



EVALUATION OF PROBIOTIC POTENTIALS OF ESSENTIAL OILS FROM FIVE NASARAWA STATE SPICES ON HUMAN GUT MICROBIOTA

***DENNIS DOGARA YAKUBU; **PROF. C.U UMEDUM; & ***PROF. S.A DIMEJESI**

*Department of Microbiology, Tansian University Umunya Anambra State, Nigeria. ORCID No; 0000-0000-4626-7646. **Department of Medical Laboratory Science, Chukwuemeka Odumegwu Ojukwu University. Anambra State, Nigeria. ***Department of Microbiology, Tansian University Umunya Anambra State, Nigeria.

Corresponding Author: yakubudennis@gmail.com

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Abstract

Plant-derived medicines are an integral part of complementary and alternative medicine worldwide that are used for therapeutic purposes by up to 75% of the general population. This research, titled "Probiotic

Keywords: Probiotics, Essential oils, Nasarawa State Spices, Gut microbiota, GC-MS, Nutraceuticals.

Potentials of Essential Oils from Selected Nasarawa

INTRODUCTION

Background of the Study

Plants in nature have served as a reservoir for therapeutic agents for thousands of years, and since the beginning of man. In Nasarawa State in Nigeria, almost all plants are medicinal and exploitation of their uses particularly in indigenous medicine is very well known and established profession (Arshao *et al.*, 2024).

Medicinal plants also have applications as pharmaceuticals, nutraceuticals, cosmetics and food supplement. Plant-based ingredients, which were used for therapeutic purposes from time immemorial, have proved to be useful. *Zingiber officinale* (Ginger) *Allium sativum* (Garlic) and many other herbs are among medicinal plants in traditional medicine. Due to the emergence of antibiotic-resistant strains of microorganism, attention is being diverted toward the use of traditional plants for their

State Spices on the Human Gut Microbiome,” investigated the biological and therapeutic effects of essential oils (EOs) extracted from Clove (*Syzygium aromaticum*), Ginger (*Zingiber officinale*), Onion (*Allium cepa*), Garlic (*Allium sativum*), and Turmeric (*Curcuma longa*) on gut microbiota using in-vitro models. Essential oils were extracted through hydro-distillation and steam, with yields ranging from 1.5–1.7% (highest in Onion oil). GC–MS analysis revealed major bioactive compounds—Eugenol in Clove, Zingiberene and Citral in Ginger, Diallyl disulfide/trisulfide in

Garlic and Onion, and α/β -Turmerone in Turmeric—indicating rich phytochemical diversity and strong bioactivity potential. Microbiological analyses assay of fecal samples identified diverse gut bacteria, including beneficial (*Lactobacillus*, *Bifidobacterium*, *Akkermansia*) and pathogenic (*E. coli*, *Salmonella*, *Staphylococcus*) species. Treatment with graded EO concentrations (0.1–1.0%), revealed beneficial bacteria maintained high viability (93–99%), while pathogenic strains showed significant inhibition (40–74%), particularly under Garlic

and Clove oil treatments. Statistical analyses (ANOVA, $p \leq 0.05$) confirmed significant effects across treatments, except for Onion oil, which showed minimal activity. Overall, the study demonstrates that essential oils from five (5) Nasarawa State spices possess probiotic potentials, capable of enhancing beneficial gut flora, suppressing pathogens. The findings provide a scientific foundation for developing natural nutraceuticals and functional foods to support gut health.

antibacterial and medicinal properties (Chouchan *et al.*, 2017). Plant-derived medicines are an integral part of complementary and alternative medicine worldwide that are used for therapeutic purposes by up to 75% of the general population. The use of plant therapy against metabolic diseases in which microbiota plays an important role, such as obesity is increasing (Rozza and Pellizzon, 2013). The potential of fruits, aromatic plants and spices for improving human health is primary owing to their essential oils (EOs) which harbor various biological activities (Unusan, 2020).

Since the dawn of civilisation, medicinal plants have been a dependable source of new drugs and treatments for diseases. A detailed review of the literature has indicated that *Curcuma longa* L. or turmeric (of Zingiberaceae family) is a widely applied plant in herbal medicine and commonly used as a panacea with its extensive range of pharmacological activities. Turmeric is a plant distributed throughout tropical and subtropical regions of the world. Curcumin, which is a vital spice for all humanity and has specific human use that is known to the Eastern people (Ravindran *et al.*, 2007). Besides the spice uses, it is used as traditional medicine in Asian countries especially India, Bangladesh and Pakistan due to its several medicinal values (Chattopadhyay *et al.*, 2004). Modern traditional medicine says its powder has effect on gastrointestinal diseases particularly for biliary and hepatic disorder, diabetic wounds, rheumatism, inflammation, sinusitis, anorexia

coryza and cough. Recent studies have authenticated turmeric as anticancer, anti-diabetic, antioxidant, hypolipidemic, anti-inflammatory, antimicrobial, anti-fertility, anti-venom, hepatoprotective, nephroprotective, anticoagulant, etc. The plant has also shown to possess anti HIV activity to combat AIDS. These medicinal properties of turmeric caused it to be considered as a spice with multifunctional medicinal properties (Hamid., 2014).

Highest proportions of the rural and urban population resort to natural food ingredients, particularly because of their availability. Spices are a large group of such natural ingredients, and include dried seeds, fruits, roots, rhizomes, barks, leaves, flowers and any other vegetative substances used in a very small quantity as food additives to colour, flavour or preserve food (Burt., 2004). Spices are fragrant, aromatic and pleasant.

Spices contain tannins, resins, pigments, volatile, essential and fixed oils which contribute to flavoring occur in traces and constitute only a small fraction of the dry matter (Ravindram, *et al.*, 2002).

Some well-known spices of commerce include; red pepper, onions, sage, ginger, nutmeg, clove, cinnamon, mustard, curry, turmeric, rosemary and garlic. Spices add flavor, relish and pungency to diets. Most spices are fragrant, aromatic and pleasant. Spices in food also exert such secondary effects as salt and sugar reduction, prevention of spoilage and improvement of texture (Ravindran *et al.*, 2002).

Ginger can be available in different commercial products like cookies, candy, teas, tinctures, sodas, jam, beer, capsule and syrup (Murcia *et al.*, 2004). The chief active constituents of ginger are volatile oil (Zingiberene, Zingiberol, D-camphor), Shogaols, Diarylheptanoids, Gingerols, and Ginger flavonoids (Ozcan, *et al.*, 2009). Garlic is a spice that has been used to treat many conditions traditionally for thousands of years (Singh and Shukla, 2009). The root bulb of garlic has a high concentration of sulfur containing compounds among which allicin appears to be among the most active compounds (Akinmoladun., 2020). Ginger (*Zingiber officinale*) is a flowering plant whose rhizome, ginger root or ginger, is widely used as a spice and a folk medicine (Ali, *et al.*, 2008). Raw ginger is chewed to stimulate the flow of saliva and to relax congested nostrils. Ginger tea is prescribed for cough, colds and influenza.

The phytochemicals are plant metabolites, non-nutrient compounds that are biologically active when consumed by human. Many phytochemicals are health-promoting and are of disease preventive (Sharma, *et al.*, 2019).

Typical phytochemicals with antioxidant and antibacterial activities include polyphenols, phenolic acids and their derivatives, flavonoids, phospholipids, ascorbic acid, carotenoids and sterols. A number of exotic spices of international recognition with known Phytochemical constituents have been proven to be good natural antioxidants, antimicrobial and health-promoting agents (Raghavan, 2006). Some of such internationally recognized spices include chili pepper, garlic, onion, anise, cinnamon, ginger, curry, rosemary and nutmeg (Freeman, 2019).

Essential oils (EOs) are a complex mixture of volatile compounds that are produced by aromatic plants as secondary metabolites. They are present in all plant organs and their main role is to defend plants from aggressions by bacteria, fungi and viruses, but also by

insects (Sharma *et al.*, 2019). There are huge amounts of EOs obtained from different plants around the world, and most of them have been at least partially characterized for their antimicrobial activity against Gram-Negative bacteria, but also against other microorganisms such as fungi and virus. The antimicrobial strategies of EOs have been to develop multi-target mechanisms of action which make it very difficult for microorganisms to become resistant to these compounds (Sharma *et al.*, 2019). There are enormous potentials for the possible use of EOs as therapeutic agents against human pathogen.

The human gut microbiome, a complex ecosystem of trillions of microorganisms, plays a critical role in maintaining host health by regulating metabolism, immunity, and protection against pathogens (Dhifi., 2016). Dysbiosis, or imbalance in the gut microbiome, has been linked to numerous diseases, including inflammatory bowel disease (IBD), obesity, diabetes, and neurological disorders (Gresse, 2019). As the global burden of these diseases rises, there is an urgent need for innovative therapeutic strategies to restore gut microbiome balance and enhance immune function.

Statement of the Problem

The human gut microbiome plays a crucial role in maintaining overall health, influencing digestion, immune function, and even mental well-being. Recent research highlights the potential of dietary interventions to modulate the gut microbiome positively (Clemente, 2012). Among these interventions, essential oils derived from various spices are emerging as promising candidates due to their probiotic properties (Hamid, 2014; Swamy, *et al.*, 2016).

In Nasarawa State - Nigeria, a diverse range of spices with significant cultural and culinary importance are known for their therapeutic benefits. However, there is a lack of comprehensive scientific understanding regarding the specific effects of essential oils from these Nasarawa State spices on the human gut microbiome (Nazzaro, 2013).

Despite anecdotal evidence suggesting their beneficial effect, there is limited empirical data on how these essential oils influence gut microbiota composition and function. This study aimed at addressing the gap in knowledge by exploring probiotic potentials of essential oils extracted from selected Nasarawa State spices.

Specific Objectives for the Study are:

1. To extract essential oil from the five spices selected: a) using Steam distillation and hydro-distillation and b), To characterize the EOs extracted.
2. To Isolate and identify the gut microbiota from human stool.
3. To identify the antimicrobial effects of essential oils from selected Nasarawa State spices on gut microbiota composition.
4. To assess the probiotic potential of these essential oils by determining their ability to promote the growth of beneficial gut bacteria.

Selection of Plant Spices For Extraction of Essential Oils

Traditionally five (5) used Spices in Nasarawa State cuisine with known medicinal properties and readily available in the Markets were selected for this study, also with previous research documented or potential bioactive compounds in them which include: **Cloves** (*Syzygium aromaticum*), **Onion** (*Allium cepa*), **Ginger** (*Zingiber officinale*), **Turmeric** (*Curcuma longa*) and **Garlic** (*Allium sativum*). All the plant spices materials were washed and rinsed with distilled water to remove debris. Each of the plant material was cut into pieces separately before extraction. Extraction of the essential oils from these spices were then, carried out by steam distillation and hydro-distillation methods respectively.

Analysis of chemical composition of the essential oils using Gas Chromatography-Mass Spectrometry (GC-MS) was performed.

Extraction of Essential Oils (EOs)

Extraction of essential oils from Cloves, Ginger and turmeric were done by steam distillation. While, Onion and Garlic essential oils were extracted by hydro-distillation. A total of 6.5kg, of each of the plant material was used for the extraction. 200g pieces of Cloves was loaded into the distillation flask, which is connected to a round bottom flask containing water as described by Giwa, *et al.*, (2018). The flask was connected to a condensing unit with its tube. Heating mantle was used for heating the water in the round bottom flask. The essential oil was extracted as the steam passed through the Cloves pieces in the distillation flask. This process was repeated for Ginger and Turmeric separately.

Similarly, essential oils were extracted from Onion and Garlic by the process of hydro-distillation using the cleavenger apparatus. Five hundred grams of each of these plant materials were placed in a round bottom flask and filled with water to about three quarter full separately as described by Obidi. *et al.*, (2013). The distillation apparatus was connected to the flask. A separating funnel was used to collect the essential oil as it floats on top of water. After distillation process, the product obtained was a mixture of oil and water. The separating funnel tap was carefully opened to separate water from the oil. The essential oil was dried over anhydrous sodium sulfate and stored in amber colored bottles.

Composition of Essential oils by (GC-MS)

Composition of essential oils by Gas Chromatography-Mass Spectrometry (GC-MS) of the selected spices namely: Cloves, Ginger, Onion, Garlic, and Turmeric analysis was conducted using a Hewlett-Packard 6870 gas chromatograph interfaced with a Hewlett-Packard Mass Spectrometer 5973 (Agilent Technologies, Palo Alto, California, USA) equipped with an HP-SMS capillary column (30m × 0.32mm. i.d. 0.25µm film thicknesses). The column temperature was held at 45°C for 8min and then increase to 250°C at a rate of 2°C/min and held at 250°C for 16min. For GC-MS detection, an electron ionization system with ionization energy of 70eV was used over a scan range of 29-550m/z. Helium was used as the carrier gas at a constant flow rate of 1ml/min in split mode 1:20

with an injection volume of 1µl of essential oils. Detector and injector temperature were 280 and 250°C respectively. The ion source temperature was 175°C.

The EO compounds were tentatively identified based on linear retention index (RI) and by comparison of mass spectra with MS data of reference compounds. The linear RIs were determined for all constituents by co-injection of the samples with a solution containing the homologous series of *n*-alkenes (C₈-C₂₄) (Fluka, Buchs/sg. Switzerland), on HP-SMS column. The components were identified by matching their recorded mass spectra with the data bank Mass Spectra and further confirmed by comparison with values in the literature (Adams. 2007 and NIST libraries). The relative proportions of the chemical constituents of EOs were expressed as percentages obtained by peak area normalization and hence the components obtained.

Stool sample collection

A total of sixty (60) fresh fecal samples were collected randomly from volunteers aseptically for the isolation of gut bacteria. The fresh fecal samples were collected into sterile specimen bottles immediately after defecation and stored in air tight bags. The samples were then transported to the medical laboratory in IMAPOLY-Lafia Nasarawa State medical center. The stool samples were preserved in the refrigerator at 4°C.

Stool sample processing and isolation of gut bacteria

Each of the samples was mechanically mixed with a sterile spatula and a sterile loop was used to deliver a loop full of the stool sample into selenite broth and peptone water as described by Abubakar *et al.*, (2020). These were incubated at 37°C aerobically for 24hrs. An aliquot from the Selenite broth was sub-cultured unto *Salmonella shigella* agar. While an aliquot from Peptone water was sub-cultured into each of these media (MRS agar, MacConkey, Simmon citrate agar, Triple sugar iron agar, Sulphide indole motility medium, Blood agar and Eosin methylene blue. One set of inoculated plates were incubated at 37°C for 24hrs and the second set were incubated at 37°C for 5days in an anaerobic chamber. The plates were then observed for bacteria growth and isolates were characterized.

Characterization and identification of isolates

After incubation the isolates were characterized and identified using the standard procedures including colonial characteristics, Gram-stain reaction and other biochemical tests viz: Triple sugar iron test, Nitrate test, Catalase, Oxidase, Citrate, Urease, Sulphide indole motility test, Methyl red and VP test.

Preparation of essential oils solutions

Each of the five (5) essential oils was diluted in Dimethyl Sulfoxide (DMSO) to produce V/V. These were further diluted to working concentrations of 0.1%, 0.25%, 0.50%, 0.75% and 1% in sterile broth media immediately before use (Kalembe. & Kunicka, 2003; Bakkali *et al.*, 2008).

Treatment of Gut Bacteria

Treatment of the gut bacteria with essential oils was carried out as described by Lynch and Pederson., (2016). Each bacteria strain suspension was adjusted to optical density of $OD_{600}=0.1$ approximately 10^7 CFU/ml. A volume of 100 μ L of bacteria suspension was added to each of a sterile 96-well micro titer plates. Essential oil dilutions were added to each of the wells. DMSO was used as negative control, while Ampiclox was used as positive control.

The plates were sealed and incubated at 37°C under aerobic and anaerobic condition for 24hrs for aerobic and anaerobic bacteria respectively.

The bacteria growth was determined by measuring the optical density at 600nm using a micro plate reader. Viable cell count was performed by plating serial dilutions on Mueller Hinton agar plates and incubated at 37°C for 24hrs after which colony forming units were counted representing viable cell count.

RESULTS AND DISCUSSION

From the study carried out on the evaluation of probiotic potentials of essential oils extracted from five selected Nasarawa State spices (*Cloves, Ginger, Onion, Garlic and Turmeric*) and their effects on the human gut microbiome through in-vitro process, hence the following results and data were obtained.

Table 1 shows yield in percentage for each of the five essential oils extracted. Viz: Ginger 1.5%, Onions 1.7%, Garlic 1.6%, Cloves 1.5% and Turmeric 1.5%.

Table 1: Percentage yield of Essential Oils Extracted.

Sample Plant	Mass of Plant	Essential Oil	Percentage %
Material	sample Used (kg)	Obtained (ml)	Yield
Ginger	6.5	9.75	1.5
Onions	6.5	10.85	1.7
Garlic	6.5	10.70	1.6
Cloves	6.5	9.50	1.5
Turmeric	6.5	9.65	1.5

Table 2 shows the physical properties for each of the essential oils extracted as follows: Ginger has density of 0.89, Onions 0.94, Garlic 1.06, Cloves 1.05 and Turmeric 0.92 respectively, while specific density of Ginger is 0.86, Onions 0.93, Garlic 1.05, Cloves 1.04, and Turmeric 0.91. For odour and appearance Ginger has fresh spicy sweet and greenish-yellow colour, Onions strong pungent and pale-yellow colour, Garlic strong sharp and yellowish in colour, Cloves strong spicy sweet odour with light brownish colour while Turmeric has warm earthy odour and orange yellow.

Table 2: Some Physical Properties of Essential Oils Extracted.

Essential Oil	Density (g/ml)	Specific Density	Odour	Appearance
Ginger	0.89	0.86	fresh spicy sweet	greenish-yellow
Onions	0.94	0.93	strong pungent	pale yellow
Garlic	1.06	1.05	strong sharp	yellowish
Cloves	1.05	1.04	strong spicy sweet	light brownish
Turmeric	0.92	0.91	warm, earthy	orange yellow

Table 3: shows identified components by GC-MS to RI_{exp} = Experimental Retention Index. RI_{lit} = Literature Retention Index

Table 3: Identified Components by GC-MS of Essential Oils Extracted from the 5 Spices in the study.

Spice	Components	RI _{exp.}	RI _{lit}	%
1). Cloves	Eugenol	1352	1361	87
	Eugenol acetate	1521	1530	05
	Caryophyllene	1407	1412	03
	α – Humulene	1444	1450	02
	Caryophyllene oxide	1461	1470	01
2). Ginger	Zingiberene	1435	1441	35
	α – Curcumene	1404	1411	20
	β – Sesquiphellandrene	1453	1461	12
	β – Elemene	1386	1592	03
	Camphene	945	951	10
	Citral	1262	1265	18
	α – Pinene	923	930	02
3). Onions	Dipropyl disulfide	1278	1280	35
	Diallyl disulfide	1136	1140	30
	Methylpropyl disulfide	905	950	12
	Diallyl trisulfide	1310	1320	10
	Dimethyl trisulfide			05
	Dipropyl trisulfide			08
4). Garlic	Diallyl disulfide	1136	1140	35
	Diallyl trisulfide	1310	1320	20
	Allyl methyl disulfide	945	950	---
	Methyl allyl trisulfide	1220	1245	---

Spice	Components	RIexp.	RIlit	%
5). Turmeric	α – Turmerone	1550	1560	45
	β - Turmerone	1562	1565	20
	D - Limonene	1028	1030	21
	α – Zingiberene	1440	1445	03
	Curlone	1480	1484	11

Tale 4: Biochemical characterization and identification of human gut bacterial isolates.

S/N	Gr	Shp	Cat	Ind	Ur	Mr	Vp	Oxi	Cit	Gas	Mt	Nit	H ₂ S	A	Co	Org
1	-	Rod	+	+	-	+	-	-	-	+	+	-	-	+	+	<i>E. coli</i>
2	-	Rod	+	+	-	+	-	-	-	+	+	-	-	+	+	<i>E. coli</i>
3	-	Rod	+	+	-	+	-	-	-	+	+	-	-	+	+	<i>E. coli</i>
4	-	Rod	+	-	-	-	-	-	-	-	-	-	-	+	-	<i>Bact. Sp</i>
5	-	Rod	+	-	-	-	-	-	-	-	-	-	-	+	-	<i>Bact. Sp</i>
6	+	Cocci	-	-	-	+	+	-	-	+	-	-	+	+	-	<i>Enter.sp</i>
7	+	Cocci	-	-	-	+	+	-	-	+	-	-	+	+	-	<i>Enter. Spp</i>
8	+	Rod	-	-	-	-	-	-	-	+	-	-	-	+	-	<i>Lact. Sp</i>
9	+	Rod	-	-	-	-	-	-	-	+	-	-	-	+	-	<i>Lact. Sp</i>
10	-	Cocci	-	-	-	-	-	-	-	-	-	-	-	-	-	<i>Akk. Sp</i>
11	+	Rod	-	-	-	-	-	-	-	-	-	+	-	+	-	<i>Cl. Sp</i>
12	+	Rod	-	-	-	-	-	-	+	+	+	-	+	+	-	<i>Sal.sp</i>
13	+	Cocci	+	+	+	+	-	+	+	+	-	+	-	+	+	<i>Staph. Sp</i>
14	+	Rod	-	-	-	+	-	-	-	+	-	-	-	+	-	<i>Bifido. Sp</i>
15	-	Rod	+	+	-	+	-	-	-	+	+	-	-	+	+	<i>E. coli</i>
16	-	Rod	+	+	-	+	-	-	-	+	+	-	-	+	+	<i>E. coli</i>
17	-	Rod	+	+	-	+	-	-	-	+	+	-	-	+	+	<i>E. coli</i>
18	-	Rod	+	-	-	-	-	-	-	-	-	-	-	+	-	<i>Bact. Sp</i>
19	-	Rod	+	-	-	-	-	-	-	-	-	-	-	+	-	<i>Bact. Sp</i>
20	+	Cocci	-	-	-	+	+	-	-	+	-	-	+	+	-	<i>Enter. Sp</i>
21	+	Cocci	-	-	-	+	+	-	-	+	-	-	+	+	-	<i>Enter. Sp</i>
22	+	Rod	-	-	-	-	-	-	-	+	-	-	-	+	-	<i>Lact. Sp</i>
23	-	Rod	-	-	-	-	-	-	-	+	-	-	-	-	-	<i>Lact. Sp</i>
24	-	Cocci	-	-	-	-	-	-	-	-	-	-	-	-	-	<i>Akk. Sp</i>
25	+	Rod	-	-	-	-	-	-	-	-	-	-	+	+	-	<i>Cl. Sp</i>
26	+	Rod	-	-	-	-	-	-	+	+	+	-	+	+	-	<i>Sal. Sp</i>
27	+	Cocci	+	+	+	+	-	+	+	+	-	+	-	+	+	<i>Staph. Sp</i>
28	+	Rod	-	-	-	+	-	-	-	+	-	-	-	+	-	<i>Bifido. Sp</i>
29	+	Cocci	+	+	+	+	-	+	+	+	-	+	-	+	+	<i>Staph. Sp</i>
30	-	Rod	+	+	-	-	-	-	+	+	+	-	+	+	-	<i>Prot.sp</i>

Key: + = Positive, - = Negative. Shp.=Shape, Gr = Gram reaction, Cat.= Catalase, Coa.= Coagulase, Ind.= Indole, Ur.= Urase, Mr. = Methyl Red, Vp.= Vogue Proskaure, Oxi.= Oxidase, Cit.= Citrate, Mt= Motility, Nit.= Nitrate, H₂S= Hydrogen Sulfide, A.= Acid, Org.= Organism, *L. bacillus* = *Lactobacillus spp.*, *Bact. Spp* = *Bacteriodes*, *Cit. spp* =

Citrobacter spp., *Prot. spp* = *Proteus spp.*, *Ent. Spp* = *Enterococcus spp.*, *Cl. Spp* = *Clostridium spp.*, *E. coli* = *Escherichia coli.*, *Akk. Spp* = *Akkermansia spp.* *Staph. Spp* = *Staphylococcus spp*, *Bifido. Spp.* = *Bifidobacterium spp.*, *Sal. Spp.* = *Salmonella spp.*

Table 5: Mean values of effect of treatment on Gut Bacteria at different concentrations (%) of EOs.

S/N	Org.	V-count before treatment	Cloves	Ginger	Onions	Garlic	Turmeric Viability (%)	% Reduction after treatment
1	Lacto spp	8.50	7.928	8.274	8.276	8.412	8.438 93.27 99.8 97.37 98.97 99.27	Cloves 6.73% 0.20% Onion 2.63% Garlic 1.03% Turmeric 0.73%
2	Bact. Spp	8.30	8.212	8.258	8.274	6.596	8.408 98.94 99.94 99.69 79.53 101.3	Cloves 1.06% 0.06% Onions 0.31% Garlic 20.53% Turmeric - 1.30%
3	Entero spp	8.40	7.316	7.55	7.3	6.51	8.208 87.10 89.88 86.09 77.50 97.72	Cloves 12.90% 10.12% Onions 13.09% Garlic 22.50% Turmeric 2.28%
4	Clostri spp	8.20	6.296	6.662	6.836	6.284	6.584 76.78 81.45 83.37 76.64 80.29	Cloves 23.22% 18.55% Onions 16.63% Garlic 23.36% Turmeric 19.71%
5	Bifido. Spp	8.40	8.056	7.886	7.956	7.346	8.192 95.91 93.81 94.72 87.45 97.52	Cloves 4.09% 6.19% Onions 5.28% Garlic 12.55% Turmeric 2.48%
6	Akk. Spp	6.50	4.794	6.254	4.948	6.450	6.242 73.75 96.22 76.12 61.65 96.03	Cloves 26.25% 3.78% Onions 23.88% Garlic 0.77% Turmeric 3.97%
7	E. coli	9.00	5.086	5.854	6.106	3.64	4.588 56.51 65.05 67.85 40.45 50.98	Cloves 43.49% 34.95% Onions 32.15% Garlic 59.55% Turmeric 49.02%.
8	Staph. Spp	8.10	4.4	4.942	5.308	4.654	6.788 54.32 59.54 65.53 57.46 83.81	Cloves 45.68% 40.46% Onions 34.47% Garlic 42.54% Turmeric 16.19%

S/N	Org.	V-count before treatment	Cloves	Ginger	Onions	Garlic	Turmeric Viability (%)	% Reduction after treatment
9	Prot. spp	7.90	6.548	4.758	6.284	6.588	5.644 82.89 60.23 79.55 83.39 71.44	Cloves 17.11% Ginger 39.77% Onions 20.45% Garlic 16.61% Turmeric 28.56%
10	Citro. Spp	7.60	4.77	6.482	4.852	5.72	6.662 62.76 85.29 63.84 75.26 87.65	Cloves 37.24% Ginger 14.71% Onions 36.16% Garlic 24.74% Turmeric 12.34%
11	Salm. Spp	8.90	3.246	3.898	4.384	2.33	5.384 36.47 43.80 49.26 26.18 60.50	Cloves 63.53% Ginger 56.20% Onions 50.74% Garlic 73.82% Turmeric 39.50%

Key: *Lacto. bacillus* = *Lactobacillus spp.*, *Bact. Spp* = *Bacteriodes*, *Citro. spp* = *Citrobacter spp.*, *Prot. spp* = *Proteus spp.*, *Entero. Spp* = *Enterococcus spp.*, *Clostri. Spp* = *Clostridium spp.*, *E. coli* = *Escherichia coli.*, *Akk. Spp* = *Akkermansia spp.* *Staph. Spp* = *Staphylococcus spp*, *Bifido. Spp.* = *Bifidobacterium spp.*, *Salm. Spp.* = *Salmonella spp.*

DISCUSSION

Table 1: shows yield in percentage for each of the five essential oils extracted. Viz: Ginger 1.5%, Onions 1.7%, Garlic 1.6%, Cloves 1.5% and Turmeric 1.5%. All the values obtained fall within the standard values, except for Ginger and Garlic which are slightly higher. This may be attributed to the varieties used and environmental conditions as well as extraction method (Sharma *et al.*, 2017).

Table 2: shows the physical properties for each of the essential oils extracted as follows: Ginger has density of 0.89, Onions 0.94, Garlic 1.06, Cloves 1.05 and Turmeric 0.92 respectively, while specific density of Ginger is 0.86, Onions 0.93, Garlic 1.05. Cloves 1.04, and Turmeric 0.91. For odour and appearance, Ginger has fresh spicy sweet and greenish-yellow colour, Onions strong pungent and pale-yellow colour, Garlic strong sharp and yellowish in colour, Cloves strong spicy sweet odour with light brownish colour while Turmeric has warm earthy odour and orange yellow. These properties are typical of the five oils studied. These properties are influenced by storage conditions and location, varieties and extraction method (Shahid Ud-Daula & Basher, 2019).

Also in Table 2, components of Garlic are shown to be the following: Diallyl disulfide, Diallyl trisulfide, Allyl methyl disulfide and Methyl allyl trisulfide with Diallyl disulfide as the highest component having 35%. Several studies revealed that the major active components of Garlic which are Organosulfur compounds including; Allicin (Diallyl thiosulfonate) and Diallyl disulfide which are responsible for the characteristic odour and pharmacological activities (Lanzotti, 2006).

Table 3: shows the identified major components by GC-MS of the five essential oils extracted. In this study the GC-MS analysis revealed the presence of five major compounds in Cloves: Eugenol, Eugenol acetate, Caryophyllene, α -Humulene and Caryophyllene oxide and Eugenol as the most dominant compound with (87%). Study by Yeddes *et al.*, (2022) showed cloves to have eight compounds with Eugenol as the dominant with (87.3%) which is similar to that obtained in this study.

The chemical components of Ginger essential oil as indicated in Table 3 include; Zingiberene, α -Curcumene, β -Sesquiphellandrene, β -Elemene, Camphen, Citral and α -Pinene. Zingiberene has the highest constituent followed by α -curcumene with 35% and 20% respectively as obtained in the study. Noori (2018) and Sharma (2016) in their study revealed the main component of Ginger essential oil was Zingiberene.

Onions has the following components as obtained in table 3 namely; Dipropyl disulfide, Diallyl disulfide, Methylpropyl disulfide, Diallyl trisulfide, Dimethyl trisulfide and Dipropyl trisulfide with Dipropyl disulfide as the major component with 35% (Zhou *et al.*, 2017).

Turmeric in Table 3: shown the following components namely; α -Turemerone, β -Turemerone, D-Limonene, α -Zingibrene and Curlone with α -Turemerone as the major component with 45%. Guimaries, *et al.*, (2020), reported that the major components identified by GC-MS were α -Turemerone (40%), α -Turemerone (10%) and Curlone (23%). While Nasri *et al.*, (2014), reported Turmeric possesses Sesquiterpenes (53%), Zingiberene (25%), α -Phellandrene (1%), Sabinene (0.6%), Cineol (1%) and Borneol (0.5%). Curcumin is responsible for the yellow colour in Turmeric.

Table 4: shows the isolated gut bacteria as indicated by their characteristics using both morphological and biochemical characterization. The organisms isolated include; = *Lactobacillus spp.*, *Bacteriodes Spp*, *Citrobacter spp.*, *Proteus spp.*, *Enterococcus spp.*, *Clostridium spp.*, *Escherichia coli.*, *Akkermansia spp.*, *Staphylococcus spp.*, *Bifidobacterium spp.*, and *Salmonella spp.* Gut microbiota is made up of diverse group of organisms and constantly dynamic with bacteria being majority (Zaky *et al.*, 2021). This finding is consistent with several studies on gut bacteria.

Study by Abubakar *et al.*, (2020), showed similar bacteria isolated from human gut. Even though not all organisms were detected, this could be due to the method used for the isolation and detection of the gut microbiota. In the present study *E. coli*, *Bacteriodes spp.*, *Lactobacillus spp.*, *Bifidobacterium spp.*, account for 53.3% with each of them 13.33%. These bacteria represent the dominant species found in the human gut. *Lactobacillus spp.*, *Bifidobacterium spp.*, and *Bacteriodes spp.*, are associated with modulation of host immunity and gut health (Wexler, 2007). *E. coli* is a commensal in the gut (Madigan *et al.*, 2018). Other organisms which include; *Akkermansia spp.*, *Clostridium spp.*, *Citrobacter spp.*, *Enterococcus spp.*, and *Salmonella spp.*, with each accounting for 6.6%. While *Proteus spp.*, 3.3% and *Staphylococcus spp.*, 10%. The presence of pathogens which are *Salmonella*, *Proteus*, *Enterococcus* and *Citrobacter spp.* is an indication that healthy individual are harbouring these pathogens and are at an asymptomatic stage. As collaborated by Abubakar *et al.*, (2020).

Essential oils (EOs) from culinary spices exert biologically active effects on microbes. They frequently display **bactericidal/bacteriostatic actions** against pathogens while exerting variable effects on commensal and probiotic species. Interpretation requires careful attention to species-specific responses and concentrations used. Table 5 provides percent reductions (viability changes) in eleven gut bacterial groups following treatment with clove, ginger, onion, garlic and turmeric EOs — allowing species-level comparisons of antimicrobial potency and selectivity.

Clove essential oil showed strong antibacterial activity, with the following effect; *Salmonella spp.* (63.53% reduction), *E. coli* (43.49%), *Staphylococcus spp.* (45.68%), *Citrobacter spp.* (37.24%) were highly suppressed. This aligns with reports that **eugenol disrupts bacterial membranes and inhibits enzyme activity** (Marchese, *et al.*, 2017). CEO exhibited moderate suppression of probiotic as shown; *Lactobacillus* (6.73%), *Bifidobacterium* (4.09%), and *Akkermansia* (26.25%) were less affected compared to pathogens. The implication is that COE is **selective**, showing **strong anti-pathogen activity while sparing beneficial bacteria** to some degree, hence **beneficial for controlling enteric infections like *Salmonella* and *E. coli***.

Ginger Essential Oil (GEO) exhibited mild-moderate antibacterial effect, by reducing *E. coli* (34.95%), *Salmonella* (56.20%), *Staphylococcus* (40.46%), and *Proteus* (39.77%). While preserving beneficial species by minimal reduction in *Lactobacillus* (0.20%), *Akkermansia* (3.78%), *Bifidobacterium* (6.19%). This suggest probiotic friendly activity. Gingerols is one of the major components of GEO and is capable of altering bacterial quorum sensing and biofilm formation without causing drastic probiotic depletion (Grzanna. *et al.*, 2005). GEO is a good immunomodulator and gut microbiota stabilizer by moderately inhibiting pathogens while, largely sparing beneficial microbes.

Onion essential oil (OEO) showed moderate, balanced activity but not as strong as garlic and cloves EOs. The effect of OEO reduction on viability of the bacteria varied with *E. coli* (32.15%), *Salmonella* (50.74%), *Staphylococcus* (34.47%), *Citrobacter* (36.16%). OEO had moderate inhibition effect of probiotics: *Lactobacillus* (2.63%), *Bifidobacterium* (5.28%), *Akkermansia* (23.88%). OEO is rich in **thiosulfinates and propyl sulfides** which can interfere with bacterial sulfhydryl metabolism and oxidative balance (Griffiths *et al.*, 2002). This result is indicative of broad spectrum activity of OEO.

Garlic Essential Oil (GEO) is rich in Allicin and Diallyl sulfides. GEO exhibited strong and marked inhibition of *Salmonella* (73.82%), *E. coli* (59.55%), *Enterococcus* (22.50%), *Staphylococcus* and *Clostridium* (23.36%). While the percentage reduction on probiotic showed: *Lactobacillus* (1.03%), *Bifidobacterium* (12.55%), *Akkermansia* (0.77%). Though *Akkermansia* is preserved well, others are reduced more strongly compared to onion/ginger. Allicin rapidly penetrates cells, inhibits thiol-containing enzymes, and triggers oxidative stress (Ankri & Mirelman, 1999). **Turmeric Essential Oil (TEO) rich in Curcumin and Turmerones exhibited mild antibacterial activity against pathogens and preservation/enhancement of probiotics, reduction in cell viability were: *E. coli* (49.02%), *Salmonella* (39.50%), *Proteus* (28.56%), *Staphylococcus* (16.19%), and *Citrobacter* (12.34%).** Others are *Lactobacillus*

(0.73%), *Bifidobacterium* (2.48%) showed minimal reduction. *Akkermansia* viability was high (96.03%), indicating **supportive prebiotic-like effect**.

Turmeric compounds act as **antioxidants, anti-inflammatories**, and regulate bacterial metabolism without strongly killing commensals (Jagetia and Aggarwal, 2007). Turmeric oil is **gut-protective**, enhancing beneficial microbes while moderately suppressing pathogens.

Conclusion

The findings demonstrate that the oils exhibit varying yields and chemical composition, largely influenced by environmental, varietal, and methodological factors. GC-MS profiling confirmed the dominance of bioactive compounds such as eugenol, zingiberene, diallyl disulfide, dipropyl disulfide, and α -turmerone, validating their pharmacological significance.

The analysis of stool samples and microbiota composition confirmed the complexity and diversity of gut ecosystems, highlighting both beneficial and pathogenic microorganisms. Essential oils displayed concentration and species specific antimicrobial effects as well as balance microbiome preserving and modulatory properties.

The study established that the essential oils, particularly ginger and turmeric, hold promise as natural modulators of gut microbiota responses, supporting their application in preventive nutrition, probiotic enhancement, and therapeutic interventions for gut health management.

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