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Review Article

Assessment of *Marrubium vulgare* hydro-alcoholic extract's biological activities

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ABSTRACT

Marrubium vulgare is a tall, robust herbaceous perennial plant originating from Asia and Mediterranean and currently distributed throughout North, South America, Europe, Mediterranean and west Asia. *M.vulgare* thrives in any type of the soil but it prefers light calcareous, dry soils where it is sunny and warm at an altitude between 1500 –2400 m. This plant is becoming increasingly important because it is currently cultivated in different countries to be used as a source of medicine and food flavors. Also,it has more than 54 different phytocompounds such as polyphenols, monoterpenes, diterpenes and essential oils. Marrubin was the first isolated diterpenes and it's characterized as the chemotaxonomic marker for the genus *Marrubium.* According to different studies, these compounds are responsible for antioxidant, antimicrobial, antifungal, analgesic, and anti-inflammatory, wound healing and anthelmintic activities. Due to these biological activities, hydroalcoholic extracts of *Marrubim vulgare* have been exploited for their therapeutic nature by traditional healers to cure several illnesses in Algeria. This review is to assess the different studies of hydroalcoholic extracts of *Marrubium vulgare* for different biological activities.

Introduction

The World Health Organization (*WHO, 2023*) center for traditional medicine estimates that more than 80% of the world's population uses herbal medicines to treat a variety of illnesses; therefore, it is important to make the advantages and potential risks of using herbal remedies clear. Several investigations have been carried out through the extraction, separation, and purification of the contents to elucidate the various biological activities. To completely extract the active components from plants, several extraction techniques have been developed. However, the harvesting season, plant development stage, geographical origin, drying, storage and extraction process have significantly impacted on extraction yield [1].

Marrubium vulgare is an important herbaceous plant that belongs to the *Lamiaceae* family, which has spread around the world from its native origins in Asia and the Mediterranean. *M.vulgare* is a tall robust herbaceous perennial plant having hairy roundish leaves, white flowers , numerous quadrangular ,erect stems of height 12–18 inches and fibrous roots[2]. *M. vulgare* derives its name from the Hebrew word "marrob"which means "bitter juice"and "vulgare" which means "common or known as" [3]. In context of a bitter remedy, this plant is used in Algeria as a traditional medicine to cure common ailments of the digestive tract,

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diabetes, bronchitis, diarrhea, rheumatism, colds and cough [4]. *M. vulgare* has several biological properties such as hypoglycemia, anti-oxidant, analgesic, wound healing, anti-inflammatory, anti-hypertensive, hypolipidemia [5], anti-microbial [6], insecticidal [7], anthelminthic and mosquitocidal properties [8]. These properties have been linked to the presence of different active compounds in the different parts of this herbal plant. Phytochemical analysis reports that *M. vulgare* contains more than 54 secondary metabolites such as labdane diterpenes and flavonoids [9]. Hydro-alcoholic and aqueous extracts are the most used extracts to obtain active compounds from plants by traditional healers [3]. This interests us to understand the different scientific evidence published on these extracts. This article is aimed at reviewing the different studies on the phytochemical composition and biological activities of the hydro-alcoholic extract of *Marrubium vulgare* to make a clear understanding of this herb's potential benefits.

1. Phytochemical composition

Due to the increased use of herbal remedies, research has intensified in search for answers on how plant-derived active compounds contribute to the drug market. Several active compounds have been reported to have significant pharmacological effects, with their compositions varying across species due to factors such as plant's geographical origin, climatic conditions, soil nutrients, vegetative phase, sampling season periods and quantification methods [10]. *M.vulgare* is reported to have more than 54 secondary metabolites from different parts contributing to its biological activities .These compounds include diterpenes, monoterpenes, essential oils, phenolic compounds and others.[2].

1.1 Diterpenes

Diterpenes are predominately phytochemicals compounds of *M. vulgare* and Marrubin is the first diterpene isolated from *M. vulgare* [11]. Marrubin's synergy effects with other compounds are reported to greatly contribute to the pharmacological activities of *M. vulgare*. Marrubin accumulates to maximum concentrations in the plant's aerial parts before flowering [12].

1.2 Essential oils

According to the different studies , essential oils extracted from the aerial parts of *M.vulgare* (leaves and flowers) are dominated by sesquiterpenes, oxygenated hydrocarbons, oxygenated monoterpenes and other compounds (*Pouris et al., 2021 Yabrir, 2019).*An essential oil of pale yellow color with 29 compounds has been reported from aerial parts of Algerian *M.vulgare* with several constituents: α bisabolene (36.3%), caryophyllene (7.8%), phytol (6.2%), nonacosane (4.0%), heptacosane (3.3%), nonanal (2.8%), 6, 10, 14-trimethyl-2-pentadecanone (2.6%) and humulene (2.0%) [4]. Other light yellow essential oils from aerial parts of Tunisian horehound species exhibit a high portion of α-bisabolene at 28.3%, β-bisabolene (25.4%), (E) farnesene (8.3%) and caryophyllene (11.1*%)* [13]. A GC-MS analysis carried out on *M. vulgare* collected from different locations of Yemen reveals Z-caryophyllene (10.95%), octadecanol (10.44%) and α-bisabolene (9.72%) [14]. Another study of 8 populations from different climatic backgrounds revealed that essential oils had 42 compounds with hydrocarbon sesquiterpenes frequent in all the populations and β-bisabolene dominant of the compounds [15]. The research results above confirm the presence of the different essential oils obtained from *M. vulgare* extracts with varying concentrations depending on the different climatic and geographical zones.

1.3 Phenolic compounds

Phenolic compounds are very important secondary metabolites found in the plant kingdom. These are among the most common compounds found in different extracts of *Marrubium vulgare.* Reports show that phenols and flavonoids are the most abundant classes, with their quantities varying from one extract to another [2]. Several studies use Gallic acid and quercetin as standards to quantify the total phenolic acid and flavonoid concentrations in the different extracts. A study done from Morocco's *M. vulgare* species show a remarkable phenolic compound yield of (112.09 ± 4.77 mg GAE/DW) for *M. vulgare* ethanol extracts (MVE) and *M. vulgare* acetonic extract (MVA) (98.77 ± 1.68 mg GAE/DW) [16]. A study on *M.vulgare* leaves using UV methods showed the total phenolic content and total flavonoid content were 6.02 0.01 mg/g (Gallic acid extract/dry weight) and 45.21 0.01 mg/g (CE/DW) respectively [12]. An ethanol extract from the aerial part of this plant obtained from the southeastern part of the Republic of Serbia yields a total phenolic and total flavonoid of 59.87 7.31 mg of Gallic acid equivalents/g of dry extract and 14.47 0.54 mg of quercetin equivalents/g of dry extract, respectively [17]. The aerial parts of *M. vulgare* collected from Wadi karma, Saudi Arabia, extracted with this method yielded total flavonoid content of 15.53mg quercetin equivalent/g of dry plant materials [5] .Phyto analysis of 8 populations of

Tunisian *Marrubium vulgare* reveals these populations are rich in phenolic compounds from (20.8 to 44.65mg GAE/g DW), with total flavonoid content from 8.91 to 37.48mg RE/g DW [15]. Results from different studies indicate that *M. vulgare* has significant quantities of phenolic compounds such as flavonoid compounds as luteolin, apigenin, quercetin, isoquercitrin, vitexin [18] . Variations in the flavonoids and gallic acid of the same plant samples are attributed to the different extraction methods, solvents, temperature, and other factors [19]. Phenolic compounds from *Marrubium vulgare* have been tested for different biological activities such as antibacterial, antioxidants, hypoglycemic, hyperlipidemia.

2. Biological activities

2.1 Antioxidant activities

Due to the presence of significant concentrations of phenolic compounds, *Marrubium vulgare* has demonstrated a scavenging potential against free radicles that cause lipid peroxidation, protein oxidation, enzyme inhibition, and DNA fragmentation [20]. The anti-oxidant property is important to decrease the aging, and pathogenesis of diabetes, cancer, and liver diseases [21]. In vitro antioxidant properties of hydro-alcoholic extract of 70% ethanol were done on aerial parts of *M. vulgare* showed remarkable neutralization of DPPH, OH, and NO free radicals exhibiting a strong antioxidant potential with IC_{50} = 13.41µg/ml, 63.99µg/ml, and 64.86µg/ml for DPPH, OH^-, NO test systems respectively [17]. The anti-oxidant property of different concentrations of the air-dried leaves of *M. vulgare* extracted with a solution of 80 % methanol showed EC_{50} of 38.56 ± 0.10 µg/mL determined by DPPH essay using a green tea-standardized extract (Greenselect®, Indena S.p.a) (EC50 = 4.6 µg/mL)[12]. Microwave-assisted extraction of *Marrubium vulgare* with solvent ethanol: water (1:1) showed an ability to scavenge for DPPH-free radicals in vitro with IC 66.28±0.6µg/ml relative to the 84.14 ± 0.7 µg/mL of the extract obtained by the conventional method [22]. In addition, hydro-alcoholic extract of *M. vulgare* leaves 70% ethanol tested the ability to reduce the power of iron Fe^{3+} to Fe^{2+} using ferric reducing capacity (Ferric Reducing Antioxidant Power Assay, or FRAP) showed (EC50 = 4.51 ± 0.5 mg/mL) [16].The anti-oxidant potential of the different DPPH essays varies mainly because of the sampling locations and the extraction solvents used [23]. All the findings of the antiradical potential of *M. vulgare* extracts are in vitro, so there is a need to direct research toward *in vivo* experiments to exhaust their full anti-oxidant capacity [3].

2.2 Antibacterial activities

Reports summarized in (table 1) have shown the efficacy of the different constituents of *M. vulgare* hydro-alcoholic extracts against several micro-organisms. However it has been noted that they are more efficient against bacteria gram-positive than a gram-negative [24]. The gram-negative bacteria resistance against these extracts can best be explained by the presence of the outer layer that acts as a barrier to entry. A study of Hydro-alcoholic extract of *M. vulgare* leaves with 70% ethanol using the disc diffusion method showed an inhibition activity against; *Bacillus subtilis*, *Escherichia coli, Salmonella enterica* with a zone of inhibition ranging from 7.33 ± 0.33 mm to 11.66 ± 0.66 mm [16]. A study done on *M.vulgare* leaves from three different locations of Algeria using 70% methanol against *Escherichia coli* ATCC 25922, *Bacillus cereus* ATCC 10876, and *Proteus mirabilis* ATCC 35659.Results show that Lyophilized *M.vulgare* flavonoids extract dissolved in different concentrations of pure dimethyl formamide (DMF) exhibited varying zones of inhibition diameter ranging from 7.5 to 34.3 mm and minimum inhibitory concentration (MIC) ranging from 25 to 100µg/ml.In conclusion, flavonoids in *M.vulgare* extracts showed good antibacterial activity against all tested strains with remarkable sensitivity percentages [25]. Therefore anti-bacterial activity proven in the extract of *M. vulgare* is likely induced by the synergistic action in chemical ingredients present in the extracts.

2.3 Antifungal activity

Extracts from *M.vulgare* demonstrate a significant antifungal activity varying from one fungi to another, this makes it a prospect biofungicide. If exploited it would be an alternative to prevention of fungal infection in agricultural sector [6].A study of hydroalcoholic extract of *M. vulgare* leaves summarized in (table 1) showed an inhibition activity against *Candida albicans* and *Aspergillus niger* with varying inhibition zone ,MIC and minimum bactericidal/fungicidal concentrations (MBE)[26]*)*.

2.4 Anti-inflammatory activity

Several studies summarized in (table 1) have demonstrated the ability of *M. vulgare* extracts to inhibit different pro-inflammatory agents. This could be due to the presence of phenolic compounds and phenypropanoid esters that have been reported to have effect on cyclooxygenase-

catalyzed prostaglandin and cyclo-oxygenase-2 (cox-2) [2]. A particular investigation of hydroalcoholic extract (70% ethanol) of *M. vulgare* leaves against inflammation induced by carrageenan in albino male mice with 500mg/kg of the extract administered orally showed a significant reduction in the size of the paw at $P< 0.05$ after 6hrs of carrageenan injection. *M. vulgare* extract also showed the inhibition of edema at 47.65% with the reference indomethacin producing 54.55% inhibition [26]. Based on these results, *M.vulgare* can be considered a prospect in the development of anti-inflammatory drugs[27].

3 Anti-diabetic activity

Diabetes is one of the common diseases where *M. vulgare* extracts are used to cure by traditional healers [28]. A study done on methanol, water and butanol extracts of *M. vulgare* carried out albino male rat-induced autoimmune diabetes mellitus. The result demonstrates a significant decrease in total serum cholesterol ranging from 8.43%, 14.3% and 36.6% of the different extracts. The results further demonstrated a significant decrease of the levels of serum Low-Density Lipoprotein (LDL), serum triglycerides ,the activity of interferon-gamma (IFN-γ),Tumor Necrosis Factor (TNF-α) and Nitric oxide (NO) [29]. This anti-diabetic activity of the *M. vulgare* extract may be due to the secondary consequence of its anti-inflammatory effects since it significantly decreases of the pro-inflammatory mediators such as TNF α-, IFNγ and NO [29]. Results from different studies suggest that the antidiabetic activity of M.vulgare extracts may be due to the extrapancreatic mechanisms [3]. According to [28], diterpenes, flavonoids and phenylpropanoid esters may also play a role in the anti-diabetic activity or the diabetes concomitant effects on animal mode.

4 Wound healing

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Wound healing is a natural mechanism that is supported by cell proliferation, differentiation and collagen formation for tissue regeneration [26]. Several individuals cannot regenerate these tissues due to a lack of nutrients (vitamins A and C) essential to cellular differentiation and immune functions. A study of methanolic extract using 80% methanol set with microwave-assisted extraction of *Marrubium vulgare* demonstrates cell viability percentages of *in vitro* human dermal fibroblast (HDFs) ranging from 5–500 µg/mL of the extract showing the capability to improve cell proliferation. 5µg/mL of the extract reached sub-confluence at 24-hr cells and complete confluence after 45 h this proves that *M. vulgare* extract exhibits a wound healing property [12]. A study of the ointment made from 1g of hydro alcoholic *M. vulgare* extract +9-g of Vaseline tested against burn-induced wounds on rats shows complete wound closer after 21 days with wound contractions (97.78 +/– 4.95%) [26]. Depending on the sampled results, extracts of *M. vulgare* accelerate the healing process of wounds by promoting cell migration and fibrosis proliferation and differentiation [12]. Also, this is linked to the presence of flavonoids and condensed tannins highlighted for the potent vasoconstriction property [3].

5 Analgesic property

A study of an oral administration (500mg/kg) of hydro-alcoholic extract tested on intraperitoneal injection of acetic acid-induced pain on albino male mice shows a significant reduction in pain of *M.vulgare*.The extract presented (58.8 ± 6.64) greater than the negative control (97.8 ± 6.24) with inhibition of contraction of 39.62 ± 7.31% less than that of tramadol (45.86 ±4.38%) [26]

Table: 1 summarizes the different biological activities of *Marrubium vulgare* extracts

Activity	Plant part	Type of extraction	Experimental methods	Control (+/-)	Doses	Results	References
Antibacterial	Leaves	Ethanol: water (7:3) Methanol: water (7:3)	Disc diffusion method (*Bacillus subtilis, Staphylococcus aureus, Salmonella enterica* and *Escherichia coli)* Disc diffusion method (*Escherichia coli, Bacillus subtilis* and *Proteus mirabilis)*	+ve: Tetracycline (0.02mg/disc) +ve Chloramphenicol +Gentamicin +Aztreonam +Nalidixin acid +Ceflazidime +imiprenem	10 µL 10mg/disc 40µL/disc	An activity against all strains with inhibition zones (7.33 ± 0.33–11.66 ±0.66) mm MIC of 1.87mg/ml against S.aureus,2.5mg/ml of B subtilis ,E.coli and 5mg/ml for S.enterica The inhibition zone varied from 7.5-34.3mm MIC 25 and 100 µg/mL; revealing strong antibacterial inhibition.	[16] [25]
Antifungal	Leaves	Ethanol: water (7:3)	Disc diffusion method (*Candida albicans, Aspergillus niger)*	+ve:Imazalil (0.02mg/disc)	10µl of the extract (10mg/disc)	The *M .vulgare* ethanolic extract had (MIC of 10mg/ml) and 1.75 mg/ml , MFE of >10 and 2.5mg/ml for an *A.niger, C.albicans* respectively	[16]
Antioxidant antioxidant	Leaves leaves Leaves Leaves	Ethanol: water (7:3) Methanol: water (8:2) Ethanol: Water (1:1) Ethanol: Water (7:3)	In vitro DPPH assay Fenton reagent assay Griess reagent assay In vitro DPPH assay In vitro DPPH assay Ferric reducing antioxidant power method (FRAP)	-ve Blanks +ve: Green tea-standardized extract (EC50 = 4.6 µg/mL) +ve: Ascorbic acid +ve: Ascorbic acid	0-3000µg/mL 2.5-120µg/mL 20-140µg/ml	The strongest antioxidant potential in DPPH of IC_{50} = 13.41µg/ml , IC_{50} = 63.99µg/ml, 64.86µg/ml for, OH^-, NO test systems respectively The extract shows an EC_{50}= 38.56± 0.10µg/mL MAE extract significantly reduced the IC_{50} to 66.28 ± 0.6 µg/mL relative to the 84.14 ± 0.7 µg/mL of the conventional extract *M. vulgare* extract antioxidant activity EC_{50} 4.51 ± 0.5 mg/mL	[17] [12] [22] [16]

Anti-inflammatory	Leaves	Ethanol: water	Carrageenan-induced paw inflammation test	-ve: NaCI (0.9%) +ve:Indomethacin (10mg/kg)	500mg/kg	At 6hrs, *M. vulgare* extract shows a significant reduction in the size of the paw ($p<0.05$), presenting inflammation inhibition %(47.65%)	[26]
Anti-diabetic	Whole plant	Methanol Water butanol	Cyclosporine A-induced autoimmune Diabetes mellitus –type 1	-ve:Normal saline	2mg/ml-methanol and water 1mg/ml-butanol	A significant decrease in total serum cholesterol, serum LDL cholesterol, Serum triglycerides and the activity of IFN-γ, TNF-α by, Nitric oxide (NO) in all extract	*(Maraia et al, 2014)*
Wound healing	Leaves	Methanol: water (8:2)	Wound healing test toward dermal fibroblasts	+ve: Complete medium -ve : Vaseline	5µg/ml	The extract obtained a human cell fibroblast sub-confluent after 24hrs and complete confluence after 48 hrs.	[12]
	Leaves	Ethanol: water (7:3)	Wound healing test toward Burn induced wounds	+ve: Madecassol (1%)	1g of the extract	On day 21, *M. vulgare* extract shows a significant wound Contraction (97.78 ± 4.95%)	[26]
Analgesic properties	leaves	Ethanol: Water (7:3)	Acetic acid method	+ve Tramadol (50mg) - ve: NaCI(0.9%)	500mg/kg	The *M. vulgare* extract had abdominal contractions of (58.8 ± 6.64) and contraction inhibition % (39.62 ± 7.31%)	[26]

+ve: positive control, -vet: negative control, MIC: minimum inhibitory concentration, MFC: minimum fungal concentration, DPPH: 2, 2-diphenyl-1-picrylhydrazyl, OH: hydroxyl ions, NO: nitric oxide, IC_{50} : inhibitory concentration, EC_{50}: effective concentration, LDL: low-density lipoprotein, IFN γ:interferon gamma, TNFα: tumor necros

Conclusion

The biological activities of *M. vulgare* are none exhaustive however more research is needed to be done to get better scientific evidence about the different pharmacological purposes. Hydro-alcoholic extracts of *M. vulgare* have mainly been on the air-dried leaves and aerial parts leaving research gaps for investing in the other parts such as roots and stems. Hydro-alcoholic extracts are highly preferred due to the alcohol-soluble extractive value being higher than other solvents suggestive most plant constituents are soluble in alcohol. Methanol extracts show a better extraction yield compare to ethanol extracts and higher values of the same biological activities. *M. vulgare* extracts are promising prospects in the drug industry therefore, purification of these active compounds and more in vivo experiments should be done.

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this work

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