The Journal of Medical Research

Research Article

JMR 2022; 8(1):19-22
January- February
ISSN:2395-7565

www.medicinearticle.com
Received:17-12-2021
Accepted:29-01-2022

Phytobiotic Potential of Flower Essential Oil of *Melissa officinalis* on Multi-Drug Resistance Bacteria common to Human and Animal

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ABSTRACT

Essential oils are natural therapeutic agents, as they are rich sources of terpenoids and polyphenols. This study was aimed at evaluation of the synergistic potential of the phytochemicals in the essential oil obtained from the flower of *Melissa officinalis* on clinically isolated uropathogens. The research led to identification of various organic compounds from the essential oil of the vegetable part investigated. The main constituents identified were: phytol (49.9%), τ-muurolol (13.0%), l-calamenene (8.8%), octyl butyl phthalate (8.6%), β- ionol (4.6%), and thymol (4.0%). In the antibacterial study, the highest inhibitory effect of the essential oil was observed against *P. aeruginosa* (20 mm), The results indicated that the flower essential oil has high antimicrobial potential and could be alternative sources of natural therapeutic agents. The sample can be exploited as natural antimicrobial and antioxidant in real pharmaceutical and pharmacognosy systems.

Keywords: *Melissa officinalis,* Flower Essential Oil, Secondary Metabolites, Phytobiotic, Pathogen.

INTRODUCTION

Essential oils derived from plants are important source of medicinally active substances industrially used in drugs and foods productions as preservatives and addictives [1-3]. Essential oils remain the dominant form of therapy in most countries. Use of synthetic drugs and foods additives imposed some dangerous side effects, therefore, the need for alternative and safe natural preservatives and addictives with antioxidant properties. Essential oils are well known to be used as natural antioxidant agents, phytobiotics, antimicrobial agents as they are rich sources of terpenoids and polyphenols [4]. The presence of phenolic compounds in essential oils is oftentimes responsible for its potent antioxidant and antimicrobial properties [5,6]. Essential oils are natural products with abundant polyphenols which are good antioxidant [4]. Essential oils have long been known to in treatment of respiratory and microbial health problems. Many scientific studies have shown that they have activities against SARC-CoV-2 virus. Due to the lipophilic nature of essential oils they have ability to penetrate viral membranes easily leading to membrane disruption. Moreover, essential oils contain multiple active secondary metabolites that can act synergistically on multiple stages of viral replication and also induce positive effects on host respiratory system including bronchodilation and mucus lysis [7]. Based on the high antimicrobial and antioxidant ability of these natural products, they can be beneficial for healing all types of wounds. Pharmacological potential is the primary essential measure for assessing the health benefits of natural products for humans and animals. Recently, the idea that natural products are safer than commercially produced synthetic drugs has acquired traction and contributed to a massive rise in phytopharmaceutical applications [6,8]. Epidemiological studies have shown that the plants abundant in active secondary metabolites with antioxidant and antimicrobial properties can be exploited for bioactive pharmaceutical compounds. The pharmacology and pharmacognosy activities of medicinal plants is generally studied with respect to phytochemicals such as polyphenol content and their free radical scavenging assays as they may be responsible for various medicinal potential [9-11]. Natural products from plants are globally known as effective detoxifying agents, daily consumption of fresh vegetables and fruits help in discharging of toxins and waste from body of human and animals [12]. The fruit and leaves of medicinal plants are loaded with soluble fiber and antioxidants, making them excellent laxative. The plant bears some amazing medicinal qualities, like serving as an insecticide and abortifacient [11,13,14]. Natural plant extracts are used as herbal medicine for the treatment of human infectious diseases. Globally, there is an increase in prevalence of infectious diseases caused by bacteria as a major public health problem [15-19]. Medicinal plants and their metabolites are the natural sources to be used as antimicrobials. Researchers have reported the medicinal use of phytochemicals as natural sources to be used as antimicrobial agents against different types of bacteria pathogens, including food borne pathogens [20,21].

Melissa officinalis is a member of the Lamiaceae family. It is a perennial herbaceous plant which vastly cultivated worldwide for its nutritional and therapeutic properties. The leaves with lemon scent and taste

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are used as herbal drug, because of its active component. It produces white/pink pale flowers annually; it possesses hairy root system with many lateral roots [21,22]. Pharmacologically, the plant is used as natural anti-oxidative, anti-cancer, anti-oedema, anti-obesity, anti-spasmodic agent [23]. It was reported that the medicinal uses of the plant gives no side effects; it can be used topically or orally in recommended doses for a month in otherwise healthy adults and poses no danger when consumed in amounts recommended in foods. Vegetable has been generally regarded as safe (GRAS) foods and drugs substances globally. Many studies confirmed its anti-oxidative effects; therefore, its potential in preventing and treating oxidative stress-related diseases [24,25]. The aim of this study was to evaluate the synergistic potential of the phytochemicals in the flower essential oil of *Melissa officinalis* as phytobiotic agents on uropathogens commonly affecting human and animals.

MATERIALS AND METHODS

Collection of Sample

The samples used in this study were collected from Ota, Nigeria and they were identified as *Melissa officinalis.*

Preparation of Flower Essential Oil

The fresh flowers were pulverized and the essential oil was obtained by hydro-distillation using all-glass Clevenger-type apparatus [26]. The essential oil collected was then stored in vial in a refrigerator to prevent evaporation.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The chemical composition of the essential oil was analysed using a coupling system of Shimadzu QP2010 series gas chromatography with Shimadzu QP2010 plus mass spectroscopy detector (GC-MS) [3].

In vitro Agar-Well Antibacterial Assay

The antimicrobial potential of the essential oil solutions of different concentrations (1000, 500 and 250 µgml-1), of the essential oil against all the strains tested *(Streptococcus agalactiae, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhimurium and Serratia marcescens),* was determined using the agar-well diffusion technique [27]. as described by CLSI guidelines. The plates were allowed to stay in a refrigerator for 1 hour to allow proper diffusion of the extract solution into the medium. A synthetic antibiotic nitrofuratoin (300 µg/disc), was used as a positive control. The plates were then incubated at 37℃ for [18-24]. h before visual assessment of the inhibition zones. The zone of inhibition was measured to the nearest size in millimetre (mm) using a ruler [27,28].

RESULTS AND DISCUSSION

Chemical Constituent of the Flower Essential Oil of *Melissa officinalis*

A total of fourteen [14]. chemical components were identified in the essential oil obtained from the flower of *M. officinalis,* accounting for (99.2%), of the total components in the extract (Table 1), and the main constituents identified were phytol (49.9%), τ-muurolol (13.0%), l-calamenene (8.8%), octyl butyl phthalate (8.6%), β- ionol (4.6%) and thymol (4.0%). Phytol (3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol), is an acyclic monounsaturated diterpene alcohol, present in vitamin E, K and other tocopherols. Phytol is an odoriferous compound present in the essential oil and is often used in much fragrance, cosmetic and non-cosmetic substances. Phytol is a major component of some essential oils obtained from medicinal plants used as a fragrance and a potential candidate for a broad range of applications in the pharmaceutical and biotechnological industry. Therapeutically, phytol has shown many pharmacological and pharmacognosy potential such as antioxidant, anti-cancer, anti-inflammatory, anxiolytic, metabolism-modulating, cytotoxic, antioxidant, antinociceptive, immune-modulating, antinociceptive, anti-allergic, antimicrobial, autophagy- and apoptosis-inducing activities. Researchers have reported that phytol is an excellent immunostimulant, superior to a number of commercial adjuvants in terms of long-term memory induction and activation of both innate and acquired immunity [29-31].

Table 1: Chemical Composition of the Flower Essential Oil of *M. officinalis*

Compound	Retention Index	Percentage Composition
2,2-dimethylbutane	533	0.5
2,2,5,5-tetramethylhexane	846	1.0
1-chlorooctane	1042	0.8
thymol	1262	4.0
3-fluoro-5-trifluoromethylbenzoic acid, neopentyl ester	1296	1.3
cis-muurola-4(14),5-diene	1435	1.5
β-sesquiphellandrene	1446	2.6
β- ionol	1484	4.6
ethyl 4-isopropenyl-6-methyl-2-oxo-6-heptenoate	1487	0.8
l-calamenene	1537	8.8
τ-muurolol	1580	13.0
1-(1,3a,4,5,6,7-hexahydro-4-hydr oxy-3,8-dimethyl-5-azulenyl)-ethanone	1758	2.0
phytol	2045	49.9
octyl butyl phthalate	2434	8.6
Percentage Total		**99.4**

Antimicrobial Activities

The antimicrobial activities of the flower essential oil of *M. officinalis* against Gram-positive bacteria and Gram-negative bacteria were shown in figure 1-2, The essential oil showed variable activities against tested bacteria at different concentrations. The essential oil was effective on all bacteria tested. The highest inhibitory effect of the essential oil was observed against *P. aeruginosa* (20 mm), as depicted in Figure 1. Other highly susceptible bacteria at 1000 - 250 µgml^{-1} were *S. aureus* (13 mm), *S. marcescens* (11 mm), *S. agalactiae* (10 mm), and *S. typhimurium* (10 mm), *S. aureus* only showed resistance to the essential oil solution at 250 µgml^{-1}. The activity nitrofuratoin (300 µg), (a synthetic antibacterial disc), was shown in figure 2. The zones of inhibition of nitrofuratoin ranges between 15-19 mm on the tested bacteria. It is noteworthy, the flower essential oil have similar activities compared to the standard antibiotic used in this study as positive control. Comparatively, the flower essential oil from *M. officinalis* investigated in this study has antibacterial potential compared to the leaves essential oil of *Rosmarinus officinalis* from Ethiopia against *Escherichia coli* and *Staphylococcus aureus* with the zones of inhibition ranging between (10-13.7), mm [32].

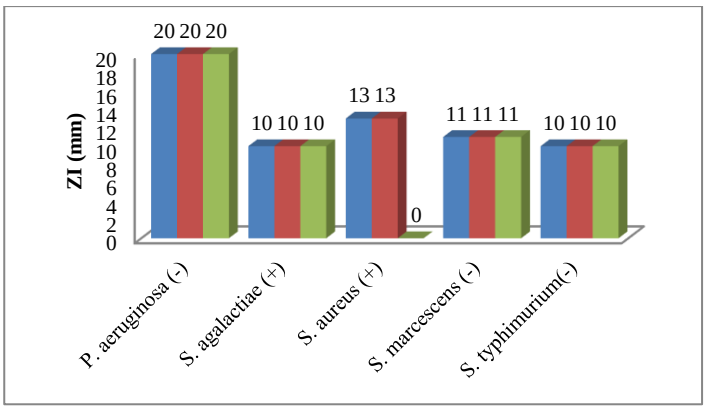

Figure 1: Zones of Inhibition (mm) showing the Antibacterial Activity of Flower Essential Oil of against Gram-positive and Gram-negative bacteria

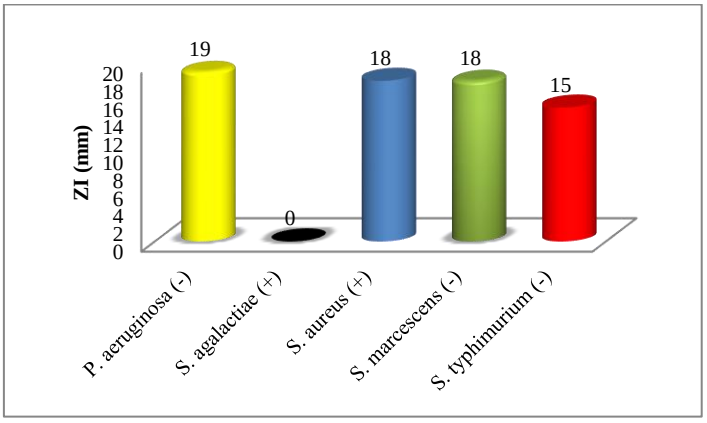

Figure 2: Zones of Inhibition (mm) showing the Antibacterial Activity of Nitrofuratoin (300 µg) of against Gram-positive and Gram-negative bacteria

The upsurge in the prevalence of side effects of many synthetic antimicrobial agents and incidence of multi-drug resistant bacteria and pests has triggered researchers onto the studies for plant based antimicrobial and antioxidant of therapeutic and pesticidal potential. It is noteworthy that secondary metabolites in essential oils from odoriferous medicinal vegetables can act as phytobiotics, antibiotic natural alternative and direct antioxidants in human and animals (poultry) by blocking ROS generation and therefore can inhibit the planned cell death pathways [11,33].

CONCLUSION

This study led to identification of various organic compounds from the essential oil of the part of vegetable investigated, terpenoids and phenolic compounds were found to be the major constituents of the essential oil. Medicinal potential of flower of this medicinal flower was established. The results indicated that the plant has high medicinal potentials and they could be alternative sources of natural therapeutic agents. It has been ascertained in this study that the part of the plant investigated possess useful medicinal organic compounds with excellent therapeutic potential. However, further studies on the clinical trials and investigation of the mechanisms by which the plant exert their medicinal activities. Individual phytochemicals responsible for antimicrobial activities of the plant should be isolated and characterized. Modes of antibacterial activities of these phytochemicals should be clinically studied for broader application. Therefore, more studies are essential to diagnose clinical effectiveness and potential of the phytochemicals identified in the essential oil investigated for antimicrobial in a safe dose. The sample can be exploited as natural antimicrobial and antioxidant in real pharmaceutical and pharmacognosy systems.

Conflicts of interest

None declared.

Financial support

None declared.

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