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Evaluation of Secondary Metabolites and Antibacterial Potency of the Root Extract of *Lemon balm* (Lamiaceae) Vegetable against Multidrug Resistant Bacteria

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Abstract

Lemon balm (Lamiaceae) vegetable is known for its aromatherapeutic properties is being used in curring many diseases. The aim of this study was to scientifically screen the medicinally active secondary metabolites and antibacterial potency of *Lemon balm* vegetable grown in Nigeria. Gas chromatography-mass spectrometry (GC-MS) was used to analyse the extract while the antibacterial screening was carried out using agar-well diffusion method. Thirty-four (34) therapeutically active organic compounds were identified in the root extract with terpenoids and phenolic compounds representing the major class, including 2,3-bis[(9E)-9-octadecenoyloxy]propyl (9E)-9-octadecenoate (24.6%) as the major component, methyl (11E)-11-octadecenoate (11.7%), dipalmitin (10.7%), α-methyl-D-galactopyranoside (7.7%), eudesm-7(11)-en-4-ol (4.4%), 1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol (3.0%), isoborneol (2.9%) and 2,6-cresotaldehyde (2.7%) as well as other minor compounds. The highest inhibitory effect of the root extract was observed against Proteus mirabilis which showed a zone of inhibition of 19 mm. The result evidently showed that the sample have several therapeutically active secondary metabolites that can be applied in the treating bacteria diseases.

Keywords: *Lemon balm*, Lamiaceae, GC-MS, Secondary metabolites, Antibacterial potency.

INTRODUCTION

Medicinal plants in the *Lamiaceae* family are important to humans and animals; they are useful as flavour, fragrance, preservative or therapeutic values. *Lamiaceae* family are known as a major class of odourifeous plant and major sources of essential oils. They are also known for their aromatherapeutic properties and being used to curemany diseases [1-5]. *Lamiaceae* are distributed nearly globally, and many species are cultivated for their medicinal purposes [6, 7]. *Lemon balm* (*Melissa officinalis*) is a lemon-scented perennial vegetable that comes from the family *Lamiaceae*. *Lemon balm* has traditionally been used to improve mood and cognitive function [8, 9]. It is a plant cultivated in some parts of Nigeria and other parts of West Africa. The aerial parts of *Lemon balm* are used traditionally as consumable vegetable as well as for functional gastrointestinal disorders [10, 11]. *Lemon balm* is said to soothe symptoms of stress, helps one to relax, and in boosting the mood. Medicinally, *Lemon balm* has been said to possess mitigative and detoxication potential [12, 13]. Likewise, it is a famous anti-ageing, antioxidant, anti-inflammatory, anti-cancer, menstrual-inducing natural product [2, 14]. *Lemon balm* plant is also used to help reduce symptoms of anxiety, such as nervousness and excitability [15, 16]. It was also reported that *Lemon balm* helps relieve restlessness and sleep disorders such as insomnia. It may help treat cold sores, indigestion, frequent abdominal pain and discomfort. *Lemon balm* vegetable also helps relieve feelings of nausea, minimize menstrual cramps, ease headache pain and lessen toothache pain [17].

Natural products originated from plants most especial edible vegetables have contributed to the quick development of pharmaceutical and food industries. They are sources of cheap raw materials used for the production of fine chemicals, cosmetics, pharmaceuticals, drugs etc [18, 19]. Medicinal plants have been used traditionally in treatment number of chronic diseases such as reactive oxygen/nitrogen related diseases. The natural vegetables are considered as rich resources of phytochemicals, nutrition and as a result of this they are in many cases recommended for their therapeutic values, which makes most drug

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and related industries used them as preservatives, drug development and synthesis [20, 21]. Vegetables have been used as flavouring agents, condiments and natural preservatives, anti-diabetic, anti-oedema, to treat skin diseases and epidemics [22, 23]. The struggle against the wide spread of bacteria and viral infections is still a major concern globally. Pathogenic microorganisms are developing several resistances to existing synthetic antibiotics at an alarming rate by the development of multi-drug resistant (MDR) phenotypes [24, 29]. Globally, food spoilage caused by microorganisms still affects all types of food, causes food waste and loss on a large scale [28, 30]. Food borne disease is another pervasive problems associated with food safety which are traceable to the caused by consumption of contaminated food items, which has been raising major safety questions to public health most especially in the developing countries [31, 33].

The present study was aimed at providing more scientific information on the secondary metabolites and antibacterial potency of *Lemon balm* vegetable grown in Nigeria.

MATERIALS AND METHODS

Identification of the Plant Sample

Fresh sample of the plant investigated was collected at Ota, Ogun State, Nigeria and it was identified as *Lemon balm* (*Lamiaceae*).

Preparation of Extract

The pulverised sample material was extracted for at least 3 days by maceration using methanol and ethyl acetate (2:1) at room temperature with periodical shaking. After filtration, the solvent was evaporated using Uniscope water bath. The concentrated extract was refrigerated until used. The extraction and dilution procedures were protected from contamination [2].

Phytochemical Analysis using Gas Chromatography–Mass Spectrometry

The qualitative and quantitative analysis of the extract was carried out by using GC-MS QP2010 Plus (Shimadzu, Kyoto, Japan) system at the Shimadzu Training Centre for Analytical Instruments (STC) Lagos, Nigeria. The analytical specifications of the GC-MS was used as in our previously study.

Screening of *In vitro* Antibacterial Properties: Agar well diffusion assay

The antimicrobial potential of the extract obtained from *Lemon balm* vegetable against some clinically isolated MDR bacteria strains was determined using the Agar-well diffusion technique as described by CLSI guidelines [2, 8]. Three Gram positive and four Gram negative bacteria were employed in the antibacterial test. The Gram-positive bacteria were *E. faecalis, S. agalactiae, S. aureus* while the Gram-negative bacteria were *E. coli, K. pneumonia, P. mirabilis* and *S. typhimurium.* 0.5 McFarland solution was used to inoculate the agar plates. The extract was loaded onto the sterile wells at a concentration of 1000, 500 and 250 $\mu g ml^{-1}$. Positive control for the experiment was antibioticdiscs containing standard antimicrobial agents; Nitrofurantion (300 µg per disc).

RESULTS AND DISCUSSION

The chemical composition of the root methanolic/ethyl acetate extract of *Lemon balm* vegetable isdepicted in Table 1. Thirty-four (34) organic compounds were identified in the root extract with terpenoids and phenolic compounds representing the major class, including2,3-bis[(9E)-9-octadecenoyloxy]propyl (9E)-9-octadecenoate (24.6%) as the major component, methyl (11*E*)-11-octadecenoate (11.7%),dipalmitin (10.7%),*α*-methyl-D-galactopyranoside (7.7%), eudesm-7(11)-en-4-ol (4.4%), 1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol (3.0%), isoborneol (2.9%) and 2,6-cresotaldehyde (2.7%)as well as other minor compounds. It is worth mentioning that this is, in fact, the first time a chemical analysis of the extract from the root part of *Lemon balm* vegetable grown in Nigeria will be reported.

Table 1: Chemical composition of the root extract of *Lemon balm* vegetable

Compound	Retention Index	Percentage Composition
dimethyl sulfoxide	691	0.1
2-propen-1-ol	552	0.3
but-3-enyl (*E*)-2-methylbut-2-enoate	1058	1.0
2-isopropylcyclohexanol	1103	0.3
1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol	1138	3.0
p-hydroxyphenol	1122	1.0
isoborneol	1140	2.9
DL-dihydro-3-hydroxy-4,4-dimethyl-2(3H)-furanone	1148	1.0
3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	1269	0.3
syringol	1279	1.0
p-vinylguaiacol	1293	1.1
β,β′-iminodipropionitrile	1307	1.0
2,6-cresotaldehyde	1316	2.7
aromandenrene	1386	0.5
patchouli alcohol	1420	1.3
β-eudesmene	1464	1.2
(+)-*δ*-cadinene	1469	1.5
isocaryophyllene	1494	1.0
viridiflorol	1538	0.5
3-(4-hydroxybutyl)-2-methylcyclohexanone	1554	1.3
α-humulene	1579	1.7
tau-muurolol	1580	2.4
α-cadinol	1663	2.6
eudesm-7(11)-en-4-ol	1647	4.4
α-methyl-D-galactopyranoside	1714	7.7
palmitic acid, methyl ester	1878	2.2
methyl (11*E*)-11-octadecenoate	2085	11.7
cis-9,*cis*-12-octadecadienoic acid	2183	0.4
dihydro-torulosol	2269	0.7
cis-8,11,14-eicosatrienoic acid	2390	0.7
palmitic acid-β-monoglyceride	2498	2.0
glycerol 2-monooleate	2705	2.2
methyl melissate	3270	2.6
dipalmitin	4013	10.7
2,3-bis[(9E)-9-octadecenoyloxy]propyl (9E)-9-octadecenoate	6149	24.6
Percentage Total		**99.6**

***In vitro* Antimicrobial Activities**

The antimicrobial activities of the root of *Lemon balm* against Gram-positive bacteria and Gram-negative bacteria are shown in figure 1-2. The root extract showed variable activities against the tested bacteria. The extract showed varying levels of sensitivity on all bacteria tested. The activities ranged as follows: Resistant (-), not sensitive (<8 mm), sensitive (9–14 mm), very sensitive, (15–19 mm) and ultrasensitive (>20 mm). The highest inhibitory effect of the root extract was observed against *P. mirabilis* (19 mm) at 1000 μgml^{-1} as depicted in figure 1. Other organisms tested such as *E. faecalis* (+), *S. agalactiae* (+), *S. aureus* (+), *E. coli* (-), *K. pneumonia* (-), *P. mirabilis* (-), *S. typhimurium* (-) have very low sensitivity to the extract. The tested bacteria were found to be sensitive to Nitrofurantion (300 µg per disc) synthetic antibiotics. The variation in the activities the sample towards Gram-negative and Gram-positive bacteria is due to the differences in their cell wall structure. The cell wall of Gram-positive bacteria consists of a thick outer peptidoglycan layer (70–100) which comprises of two polysaccharides, N-acetyl-glucosamine and N-acetyl-muramic acid cross-linked by peptide side chains and cross bridges [34, 35]. This outer layer of Gram-positive bacteria is not an effective permeability barrier which makes it to be more susceptible to the sample while Gram-negative bacteria have a thin peptidoglycan layer plus an outer phospholipidic membrane unlike Gram-positive bacteria[36,37]. Resistance from some Gram-negative bacteria used against the sample may be due to the secretion of the lactamase enzyme in the periplasmic space between the thin outer membrane and the cytoplasmic membrane[38-40]. *Proteus mirabilis* is well-known for its potential to robustly swarm across surfaces in a striking bulls'-eye pattern [41]. *P. mirabilis* is clinically a major cause of respiratory tract infections, community-acquired and catheter-associated UTI, cystitis, pyelonephritis, meningitis, prostatitis, wound infections, burn infections, chronic suppurative otitis media, eye infections etc. The understanding of the mechanism of microbial action of natural products from vegetables is the first step in the optimal utilization of the secondary as natural antimicrobial agent [42, 45].

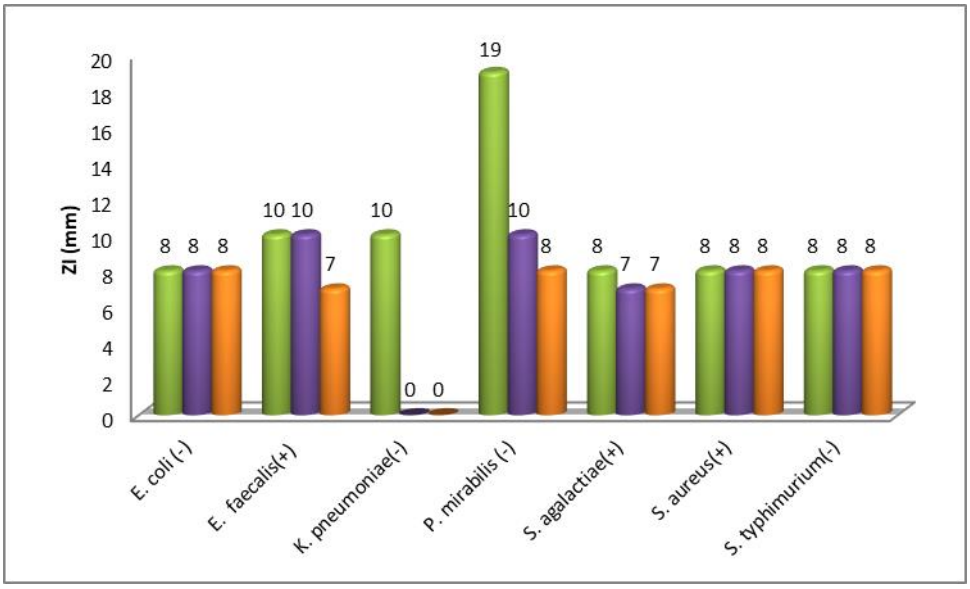

Figure 1: Zones of inhibition (mm) showing the antimicrobial potential of root extract of *Lemon balm* vegetable against gram-positive and gram-negative bacteria

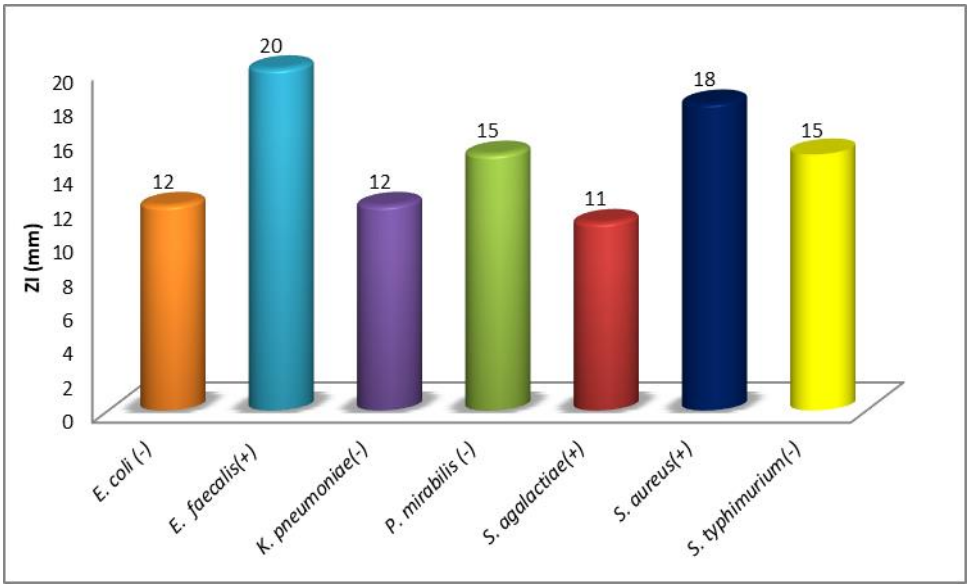

Figure 2: ZI (mm) showing the nitrofurantoin (300 µg per disc) susceptibility test on the gram-positive and gram-negative bacteria

CONCLUSION

GC-MS analysis of the root extract of *Lemon balm* showed the presence of the therapeutically active compounds. The *Lemon balm* root extract had a good potential for health uses against some pathogens. The extract showed antimicrobial activity, particularly against *Proteus mirabilis*. The potency of the investigated sample on the test bacteria was due synergistic potential of the concern secondary metabolites present in the extract. The promising antimicrobial activity of this pharmacologically active sample needs further multipronged study to implement its use as a novel therapeutic agent for treating ailments with drug resistant pathogens.

Conflict of Interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of research reported.

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