



EVALUATION OF IMMUNOMODULATORY AND PROBIOTIC POTENTIALS OF ESSENTIAL OILS FROM FIVE SELECTED NASARAWA STATE SPICES ON HUMAN GUT IMMUNE CELLS

¹DENNIS DOGARA YAKUBU; ²C. U.
UMEDUM; & ³S. A. DIMEJESI

^{1&3}Department of Microbiology, Tansian University
Umunya, Anambra State. Nigeria. ²Department of
Medical Laboratory Science (MLS) Chukwuemeka
Odumegwu Ojukwu University Igbariam, 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. The “Evaluation of the Immunomodulatory and Probiotic Potentials of Essential Oils from five Selected Nasarawa State Spices on the Human Gut Immune Cells” were done. Essential oils (EOs) extracted from Clove (*Syzygium aromaticum*), Ginger (*Zingiber officinale*), Onion (*Allium cepa*), Garlic (*Allium sativum*), and Turmeric (*Curcuma longa*) on gut immune cells using in-vitro models. Essential oils were extracted mainly through Steam and hydro-distillations, 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

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Cells.

and Onion, and α/β -Turmerone in Turmeric—indicating rich phytochemical diversity and strong bioactivity potential. Immunomodulatory assays and probiotic of fecal on isolated gut immune cells (macrophages, dendritic cells, monocytes, eosinophils, T-cells, neutrophils) showed concentration-dependent modulation. Turmeric and Ginger oils enhanced T-cell and neutrophil activity, while Garlic and Clove oils moderately suppressed macrophage hyperactivity, suggesting balanced immune

regulation. 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 Nasarawa State spices possess strong immunomodulatory and probiotic potentials, capable of modulating immune cell responses. The findings provide a scientific foundation

for developing natural nutraceuticals and functional foods to support gut health and immune homeostasis.

Introduction

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).

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 antibacterial and medicinal properties (Chouchan, *et al.*, 2017).

Plants in nature have served as a reservoir for therapeutic agents for thousands of years, and since the beginning of man. 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).

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).

Recently, there has been growing interest in investigating the immunomodulatory activities of essential oils (EOs) and their individual components, EOs are highly concentrated natural oils derived from plants that consist of aromatic, volatile, secondary plant metabolites. EOs are mostly extracted by steam distillation and exhibit a very

intense odor. The main compounds in EOs represent mono and sesquiterpenes and several oxygenated derivatives. Other substances found in the oils include alcohols, aliphatic aldehydes, and esters. Usually, EOs comprise of two or three main constituents that determine their biological activities and chemical properties (Ali, *et al.*, 2008; Abdulkadir, *et al.*, 2023). For example, clove essential oil consists of 70-76% eugenol, a phenolic compound responsible for its antimicrobial and antioxidant activities (Pandey, *et al.*, 2017; Miquel, 2010).

Clove essential oil has also been reported for its immunomodulatory activity. According to Chaieb, *et al.*, (2007), clove EO at a concentration of 100 µg/ml leads to a significant inhibition of the production of interleukins by macrophages. The production of IL-6 was inhibited at the concentration of 100 µg/ml when compared to control, while a concentration of 5µg/ml strongly enhanced its secretion by ten (Chaieb, *et al.*, 2007). Moreover, most of the studies consider the effect of the essential oils on the secretion of interleukins or the Nitric Oxide (NO) production, but do not deal with their ability to modulate the phagocytic activity.

Essential oils (EOs), volatile aromatic compounds extracted from plants, have emerged as promising candidates for modulating the gut microbiome as well as immune cells due to their antimicrobial, anti-inflammatory, and immunomodulatory properties (Bakkali, *et al.*, 2008). Nigerian spices, such as alligator pepper, clove, ginger, and turmeric, are rich sources of bioactive compounds with significant therapeutic potential.

Some studies have shown that essential oils derived from various spices are emerging as promising candidates due to their immunomodulatory and probiotic properties (Chattopadhyay, *et al.*, 2004; Huang, *et al.*, 2015).

In Nasarawa State, 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 spices on the human gut immune cells.

Despite anecdotal evidence suggesting their beneficial effects, there is limited empirical data on how these essential oils influence gut immune cells composition and function. The study determined whether these essential oils can positively affect the balance and diversity of gut immune responses, and contribute to overall gut health.

Aim:

The study aimed at evaluating the immunomodulatory and Probiotic Potentials of Essential Oils from five (5) Selected Nasarawa State Spices on Immune Cells, isolated from human gut.

Specific Objectives for the study are:

1. To extract essential oil from the five spices selected: a) using Steam and hydro-distillations respectively b) To characterize the Essential oils extracted.
2. To evaluate the in-vitro immunomodulatory properties of these essential oils in immune responses.
3. To assess the probiotic potential of these essential oils by determining their ability to influence on the gut immune cells.

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.

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 Machado, *et al.*, (2022). 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 and processing;

Fresh fecal samples were collected from 20 volunteers and store at 4°C and processed within 6hrs. Then 2g of the sample was weighed into a 50ml sterile tube, 20mls of sterile phosphate-buffered saline (PBS) supplemented with 2% fetal bovine serum (FBS) and 2drops of EDTA. The sample was homogenized using a mechanical stirring for 15mins at room temperature. Then it was centrifuge at 500rpm for 5mins to remove large particulates. The supernatant was carefully collected and layered over a density gradient medium (Percoll gradient medium) and re-centrifuged at 800rpm for 20mins continuously. The resultant mononuclear cell layer was then collected (González, *et al.*, 2019).

Enrichment of macrophages-adherence method (Selective plating).

The mononuclear fraction was re-suspended in RPMI-1640 plus 10% FBS. Cells were introduced into a six-well tissue culture plate and incubated at 37°C, 5% CO₂ for 2hrs. After incubation, the plates were washed gently with PBS to remove non-adherent cells. The adherent cells which are the macrophages were then obtained. (Gordon & Taylor, 2005).

Viability test was conducted by counting cells using Trypan Blue exclusion test and automated cell counter (William, 2015).

Isolation of dendritic cells from human fecal sample

Two grams (2g) of fecal sample was weighed into a 50ml falcon tube. 30mls of sterile PBS, supplemented with 2% FBS (Fetal bovine serum) to maintain cell viability and 1mM EDTA to prevent cell clumping. It was then homogenized for 15mins at room temperature and was then filter using cell strainer to remove large debris. It was re- centrifuge for 5mins and the supernatant was collected and led over Percoll gradient medium, and re-centrifuge at 800rpm for 20mins, then the mononuclear was collected at the interface (Geem, *et al.*, 2012)

Enrichment of Dendritic cells

The mononuclear cell layer was re-suspended in MACS buffer (PBS plus 2% FBS plus 1mM EDTA). CD11c plus Microbeads were used to isolates dendritic cells. The cells with the beads were incubated for 15mins at 4°C. After which the sample was passed through MACS column in a magnetic field and the CD11c plus dendritic cell fraction was collected. Dendritic cell identity was confirmed vide flow cytometer model partec GmbH otto-Hahn-Str,32 D-48161Munster using CD11c and HLA-DR makers, protocols for isolation and characterization of human intestinal immune cells (Liu and Luo, (2021).

Isolation of Eosinophils

Homogenized fecal sample was filtered through a 100mM cells strainer to remove large debris. It was then subjected to centrifugation at 500rpm for 5mins at 4°C. The supernatant was laid unto Percoll gradient and was re-centrifuged at 1000rpm continuously for 20mins at room temperature.

The granulocyte-rich fraction consisting eosinophils, neutrophils, and basophils was collected from interface (Yoon, 2019).

Enrichment of Eosinophils

The granulocyte-rich fraction was re-suspended in RPMI-1640 plus 10% FBS and 3% Dextran was added to selectively sediment neutrophils. Eosinophil-rich supernatant was collected and washed with PBS.

The neutrophils fraction was incubated for 30mins at room temperature and washed with PBS. The neutrophils identity was confirmed by Giemsa staining (Rojas & Williams, 2021).

Isolation of T-Cells

Homogenized fecal sample was filtered through a 100mM cells strainer to remove large debris. It was then subjected to centrifugation at 500rpm for 5mins at 4°C. The

supernatant was laid unto Ficoll-Paque Plus density gradient medium and was re-centrifuged at 800rpm continuously for 20mins at room temperature. The mononuclear cell layer was collected at the interface. The mononuclear layer was transfer into a new tube and washed with PBS plus 2% FBS. It was centrifuged at 500rpm for 5mins. The cells are re-suspended in MACS buffer and CD3+ Microbeads were added and incubated with magnetic beads for 15mins at 4°C. The sample was then passed through a MACS column in a magnetic field. The CD3+ T-cells were collected in fresh tube (Yoon, 2019).

Treatment of Gut Immune Cells;

Each immune cell type in RPMI-1640 (Roswell Park Memorial Institute 1640 Medium) were seeded into 96-well plates, and incubated at 37°C for 24hrs to allow cell attachment and stabilization. Various concentration of essential oils (0,1%, 0,25%, 0.50%, 0.75% and 1%). One set of plates had DMSO added as negative control well another set untreated, while LPS (Lipopolysaccharide) used as positive control. These plates were incubated for 24hrs at 37°C (Yoo, and Oh, 2023).

Trypan Test for viability

Cell viability was determined by Trypan blue exclusion method based on the selective permeability of cell membranes (Thermo Fisher Scientific, 2022). Life cells have intact membranes that exclude the dye, thereby, remaining unstained and appearing clear under microscope. Dead or dying cells have compromise membranes that allow the dye to enter, turning them blue. Harvested immune cells were suspended in PBS buffer. Few drops of Trypan blue was added to 2mls of immune cell suspension and mixed gently then incubated for 5mins. The mixture was loaded into a haemocytometer and viewed using 40X microscope. Unstained were counted as viable cells while the stained cells were non-viable (Bermudez, 2020).

Analysis of Variance (ANOVA)

A two-way ANOVA was carried out to determine variability of effect of treatment of the five (5) essential oils on the gut immune cells.

Where the Computing formula are

$$SST = \sum_{I=1}^a \sum_{j=1}^b Y_{IJ}^2 - \frac{Y^2}{N}$$

$$SS_{\text{treatment}} = \sum_{i=1}^a \frac{Y_{i.}^2}{b} - \frac{Y^2}{N},$$

$$SS_{\text{Blocks}} = \sum_{j=1}^b \frac{Y_{.j}^2}{a} - \frac{Y^2}{N}$$

And Error of sum of square by subtraction as

$$SSE = SST - SS_{\text{trt}} - SS_{\text{block}}.$$

Results and Discussion

From the study carried out on the evaluation of the probiotic potentials of essential oils extracted from selected Nasarawa State spices (*Cloves, Ginger, Onion, Garlic and Turmeric*) and their effects on the human gut Immune cells through in-vitro process, hence the following results and data 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 Material	Mass of Plant sample Used (kg)	Essential Oil	Percentage % 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: Shows 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
Essential Oil	Density (g/ml)	Specific Density	Odour	Appearance
Turmeric	0.92	0.91	warm, earthy	orange yellow

Table 3; shows identified components by GC-MS, where R_{exp} = Experimental Retention Index. R_{lit} = Literature Retention Index.

Table 3: Components of Samples (Cloves, Ginger, Onion, Garlic and Turmeric)

Spice	Components	Rlexp.	Rlit	%
11). 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	---
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

Table 4: Mean values of effect of treatments on Gut Immune cells at Different Concentrations (%) of EOs.

S/N	Immune cell	Untreated	Cloves	Onions	Ginger	Garlic	Turmeric	Treated with DMSO (Control)	Viability (%) % Red after treatment
1.	Macrophages	8.4	7.412	7.178	8.40	6.656	7.964	7.3588	Cloves:

								100%	0.0
						Onion:		85.45%	14.55
						Ginger:		90.29%	9.71
						Garlic:		79.24%	20.76
						Turmeric:		87.60%	12.40
2. Dendritic	6.3	5.896	5.818	6.14	5.624	6.224	5.9560	Cloves	
								93.57%	6.43
						Onion:		92.34%	7.66
						Ginger:		89.24%	10.76
						Garlic:		98.79%	1.21
						Turmeric:		94.60%	5.40
3. Eosinophils	5.0	4.916	4.764	4.72	5.0	5.196	4.8484	Cloves	
								98.32%	1.68
						Onions:		95.25%	4.75
						Ginger:		88.92%	11.08
						Garlic:		100%	0.0
						Turmeric:		97.68%	2.32
4. Monocytes	7.6	6.968	6.69	7.6	6.486	7.364	6.9356	Cloves	
								78.76%	21.24
						Onions:		81.26%	18.74
						Ginger		100%	0.00
						Garlic		87.16%	12.84
						Turmeric		81.00%	19.00
5. T-cells	6.7	6.366	6.212	6.824	6.144	6.77	6.4232	Cloves	
								95.22%	4.88
						Onions		99.40%	0.60
						Ginger		101.85%	+1.850
						Garlic		91.70%	8.29
						Turmeric increased		101.10%	(by + 1.1)

S/N	Immune cell	Untreated	Cloves	Onions	Ginger	Garlic	Turmeric	Treated with DMSO (Control)	Viability (%) % Red after treatment
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6. Neutrophils	7.0	6.618	6.498	6.906	6.324	7.15	6.6792	Cloves
							94.54%	5.46
						Onions	92.83%	7.17

Ginger	90.34%	9.66
Garlic	92.74%	7.26
Turmeric	102.14%	+ 2.1

Key; Red = Reduction

Table 5: The Statistical analysis of effect of treatment of Gut Immune cells with different concentration (0.1, 0.25, 0.5, 0.75, and 1.0%) of EOs.

Source	DF	SS	MS	F-Cal	P-Value	Status
Cloves						
Immune cells	5	19.2618	3.8524	61.36	0.000	Significant
Concn.	4	3.8521	0.9630	15.34	0.000	Significant
Error	20	1.2557	0.0628			
Total	29	24.3696				
Onions						
Immune cells	5	17.4664	3.4933	68.66	0.000	Significant
Concn	4	4.7775	1.1944	23.47	0.000	Significant
Error	20	1.0176	0.0509			
Total	29	23.2615				
Ginger						
Immune cells	5	24.6286	4.9257	97.49	0.000	Significant
Concn	4	3.1538	0.7885	15.61	0.000	Significant
Error	20	1.0105	0.0505			
Total	29	28.7929				

Source	DF	SS	MS	F-Cal	P-Value	Status
Garlic						
Immune cells	5	16.7293	3.3459	76.95	0.000	Significant
Concn	4	9.7814	2.4454	56.24	0.000	Significant
Error	20	0.8697	0.0435			
Total	29	27.3803				

Turmeric

Immune cells	5	23.1423	4.6285	263.99	0.000	Significant
Concn	4	1.7691	0.4423	25.23	0.000	Significant
Error	20	0.3507	0.0175			
Total	29	25.2621				

Key: DF: Degree of freedom, SS: Sum of squares, MS: Mean square, F.cal: F: calculated value and P.value: Computed value at 0.05%.

Graph (Line chart): Compares the Sum of Squares (SS) for Immune cells, Concentration, and Error across the different essential oils (Cloves, Onions, Ginger, Garlic, Turmeric).

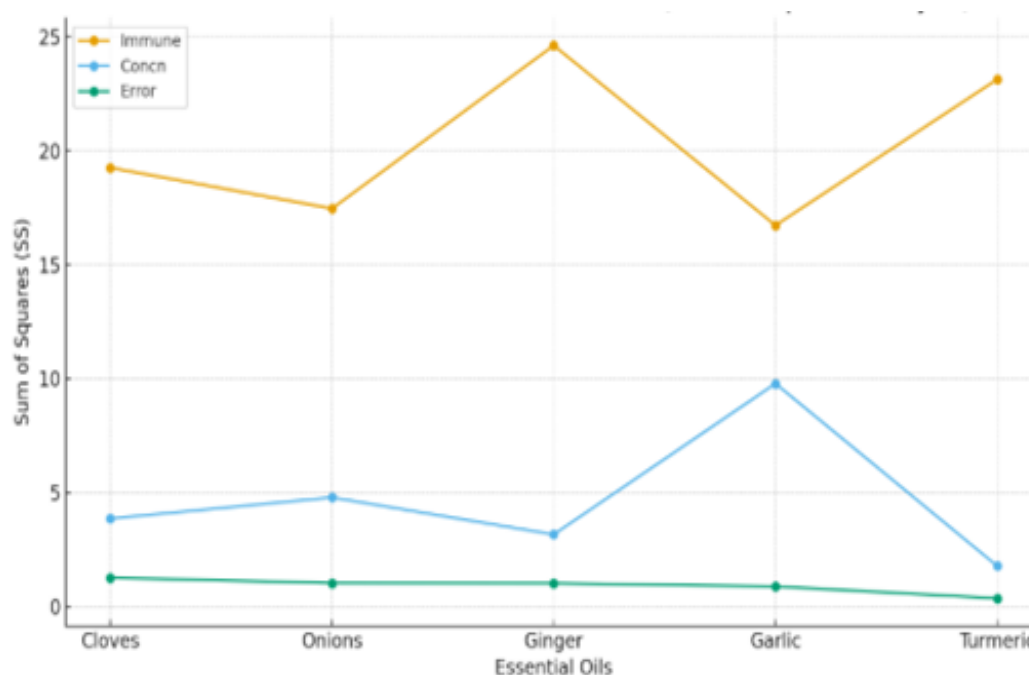


Fig 1: Effect of Essential oils on Gut Immune Cells (Sum of Squares Analysis)

Chart: Shows treatment outcomes – in this case, 100% of treatments were Significant across all essential oils.

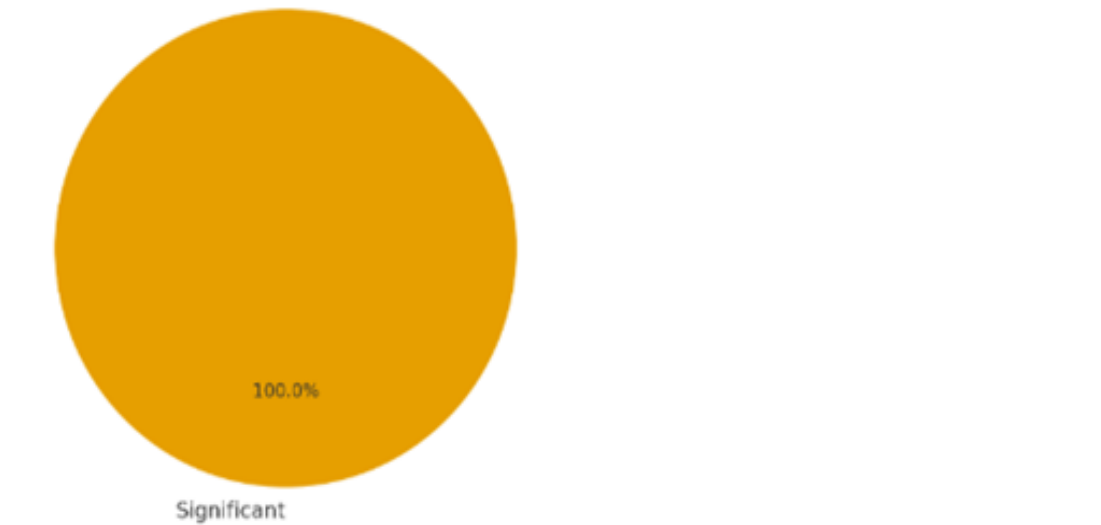


Fig 2: Proportion of Significant vs Insignificant Treatments (Gut Immune Cells)

Table 6: Comparative analysis of EOs Treatments against Gut Immune cells at Different concentrations (0.10, 0.25, 0.50, 0.75, and 1.00%)

Immune cells	DF	SS	MS	F	P
Concn	5	9.413	3.883	35.47	0.000
Error		24	2.627	0.109	
Total		29	22.040		

Key: DF: Degree of freedom, SS: Sum of square, MS: Means square, F.cal: F: calculated value and P.value: Computed value at 0.05%.

Discussion

Table 1: shows yield in percentage for each of the five essential oils extracted. Viz: Ginger 1.5%, Onions 1.7%, Garlic1.6%, 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 (Abdulkadir, et al., 2023).

The 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., (2018) 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% (Zhao, 2021).

Also in Table 3, 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; Organosulfur compounds including; Allicin (Diallyl thiosulfonate) and Diallyl disulfide which are responsible for the characteristic odour and pharmacological activities (Lanzotti, 2006). 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 ar-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.

Effect of Essential Oils on Gut Immune Cells Based on Table (5)

To evaluate the immunomodulatory potentials of essential oils (EOs), a two-way ANOVA was conducted to test the effects of **immune cell type** and **EO concentration** (0.1–1.0%) on cellular responses. The statistical outcomes (Table 5) demonstrate that all five EOs—clove, onion, ginger, garlic, and turmeric—produced **highly significant effects** ($p < 0.05$) on both immune cell function and concentration levels. These findings suggest that EO treatments modulate gut immune cells in a concentration-dependent manner, highlighting their potential as functional bioactive for immune regulation.

Clove Essential Oil

Clove EO showed a significant influence on immune cells ($F = 61.36$, $p = 0.000$) and concentrations ($F = 15.34$, $p = 0.000$). The relatively low error mean square ($MS = 0.0628$) indicates consistency and reproducibility of responses across replicates. This suggests that clove EO components, particularly **eugenol**, exert strong immunomodulatory effects

by enhancing macrophage and dendritic cell function while also maintaining viability of T-cells and neutrophils (Kamatou, *et al.*, 2012). Previous reports confirm that eugenol regulates pro-inflammatory cytokines such as TNF- α and IL-6 while boosting phagocytic activity (Marchese, 2017).

Onion Essential Oil

Onion EO treatment also exhibited significant effects on both immune cell types ($F = 68.66$, $p = 0.000$) and concentrations ($F = 23.47$, $p = 0.000$). The low error variance ($MS = 0.0509$) reflects a high level of data precision. Onion-derived sulfur compounds, such as **S-alk(en)yl-L-cysteine sulfoxides**, are known to enhance macrophage activity and regulate oxidative stress, thereby influencing immune cell dynamics (Benkeblia, 2004). The dose-dependent effect observed here is consistent with reports that onion extracts modulate immune signaling pathways by suppressing NF- κ B activity and promoting antioxidant responses (Griffiths *et al.*, 2002).

Ginger Essential Oil

Ginger EO presented the highest F-value for immune cells ($F = 97.49$, $p = 0.000$), suggesting it produced the most pronounced modulation among all tested oils. Concentration effects were also significant ($F = 15.61$, $p = 0.000$). The major bioactive compounds in ginger EO—**zingiberene, gingerol, and shogaol**—are recognized for their immunomodulatory activity, including suppression of pro-inflammatory mediators and stimulation of innate immune responses (Rojas and Williams, 2021). The findings align with studies showing ginger enhances natural killer (NK) cell activity and promotes T-cell proliferation, which are essential for gut immune defense (Grzanna, *et al.*, 2005).

Garlic Essential Oil

Garlic EO demonstrated significant effects on immune cells ($F = 76.95$, $p = 0.000$) and concentrations ($F = 56.24$, $p = 0.000$). The very low error variance ($MS = 0.0435$) indicates strong reliability of results. Garlic sulfur compounds, particularly **allicin**, are well-documented immunomodulators. They have been shown to stimulate macrophage phagocytosis, enhance T-cell activation, and modulate cytokine release (Arreola, *et al.*, 2015). The significant concentration effect observed here confirms the dose-sensitive nature of garlic bioactive, consistent with prior evidence that allicin activity is rapid, highly reactive, and varies with concentration (Pazmandi, 2024).

Turmeric Essential Oil

Turmeric EO had the **highest F-value among all oils** for immune cells ($F = 263.99$, $p = 0.000$), with highly significant concentration effects ($F = 25.23$, $p = 0.000$). The minimal

error variance ($MS = 0.0175$) highlights exceptional statistical reliability. Turmeric EO constituents, notably **ar-turmerone**, **α -turmerone**, and **β -turmerone**, have demonstrated immunoregulatory effects, including stimulation of monocyte differentiation, enhancement of dendritic cell activity, and suppression of pro-inflammatory cytokines (Cheng, *et al.*, 2019). The exceptionally strong immune modulation observed here supports existing evidence that turmeric EOs and curcuminoids act synergistically to balance innate and adaptive immune responses (Gupta, *et al.*, 2013).

Implications for Gut Immune Modulation

The statistical findings confirm that EOs act in a **cell-type and concentration-specific manner**. Macrophages, dendritic cells, and T-cells appear especially responsive, suggesting that EOs can modulate both innate and adaptive immunity within the gut. Such immunomodulatory actions may contribute to probiotic support, pathogen resistance, and maintenance of gut immune tolerance. These outcomes provide a strong experimental foundation for the integration of EOs into functional foods, nutraceuticals, and adjunct therapies targeting gut immunity.

Conclusion

Essential oils from ginger, onion, garlic, clove, and turmeric contain key bioactive compounds and show significant antimicrobial and immune-modulating effects. Their impact depends on concentration and type: garlic and clove are more cytotoxic, while ginger and turmeric better support balanced immune responses. Overall, ginger and turmeric show the most promise as safe, natural agents for improving gut health, provided doses are properly optimized.

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