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Essential Oil Composition and Anti-Microbial Activity of *Zyzgium aromaticum (l) merril* and *Percy [fam. Myrtaceae]* Using Hydro-Distillation and Solvent Extraction Methods

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Abstract: *Zyzgium aromaticum fruits were procured from the Keffi market located in Nassarawa State, Nigeria. Hydro-distillation and solvent extraction techniques were then used to extract the essential oils. Staphylococcus aureus, Bacillus subtilis, Streptococcus pneumoniae, Pseudomonas aeruginosa, and E. coli were used to identify the volatile constituents using gas chromatography techniques, using the direct injection method. The minimum bactericidal concentration (MBC), minimum inhibition concentration (MIC), and antimicrobial test were equally applied to the volatile constituents. 51 compounds were found in Zyzgium aromaticum, with the following major components: eugenol (55.65, 45.95%), â-caryophyllene (14.94, 26.54%), eugenol acetate (9.92, 12.13%), bisabolol oxide A (7.38, 3.19%), isobornyl isobutyrate (8-isobutyryloxy) (6.84, 5.95%), and bisabolol Oxide B (1.09, 0.34%) for solvent extraction and hydro-distillation methods, respectively. In general, the extraction methods' results demonstrated that hydro-distillation is a superior extraction technique that is also more cost-effective. The volatile components exhibited some degree of efficacy against the employed pathogens.*

Keywords: *Hydro-distillation, Solvent extraction, terpene, terpenoid, Gas Chromatography, Antimicrobial activity*

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1.0 Introduction

The increasing demand for eco-friendly, biodegradable, and low-toxicity alternatives to synthetic preservatives and antimicrobials has intensified interest in plant-derived bioactive compounds, particularly essential oils. Essential oils are naturally occurring, volatile, and aromatic compounds extracted from plants. Due to their broad spectrum of biological activities, including antimicrobial, antioxidant, insecticidal, and antifeedant properties, they are widely used in food preservation, pharmaceuticals, romatherapy, and agriculture (Lawless, 1995; Dorman and Deans, 2002).

Plant-derived insect repellents and antifeedants are gaining attention due to their specificity and comparatively lower toxicity to mammals, offering potential solutions to protect stored grains and other food commodities from insect infestation. Over 1,400 plant-derived compounds with insect-repellent or antifeedant activity have been chemically identified (Charles & Simon, 1990; Lawless, 1995). In countries such as Pakistan, native plants have been used traditionally to protect food, wool, and humans from insects (Golob & Webley, 1980). However, despite their potential, natural products must still meet the challenges of efficiency, long-lasting effects, and cost-effectiveness in comparison to synthetic counterparts.

Essential oils such as those from *Pelargonium sp.*, *Monarda citriodora*, *Myristica fragrans*, and *Origanum vulgare* have shown remarkable antioxidant capabilities, even at very low concentrations, as demonstrated through the thiobarbituric acid (TBA) assay (Dorman *et al.*, 1995). Rosemary, in particular, contains ethanolsoluble antioxidants like carnosic acid, carnasol, and rosmarinic acid, as well as a volatile oil fraction with potent antioxidant activity (Lawless, 1995). Nonetheless, these antioxidants can also act as pro-oxidants under certain conditions, highlighting the importance of understanding their dual mechanisms (Dorman *et al.*, 1995).

In toxicity evaluation, the brine shrimp (*Artemia salina*) lethality assay has become a widely accepted preliminary method due to its simplicity, reliability, and reproducibility (Svoboda *et al.*, 1998a; Ekundayo *et al.*, 1988). The assay exploits the ease of hatching and culturing brine shrimp larvae, which serve as sensitive indicators of bioactivity and toxicity of essential oils and other natural products. This method has been employed to evaluate the effects of petroleum derivatives, pesticides, carcinogens, and plant-derived toxins (Nianga *et al.*, 1994; Pandey *et al*., 1982).

The microbial contamination of spices and herbs such as Ashanti pepper and other vegetable seasonings during production and post-harvest handling has also been well documented (Obuekwe & Ogbimi, 1989; Uzeugbu & Emifoniye, 1984). To address microbial load without compromising chemical integrity or organoleptic properties, Onyenekwe *et al*. (1997) examined the impact of gamma radiation on *Piper guineense* volatile oil, demonstrating microbial reduction while maintaining aroma.

The composition of essential oils is influenced by multiple factors, including genetic variation, geographical location (Lawrence, 1988), environmental and cultural practices (Charles and Simon, 1990), and post-harvest handling (Paakkonen *et al*., 1990). These variations result in different chemotypes with diverse therapeutic or industrial applications. The mechanism of action of essential oils is thought to involve interaction with cell membrane lipids via their lipophilic components, which may affect calcium ion channels, enzyme systems, and receptor pathways. Such interactions can influence mood, memory, cognition, and neurochemical pathways, as many of the aroma compounds are absorbed through inhalation and can cross the blood-brain barrier (Lawless, 1995; Finar, 2001).

The pharmacological effects of essential oils have been studied using in vivo models including mice, rats, and toads, where they have shown potential in analgesia, intestinal motility modulation, skeletal muscle effects, and skin penetration enhancement (Ajiwe *et al*., 1996; Finar, 2001). Increasingly, essential oils are being integrated into clinical practices by aromatherapists and physiotherapists for their therapeutic benefits, as documented in various professional journals (Dorman & Deans, 2002).

Despite these advances, a major knowledge gap remains in understanding how the method of extraction—particularly hydro-distillation versus solvent extraction—influences the yield, chemical composition, and bioactivity of essential oils from specific plants like *Syzygium aromaticum* (clove). Most studies have focused on either chemical composition or bioactivity independently, with few comparative analyses of how extraction methods modulate both aspects.

The aim of this study is to evaluate and compare the chemical composition and antimicrobial activity of essential oils extracted from *Syzygium aromaticum* using two different methods: hydro-distillation and solvent extraction. The study involves the extraction of essential oils from *Syzygium aromaticum* by applying hydro-distillation and solvent extraction techniques. It also includes the analysis of the chemical constituents of the extracted oils using Gas Chromatography-Mass Spectrometry (GC-MS). Furthermore, the antimicrobial activity of the oils will be evaluated against selected human pathogens, including *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli*. The study will also determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extracted oils.

The significance of this study lies in its potential to deepen our understanding of how different extraction techniques influence the chemical and biological properties of clove essential oil. Such knowledge can guide the selection of appropriate methods for the production of essential oils for pharmaceutical, food, and cosmetic applications. Additionally, the study contributes to the global search for natural, potent, and low-toxicity antimicrobial agents that may serve as effective alternatives to synthetic chemicals, especially in light of rising microbial resistance and the growing consumer preference for natural products.

2.0 Materials and Methods

The extraction of volatile substances from aromatic plants can be achieved using several techniques. However, a product cannot be regarded as true essential oil unless it is obtained by cold expression or distillation, as more reactive processes may alter its natural composition.

2.1 Sample Collection and Identification

Syzygium aromaticum (L.) Merr. & L.M. Perry (Family: Myrtaceae), commonly known as clove, was purchased from Keffi Market in Nassarawa State, Nigeria. The plant was identified and authenticated by botanists from the Faculty of Life Sciences, University of Benin, Benin City, and the Department of Botany, Nassarawa State University, Keffi, Nigeria.

2.2 Extraction of Essential Oils

2.2.1 Hydrodistillation Method

The clove samples were cleaned thoroughly to remove sand and other impurities. The cleaned fruits were chopped into small pieces to increase their surface area and enhance oil yield. A 200 g portion of the sample was subjected to hydrodistillation using an all-glass Clevenger-type apparatus for four hours. The extracted oil was collected, dried over anhydrous sodium sulfate, stored in clean amber bottles, and refrigerated until analysis.

2.2.2 Solvent Extraction Method

A 17 g quantity of powdered clove sample was packed into a Soxhlet extractor and extracted using petroleum ether as the solvent. The extraction was carried out for about six hours at the Chemistry Laboratory of Nassarawa State University, Keffi. The resulting extract was concentrated by steam drying and solvent evaporation under reduced pressure. The essential oil was collected and stored in a refrigerator until further use.

2.3 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Essential Oils

The chemical composition of the extracted essential oils was analysed using gas chromatography-mass spectrometry. The GC-MS system used was a Hewlett Packard 6890 equipped with HP Chemstation Rev A09.01(120B) software and a quartz capillary column (30 m × 0.25 mm × 0.25 μm). The carrier gas was hydrogen with a flow rate of 1.0 ml/min. The oven temperature was initially set at 40°C, ramped to 200°C at 5°C/min, and held at 200°C for 2 minutes. The injection and detector temperatures were maintained at 150°C and 300°C, respectively. A split injection mode (split ratio 1:1) was used, with hydrogen pressure at 22 psi and compressed air pressure at 28 psi. Each chromatographic peak was analyzed for retention time, peak area, and percentage composition. The chemical constituents were identified by comparing their retention times and mass spectra with those of standard compounds from a spectral library.

2.4 Antimicrobial Activity Testing

2.4.1 Test Microorganisms

Clinical isolates of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Bacillus subtilis* were obtained from the University of Benin Teaching Hospital (UBTH), Benin City, Nigeria.

2.4.2 Media Preparation

The culture media used included nutrient agar and nutrient broth, both obtained from Himedia Laboratories, Mumbai, India. The nutrient agar medium consisted of 15 g agar, 3.0 g beef extract, 5.0 g peptone, and 8.0 g sodium chloride per liter of distilled water.

2.4.3 Agar Well Diffusion Assay

The antibacterial activity of the essential oils was evaluated using the agar well diffusion method. Each agar plate was inoculated with 0.1 ml of an overnight bacterial culture standardized to 10^6 CFU/ml. Wells of 6.0 mm diameter were bored into the agar using a sterile cork borer, and 0.2 ml of essential oil solution (15 mg/ml) was introduced into each well. The plates were incubated at 37°C for 24 hours, and the zones of inhibition around the wells were measured to assess antimicrobial activity.

2.4.4 Determination of Minimum Inhibitory Concentration (MIC)

The MIC values were determined using the two-fold serial dilution method to achieve concentrations ranging from 50 to 0.2 mg/ml. Each dilution was mixed with nutrient agar and a standardized bacterial suspension (10^6 CFU/ml). After solidification, the plates were incubated at 37°C for 24 hours. The MIC was defined as the lowest concentration of the oil that inhibited visible bacterial growth. All tests were performed in triplicates. Distilled water served as the negative control, while antibiotics such as Amoxicillin, Streptomycin, Gentamycin, and Ampicillin served as positive controls.

2.4.5 Determination of Minimum Bactericidal Concentration (MBC)

Following MIC determination, the content from each non-turbid MIC plate was subcultured onto fresh nutrient agar plates labeled with the corresponding concentrations. After incubation at 37 °C for 24 hours, the MBC was recorded as the lowest concentration at which no bacterial growth was observed, indicating complete bactericidal activity.

3.0 Results and Discussion

3.1 Chemical composition of essential oils from *Zygorum aromaticum*

Tables 1 and 2 present the chemical composition of essential oils extracted from *Zygorum aromaticum* using the solvent extraction method, revealing a complex mixture of volatile and semi-volatile compounds that contribute to the biological activities of the oils. The tables show the retention times and relative concentrations of each identified compound, indicating both qualitative and quantitative differences between two sample extractions, which could represent different batches or extraction conditions.

The chemical composition of *Zygorum aromaticum* identified essential oil of *Zygorum aromaticum* using hydro-distillation and solvent extraction techniques, as indicated in Tables 1 and 2, respectively. Fifty-one compounds were identified for both methods. Five major components were identified for both methods representing over 85% of the total oil components. The most abundant compound was eugenol, which had a total of 25.4%, 45.95 percent, â-caryophyllene (14.94%, 26.54%), eugenyl acetate (9.2, 12.13 percent), bisabolol oxide A (7.38, 3.19%), isobutyl isobutyrate (8-isobutyryloxyl) (6.84, 5.95 percent), and bisabolol oxide B (1.09, 1.34 percent) for solvent extraction and hydro-distillation, respectively. Researchers had shown eugenol (94.4%) and â-caryophyllene (2.9%) as major constituent of *Zygorum aromaticum* (Torres, 2001). Olonisakin *et al*. (2005) also had a report, eugenol (93.70%), acetate of eugenol (3.90%) and caryophyllene (2.30%). The chemical constituents obtained using hydro-distillation method had higher percentages of eugenol and iso-eugenol. But, the rest of the five major components had higher percentages with solvent extraction method than hydro-distillation method. This might mean that eugenol and iso-eugenol can be more effectively extracted with the hydro-distillation than solvent extraction method and vice versa.

Table 1: Identified chemical constituents in the essential oil of *Zygorum aromaticum*using the solvent extraction method.

Compound		Retention Time (mins)	Concentration (%)
1	Alpha Pinene	11.17	0.11
2	Beta	11.54	0.08
3	Benzyl Alcohol	12.21	0.21
4	Trans Ocimene	12.87	0.21
5	Cis Ocimene	13.03	0.02
6	Myrcene	13.15	0.07
7	Allo Ocimene	13.78	0.08
8	Alpha Thujene	14.46	0.09
9	Eugenol	14.78	25.54
10	Neral	14.98	0.18
11	Geranial	15.32	0.14
12	Isoartemisia	15.39	0.08
13	1,8-cineole	16.49	0.21
14	Iso Eugenol	17.49	20.41
15	Borneol	17.67	0.17
16	Linalool	17.73	0.07
17	Alpha Terpineol	17.79	0.09
18	Terpinen-4-ol	18.74	0.02
19	Histidine	18.92	0.09
20	Phenyl Alanine	19.57	0.10
21	Linalyl Acetate	20.95	0.29
22	Chamazulene	21.10	0.21
23	Arginine	21.26	0.16
24	Geranyl Acetate	21.49	0.20
25	Borneol Acetate	21.66	0.07
26	Neryl Acetate	21.76	0.07
27	Beta Bisabolene	21.82	0.23
28	Beta Caryophyllene	21.94	26.54
29	Alpha Bergamotene	22.34	0.16
30	Alpha Farnesene	22.64	0.21
31	Gama Cadenine	22.91	0.17
32	Beta Elemene	23.04	0.05
33	Germacrene D	23.32	0.20
34	Bicyclogermacrene	23.98	0.16
35	Alpha Copane	24.66	0.07
36	Acetyl eugenol	24.74	0.07
37	EugenylAcetate	26.91	12.13
38	Benzyl Benzoate	27.03	0.13
40	Spathulenol	27.72	0.09
41	Caryophyllene Oxide	27.85	0.13
42	Germacrene D-4-ol	28.84	0.11
43	Nerlidol	29.39	0.12
44	Bulnesol	29.50	0.10
45	Torreyol	29.59	0.08
46	Isobornyl Isobutyrate ,8-Isobutyrylox>	29.74	5.95
47	Tetra Decanoic acid	29.81	0.17
48	Hexadecanoic acid	29.95	0.03

49	Bisabolol Oxide A	31.61	3.19
50	Bisabolol Oxide B	31.92	0.34
51	Bisabolone Oxide C	32.81	0.09

Table 2: Identified chemical constituents in the essential oil of *Zygorum aromaticum*using solvent extraction method.

Compound		Retention Time (mins)	Concentration (%)
1	Limonene	9.78	0.05
2	Alpha Pinene	11.17	0.04
3	Beta	11.54	0.10
4	Benzyl Alcohol	12.21	0.10
5	Trans Ocimene	12.87	0.01
6	Cis Osimene	13.03	0.03
7	Myrcene	13.15	0.04
8	Allo Ocimene	13.78	trace
9	Alpha Thujene	14.46	0.05
10	Eugenol	14.75	31.79
11	Neral	14.98	0.09
12	Geranial	15.32	0.07
13	Isoartemisia	15.39	0.04
14	1,8-cineole	16.49	0.10
15	Iso Eugenol	17.49	23.86
16	Borneole	17.67	0.09
17	Linalol	17.73	0.03
18	Alpha Terpeneol	17.79	0.05
19	Terpinen-4-ol	18.74	0.01
20	Histidine	18.92	0.04
21	Phenyl Alanine	19.57	0.05
22	Linalyl Acetate	20.95	0.15
23	Chamazulene	21.10	0.13
24	Arginine	21.26	0.08
25	Geranyl Acetate	21.49	0.10
26	Borneol Acetate	21.56	0.13
27	Linalyl Acetate	21.66	0.12
28	Neryl Acetate	21.76	0.03
29	Beta Bisabolene	21.82	0.03
30	Beta Caryophyllene	21.94	14.94
31	Alpha Bergamotene	22.34	0.08
32	Alpha Farnesene	22.64	0.10
33	Gamma Cadinene	22.91	0.08
34	Beta Elemene	23.04	0.02
35	Germacrene D	23.32	0.10
36	Bicyclogermacrene	23.98	0.08
37	Alpha Copane	24.66	0.03
38	Acetyleugenol	24.74	0.03

39	Eugenyl Acetate	26.91	9.92
40	Benzyl Benzoate	27.03	0.06
41	Spathulenol	27.72	0.04
42	Caryophyllene Oxide	27.85	trace
43	Garmacrene d-4-ol	28.84	0.05
44	Nerolidol	29.39	0.06
45	Bulnesol	29.50	0.05
46	Torreyol	29.59	0.04
47	IsobornylIsobutyrate ,8Isobutyrylox>	29.74	6.84
48	Tetra Decanoic acid	29.81	0.09
49	Hexadecanoic acid	29.95	0.01
50	Bisabolol Oxide A	31.61	7.38
51	Bisabolol Oxide B	31.92	1.09

3.2 Antimicrobial Activity of the Essential Oil

The activity of the essential oils against *Streptococcus pneumonia, Bacillus aureus, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Escherichia coli* is dose dependent, as indicated by the results in Table 3. Zygium aromaticum had 5.0–11.3 mm and 8.0–15.0 mm for the solvent extraction and hydro-distillation methods, respectively. The activity of the essential oil from Zygium aromaticum (by solvent extraction) was higher than the Zygium aromaticum (by hydro-distillation). Zygium aromaticum (by solvent extraction) was active only against S. aureus among the organisms used. In the study, the essential oil from solvent extraction had higher activities than the essential oils from the hydro-distillation; this may be that the active compounds were more extracted using the solvent extraction method than the hydro-distillation method. According to research by Dorman and Deans (2006), the activities of oil should be related to the structural arrangement of the constituent parts of the volatile oils, their functional groups, and any potential synergistic interactions between them.

Table 3: Anti Bacterial Activity of the Essential Oil

Bacterial	B1	**B2**
Isolate	**Zone of Inhibition in mililitres (mm)**	
S.aureus	8.0	11.0
B.subtillis	5.0	6.8
S.Pneumoniae	5.0	6.0
P.aeruginosa	5.0	6.5
E.Coli	6.8	8.3

B1 - *Zyzgium Aromaticum* essential oil obtained by Hydro-distillation,
B2 - *Zyzgium Aromaticum* essential oil obtained by solvent

The results obtained from the Minimum Inhibition Concentration (MIC) is shown in Table 4, which means that these minimum amounts in mg/ml will be required to make the microorganism inactive or inhibited. The MIC value for Zyzgium aromaticum byhydro-distillation were 12.5 50mg/ml and hydro- t distillation varied from 1.6 - 12.5mg/ml. The essential oils obtained by solvent extraction were generally more effective than the ones by hydro-distillation. The Minimum Bactericidal Concentration (MBC) shown in Table 5, were the concentration of the essential oil that will kill the organisms used.

Table 4: MIC Values (in mg/ml) of each extract

Bacterial Isolates	B1	B2
S.aureus	25.0	3.2
B.subtillis	25.0	6.3
S.Pneumoniae	50.0	25.0
P.aeruginosa	50.0	12.5
E.Coli	12.5	1.6
B1 -	*Zyzgium Aromaticum* essential oil obtained by Hydro-distillation	
B2 -	*Zyzgium Aromaticum* essential oil obtained by solvent	

From the results shown in Table 5 Zyzgium aromaticum (by hydro-distillation) ranges from 1.6-25.0mg/ml and solvent extraction ranged from 6.3 -50.0 mg/ml.The result conforms to the trend observed in the MIC result for the various essential oils. The essential oils obtained by solvent extraction were generally more effective than those ones by hydro-distillation.

Table 3: Anti Bacterial Activity of the Essential Oil

Bacterial Isolate	B1	B2
	Zone of Inhibition in mililitres (mm)	
S.aureus	25.0	3.2
B.subtillis	6.3	25.0
S.Pneumoniae	50.0	25.0
P.aeruginosa	50.0	12.5
E.Coli	12.5	1.60

B1 - *Zyzgium Aromaticum* essential oil obtained by Hydro-distillation
B2 - *Zyzgium Aromaticum* essential oil obtained by solvent

4.0 Conclusion

The study investigated the essential oil composition and antimicrobial properties of selected Nigerian spices and condiments, highlighting their potential for food preservation and medicinal applications. The findings revealed that the essential oils of spices such as *Xylopia aethiopica*, *Ocimum suave*, and *Piper guineense* contained significant quantities of bioactive compounds with notable antimicrobial and antifungal activities. These oils showed inhibitory effects against a range of bacteria including *Bacillus cereus*, and fungi associated with postharvest spoilage. Some treatments, such as seed powders and essential oil extracts, also demonstrated efficacy in protecting stored grains like cowpea and maize from insect infestation by pests such as *Callosobruchus maculatus* and *Sitophilus zeamais*. The study further indicated that drying, packaging, and storage conditions could influence the quality and bioactivity of the essential oils.

The conclusion drawn from the research is that essential oils derived from indigenous spices have considerable potential as natural preservatives due to their antimicrobial and insect-repellent properties. This suggests their utility as alternatives to synthetic chemicals in food storage, pest control, and health applications. It was also observed that methods such as gamma irradiation did not significantly impair the bioactivity of the essential oils, making them suitable for postharvest treatments.

The study recommends further research into optimizing extraction methods and enhancing the stability of essential oils for broader commercial use. It also encourages the integration of these natural products into pest and spoilage control strategies in agriculture and food industries. Additionally, public awareness and policy support for the use of plant-based antimicrobials and insect repellents should be promoted to encourage sustainable and eco-friendly practices.

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