Journal of Pharmaceutical Research Vol. 10, No. 4, October 2011 : 124-129.

RECENT DEVELOPMENTS IN PHYTOCHEMICAL AND PHARMACOLOGICAL STUDIES OF *MARRUBIUM VULGARE* L.

Masoodi MH*
Department of Pharmaceutical Sciences, Faculty of Applied Science & Technology, University of Kashmir, Hazratbal, Srinagar – 190 006, J & K, India. Ph: 09419076525.

Received on : 26.04.2011 Revised : 04.08.2011 Accepted : 04.08.2011

ABSTRACT
Marrubium vulgare possesses folklore claim for curing various ailments in the form of extract. It has been reported to possess expectorant, diaphoretic and diuretic properties. Chemical constituents such as phenylethanoid glycoside, phenylpropanoid esters, flavonoid lactates, diterpenoids, anthocyanins and many others have been isolated. The plant possesses antihypertensive, analgesic, anticancer, antioedematogenic and many other reported biological activities. The present article reviews the complete pharmacological and phytochemical work done on the plant so far.

Keywords: *Marrubium vulgare; white horehound; liver disorders; antioedematogenic; marruboside; marrubiol.*

INTRODUCTION

Marrubium vulgare L. (Lamiaceae) commonly known as 'white horehound' or 'paharigandana' has been used since ancient times for the treatment of various ailments. It thrives almost in any soil, and is naturalized in North and South America and Western Asia as far as India. It is found in Kashmir at 5,000-8,000 ft. *M. vulgare* is a tall robust herbaceous perennial herb, 40-120 cm high, with a short root stock, tough stems, branches below, bluntly quadrangular, more rounded below, densely covered especially when young, with a thick white cottony felt[1]. Leaves opposite, blade reaching an inch in length, on a long broad, woolly petiole, ovate, acute or blunt, coarsely crenate-serrate, veins very prominent on the under surface. Flowers sessile, few, crowded, forming small, very dense, rounded whorls in the axils of the upper leaves, bracts linear, woolly. Calyx tubular, oblong, cylindrical, faintly 10-ribbed, woolly externally with stellate hairs[2].

M. vulgare possesses tonic aromatic, stimulant, expectorant, diaphoretic and diuretic properties. It increases appetite and supports the function of the stomach. It is helpful for bronchitis, bronchial asthma and non-productive cough. It is also laxative in large doses and was formerly regarded as emmenagouge. The plant is of much benefit in catarrh in which there is much cough, with copious excretion of mucus, nocturnal sweats, and great prostration of strength. It was formerly much esteemed in various uterine, visceral and hepatic affections and also in phthisis[2,3]. *M. vulgare* possesses hypoglycemic, antihypertensive, analgesic, anticancer, antioedematogenic and many other reported biological activities. Various chemical constituents isolated form the plant are flavonoid lactates, diterpenoids, phenylpropanoid esters, anthocyanins, and essential oils. The present review on *M. vulgare* gives a detailed account of its phytochemical and pharmacological investigations done so far by different authors.

PHARMACOLOGICAL INVESTIGATIONS

Pharmacological studies related to this plant undertaken by various workers are given below:

Hypoglycemic activity

A detailed study was carried out regarding the hypoglycemic potential of twelve plants used in Mexico as antidiabetics. The studies were performed using 27 healthy rabbits with the gastric administration of water, tolbutamide or decoction of the antidiabetic plant before the induction of temporary hyperglycemia by subcutaneous injection of 50% dextrose solution (4 ml/kg body weight) at the beginning of the experiment. After 60 min tolbutamide and eight of the studied plants significantly decreased hyperglycemia as compared with control test. The strongest effect was yielded by *Guaiacum coulter* followed by *M. vulgare*[4].

Preliminary evaluation was carried out on the hypoglycemic effect of five Brazilian medicinal plants *(Epidendrum monsenii, Marrubium vulgare, Rheedia gardneriana, Rubus imperialis* and *Wedelia paludosa)* on alloxane-induced diabetic rats. The extract of these plants was intragastrically administered to diabetic rats. The results showed that all plants studied (except *R. gardneriana*) significantly lowered the blood glucose. These results suggest that these four medicinal plants could be an adjuvant agent in the treatment of diabetes mellitus[5].

***Correspondence :** mubashir@kashmiruniversity.ac.in, mube5090@gmail.com

Antihypertensive activity

Comparative study of antihypertensive activity of water extracts of *M. vulgare* and dihydropyridine calcium antagonist amlodipine in (SHR) spontaneously hypertensive rat was evaluated. The results demonstrated that, in addition to its antihypertensive effect, aqueous extract improved the impaired endothelial function in spontaneously hypertensive rat[6].

The hypotensive effects of the water extract of *M. vulgare* L. and *Foeniculum vulgare* L. were investigated in spontaneously hypertensive rats (SHR) and in normotensive Wistar-Kyoto rats (WKY). Oral administration of marrubium or foeniculum extract lowered the systolic blood pressure of SHR but not of WKY. *Ex vivo* as well as *in vitro*, marrubium extract inhibited the contractile responses of rat aorta to noradrenaline and to KCl (100 mM). Inhibition was greater in aorta from SHR compared to WKY and was not affected by the NO synthase inhibitor N-nitro-L-arginine. Vascular effects of foeniculum extract were less pronounced than those of marrubium and were blocked by N-nitro-L-arginine thus indicating hypotensive activity of *M. vulgare* was mediated by vascular relaxant activity[7].

Analgesic activity

Marrubiin, a furan labdane bioactive diterpene present in *M. vulgare* was structural modified to obtain more active compounds. Success was obtained in reducing the lactonic function, in the formation of marrubiinic acid and two esterified derivatives, which exhibited significant analgesic effect against the writhing test in mice[8].

Significant analgesic activity has been reported for hydroalcoholic extracts obtained from *M. vulgare* in different models of pain in mice. The extracts exhibited analgesic activity, antagonizing chemical-induced acute pain[9].

As cosmetic

M. vulgare extract-based formulation under patent No. WO: 2003105876 has been used for the treatment of acne and to reduce the appearance of cutaneous blemishes or to smooth rough skin[10].

Vasorelaxant activity

The vasorelaxant activity of marrubenol and marrubiin from *M. vulgare* has been reported by El Bardai and his co-workers. Crude extracts of the aerial parts of *M. vulgare* show potent *in vitro* inhibition of KCl-induced contraction of rat aorta. Bio-guided fractionations, spectroscopic analysis and chemical derivatization revealed the furanic labdane diterpenes marrubenol and marrubiin as the most active compounds[11].

In rat aorta, marrubenol was a more potent inhibitor of the contraction evoked by 100 mM KCl (IC50: 11.8+/-0.3 microM, maximum relaxation: 93+/-0.6%) than of the contraction evoked by noradrenaline (maximum relaxation: 30+/-1.5%) thus showing that marrubenol inhibits smooth muscle contraction by blocking L-type calcium channels[12].

Anti-inflammatory activity

Sahpaz and his coworkers carried out the isolation and identification of major phenylpropanoid esters from *M. vulgare*, (+) (E)-caffeoyl-L-malic acid, acteoside, forsythoside B, arenarioside and ballotetroside. Their anti-inflammatory activities are reported for the first time. They evaluated the inhibitory effects of these five compounds on cyclooxygenase (Cox) catalyzed prostaglandin biosynthesis activity. Only the glycosidic phenylpropanoid esters showed an inhibitory activity towards the Cox-2 enzyme and three of them: acteoside, forsythoside B and arenarioside exhibited higher inhibitory potencies on Cox-2 than on Cox-1. These results are of interest, as Cox-2 is mainly associated with inflammation and the Cox-1 inhibition with adverse side effects often observed with non-steroidal anti-inflammatory drugs[13].

Anticancer activity

Yamaguchi and his coworkers have show that extracts of *M. vulgare* (horehound) and *Prunus serotina* (wild cherry bark) exhibit anti-proliferative activity in human colorectal cancer cells. Both horehound and wild cherry extracts cause suppression of cell growth as well as induction of apoptosis[14].

Antinociceptive activity

The antinociceptive properties of marrubiin which is the main component isolated from *M. vulgare*, was analysed in some models of nociception in mice. The results showed that marrubiin exhibits potent and dose-related antinociceptive effects. The antinociception produced by the marrubiin was not reversed by naloxone when analyzed against the writhing test. In the hot-plate test, marrubiin did not increase the latency period of pain induced by the thermal stimuli. Its exact mechanism of action remains to be determined, but the results suggest that marrubiin, like hydroalcoholic extract of *M. vulgare* does not interact with opioid systems[15].

Antioxidant activity

Antioxidant activity of horehound (*M. vulgare*) grown in Lithuania was reported by Weel and his co-workers. Acetone extracts (AE), deodorized acetone extracts (DAE) and deodorized water extracts (DWE) from leaves of horehound (*Marrubium vulgare*) were tested for their antioxidant activity in rapeseed (*Brassica napus*) oil at 80°C. Weel investigated antioxidants containing extracts (AE and DAE) of sage (*Salvia officinalis*) for comparison purposes. The effect of the extracts on the edible oil stability was assessed by measuring weight gain, peroxide value, and the UV absorption. The antioxidant activity of AE and DAE of

horehound were comparable to the antioxidant activity of AE and DAE of sage. For both plants AE was shown to have better antioxidant properties than DAE[16].

Antioxidant effects of methanol extracts from six wild European Lamiaceae species have been studied with the use of three in vitro assays. The ability of scavenging free radicals was measured by DPPH reduction spectrophotometric assay. The reducing potential towards transition metals was tested by phosphomolybdenum method and the inhibition of lipid oxidation was tested by Fe/ascorbate system with photometric TBARS detection. All studied herbs exposed strong antioxidant capability, but the results were different for each species depending on the applied test. In DPPH scavenging the order from strongest to the weakest was: *Leonurus cardiaca*, *Lamium album*, ***Marrubium vulgare*****,** *Stachys officinalis*, *Lamium purpureum*, *Galeopsis speciosa*. In lipid peroxidation assay, the maximum inhibition of 78% was reached by *S. officinalis* and *M. vulgare*, whereas for both *Lamium* sp. and *L. cardiaca* slightly exceeded 70% and for *G. speciosa* reached 65%[17].

A study was conducted on 30 medicinal plants that are widely used in the Rio Grande Valley and using a two-stage Trolox based assay for analysing the total antioxidant capacity of aqueous extracts prepared from these plants. The antioxidant content of the aqueous extracts was substantial, ranging from 27 to 972 micromol Trolox equivalent per gram dry weight. An extract of the leaves of the plant *Ilex paraguensis* (Mate leaf) contained the highest amount of antioxidant, followed by the flowers of the *Rosa* sp. (Rosa de Castillo, 804 micromol/g), the bark of *Chinchona* sp. (Copalquin, 692 micromol/g), *Rumex hymenosepalus* stems (Cana Agria, 672 micromol/g) and the leaves of *M. vulgare* (Mastranzo, 560 micromol/g)[18].

Antispasmodic activity

The effects of hydroalcoholic extracts of the roots and aerial parts of *M. vulgare* in several smooth muscle preparations were studied *in vitro*. The extracts exerted antispasmodic activity which inhibited the action of neurotransmitters (acetylcholine, bradykinin, prostaglandin E2, histamine and oxytocin) with putative slections for cholinergic contractions[19].

Antifeedant activity

Studies were carried out related the antifeedant activity of marrubiin isolated from *M. vulgare* and reduced marrubiin, product prepared form marrubiin, and both were tested as antifeedants. Marrubin showed antifeedant activity for the larvae of *Spodoptera frugiperda*, while reduced marrubin did not show activity[20].

Expectorant properties

Karryev and his coworkers reported some therapeutic properties and phytochemistry of common horehound. Seven monoterpenes identified by gas-liquid chromatography in the essential oil of *M. vulgare* were as á-pinene, camphene, limonene, sabinene, p-cymol, pfenchene, and á-terpinolene. Clinical investigations indicated that the essential oil had vasodilative and expectorant effects[21].

Antioedematogenic activity

Stulzer and his coworkers described antioedematogenic profile of marrubiin the main constituent of *M. vulgare***,** a medicinal plant used in folk medicine of several countries to treat different pathologies. Marrubiin was analyzed in a model of microvascular leakage in mice ears. The results show that it exhibits significant and dose-related antioedematogenic effects[22].

Anti tubercular activity

M. vulgare was used as one of the components in the patented preparation used for the treatment of tuberculosis which was prepared by mixing Iceland moss 1 oz., Irish moss 1 oz., European centaury 1 oz., mullen leaves 1 oz., marshmallow leaves 1 oz., *M. vulgare* 1 oz., water 3 qts., boiling until concentrated to 1 qt., adding honey 1 qt., and again boiling until reduced to 1 qt. [23]**.**

Antimicrobial activity

Amal and his coworkers screened antimicrobial activity of indigenous Jordanian plant extracts dissolved in dimethylsulfoxide using the rapid XTT assay and viable count methods. XTT rapid assay was used for the initial screening of antimicrobial activity for the plant extracts. Antimicrobial activity of potentially active plant extracts was further assessed using the “viable plate count” method. Four degrees of antimicrobial activity (high, moderate, weak and inactive) against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* respectively, were recorded. The plant extracts of *Hypericum triquetrifolium*, *Ballota undulata*, *Ruta chalepensis*, *Ononis natrix*, *Paronychia argentea* and ***Marrubium vulgare*** had shown promising antimicrobial activity[24].

Insecticidal activity

The methanol extracts of eight species of medicinal plants *Origanum majorana*, *Ocimum basilicum*, *Cnicus benedictus*, ***Marrubium vulgare***, *Hyssopus officinalis*, *Salvia splendens*, *Salvia officinalis* and *Melissa officinalis* were tested for insecticidal activity in larvae of Egyptian cottonworm (*Spodoptera littoralis*). All extracts showed a certain degree of larval toxicity. The extracts significantly affected the growth indexes [relative growth rate (RGR), efficiency of conversion of ingested food (ECI) and efficiency of conversion of digested food (ECD)][25].

Antihepatotoxic activity

Ahmad and his co-workers carried out antihepatotoxic activity of different extracts of whole plant of *M. vulgare* L. against CCl_4 induced hepatic damage in male Albino Wister rats. The activity of these extracts (500-mg/kg b. w for 7 days) was compared with the standard drug silymarin (Silybon-70, 10 mg/kg b.w). The petroleum ether, chloroform and methanol extracts have shown significant antihepatotoxic activity by reducing the elevated levels of serum enzymes such as serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP) and increase in total protein (TP) indicating that the methanol extract was the most active among the three extracts as compared to standard drug silymarin[26]. These biochemical observations were also supplemented by histopathological examinations of the liver sections.

Mubashir and his co-workers investigated aqueous extract of whole plant of *M. vulgare* L. for anti-hepatotoxic activity against CCl_4 induced hepatic damage in male Wister rats. The extract in dose of 500-mg/kg body weight for 7 days was compared with the standard drug silymarin (10 mg/kg- body weight). The aqueous extract has shown significant anti-hepatotoxic activity by reducing the elevated levels of serum enzymes such as SGOT, SGPT, ALP and increasing TP respectively thus showing that aqueous extract possesses significant anti-hepatotoxic activity which was also supplemented by histopathological reports[27].

PHYTOCHEMICAL STUDIES

Extensive Phytochemical studies have been carried out on ***Marrubium vulgare***. Different chemical constituents isolated from the plant are as:

A new phenylethanoid glycoside, marruboside, has been isolated from the aerial parts of *Marrubium vulgare*. Its structure was established as 3,4-dihydroxy-□-phenylethoxy-O-[□-D-apiofuranosyl-(1□ 2)-□-L-rhamnopyranosyl-(1□ 3)]-[□-D-apiofuranosyl-(1□ 6)]-4-O-caffeoyl-□-D-glucopyranoside, on the basis of spectroscopic evidence[28].

The major compounds which were isolated from the essential oil of *Marrubium vulgare* by Asadipour and his co-workers through GC-MS analysis were caryophyllene oxide (18.67%), trans-caryophyllene (12.77%), germacrene D (10.04%), bicyclogermacrene (3.38%) and trans-anethole (3.01%)[27]. Besides, Sahpaz and his coworkers reported the isolation and identification of (+) (E)-caffeoyl-l-malic acid, acteoside, forsythoside B, arenarioside and ballotetroside from *M. vulgare*[13].

Isolation of vulgarol, â-sitosterol, lupeol, marrubiin, a labdane diterpenoid lactone, vulgarin and apigenin-O-glucoside have been reported from the aerial parts of *M. vulgare*[29,30]. Various flavonoid lactates have been isolated from leaves of *M. vulgare*. These include new natural lactoyl (2-hydroxypropionyl) flavonoids, luteolin and apigenin-7-lactates together with their 2''-O-glucuronides and 2''-O-□-glucosides. The known flavonoids vitexin, vicenin II, luteolin 7-glucoside, apigenin-7-O-glucoside, apigenin-7-(6''-p-coumaroyl) glucoside, chrysoeriol, quercetin 3-rhamnoglucoside, quercetin 3-glucoside and apigenin were also reported[31,32].

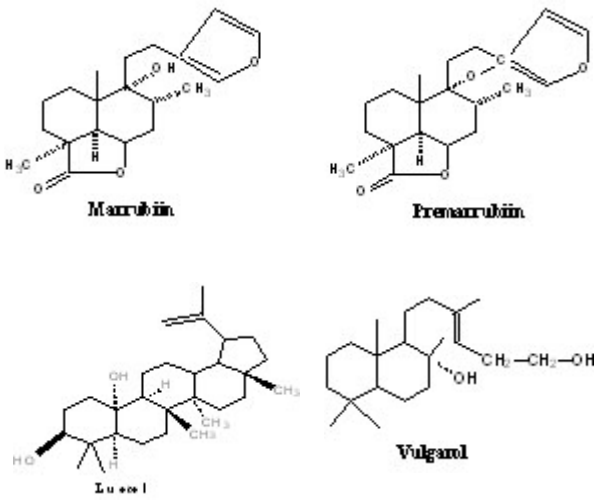
Popa and his coworkers isolated diterpenoids from the genus Marrubium [horehound]. Peregrinol (labd-13-ene-9,15-diol) was found in all 5 species of marrubium studied (*M. vulgare, M. peregrinum, M. praecox, M. leonuroides, and M. catariifolium*). Marrubiol, a new diterpenoid has also been isolated from *Marrubium vulgare*[33,34]. A new diterpenoid - premarrubiin which appears to be the major substrate from which marrubiin is generated as an artifact has been isolated from *Marrubium vulgare*[35].

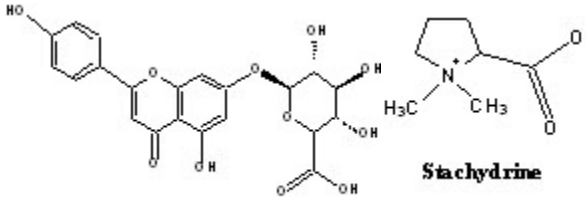
Pulatova and his coworkers while conducting the chemical study of *Marrubium vulgare* extracted stachydrine, flavonoids, anthocyanins, ascorbic acid and caffeinic acid[36]. Karryev and his coworkers reported the phytochemistry of common horehound. Seven monoterpenes isolated and identified by gas-liquid chromatography in the essential oil of *Marrubium vulgare* were as á-pinene, camphene, limonene, sabinene, p-cymol, p-fenchene, and á-terpinolene[37].

CONCLUSION

The extensive survey of literature revealed that *Marrubium vulgare* is an important medicinal plant with diverse pharmacological spectrum. Besides having the above mentioned pharmacological properties further evaluation needs to be carried out in order to explore the concealed areas and their practical clinical applications, which can be used for the welfare of the mankind.

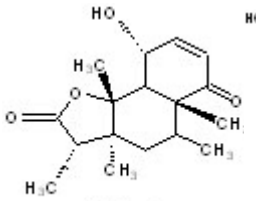
Chemical constituents of *Marrubium vulgare.*



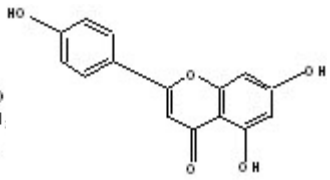


Apigenin-7-glucoside

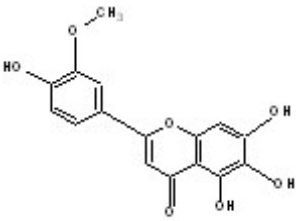
Stachydrine



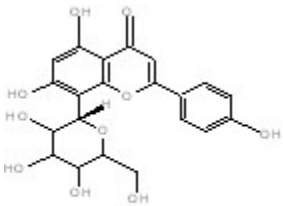
Vulgaxin



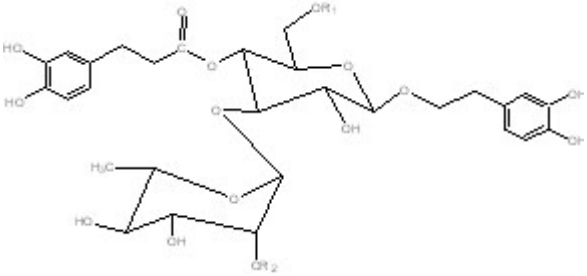
Apigenin



Chrysoeriol

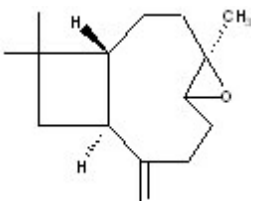


Vitexin

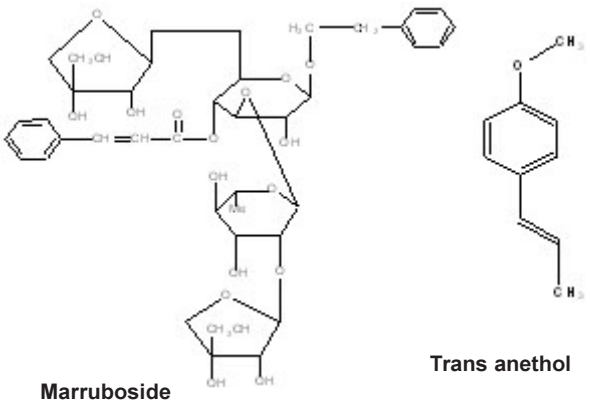


		R1	R2
1.	ACETOSIDE	H	H
2.	FORSYTHOSIDE B	B-D-APIOSE	H
3.	ARENARIOSIDE	B-D-XYLOSE	H
4.	BALLOTETROSIDE	B-D-APIOSE	A-L-ARABINOSE

Luteolin

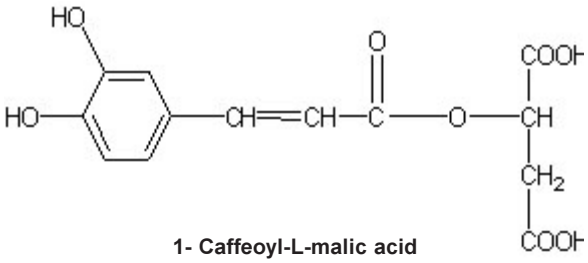


Caryophyllene oxide

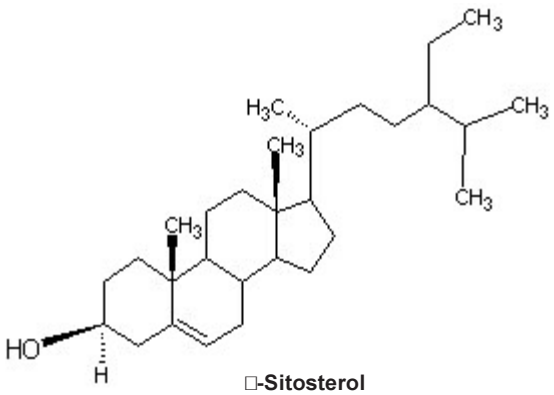


Marruboside

Trans anethol



1- Caffeoyl-L-malic acid



□-Sitosterol

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