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Estimation of the Beneficial Potential for Human Health Prevention of *Clinopodium vulgare L.* Based on Variety of Phytochemicals with Different Pharmacological Activities

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Abstract **Original Research Article**

The application of plant products as therapeutic agents is increased worldwide. The importance of medicinal plants as a source of phytocompounds with a variety of therapeutic properties and with benefitial effects in prevention of human health, is enhanced. Herbal products have been applied as an additional therapeutic sourses to traditional medicine in treatment of different diseases. *Clinopodium vulgare L.* (wild basil) is a medicinal plant from the family *Lamiaceae*, distributed all over the world. The plant is known to be used additionally to traditional medicinal drugs administered for different diseases. ***Methods:*** Searching from Medline, Pub Med, Google Scholar, Scopus, Web of Science for chemical composition and pharmacological activity of *Clinopodium vulgare L.* ***Results:*** It has been reported that phytochemicals have been isolated mainly from the aerial parts, such as leaves, flowers, and stems of *Clinopodium vulgare L.* The major triterpenoids identified in plant are betulinic acid, oleanolic acid and ursolic acid, that exhibit antimicrobial, anticancer, anti-inflammatory activity. Phenolcarboxylic acids from extract possess antibacterial properties against gram-positive and gram-negative microorganisms. It has been described that neochlorogenic acid and catechin exerts antitumor action. *vulgare L.* antioxidant potential is a result from high content of phenolic compounds, such as flavonoids. and phenolic acids as caffeic acid, chlorogenic acid, and rosmarinic acid. ***Conclusion:*** From the literature review has been proven the beneficial potential for human health of *Clinopodium vulgare L.* based on different pharmacological effects, such as antibacterial, antiviral, anticancer, antioxidant, anti-inflammatory, antihypertensive and potential for inhibition of enzymes, such as cholinesterase amd cyclooxygenase-2. One of the greatest importance is the proven sinergistic effect of plant extracts for increasing *in vitro* the antimicrobial activity of antibiotics against microorganisms. These combinations can be used as a srategy for improvement of the efficacy of antibiotics and for potential application for decreasing of microbial drug resistance.

Keywords: *Clinopodium vulgare L*. phytochemicals, pharmacological activities, human health, prevention, Bulgarian products with extraxt of *Clinopodium vulgare L*.

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INTRODUCTION

Medicinal plants exhibit different therapeutic properties and provide important benefits due potential to prevent human health and to be applied as an additional source to traditional medicine in therapy of different diseases [1]. The investigation and application of herbal products in different forms as therapeutic agents are increased worldwide [2]. Phytotherapy advantages include that products are less expensive than pharmaceutical drugs, are harmless and well tolerated by the body with better ratio of benefit to risk [3]. Phytocompounds comprise alkaloids, flavonoids, polyphenols, saponins, tannins, and terpenoids, and possess pharmacological effects against different diseases. Terpenoids from essential oils exert antibacterial, anticancer, and immunomodulatory activity [4].

I. Distribution in geographic regions of *Clinopodium vulgare L.*

Clinopodium vulgare L. (wild basil) (Figure 1.) is a medicinal plant, belonging to the family *Lamiacea* and is distributed in Europe in British Isles, Scandinavia, Balkans, and Mediterranean region [5].

The plant is growing in a large region from South Asia in Nepal to western Asia through the Afghanistan, Caucasus, Iran, Iraq, Türkiye, and can be found in the western Himalayas in Pakistan and northern part of India. *Clinopodium vulgare L.* is known to be native to North Africa in Algeria, Morocco, and Tunisia [5].

The plant has become naturalized in North America in Canada and in the United States, and is spread too in South America in southern Brazil and in New Zealand [5].

Figure 1: Clinopodium vulgare *L*

II. Chemical compounds in *Clinopodium vulgare L.*

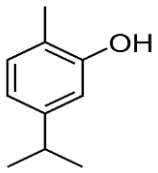
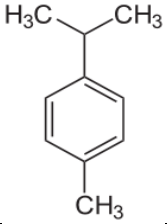
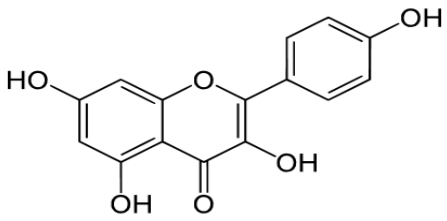
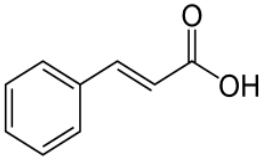
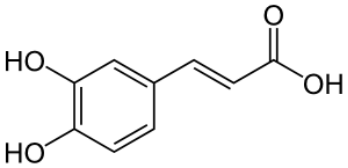
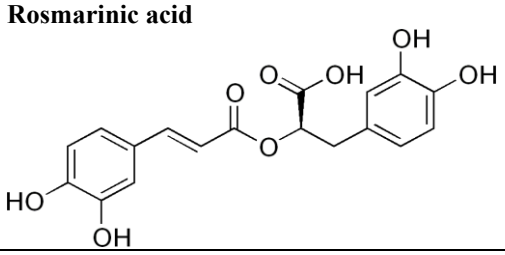
In many phytochemical studies has been investigated the presence of different chemical compounds in extracts form *Clinopodium vulgare L.* It has been studied that phytochemicals have been isolated mainly from its aerial parts, including the flowers, leaves, and stems [6].

The most important compounds are flavanoids, such as apigenin and kaempferol [7] and phenolcarbonic acid [8], such as clinopodic acids (A–Q), salvianolic acids [9], caffeic acid, chlorogenic acid, *trans*-cinnamic acid, rosmarinic acid [10], *p*-coumaric acid, ferulic acid [7,8]. Important compounds are polyphenols [11], triterpenes [12], and iridoids [9].

From the essential oil of plant by GC-MS have been analysed monoterpenes borneol, camphene, carvacrol, *p*-cymene, *α*-pinene, *β*-pinene [13,14]. The major triterpenoids identified in *Clinopodium vulgare L.* include betulinic acid from lupane type, oleanolic acid from oleanane type and ursolic acid from ursanae type, which exhibit antimicrobial, anticancer, anti-inflammatory effects. Other constituents are chlorophyll, carotenoids. fatty acids, polysaccharides, phytosterols as β-sitosterol, and tannins [9].

In Table 1. are presented chemical structures of some important compounds from *Clinopodium vulgare L.*

Table 1: Chemical structures of some componnds from *Clinopodium vulgare L.*

Carvacrol 	***p*-Cymene** 	**Kaempferol** 
Cinnamic acid 	**Caffeic acid** 	**Rosmarinic acid** 

Some of the phytochemicals in *Clinopodium vulgare L.* are summarized in clemical classess in Table 2.

Table 2: Phytochemicals in *Clinopodium vulgare L.*

Flavonoids	Phenolic acids	Essential oil Monoterpenes	Essential oil Sesquiterpenes
Apigenin	Caffeic acid	Borneol	β-Caryophyllene
Diosmetin	Chlorogenic acid	Camphene	Caryophyllene oxide
Hyperoside	Cinnamic acid	Carvacrol	Germacrene D
Isoquercitrin	p-Coumaric acid	p-Cymene	α-Humulene
Kaempferol	Ferulic acid	α-Pinene	Limonene
Luteolin	Gallic acid	β-Pinene	Linalool
Naringenin	Protocatechuic acid		Spathulenol
Quercetin	Rosmarinic acid		
Rutin	Clinopodic acids (A–Q)		

III. Pharmacological effects of *Clinopodium vulgare* L.

Clinopodium vulgare L. is known to be used as antibacterial [15,16], antiviral [9], and anticancer [10,17,18]. Other different types of biological actions, such as antioxidant [10,11,14] and anti-inflammatory [19] have been confirmed. It has been demonstrated that plant possess enzyme-inhibitory action against enzymes, such as cholinesterase [11] or can modulate cyclo-oxygenase-2 expression [7]. The plant has been applied topically for the treatment of skin diseases and can support cardiovascular and liver health [9].

III.1. Antibacterial activity of *Clinopodium vulgare L.*

It has been evaluated that phenolcarboxylic acids exert bacteriostatic properties [20]. The antibacterial activity of ethanol extract has been observed and has been proven that the plant exhibit very strong effect towards gram-positive and gram-negative microorganisms [15].

It is known that bacterial resistance is a great problem in application of antibiotics. One of the important strategies for decreasing of bacterial resistance is the development of effective combinations of different types of therapeutics, which can provide synergistic interaction, leading to increase of pharmacological potential. From different studies has been shown the sinergistic activity of plant extracts for enhansing *in vitro* the efficacy of antibiotics against microorganisms.

It has been reported that the most sensitive bacteria to extract of *Clinopodium vulgare l.* are gram-positive bacteria Steptococcus aureus ATCC 25923 and Bacillus subtillis. Synergistic action by the combinations of plant ethanol extracts with gentamicin or with cephalexin towards Bacillus subtillis has been described. It has been investigated that against Klebsialla pneumoniae, which is known to be resistant to cephalexin, the combination of acetone extract from plant and cephalexin can improve the effect of antibiotic [16].

It has been studied that for the extract obtained from the plant *Clinopodium vulgare*. *vulgare subsp. vulgare L* has been confirmed moderate antibacterial potential against Mycobacterium smegmatis and the Gram-positive bacteria Streptococcus aureus and Bacillus cereus. It has been demonstrated antimicrobial potential of plant towards the Gram-negative bacteria Yersinia pseudotuberculosis. In evaluations for antimycotic action against the Candida albicans and Saccharomyces cerevisiae did not observe antifungal activity. In other experiments it has been proven that aqueous and ethanolic extracts of plant show little effect towards Klebsiella pneumoniae and Proteus vulgaris [21].

In comparative evaluation of aqueous, methanolic, and ethanolic extracts of *Clinopodium vulgare L. subsp. vulgare* the following results have been summarised:

1. it has been reported that against Salmonella typhimurium the best antibacterial action has been obtained with ethanol extract and this potential has been stronger in comparison in the same conditions of the experiments with the activity of reference antibiotics Penicillin G 10 units and 10 μg Ampicillin
2. it has been described that for the ethanol extract has been investigated to be more effective towards Proteus vulgaris than action of Penicillin G 10 units.
3. it has been determined that for the studied ethanol extract has been confirmed to possess higher activity towards Candida albicans and Candida tropicalis and for methanol extract has been proven to be more effective against Candida krusei in comparison wih antifungal effect of 10 mg Clotrimazole [21].

III.2. Anticancer activity of *Clinopodium vulgare L.*

Aqueous extract from plant possess cytotoxic effect against HeLa (cervical carcinoma), HT-29 (colorectal carcinoma) and MCF-7 (mammary carcinoma). It has been reported that neochlorogenic acid and catechin are important for antitumor activity [10].

It has been descrited that aqueous extract of *Clinopodium vulgare L.* exhibits *in vitro* good antitumor effect against different cancer cell lines, such as:

HEp-2 - human larynx epidermoid carcinoma –

IC_{50} = 10 µg/ml
L5178Y - wild-type mouse lymphoma cells –
IC_{50} = 17.8 µg/ml for
A2058 - human metastatic melanoma –
IC_{50} = 20 µg/ml

The lower values for inhibitory concentration IC_{50} correlate with the higher potential. The determined data show that extracts exert larger activity towards HEp-2 in comparison with other investigated cells [18].

The *in vitro* effects of acidified, and lipophilic extracts of *Clinopodium vulgare L.* have been studied against CaOV (human testis cystadenocarcinoma), HeLa (human cervical adenocarcinoma). It has been confirmed that extracts exert stronger effect towards human testis cystadenocarcinoma (IC_{50} = 225 µg/ml) compared with activity against HeLa cells (IC_{50} = 360 µg/ml) [19].

It has been demonstrated that in lung cancer cells *Clinopodium vulgare L* extract can induce apoptosis [22].

In comparative study of aqueous, methanolic, and ethanolic extracts of *Clinopodium vulgare L. subsp. vulgare* the following data have been obtained:

1. it has been evaluated that all extracts suppress the growth of prostate cancer cells PC-3 in dose- and time dependent manner, due to lower values of IC_{50} aftet 48 h exposure in comparison with 24 h exposure
2. it has been observed that after 48 h exposure the antiproliferative activity of extracts is increased in the following order: ethanolic (IC_{50} = 78.9 µg/ml), methanolic (IC_{50} = 189.1 µg/ml), aqueous (IC_{50} = 114.6 µg/ml)
3. from that data is prowen the strongest anticancer effect of ethanolic, followed by methanolic and aqueous extracts
4. the mechanism of action includes the activation in PC-3 prostate cancer cells thel death pathways through the elevation of proteins, such as caspase-8 which promotes the external receptor-dependemt apoptosis and and caspase-9, which starts the internal mitochondria-controlled cell death process [21].

III.3. Antioxidant activity of *Clinopodium vulgare L.*

It has been reported that the antioxidant potential of the extracts of *Clinopodium vulgare L.* [14,23] is a result from the radical scavenging propertiers of polyphenols in plant [11]. It has been desctibed hat essential oil from *Clinopodium vulgare L.* exhibits significant antioxidant activity, which is a result of high content of phenolic compounds, such as caffeic acid, chlorogenic acid, rosmarinic acid, flavonoids. Antioxidant capacity has been demonstrated by *in vitro* radical scavenging methid as DPPH, ABTS, FRAP [14].

III.4. Antiinflammatory and enzyme inhibitory activities of *Clinopodium vulgare L.*

Inflammation is associated with oxidative stress. It is known that the suppression of xanthine oxidase can lead to the decreasing of oxidative stress. It has been demonstrated that aqueous extracts of *Clinopodium vulgare* L. exerts antiimflammatitory activity by the following mechanisms:

1. decreasing of reactive oxygen species by inhibition of xanthine oxidase
2. reduction of the levels of inflammatory cytokines, such as interleukines IL-10 and IL-10 [19].

It has been investigated that *in vitro* and *in vivo* *Clinopodium vulgare L.* extract, exert enzyme inhibitory activity [23]. It has been observed that caffeic and chlorogenic acids suppress cyclooxygenase-2 (COX-2) expression in bone marrow neutrophils [7]. Anticholinesterase potential has beeu evaluated too [11].

On the pharmaceutical market are available Bulgarian products as supplements over the counter with included a standardized extract of *Clinopodium vulgare L.* (Cat's Claw), which have been tested and are proven to have a beneficial effect for prevention and as a support for the healing process in a number of diseases.

In Figure 2. are presented supplements of Cat's Claw in forms of syrups and capsules for free radical protection.

Figure 2: Cat's Claw supplements in forms of syrups and capsules

Some of effects which can provide these supplements include:

1) Antioxidant protection.

Antioxidants help for neutralization of free radicals in the body, and by this mechanism protect cells from oxidative stress and can reduce the risk of chronic diseases, support natural antioxidant mechanisms and slows down the aging process

2) Strengthening the immune system.

Due to exhibit immunostimulating properties the product increases the immune response by supporting of natural immune functions and T-cell activity, which results in an enhanced effectivity in fighting infections and contributes to reducing the feeling of weakness and fatigue.

3) Stimulating of processes in the body.

Activation of regenerative processes
Helps maintain a stable DNA structure
Favors the normal function of cell division and physiological cell cycle
Supporting the metabolism and energy production.

In Figure 3. are illustrated supplements with *Clinopodium vulgare L.* in forms of tinctures.

Figure 3: Supplements with *Clinopodium vulgare L.* in forms of tinctures

Pharmacological activities of these supplements include:

1. Respiratory system

Tinctures relieve respiratory problems, such as coughs and bronchitis due to expectorant properties.

2. Digestive system

Supplements improve digestive problems such as bloating, and stomach discomfort and can stimulate appetite.

3. Nervous System

The plant has a mild calming effect that can help reduce stress and anxiety.

4. **Anti-inflammatory properties**

Cat's Claw contains compounds with anti-inflammatory properties that can help with various inflammatory conditions, including arthritis, muscle pain, and inflammatory diseases.

IV. CONCLUSION

Clinopodium vulgare L. exhibit different pharmacological activities, such as antibacterial, antiviral, anticancer, antioxidant, anti-inflammatory, and potential for inhibition of enzymes, such as cholinesterase and cyclooxygenase-2. These multiactivities are a beneficial potential for human health, providing possibilities for combined administration with traditional medicinal drugs for simultaneous activity against different targets in various diseases. The observed sinergistic effect of plant extracts for increasing *in vitro* the antimicrobial effect of antibiotics agains microorganisms is one of the greatest importance. These combinations can be applied as a srategy to potential application for decreasing of microbial drug resistance by improvement of the efficacy of antibiotics by plant synergistic effect.

Abbreviations

A2058	human metastatic melanoma
CaOV	human testis cystadenocarcinoma
COX-2	cyclooxygenase-2
HeLa	cervical carcinoma
HT-29	colorectal carcinoma
IC_{50}	inhibitory concentrartion
IL	interleukins
L5178Y	wild-type mouse lymphoma
MCF-7	mammary carcinoma
PC-3	prostate cancer

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statements

- The authors declared that no clinical trials were used in the present study.
- The authors declared that no experiments on humans or human tissues were performed for the present study.
- The authors declared that no informed consent was obtained from the humans, donors or donors' representatives participating in the study.
- The authors declared that no experiments on animals were performed for the present study.
- The authors declared that no commercially available immortalized human and animal cell lines were used in the present study

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Author contributions: All authors have contributed equally.

Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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