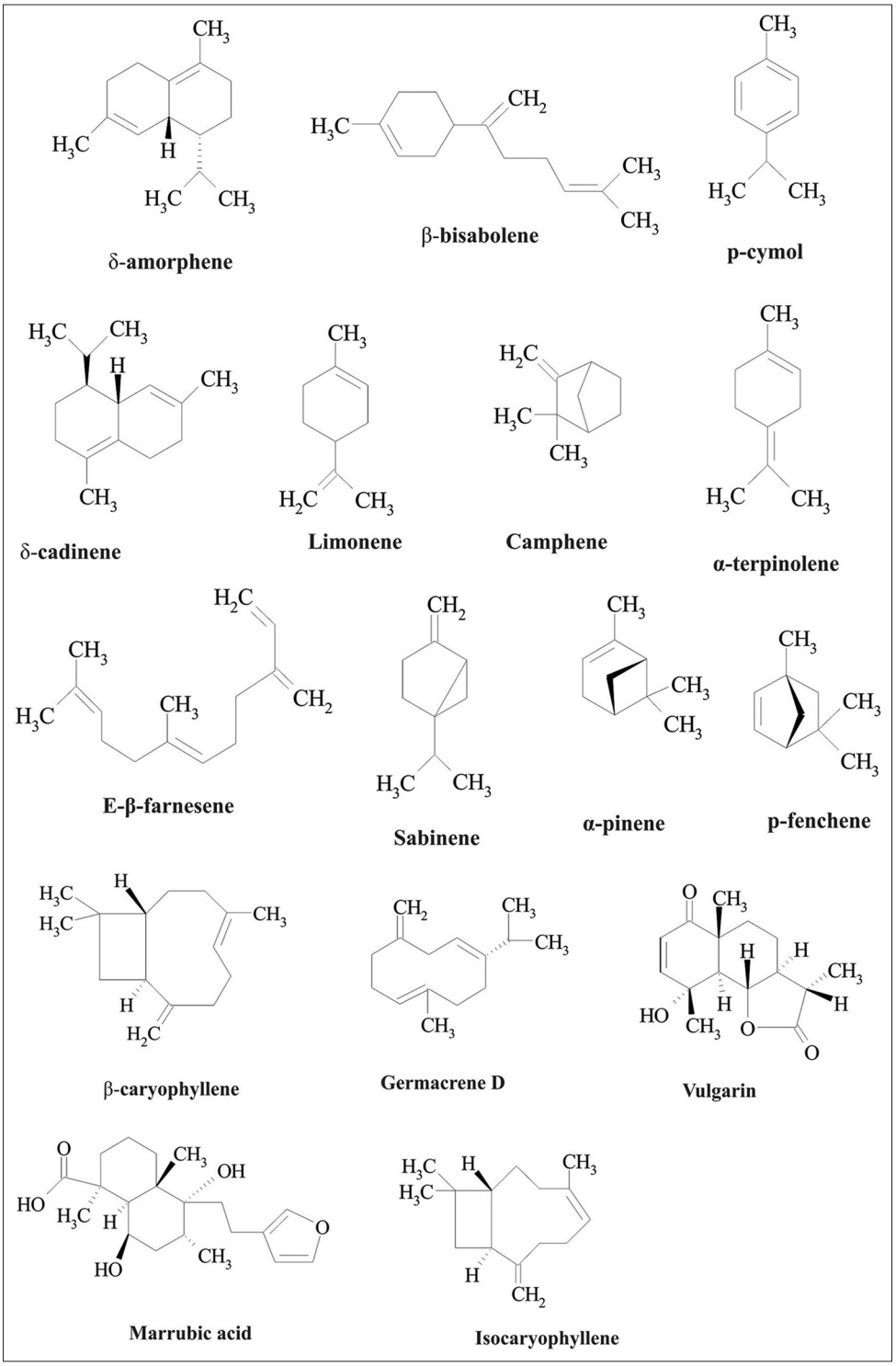

Figure 2: Chemical structure of monoterpenes and sesquiterpenes in *M. vulgare*

observed by opsonized zymosan. PMNs were collected after induction of an acute inflammatory reaction by injection of calcium pyrophosphate crystals suspension in rat pleural cavity. Hydromethanolic extract of *M. vulgare* exhibited a significant anti-inflammatory effect at 300 and 400 mg/kg/day doses. Methanolic extract of *M. vulgare* (aerial parts) was screened for anti-inflammatory effect by isoproterenol (100 mg/kg/day) induced myocardial infarction (MI) in rat model [77]. Methanol extract was administered orally with different doses of 10, 20, and 40 mg/kg/12 h concurrent with MI induction to the rats. Isoproterenol increases inflammatory response by a significant increase in myocardial myeloperoxidase activity, peripheral neutrophil count, serum levels of creatinine kinase-MB, and tumor necrosis factor-α (TNF-α). Methanol extract significantly lowered the levels of TNF-α and peripheral neutrophil count. The protective effect of *M. vulgare* extract concluded due to its anti-inflammatory property. Due to the higher content of diterpenes and polyphenols, it can use as potent antioxidant and anti-inflammatory agent for stress-induced disorders.

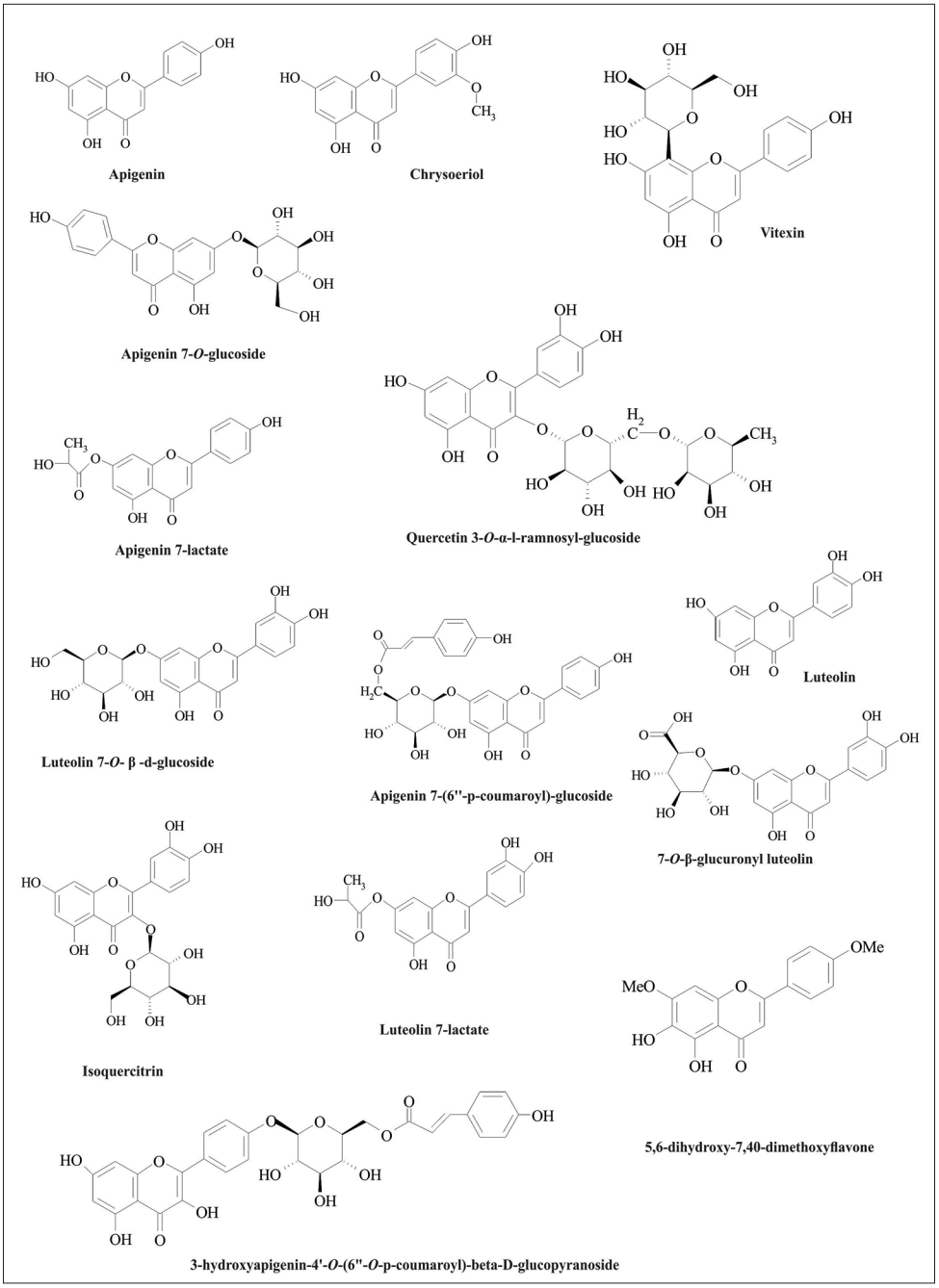

Figure 3: Chemical structure of flavonoids in *M. vulgare*

Antiedematogenic Activity

In several countries, *M. vulgare* is used for the treatment of various diseases, including gastroenterical, inflammatory, and respiratory disorders. Marrubiin showed significant and dose-related antiedematogenic effects in microvascular leakage mice model [78]. The percent inhibition for different phlogistic agents such as histamine at 13.84 mg/kg was 73.7% and carrageenan at 13.61 mg/kg was 63.0%. Marrubiin produces dose-dependent inhibition effect on bradykinin-induced microvascular extravasations of Evans blue in mouse ears, with ID_{50} value of 15.8 mg/kg and MI of about 70%. In the case of using dextran as a phlogistic agent, it produces slight inhibition of 32%. To determine the neurogenic inflammation, marrubiin was administered against the capsaicin and substance P-induced microvascular extravasation of Evans blue, which showed ID_{30} (mg/kg) of 61.95 and 38.8 and maximal inhibitions of 28.0% and 27.6%, respectively. In conclusion, the systemic administration

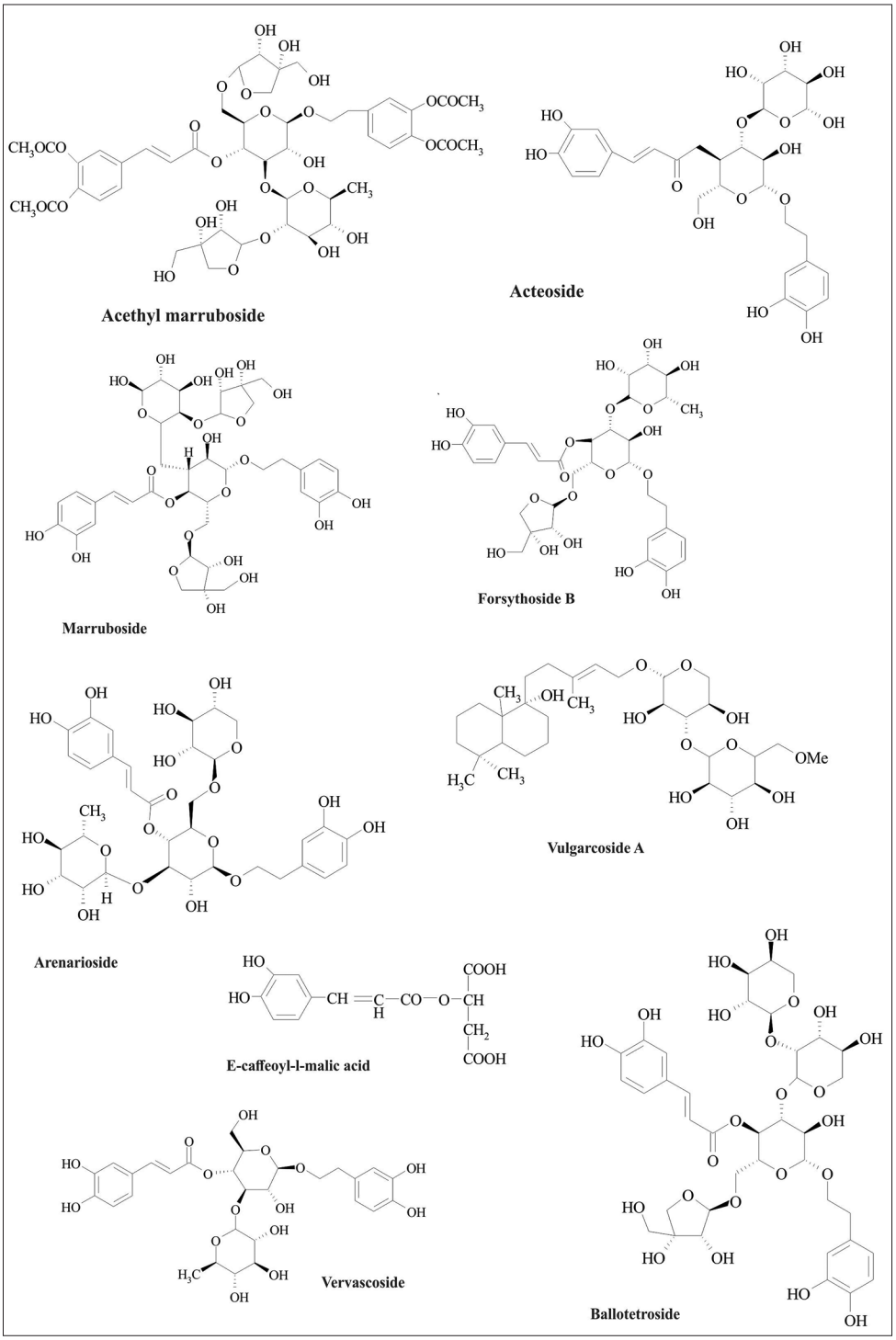

Figure 4: Chemical structure of phenylpropanoids and phenylethanoids in *M. vulgare*

of marrubiin exerts a non-specific inhibitory effect on proinflammatory agent-induced microvascular extravasation of Evans blue in mouse ear. Marrubiin at 100 mg/kg produced a substantial inhibition in ovalbumin-induced allergic edema in mice, which was comparable to the same potency as dexamethasone.

Antispasmodic Activity

M. vulgare is a small bush and native of European east Brazil. Whole parts of *M. vulgare* are used in folk medicine for the treatment of skin injury, gastroenteric disorders, kidney, and respiratory diseases. The roots and aerial parts of *M. vulgare* were evaluated for antispasmodic effects on several smooth muscles *in vitro* and found that hydroalcoholic (50% ethanol) extract exerts significant antispasmodic activity [79]. Antispasmodic effect was produced by inhibiting the action of neurotransmitters such as acetylcholine, prostaglandin E, histamine, bradykinin, and oxytocin with putative selectivity for cholinergic contractions. Effect may be attributed to the presence of steroids and terpenes. Increasing the concentration of hydroalcoholic extract (0.01-1 mg/ml) inhibit cholinergic contraction in a noncompetitive and concentration-dependent manner. A significant change found for bradykinin on guinea-pig ileum only in the presence of 1 mg/ml of extract. The hydroalcoholic extract produces inhibitory responses against acetylcholine and oxytocin-induced contractions which were characterized by parallel displacement rightward besides the reduction of maximal response.

Gastroprotective Activity

M. vulgare is used in traditional medicine for the treatment of gastrointestinal and respiratory disorders in Brazil. A diterpene and marrubiin have been isolated from methanol extract of *M. vulgare* leaves and evaluated for gastroprotective properties [80]. *M. vulgare* extract (50 and 100 mg/kg) produces a significant protective effect in ethanol-induced ulcers model in mice and effect was comparable with omeprazole (30 mg/kg). In case of indomethacin-induced ulcers, the percentage inhibition of ulcers was found significant, for the *M. vulgare* (50 mg/kg) and cimetidine (100 mg/kg) treated groups of animals. In both animal models, marrubiin (25 mg/kg) produced a significant reduction in gastric parameters when compared to control group. A significant increase was results in pH and mucus production in *M. vulgare* extract, and marrubiin treated groups. Conclusive of this study is the gastroprotection effect of *M. vulgare* extract, and marrubiin was thought to be induced by endogenous sulfhydryls and nitric oxide synthase that have vasodilator effects thereby produced gastroprotection by inhibiting gastric secretion.

Antihypertensive Activity

The aqueous extract of *M. vulgare* is widely used in traditional Moroccan medicine to lower the blood pressure. In 2001, for the first time, the antihypertensive effect of aqueous extract of *M. vulgare* was observed in spontaneously hypertensive rats (SHRs) and normotensive Wistar-Kyoto rats [81]. Oral administration of aqueous extract lowered the systolic blood pressure (SBP) of SHRs and inhibited the contractile responses of rat aorta to noradrenaline and to KCl (100 mM), in an *in vitro* study. The inhibition effect observed greater in aorta from SHR compared to Wistar-Kyoto rats. These finding indicates hypotensive effect of *M. vulgare* extracts through displayed vascular relaxant activity. Further in 2003, by bioassay-guided fractionation the furanic labdane diterpenes, marrubenol and marrubiin were the most active compounds isolated from aqueous and cyclohexane fraction from aerial parts of *M. vulgare* [82]. These compounds have been reported to elicit vasorelaxant activity. Pre-incubation of rat aorta with the cyclohexane fraction (0.064 mg/ml) evoked a dose-dependent inhibition of KCl-induced contraction while no effect showed by aqueous fraction. Marrubenol and marrubiin inhibited the contraction in concentration-dependent manner in rat aorta. Marrubenol (inhibitory concentration 50% [IC_{50}] values 7.7 ± 1.9 μM) found slightly more potent than marrubiin (IC_{50} values 24 ± 2.3 μM). In another study, the mechanism of the relaxant activity of marrubenol is related to inhibit contraction by blocking L-type voltage-dependent calcium channels in rat smooth muscle cells [83]. The relaxant property of marrubenol on rat aorta was unaffected by removal of the endothelium (IC_{50} values was 11.8 ± 0.3 μM and maximum relaxation 93.4 ± 0.6 μM). Study including the interactions between marrubenol and calcium antagonists, i.e., phenylalkylamines and 1, 4-dihydropyridines on binding sites in rat intestinal muscle cell membranes was also reported [84]. Competition binding studies indicate that marrubenol is a weak inhibitor of 1,4-dihydropyridine in membranes from intestinal smooth muscle. As marrubenol inhibited the concentration evoked by KCl depolarization of intestinal smooth muscle half-maximally at 12 μM. The interaction with the phenylalkylamine binding site seems to account for the inhibition of L-type Ca^{2+} channels by marrubenol. In addition to this, the antihypertensive effect which is comparable with amlodipine on SBP, cardiovascular remodeling, and vascular relaxation in SHR was also reported [85]. Amlodipine treatment reduced left ventricle, aortic and mesenteric artery weight.

M. vulgare improved the relaxation to acetylcholine of mesenteric artery but not by amlodipine treatment. Results showed that aqueous extract of *M. vulgare* has antihypertensive effect, as well as it also improves the impaired endothelial function in SHR. Marrubiin and marrubinol both have vasorelaxant property but less than the verapamil potency. By investigating the drug interaction effect, these two compounds can be used in combination with available vasorelaxant agents to promote the desirable effect.

In Mexico, *M. vulgare* is used to treat stomach pain, diarrhea, hypertension, and diabetes. Ethanol extract from stems, flowers, and roots of *M. vulgare* has been tested on the cumulative contraction induced by norepinephrine and extracellular Ca^{2+} at the doses of 40, 72, and 120 μg/ml extract in rats [86]. Root extract (72 and 120 μg/mL) produces dose-dependent relaxation in precontracted aortic rings with and without endothelium. This study supports the ethnobotanical use of *M. vulgare* as an antihypertensive drug for ethnomedical practices in Mexico.

In another study, methanol extract of *M. vulgare* (10, 20, and 40 mg/kg) significantly amended the electrocardiography changes induced by isoproterenol injection (100 mg/kg; sc). The extract (10 mg/kg) strongly increased left ventricular developed pressure/dt (max), and the treatment with *M. vulgare* extract 40 mg/kg, lowered the elevated left ventricular end-diastolic pressure and heart to body weight ratio [87]. Profound suppression on elevated malondialdehyde levels both in serum and in myocardium was inferred after the extract treatment.

Antidiabetic and Antihyperlipidemic Activity

M. vulgare is used for the treatment of diabetes in traditional medicine of Mexico. Antidiabetic and antidyslipidemic effects of *M. vulgare* were investigated in streptozotocin-induced diabetic rats [88]. Methanolic extract (500 mg/kg/day) of *M. vulgare* significantly lowered the blood glucose level after the treatment of 2nd week. Furthermore, *M. vulgare* extract also showed a significant increase in plasma insulin and tissue glycogen. The antidiabetic effect may possible through stimulation of insulin release from the remnant pancreatic beta cells. In conclusion, antidiabetic and antidyslipidemic effects of the *M. vulgare* may be due to its antioxidant activity. In a previous study, ethanolic extract of aerial parts of *M. vulgare* showed hypoglycemic effect on alloxan-induced diabetic rats. The results showed that ethanolic extract (300 mg/kg) of *M. vulgare* has moderate effects with percentage inhibition of 30.3 [89]. In Algeria, *M. vulgare* has been used traditionally to cure diabetes. Antidiabetic effect of aqueous extract of *M. vulgare* was evaluated on alloxan-induced diabetes on albino Wistar rats [90]. It produced antidiabetic and antihyperlipidemic effect in a dose-dependent manner. A decrease in blood glucose level has been observed by 50% at 100 mg/kg dosage and more than 60% for doses of 200 and 300 mg/kg, in addition to a significant lowering of total cholesterol, triglyceride, and lipid levels in the same extract treated animals. Results were found comparable to glibenclamide.

In Mexico, *M. vulgare* leaves have been used in a clinical trial for Type 2 diabetes. A fatty acid, 6-octadecynoic acid, is an agonist of peroxisome proliferator-activated receptor γ (PPARγ) which was identified in methanol extract of *M. vulgare*. Fibrogenesis is inhibited by PPARγ caused by hepatic stellate cells (HSCs), and ligands of these receptors were clinically used for the treatment of Type 2 diabetes. Methanol extract of *M. vulgare* was screened for activity to inhibit fibrosis in the HSC-T6 using oil red-O staining, which detects lipids accumulated in the cells [91]. Methanol extract found to have PPARγ agonist activity in a luciferase reporter assay. PPARγ regulates glucose and lipid metabolism and its synthetic agonists, such as pioglitazone, improve insulin resistance which is clinically utilized for diabetes therapy. This study concluded that methanol extracts of *M. vulgare* grown in Japan showed PPARc agonist activity comparable to the plants grown in Tunisia. Another study included the effect of whole plant of *M. vulgare* in an autoimmune diabetes mellitus-type1 induced by cyclosporine A and streptozotocin in mice [92]. Different test groups were treated daily with methanol extract (2 mg/ml), water extract (2 mg/ml), and butanol extract (1 mg/ml) of *M. vulgare*. Results showed that *M. vulgare* significantly decreases the blood glucose level, pancreatic levels of interferon gamma and nitric oxide, total cholesterol, low-density lipoprotein (LDL) and very LDL cholesterol and triglycerides and compared with diabetic mice. There are some *in vitro* or molecular studies are required to understand the mechanism involve in hypolipidemic effect of extracts. In addition, some studies are required to investigate adverse effect of crude extracts as well as individual compound when used as antidiabetic agent, especially when administered to the obese patients. The animal studies other than rats also needed before clinical trials, because the pathophysiology of disease may differ from human.

Antihepatotoxic Activity

M. vulgare is widely distributed in Saudi Arabia and has been used in folk medicine of several countries for the treatment of variety of diseases including hepatic, gastroenterical, respiratory, and inflammatory disorders. Whole plant extract of *M. vulgare* and marrubic acid exhibited a significant hepatoprotective activity [93]. The effects were observed by reducing the elevated levels of serum enzymes such as serum glutamate oxaloacetate transaminase (SGOT) by 40.16%, serum glutamate pyruvate oxaloacetate transaminase (SGPT) by 35.06%, and alkaline phosphatase (ALP) by 30.51%. The total protein (TP) levels were also increased and found comparable to the silymarin, which decreased the level of SGOT, SGPT, ALP, and increases TO levels. The results also supported by histopathological examinations of liver sections. In another study, 12 compounds of *M. vulgare* for their drug-likeness and biological activity *in silico* manner were tested for hepatoprotective activity [94]. On the basis of Lipinski's rule of five and comparison with standard drug silibinin, results showed that vulgarin showed significant antihepatotoxic activity against carbon tetrachloride (CCl_4) induced toxicity in Wistar rats. In conclusion, vulgarin is a potent hepatoprotective compound similar to the silibinin. It would be better to test on different models for hepatoprotective potential of vulgarin to proceed for clinical studies.

In vivo hepatoprotective activity of methanolic extract from *M. vulgare* (whole plant) was studied against paracetamol-induced liver toxicity in Wistar rats [95]. Methanol extract was administered at the doses 100 and 200 mg/kg/day. The toxic effects of paracetamol were significantly controlled in the extract treated groups which were manifested by the restoration of serum biochemical parameters (alanine aminotransferase, aspartate aminotransferase, ALP, albumin, total bilirubin, and triglycerides) to near normal levels. Hepatoprotective effect of petroleum ether and 70% ethanolic extracts from aerial parts of *M. vulgare* was studied on CCl_4 induced liver cells toxicity in mice [96]. Liver and kidney function parameters remained in the normal levels in ethanol extracts (0.5 ml of 250 mg/kg/day/5 days) treated groups. Administration of *M. vulgare* ethanolic extract significantly enhanced catalase and superoxide dismutase level, total antioxidant capacity with a significant reduction in lipid peroxidation.

Ethanolic extract showed potent protective effect against the damage caused by CCl_4 administration, it reduced glutamic oxaloacetic transaminase and glutamic pyruvic transaminase activities, but effect remained lower than the silymarin. The observed hepatoprotective activity may be due to the presence of flavonoids by an antioxidant mechanism. This study also supports folk use of *M. vulgare* for the treatment of hepatic affections.

Immunomodulatory Activity

Varieties of plants were used in folk medicine to treat immune-related disorders based on ethnobotanical information from Morocco. Daoudi *et al.* [97] studied the immunomodulatory activity of aqueous extract of 14 Moroccan medicinal plants including *M. vulgare*. Flower extract samples were tested for proliferation of immune cells using 3-(4, 5-dimethylthiazol-2yl)-2, 5-diphenyltetrazolium bromide (MTT) assay on the splenocytes with or without stimulation by concanavalin-A, which is a mitogenic agent used as positive control. Results observed that aqueous extract (100 μg/ml) stimulates splenocyte proliferation and bind with blood cells inducing hemagglutination [98]. *M. vulgare* showed a significant immunostimulatory activity, providing scientific explanation backing its traditional use.

Antioxidant Activity

M. vulgare is a rich source of phenolic secondary metabolites. Protective effect of aqueous extract from aerial part of *M. vulgare* was evaluated toward cardiovascular disease by protecting human-LDL against lipid peroxidation and promoting high-density lipoprotein-mediated reverse cholesterol transport in human THP-1 macrophages (Tamm-Horsfall Protein 1; a human monocytic cell line derived from an acute monocytic leukemia patient). Human-LDL was oxidized in the presence of increased concentrations of extract by incubation with $CuSO_4$ [99]. The lipid peroxidation was evaluated by conjugated diene formation and Vitamin E disappearance as well as, LDL-electrophoretic mobility. Incubation of LDL with extracts significantly prolonged the lag phase, lowered the progression rate of lipid peroxidation and reduced the disappearance of Vitamin E and electrophoretic mobility in a dose-dependent manner. Aqueous extract at concentrations ranging from 25 to 100 μg/ml induces an increase in the lag phase before the conjugated diene formation and decreased maximal rate of oxidation in a dose-dependent manner, whereas no effect has been observed on the maximal accumulation of oxidation products. These findings suggested that *M. vulgare* is a natural source of antioxidants, which reduce LDL oxidation and increases reverse cholesterol transport which can prevent cardiovascular diseases growth. Total flavonoids (0.61 mg catechin equivalents/ml), antioxidant activity, and total phenolic content (26.8 mg gallic acid/g) of *M. vulgare* leaves extract were determined using spectrophotometric methods [100]. The methanolic extract of *M. vulgare* leaves showed potent antioxidant power against 2,2-diphenyl-1-picrylhydrazyl (DPPH) (IC_{50} = 35 μg/ml) and 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) (IC_{50} = 25 μg/ml) radicals scavenging. The antioxidant activity of acetone and water extracts obtained from leaves of *M. vulgare* was tested in rapeseed (*Brassica napus* L.) oil at 80°C [51]. The effect of the extracts was assessed by measuring peroxide value, weight gain, and ultraviolet absorption. The antioxidant activity of acetone extract found better than water extract [Table 1]. In another study, total antioxidant activity of aqueous extracts of *M. vulgare* determined using a two-stage Trolox based assay [101]. Results indicated that the antioxidant potential of the aqueous extracts was 560 μmol/g Trolox equivalent/g dry weights.

In vitro, antioxidant potential of essential oil from the aerial parts of *M. vulgare* was studied in Tunisia using DPPH assay, β-carotene bleaching test and reducing power assay [102]. This investigation also directed a significant difference in the composition of essential oil of *M. vulgare* obtained from Tunisia and other countries. Authors of this study concluded that the antioxidant effect was produced through essential oil of *M. vulgare* probably due to the hydroxylated compounds and possible synergistic effect of oxygen-containing compounds. Based on these studies, essential oil from *M. vulgare* can be used as a natural food preservative and enhance the human health as a natural antioxidant. DPPH assay exhibited that, *M. vulgare* essential oil exhibited an IC_{50} value of 74 μg/ml, which is about 2 times higher than the synthetic antioxidant, i.e., butylated hydroxytoluene (BHT). In the β-carotene bleaching test, the IC_{50} of *M. vulgare* essential oil was estimated at 36.30 μg/ml compared to BHT (20.30 μg/ml). These values revealed that the antioxidant activity of *M. vulgare* oil was still less active than BHT. The reducing power of *M. vulgare* essential oil at 70 μg/ml was 0.45, which remained significantly lower than that of BHT at the same concentration.

Essential oils (0.04%), obtained from aerial parts of *M. vulgare*, were assessed for antioxidant assay by measurement of metal chelating activity, the reductive potential and free radical scavenging DPPH assay [103]. The antioxidant activity was compared with butylated hydroxyanisole (BHA) and exhibited an IC_{50} value of 153.84 μg/ml, which was higher than the BHA.

Pukalskas *et al.* [67] examined the antioxidant activity of isolated compounds, i.e., 5,6-dihydroxy-7,40-dimethoxyflavone, 7-O-b-glucopyranosyl luteolin, 7-O-b-glucuronyl luteolin, verbascoside, and forsythoside B using DPPH and ABTS+ free radical scavenging assays, and compared with rosmarinic acid. The effect of verbascoside and forsythoside B found similar to the rosmarinic acid in ABTS+ assay, which may be due to the presence of similar aglycone part. The activities of luteolin glycosides in the ABTS+ assay were observed similar. But in the DPPH assay, the antioxidant activity of 7-O-b-glucuronyl luteolin was found higher than that of its glucopyranosyl analog. This means sugar moiety can also affect biological property of compound. The butanol fraction of methanol (80%)–water (19%)- acetic acid (1%) extract showed highest antioxidant activity in the β-carotene bleaching assay and linolenic acid model. The leaves of *M. vulgare* were screened for determination of antioxidant capacity of the methanol and

acetone extracts [104]. Activity was assessed by DPPH radical scavenging assay, the scavenging activity against H_2O_2, total antioxidant capacity, and iron reducing power. Methanol extract showed 51.9- 97.15% of DPPH radical scavenging activity that indicating its use as a source of natural preservative and used for prevention of oxidative stress-related diseases.

Antimicrobial Activity

Numerous natural plant products offer antifungal, antibacterial, and antiprotozoal activities that could be used either systemically or locally in Jordanian traditional medication. Antimicrobial activity of ethanolic extract from leaves and flowers of *M. vulgare* were evaluated by rapid 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) colorimetry method and potential ethanol extract was further tested by bacterial enumeration methods [105]. *M. vulgare* showed promising antimicrobial activity against *Bacillus subtilis* and *Staphylococcus aureus*. This study also revealed a comparison between XTT and viable count methods and a nonlinear correlation was observed. A study reported moderate to the significant antibacterial activity of methanolic extract of the whole plant of *M. vulgare* against 5 out of 6 tested bacterial organisms, which was comparable to the standard ciprofloxacin [106]. This study revealed that methanolic extract of the *M. vulgare* was significantly effective against *S. aureus*, *Staphylococcus Epidermidis*, and *B. subtilis* and moderately effective against *P. vulgaris* and *E. coli*. In another study, the ethanol extract of *M. vulgare* roots tested for *in vitro* inhibition of planktonic growth, biofilm formation and adherence in methicillin-resistant *S. aureus* (MRSA) by growing biofilms for 40 h [107]. A broth microtiter dilution method was employed to determine the MIC after 18 h growth using an optical density reading using a MRSA isolate (ATCC 33593). A significant dose-dependent response for the inhibition of both biofilm formation (IC = 32 μg/ml) and adherence (IC = 8 μg/ml) was found in ethanol extract of roots.

In an alternative study, ethanol, acetone, and ether extracts of *M. vulgare* (leaves, flowers, and stems) were tested for their antimicrobial activity toward limited Gram-positive bacterial species in Turkey [108]. Among all the studies extracts ethanolic extract showed a marked antimicrobial effect against *S. aureus* and *S. epidermidis*.

The biological activity of crude extracts may differ due to geographical variation in plant and also solvents type, hence the phytochemical characterization of each extract is an important step to know about chemicals behind the activity. There is a need of some molecular studies to understand mechanism behind antimicrobial effect and to proceeds for clinical studies.

Anti-*Helicobacter pylori* effect of the methanolic extract (10 mg/ml in dimethyl sulfoxide) of *M. vulgare* was also tested using broth microdilution method and 50% minimum inhibitory concentrations (MIC) was found with a concentration more than 800 μg/ml [109]. In another tryout, antibacterial effect of *M. vulgare* essential oil against 12 bacterial and 4 fungi strains was studied by agar disk diffusion method [110]. The zone of inhibition (ZI), MIC, and concentrations inhibiting 50% of microbial growth (IC_{50}) were investigated to characterize the antimicrobial activities of essential oil. The results showed a significant activity against microorganisms especially Gram-positive bacteria with inhibition zones ranging from 6.6 to 25.2 mm and minimal inhibitory concentration values in the array of 1120-2600 μg/ml, whereas Gram-negative bacteria exhibited higher resistance.

Among Gram-positive bacteria, the significant activity of *M. vulgare* essential oil was reported through ZI against *S. epidermidis* (25.2 mm) followed by *S. aureus* 25923 (18 mm), *Enterobacter cloacae* (13.8 mm), *B. subtilis* (13.2 mm), *Micrococcus luteus* (12 mm), and *S. aureus* 1327 (12 mm). Although *Enterococcus faecalis* and *Bacillus cereus* exhibited moderate to weak activities, respectively. Antifungal activity of among four strains, *Botrytis cinerea*, exhibited the highest activity (12.6 mm). *Penicillium digitatum*, *Fusarium solani*, and *Aspergillus niger* were less sensitive to *M. vulgare* essential oils. Aqueous extracts of nine different plants from Syria were studied for their *Mycobacterium tuberculosis* inhibitory activity using the BACTEC MGIT960 susceptibility test method [111]. Out of all the studied plants, *M. vulgare* depicted significant anti-mycobacterium activity.

In an *in vitro* study, essential oil (0.04%) obtained from aerial parts of *M. vulgare* was screened for its antibacterial activity by Mueller Hinton broth dilution method against *Salmonella enteric*, *Listeria monocytogene*, *Pseudomonas aeruginosa*, and *Agrobacterium tumefaciens* [103]. MIC values ranging from 0.1 to 15 μl/ml was obtained for essential oil tested against all the bacterial strains. Following this study, essential oil obtained from flowering *M. vulgare* was also studied using GC/flame ionization detectors and GC/MS in Algeria. About 50 different components were identified in the oil [55]. The major components of the essential oil were 4, 8, 12, 16-tetramethyl heptadecan-4-olid (16.97%), α-pinene (9.37%), germacrene D-4-ol (9.61%), phytol (4.87%), piperitone (3.27%), dehydro-sabina ketone (4.12%), 1-octen-3-ol (2.35%), δ-cadinene (3.13%), and benzaldehyde (2.31%). Essential oils were also evaluated for their antibacterial activity against *M. luteus*, *B. subtilis*, *Klebsiella pneumoniae*, and *Escherichia coli*. The MIC values were found about 0.1-15 μl/ml. Methanolic extract obtained from *M. vulgare* leaves was evaluated for antimicrobial effect by measurement of inhibition zones diameter. The results showed that ZI and minimal inhibitory concentration was in the range of 13-17 mm and 12.5-25 mg/ml, respectively [100]. Antibacterial effect of ethanolic extract and essential oil of *M. vulgare* L. was studied on 17 strains of *S. aureus*. The strains were obtained from nose and throat samples and were collected from 160 healthy subjects comprising hospital staff and patients in Zabol, south-eastern Iran [112].

Essential oil was obtained by the hydrodistillation of *M. vulgare* leaves and analyzed by GC-MS. The major components were identified as γ-eudesmol, germacrene, β-citronellol, D-citronellyl formate, geranyl tiglate, and geranyl formate. The minimum and maximum MIC values of extract were

2.5 mg/ml. This study concluded that essential oil and ethanol extract of *M. vulgare* have a moderate antimicrobial activity against *S. aureus*. Sometimes, large-scale molecular studies are also required to investigate relationship between morphology of bacteria and different extracts. In addition, the compatibility of extracts with the mammalian also needs to investigate using different animal models before starting the human trial.

Anticancer Activity

Phenylpropanoid glycosides are phenolic compounds present in various plants that are used in traditional medicine. Four different phenylpropanoid glycosides (acteoside, forsynthoside B, arenarioside, and ballotetroside) and one non-glycosidic derivative (caffeoyl-1-malic acid) were tested for their chemoprotective activity. Effects of these components were evaluated against oxidized LDLs induced cytotoxicity in bovine aortic endothelial cells [113]. These compounds inhibited both copper and 2, 2-azobis (2-amidinopropane) dihydrochloride-induced *in vitro* LDL oxidation and protected the morphological aspects of the cells during incubation with oxidized LDL. In addition, in an *in vitro* study, the above components also inhibited the increased level of potent vasoconstrictor, endotthelien-1 (ET-1) when cells were incubated with Cu-LDL. These compounds were also produces inhibitory effect on Cu-LDL to induce ET-1 secretion by suppressing transcription of the ET-1 gene [114]. Yamaguchi *et al*. [115] reported the anti-tumorigenicity effect of *M. vulgare* leaves extract on human colorectal cancer cells. In this study, methanolic extract (250 μg/ml) up regulated pro-apoptotic nonsteroidal anti-inflammatory drug-activated gene (NAG-1) through a transactivation of the NAG-1 promotor. The most active compound, Ladanein, was isolated for the first time from *M. vulgare*, which displayed moderate (20-40 μM) activity against K562, K562R, and 697 human leukemia cell lines but it was inactive to MOLM13 and human peripheral blood mononuclear cells [66]. Related to the toxicity of individual compound to the normal cells have also important to a potent anticancer agent and it should be explored. The toxicity of pure compound can be minimized by synergism and specific target delivery technology.

In vitro cytotoxic assay of essential oils, against HeLa cell lines, was examined using a modified MTT assay [110]. The results obtained displayed the competence of essential oil to inhibit proliferation of HeLa cell lines under specific conditions with IC_{50} value of 0.258 μg/ml. The present studies confirmed the use of *M. vulgare* essential oil as an anticancer agent. In the cell viability assay, *M. vulgare* essential oil caused non-viability of 27% HeLa cells in a concentration of 250 μg/ml, and all cells were destroyed with concentrations higher than 500 μg/ml. The volatile oil produces the significant cytotoxic effect with IC_50 as 0.258 μg/ml toward the human tumor cell line [110].

In vitro cytotoxic activity of *M. vulgare* alcoholic extract and isolated flavonoids, viz., acacetin, apigenin, and acacetin-7-rhamnoside were tested against U251 (Ehrlich tumor cell lines) and MCF7 (human breast carcinoma cell lines). Alcoholic extract of *M. vulgare*, apigenin, acacetin, and acacetin-7-rhamnoside showed potent cytotoxic effect against breast carcinoma MCF7 cell line (effective dose 50% <20 μg/ml) [116].

In unison, methanol and n-hexanes extracts from aerial part of *M. vulgare* were also screened on a panel of human cancer cell lines including both solid and hematological cancer origins, as well as non-transformed murine fibroblasts [117]. Cell viability assay was performed for both the extracts. Cell lines were exposed to increasing concentrations of potential methanol extract. Microscopy, flow cytometry, and caspase activity assays were performed in LN229, SW620, and PC-3 cell lines. The results showed that methanolic extract has the aptitude to promote cell cycle arrest and cell death (IC_{50} 30 μg/ml). The results obtained from these studies fortify the traditional use of *M. vulgare* for the treatment of cancer.

Molluscicidal and Mosquitocidal Activity

M. vulgare is an aromatic plant widely distributed along the Mediterranean area, in the North coast of Egypt. The essential oil from aerial parts of *M. vulgare* was evaluated for its molluscicidal and mosquitocidal activities on eggs of *Biomphalaria alexandrina* and *Culex pipiens*, respectively [52]. *M. vulgare* oil showed lethal concentration 100 ovicidal activity at 200 ppm/24 h. The identified amount of oxygenated constituents was 57.50% from *M. vulgare*, while the amount of identified hydrocarbons was 32.69%. Sesquiterpenes were the major class of hydrocarbons in the amount of 32.48% while non-terpenoidal hydrocarbons were only 0.21% in *M. vulgare* oil. The major sesquiterpene hydrocarbon was γ-cadinene (17.68%). In another study, methanol extract of *M. vulgare* leaves was investigated against 4th instar larvae of the mosquito *C. pipiens* L. [118]. The results of this study showed that methanolic extracts of *M. vulgare* are toxic to 4th stage larvae of *C. pipiens* after longer exposure time for larvae. The high mortality rate (59%) was after 72 h of exposure with the dose of 900 mg/L.

Antiprotozoal Activity

M. vulgare is widely distributed and used popularly against intestinal disorders in Nuevo Leon, Mexico. An *in vitro* inhibitory activity of acetone and methanol extract from *M. vulgare* against *Entamoeba histolytica* and *Giardia lamblia*, the causative agents of amebiasis and giardiasis, respectively, was reported [119]. The extracts were found effective against *E. histolytica*; the IC_{50} was found to be 7 at a dose of 12 μg/ml and slightly moderate toxic to *G. lamblia*. Besides an *in vitro* trypanocidal activity of *M. vulgare* along with other plants used in traditional Mexican medicine for the treatment of parasitic infections was also investigated [120]. The results concluded that methanol extract of *M. vulgare* (aerial parts) was found effective against *Trypanosoma cruzi epimastigotes* and exhibited the highest trypanocidal activity, percentage inhibition was found in between 88% and 100% at a concentration of 150 μg/ml.

DRUG INTERACTION AND ADVERSE EFFECTS

The toxic effect of marrubiin has been reported with lethal dose 50% at 370 mg/kg when administered orally to rats. Marrubiin possess antiarrhythmic activity, which may also induce cardiac irregularities in larger doses. An acute toxicity study of aqueous extract from *M. vulgare* (1 g/kg) was examined on Swiss albino mice [121]. In this study, an infusion was administered orally at a dose of 1 g/kg body weight to the mice. Treated mice showed tachycardia 1 h after intake of the infusion and loss of appetite 3 h after intake of the infusion. In another experiment, five female rats were treated orally with a single dose of 2000 mg/kg extract of *M. vulgare* for an acute toxicity study [80]. After 14 days, no changes were detected in rat skin, fur, eyes, mucous membrane (nasal), central nervous system, and autonomic nervous system. The results suggested that the toxic dose of the methanolic extract of *M. vulgare* is higher than 2000 mg/kg.

The interaction of marrubenol with "classical" binding sites for calcium antagonists, namely, 1,4-dihydropyridines and phenylalkylamines, was studied in rat intestinal muscle membranes [84]. Results concluded that marrubenol (12 μM) was a weak inhibitor of 1,4-dihydropyridine binding in intestinal smooth muscle membranes. *M. vulgare* has no effects on drug metabolizing enzymes, and neither clinical nor pharmacological interactions were reported [122].

CLINICAL STUDIES

Ethnopharmacobotanical studies in Northern Sardinia have confirmed the use of *M. vulgare* in prevention and treatment of the asthmatic syndrome. A decoction made of leaves of *M. vulgare* and *Cynodon dactylon* was given for preventive treatment of perennial asthma and was administered in single dose (a glass of 25-30 ml) on an empty stomach for the prevention of asthmatic fits. The efficacy of decoction of *M. vulgare* leaves in the preventive treatment of acute asthmatic fits reported by five patients [123,124]. The effects were produced due to the presence of flavonoids could act by inhibiting the release of anaphylactic, and inflammatory mediators, with an inhibitory potency and inducing the relaxation of the bronchial smooth muscle. *M. vulgare* is generally considered to be safe when used in foods as a flavoring agent. However, there is limited scientific study related to safety, and most of information is available from animal research. Reported side effects include rash at areas of direct contact with *M. vulgare* juice, abnormal heart rate, low blood pressure, and decreased glucose level (seen in animals with high blood sugar). *M. vulgare* may cause vomiting and diarrhea.

Caution is urged in people with gastrointestinal disorders or heart disease. Caution may also be advisable in diabetes or hypoglycemic patients and for those taking drugs or supplements that affect blood sugar. In 2004, a clinical study included 43 outpatients for the effect of *M. vulgare* aqueous extract to control Type 2 diabetes mellitus [125]. All patients maintained their medical treatment and also received an infusion prepared of dry *M. vulgare* leaves for 21 days. The patients treated with *M. vulgare*, their blood sugar level was reduced by 0.64%, cholesterol and triglycerides by 4.16% and 5.78%, respectively. The results were compared between test groups and a significant difference in glucose and cholesterol diminution was observed.

CONCLUSION AND FUTURE PERSPECTIVES

The present review article on *M. vulgare* L. is an endeavor, about phytochemical profile and to brief pharmacological findings of this significant species. Myriad phytochemical, pharmacological and clinical studies performed on *M. vulgare* extracts, fractions and secondary metabolites isolated from various parts have been put forth. The existing literature evidenced that aerial parts of *M. vulgare* are extensively studied *in vitro* and *in vivo* against various disorders primarily, cancer, hypertensive, and inflammatory conditions. Diterpenoids, flavonoids, and phenylpropanoids are the major chemical constituents which have been demonstrated in *M. vulgare*. Marrubiin is a major diterpene labdane and also exists in high concentration in other traditionally important Lamiaceae species which have demonstrated excellent pharmacological properties with creditably high safety margins. It is also considered a potential biosynthetic substrate for other potent active compounds, namely, marrubiinic acid and marrubenol. The reported work includes other pharmacological studies such as analgesic activity, antinociceptive activity, antiedematogenic effect, antispasmodic effects, antidiabetic activity, gastroprotective, antioxidant, and hepatoprotective effect. The major aspiration of this review is to prompt future scientific researchers focusing broadly on phytochemical and pharmacological aspects of traditionally important natural products, and investigation leads for further chemical modification, development of novel and potent drug or drug-like moieties.

However, further improvements are required due to increasing research interest on *M. vulgare* and it is still remarkable that some gaps in our understanding of its application be present: (i) Several pharmacological studies have been reported on the leaves and aerial parts of *M. vulgare* but still, detailed scientific studies on other plant parts such as roots, endophytes, and plant secretions are essential to elaborate chemistry and medicinal assets of this important natural resource. (ii) Pharmacokinetic studies on medicinally potent secondary metabolites of *M. vulgare* are deemed to understand their absorption, distribution, metabolism, and excretion. These traits are pertinent in the process of drug development and have to be exercised. (iii) Preclinical evaluation of *M. vulgare* was done in various aspects; these claims should be scientifically evaluated clinically for better therapeutic applications. Clinical studies reported on this species are very limited and are required to explore this aspect which is crucial as the diagnosis of herbal toxicity is often based on clinical evaluations and also assists the safety and toxicity of active compounds from natural products. (iv) Several biological/pharmacological investigations are reported on crude extracts, active fractions and compounds isolated from *M. vulgare*, but still, cell/molecular level studies of potent secondary

metabolites are lacking. In addition, advanced mechanism-based studies of compounds (especially diterpenes, flavonoids, and phenylpropanoids) are mandatory to rationalize the traditional use and scientific proof beneath this. Use of natural resources especially traditionally valued plants has been a major part of researchers aiming for drug development and discoveries. The gap between the traditional medicines and the conventional mainstream medicine has been widening due to the lack of exhaustive scientific studies on natural products. Adoption of scientific proof-based medication, which backs up the use of ethnobotanicals are pertinent to find new leads for existing and emerging human ailments.

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