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Combinatorial Effects of *Syzygium aromaticum*, *Citrus limon*, and *Zingiber officinale* Extracts on Phytochemical, Antioxidant, and Antimicrobial Properties

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Abstract

This research explores the synergistic properties of lemon (*Citrus limon L.*), clove *(Syzygium aromaticum)*, and ginger (*Zingiber officinale*), examining their combined medicinal effects. Through phytochemical screening, biochemical and antimicrobial activity, it is aimed to unravel the potential applications of this unique combinations of the three plant extracts. The study involves extracting bioactive compounds from the dried rhizome of ginger, the dried fruit peel of lemon and the dried flower bud of clove, and study their additive, synergistic and antagonistic properties of two combinations of spices namely clove and lemon (C + L) and clove, lemon and ginger (C+L+G). On both the combinations of these extracts phytochemical, antimicrobial, GC-MS and FTIR studies were conducted and satisfactory results obtained. The interactions between these plant extracts were analyzed to understand how they enhance or inhibit each other's efficacy. The results of the analyses showed the presence of several primary and secondary metabolites, such as flavonoids, terpenoids, alkaloids and others. The peaks in the FTIR and GC-MS analyses of the combinations indicated the presence of standard functional groups and bioactive compounds respectively.

Key words: Additive, synergistic, phytochemical, antimicrobial, antioxidant, products

Introduction

Search for cure for various diseases including chronic illnesses have led to the discovery of various bioactive compounds present in medicinal plants. Various parts of these plants were used to treat the diseases in many traditional medicines; however, more medicinal applications are being discovered utilizing newer technologies.

The plants used in this study, *Syzygium aromaticum* (clove)*, Zingiber officinale* (ginger) and *Citrus limon L* have been used for culinary purposes as well as for their medicinal value, due to the diverse bioactive compounds present in them (1). Citrus lemon is a type of fruit which belong to *Rutaceae* family. The high fiber, vitamin C, and mineral content in lemon peel and its oil find wide range of applications in the food and flavor sector as well as in the medical sector (2). Ginger, belonging to the *zingiberaceae* family, is a flowering plant whose rhizome is used for culinary purposes. However, the antibacterial potential is used in alternative medicine such as naturopathy (3). Clove is one of the most abundant sources of phenolic components, including eugenol and eugenol acetate, this plant has significant potential for use in food, medicine, cosmetics, and agriculture (4). Clove oil is made by distilling the *Syzygium aromaticum* clove tree's flowers, stems, and leaves. It is a dark brown liquid with a flavor and scent that are rich and

aromatics. It has been used as a mild topical anesthetic since antiquity and can be used for toothache, headaches, and joint pains (5).

An essential oil is the volatile fraction that is extracted from a plant or from one or more of its parts using a physical separation method like solvent extraction, steam distillation, hydro distillation and hydro diffusion (6). Essential oils are a broad category of natural products that are used in food, industrial, and pharmaceutical products as significant sources of flavoring and aromatic compounds. Most of the compounds in essential oils are terpenes and aromatic polypropanoid compounds from the acetate-mevalonic acid and shikimic acid pathways, respectively.

The phytochemical components of the plants determine their therapeutic qualities. Alkaloids, flavonoids, tannins, saponins, steroids, terpenes, and other compounds that are found in different regions of plants are some of the significant phytochemicals. The phytochemicals with potential importance are the alkaloids, which possess antiviral, antibacterial, anti-inflammatory, and anticancer effects (7). Others include flavonoids and other secondary metabolites of phenolic type which oversee a wide range of pharmacological activities (8). Other phytochemicals of interest include the amino acids, which are a rich source of proteins (9). Terpenoids, essential in preventing cancer, also have anti-inflammatory, antioxidant, and cell cycle regulatory properties (10).

Antioxidants are regarded as significant nutraceuticals due to their numerous health benefits; A standard assay is essential for determination of antioxidant activity, which is mainly performed using DPPH,1- diphenyl-2-picryl-hydrazyl (DPPH), which is a stable free radical with an unpaired valence electron at one nitrogen bridge atom (11). Ginger extract was used in cancer patients undergoing chemotherapy for its antioxidant property (12) and in treatment of colon cancer in Wistar rat models (13). Ginger and lemon juice have been used in combination for their antioxidant potential (14). Clove extracts have also been used for its antioxidant and anticancer properties using heLa cells (15).

Citrus peel extracts have been widely used for medicinal and industrial applications. Rat models of ulcer and hepatotoxicity were used to experimentally assess the hepatoprotective/ gastroprotective effects of citrus peel in aqueous and butanol extracts, as well as hesperidin (16).

Combination of plant extracts may yield the effects as either synergistic, additive or antagonistic (17). Invitro studies have confirmed synergistic action of lemon and ginger water extract against cancer cell lines (18). The antimicrobial property of ginger and lemon oil along with neem oil has been used for its therapeutic effect on cows with mastitis (19). Clove has been used for its antimicrobial activity as a preservative in foods (20), whereas synergistic antibacterial, antifungal and antioxidant activity was observed in cinnamon and clove essentials oil (21). Synergistic antimicrobial property of clove oil and thymol was demonstrated against oral bacteria using nanoencapsulation studies (22).

In this study, combinations of clove and lemon (C+L) extracts and clove, lemon and ginger (C+L+G) are investigated to study possible additive, antagonistic or synergistic effects.

Materials and Methods

Chemicals and apparatus

Ninhydrin reagent, sulfuric acid, Molisch's regent, chloroform, green florescence, Fehling's regent, sodium hydroxide, hydrochloric acid, ferric chloride, sodium hydroxide, methanol, potassium iodide, lead acetate, dimethyl sulphoxide, acetic anhydride, nutrient agar, nutrient broth, sodium benzoate, bees wax, methyl paraben, glucose, tween 20, diphenyl 1 picrylhydrazyl (DPPH), UV–Visible spectrophotometer (Evolution 300; Thermo Scientific, UK), linear plate shaker- SM30 (Edmund Buhler GmbH, Bodelshausen, Germany), Fourier transform infra-red spectroscopy (Thermo – FTIR -002 instrument).

Collection of material

The lemon peels, clove buds and ginger were collected from the local market in Oman and dried. They were then separately ground thoroughly using a mortar and pestle and then using a blender. The fine powder obtained was used for the preparation of extracts.

Preparation of plant extracts

About 30 g of each of the powdered sample (ginger, lemon peel and cloves) were placed in conical flasks and 300 ml of methanol (99%) was added into each. The conical flasks were then covered tightly and placed into a Thermos incubator shaker for about 48 hours (37°C, 3000 RPM). Thereafter, the solutions were filtered, and the filtrates were transferred to the roto evaporator to evaporate methanol for 24 hours at 60°C. The resultant crude extracts were then stored in refrigerator at 4°C.

Phytochemical Screening:

Phytochemicals, derived from the Greek word phyton, which means "plant," are substances found naturally in plants that can have either beneficial or detrimental effects on health. The richest bio reservoirs of diverse phytochemicals are medicinal plants, which are used to treat a variety of illnesses and maladies. The phytochemical components of the plants determine their therapeutic qualities. Alkaloids, flavonoids, tannins, saponins, steroids, terpenes, and other compounds that are found in different regions of plants are some of the significant phytochemicals [10]. Two different combinations were selected to study the synergistic, additive or antagonistic activity. The extracts were clove and lemon (C & L) and clove, lemon, and ginger (C, L and G).

Phytochemical analysis:

Test for alkaloids

Alkaloids were tested using the standard method of Wagner's regent. The two test tubes containing the extracts as mentioned above were added to 1 ml of Wanger's regent. The formation of a brown color showed the presence of alkaloids.

Test for flavonoid

The two test tubes containing the extracts as mentioned above were added to 1 ml of concentrated H_2SO_4. The dark brown solution was taken as evidence for the presence of flavonoids.

Test for carbohydrates

Mixtures of different extracts in tube 1 (C+L) and tube 2 (C+L+G) were taken in separate test tubes and 3 drops of alpha Naphthol was added and mixed well. Sulphuric acid was added along the walls of the test tube. Purple ring at the junction of two liquids showed the presence of carbohydrates.

Test for protein

1% of sodium hydroxide was prepared and 1 ml of it was added to test tubes containing the two extract combinations. A few drops of copper sulphate solution were gently added. The solution turning to purple color indicated the presence of protein.

Test for reducing sugars

About 1ml each of Fehling's reagent type 1 and type 2 were added to the extracts in tube 1 and 2 for both the extract combinations. The mixture was kept in a hot water bath for two minutes. The presence of reducing sugars in the samples were indicated by a brick red color.

Test for amino acid

The two different plant extracts combinations were taken, and excess of ninhydrin regent was added, and solutions were mixed well. The solutions were placed in a boiling water bath for about 3 minutes. A bluish-black color indicated the presence of amino acids.

Test for terpenoid

2 ml of Chloroform was added to two test tubes containing the extract combinations.

About 3ml of concentrated sulphuric acid was added to the resulting solution. Formation of a reddish-brown layer indicated the presence of terpenoids.

Test for steroid

2 ml acetic anhydride was added to the two different test tubes containing the extract combinations. Further, 2ml of concentrated sulphuric acid was added. The deep brown color showed the presence of steroids.

Test for tannins

About 0.5 of ferric chloride was dissolved in 10 ml of distilled water. 1ml of the solution was added to test tubes containing the plant extract combinations. Formation of a green color showed the presence of tannins.

Test for coumarins

10g of sodium hydroxide was dissolved in 100 ml of distilled water. 1 ml of sodium hydroxide was added to two different test tubes containing the mixtures of plant extracts, followed by 1ml of chloroform. Formation of yellowish color indicated the presence of coumarins.

Test for phenols

Lead acetate solution was prepared by dissolving 2g of lead acetate in 20 ml of distilled water. Then few drops of the reagent were added to the two test tubes containing the extracts. The formation of a white precipitate in the aqueous extract indicated the presence of phenols.

Saponins test

The extracts were prepared in two different tubes, tube 1 containing clove and lemon and tube 2 containing clove, lemon, and ginger. 1ml of each extract was mixed with 9 ml of distilled water. The solutions were shaken vigorously for 5 minutes and allowed to stand for 30 minutes. The formation of honeycomb or foam in the upper layers of the test tube indicated the presence of saponins.

Phlobotanins test

1 ml of concentrated hydrochloric acid was diluted in 50 ml of distilled water, then 1 ml of it was added to test tubes containing the extract combinations. The formation of a yellow color indicated the presence of Phlobotanins.

FTIR analysis

A small amount of extract 1 containing clove and lemon (C+L) and another extract clove, lemon and ginger (C+L+G) were introduced into the FTIR spectrometer. Each plant extract combination was placed individually on the sample holder. Upon exposure to the infrared (IR) rays inside the spectrometer, the device measured the absorbance of different IR frequencies by the samples. The spectrometer was connected to a computer-based software system that controlled the device and handled the data processing. The resulting spectra were displayed as graphs showing the relationship between transmittance (or absorbance) and frequency. These graphs revealed the functional groups present in each extract based on the characteristic peaks observed.

GC- MS analysis

The GC-MS analysis was conducted using a split less injection method, where 1µl of the extracts of both combinations were injected into the GC-MS assembly, equipped with a column and a mass detector. Several criteria were applied to analyze the two extracts as described above. The column oven temperature was set to 100°C, maintaining a pressure of 175.1 kPa, while the purge flow was maintained at 3.0 ml/min. The total flow rate was 16.3 ml/min, with a column flow of 1.21 ml/min, and a linear velocity of 28.9 cm/sec. The mass detector initiated its operation at six minutes and concluded at forty-nine minutes. The sample, mixtures containing clove and lemon, and Clove, lemon and ginger were inserted into the instrument and results were tabulated and analyzed (23).

Antimicrobial assay

Antibacterial assay was conducted for both the plant extract combinations using Kir-

by Bauer method by taking two Gram positive (*Bacillus subtilis* and *Staphylococcus aureus*) and two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). The two combinations of methanolic extracts (clove and lemon) and (clove, lemon and ginger) were then tested against the bacteria to determine the zones of inhibition.

Antioxidant activity (DPPH assay)

Stock solution was prepared by measuring 0.012 g of dark colored crystalline powder of 2.2-Diphenyl-picrylhydrazylradical (DPPH) with 50 ml of methanol. In one test tube the control was prepared by adding 3ml of methanol and 1ml of DPPH. 20 µl and 50µl of two extract combinations ware added to two test tubes with 1 ml of DPPH and 3 ml of methanol. For 30 minutes the tubes were incubated in dark place. The absorbance of the tubes was measured using a spectrophotometric at λ_{max}= 517 nm. DPPH free radical inhibition was calculated using the equation below.

(%) antioxidant classes = $(A_0 - A_S/A_0 \times 100)$

Where A_0 the absorbance of blank at 517 nm of racial (DPPH) in absence of antioxidant, and A_S absorbance of sample.

Preparation of shampoo

0.2% Sodium Benzoate was added in a 250 ml beaker. Three grams of salt (NaCl), 40 ml of Tween 20, and 1 ml of a mix of extracts (from cloves, lemons, and ginger) were added to the sodium benzoate solution. The mixture was stirred together, and then water was poured in until it reached a total of 100 ml. Shampoo was used for antimicrobial assay.

Results and Discussion

Phytochemical Screening

Phytochemical analysis of the extract combinations clove with lemon (C+L) and clove with lemon and ginger (C+L+G) were performed for the identification of various primary and secondary metabolites (Table 1).

Table 1: Results of Phytochemical Screening

Test/ compilation	C+L	C+L+G
Amino acid	+++	+++
Carbohydrate	+++	+++
Steroids	++	+++
Sugar	++	++
Protein	++	++
Alkaloid	+	+
Terpenoids	+++	+++
Flavonoids	+++	+++
Tannins	+++	+++
Phytosterol	+	+
Phlobatannins	+	++
Coumarins	++	++

(C= Clove, L= Lemon, G=Ginger)

FTIR Analysis

To identify the active functional groups, present in the extract combinations (C+L and C+L+G) a FTIR analysis was conducted. Results of analysis have shown that standard functional groups like the carboxylic group, nitro group and others were found in the combination of three plant extracts (Table 2 Figure 1).

Table 2: FTIR analysis of methanolic extract of C+L+G.

Frequency	Functional group
3289.53	alkyne
2921.81	Alkane
1604.93	α, β-unsaturated ketone
1512.96	Nitro
1431.02	Carboxylic acid
1366.74	Phenol
1267.05	Alkyl aryl ether
1197.50	Sulfonamide
1148.93	Alphatic ether
996.15	Alkane
594.43	Halo compound
568.14	Halo compound

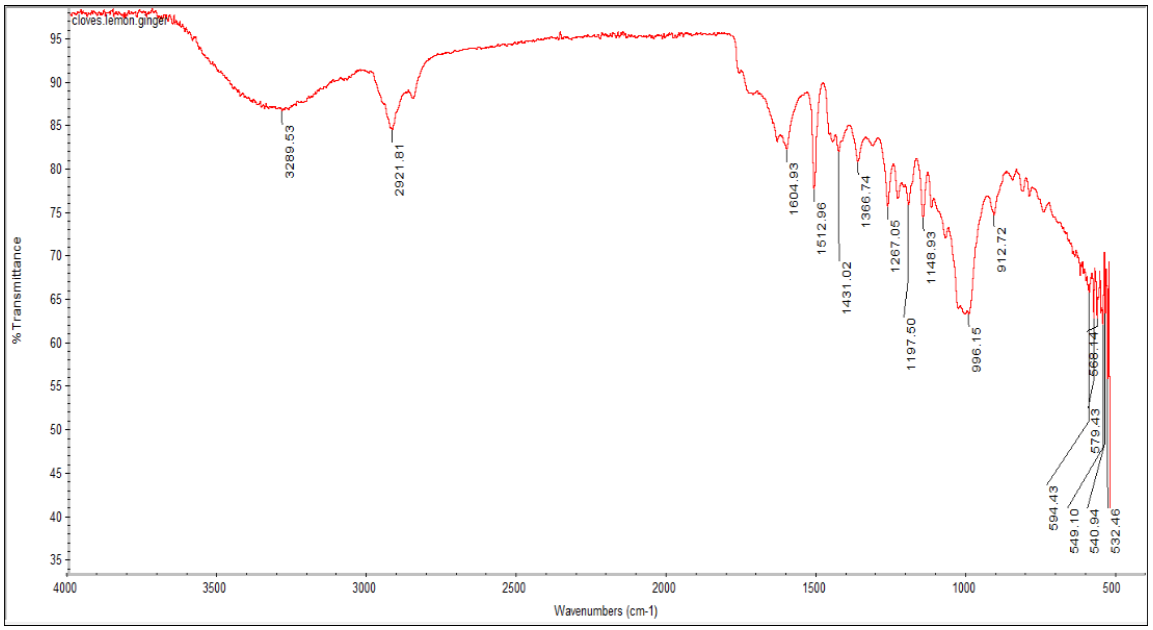

Figure 1: FTIR result of combinations of clove, lemon peel and ginger.

However, the functional groups in the combination C+L were more than that of C+L+G, which indicates an antagonism, when the three plant samples are mixed. (Table 3 and figure 2).

Table 3: FTIR analysis of methanolic extract of C+L

Frequency	Functional Group
3351.05	Secondary amine
1728.38	Unsaturated ester
1605.25	α,β-unsaturated ketone
1512.06	Nitro compound
1431.22	Carboxylic acid
1366.72	Sulfonamide
I316.40	Aromatic amine
1266.68	Aromatic ester
1196.88	Aliphatic ether
1120.98	secondary alcohol

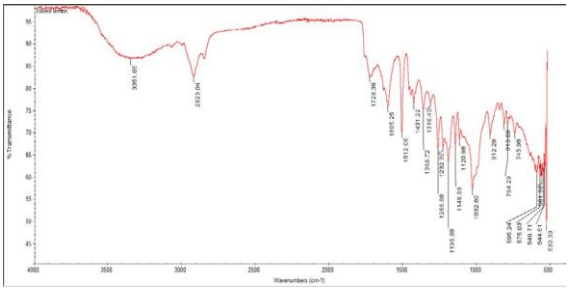

Figure 2: FTIR analysis of combination of clove and lemon peel.

GC- MS analysis

Gas Chromatography -mass spectrometry analysis was performed on the two extract combinations for the determination of bioactive compounds and various fatty acids. The analysis showed that both extracts have significant number of bioactive compounds, however the extract with the three samples (C L and G) more potential in terms of the compounds identified when compared to the clove and lemon extract. The significance of the compounds obtained is mentioned in the tables and discussion part of the manuscript (Table 4 and 5 and Figures 3 and 4).

Table 4: GC-MS analysis of methanolic extract of C+L

S.No	Retention time (min)	Peak value	% of total	Compound identified
1	5.449	10272269	2.409%	2,5-Furandione
2	7.848	1463023	1.719%	Furan
3	10.760	607307	0.325%	Terpinene -4-ol
4	11.004	473960	0.312%	Alpha-Terpineol
5	11.729	640931	0.679%	5-Hydroxymethylfurfural
6	12.165	356546	0.451%	Benzaldehyde
7	13.686	31930860	44.459%	Phenol
8	13.898	1196986	0.628%	Copaene
9	14.533	23394067	14.966 %	Caryophyllene
10	14.950	3977709	2.235 %	Humulene
11	15.187	361355	0.325%	Azulene
12	15.841	26472962	27.974%	Phenol
13	16.970	200762	0.386%	Butyric acid
14	20.525	824587	0.437%	n-Hexadecenoic acid
15	22.128	651973	0.862%	9,12-Octadecadienoic acid
16	36.088	287722	0.591%	Gamma and beta – Sitosterol

Table 5: GC-MS analysis of methanolic extract of C+L+G

	Retention time (min)	Peak value	% of total	Compound identified
1	7.848	1747859	4.393%	Furan
2	11.011	533473	0.489%	Terpinol
3	11.703	780863	1.207%	5-Hydroxymethylfurfural
4	13.647	23587163	34.712%	Eugenol
5	14.507	10600723	8.426%	Caryophyllene
6	14.950	1534897	2.137%	Humulene
7	15.270	2907508	2.081%	Benzene
8	15.437	1984547	1.520%	Cyclohexadiene
9	15.809	18822368	21.659%	Phenol
10	17.002	224620	0.587%	Propanedioic acid
11	17.535	215253	0.540%	Diethyl adipate
12	18.266	509423	0.518%	2-cyclopenten
13	20.513	1324840	1.159%	n-Hexadecanoic acid
14	22.186	1057920	1.987%	Octadecnoic acid
15	23.546	7822832	6.841%	Methoxyphenyl
16	24.348	1665689	1.447%	Gingerol
17	26.901	1745703	1.849%	3,4-dimethoxyphenol
18	36.082	157421	0.513%	Gamma and beta – Sitosterol

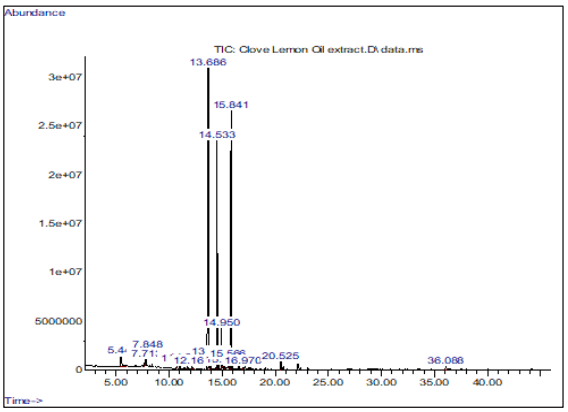

Figure 3: GC-MS analysis of methanolic extracts of Clove and Lemon

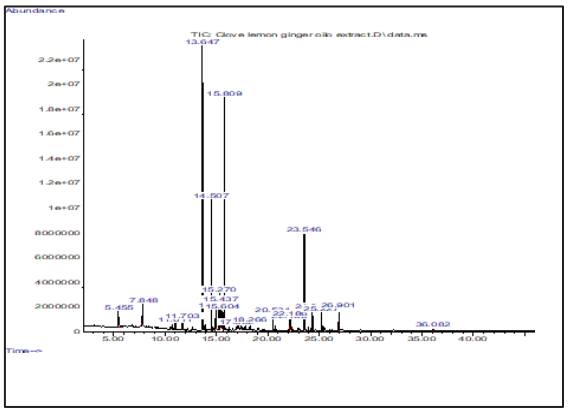

Figure 4: GC-MS analysis of methanolic extracts of clove & lemon with Ginger

Antimicrobial activity

To assess the anti-microbial potential of the two extracts, four different bacteria were selected. Two of them were *Staphylococcus aureus* and *Bacillus subtilis* (Gram positive bacteria and *E. coli and Pseudomonas aeruginosa* (gram negative bacteria), were selected and analyzed. The results have been thoroughly interpreted in the discussion section of the manuscript. (Table 6).

Table 6: Antimicrobial activity of the methanol extracts of the two combinations (units in millimeter)

Extract	Staphylococcus aureus			Bacillus subtilis			E. coli			Pseudomonas aeruginosa		
Conc.	Control	50µl	100µl	Control	50µl	100µl	Control	50µl	100µl	Control	50µl	100µl
Methanol Extraction (C+L)	21	34	35	21	26	34	12	21	24	18	18	24
Methanol Extraction (C+L+G)	21	33	35	21	30	31	12	21	24	18	25	21

conc.= concentration, Control (Gentamicin antibiotic disk) C= clove, L=lemon, G= ginger.

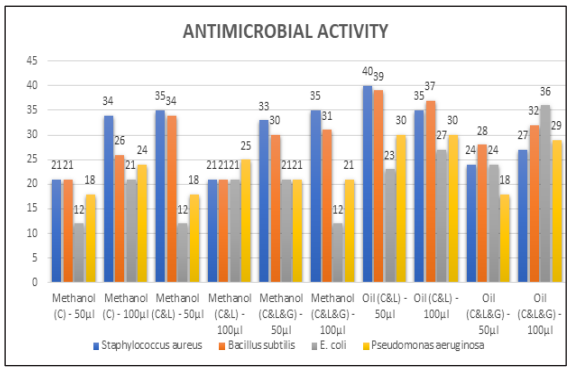

Figure 5: Antimicrobial activity chart of methanolic extract of the two combinations

The zones of inhibition related to both the bacteria are summarized in the form of a histogram for clear understanding and comparison (Figure 5).

DPPH assay

To understand the radical scavenging and % antioxidant activity DPPH assay was performed and the results obtained with the two combinations are summarized in Table 7

Table 7: DPPH assay for determining antioxidant activity

Methanolic Extract	Volume (µl)	% antioxidant activity
C+L	20	97.05%
C+L+G	20	94.05%

Antimicrobial activity of cream and shampoo

One of the essential objectives of the manuscript was to prepare value added products from the two extract combinations. The methodology related to the preparation has been discussed in the materials and methods section of the manuscript and is shown in Figure 6. The results related to antimicrobial activity of the prepared cream and shampoo can be seen form Table-8.

Table 8: Antibacterial activity of the shampoo and cream

Bacteria	Control	Shampoo	Cream
Escherichia coli	20mm	26mm	26mm
Bacillus	20mm	29mm	24mm

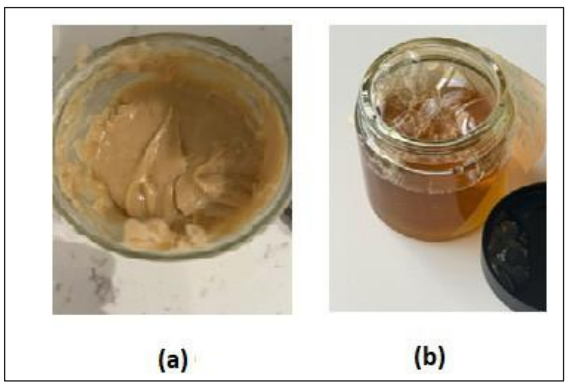

Figure 6: Preparation of (a) Cream and (b) Shampoo from methanolic extracts

Discussion

Phytochemical screening

Results of the phytochemical screening tests as shown in Table 1 provides valuable insights into the composition and potential bioactivity of the extracts of cloves, lemon, and ginger, in two different combinations (clove with lemon and the other one was clove with lemon and ginger). When the three combinations were included, there were potentially high concentrations of the phytochemicals when compared to the two extract combinations of clove and lemon. Nitrogen-containing compounds and alkaloids with potential pharmacological activity were found in both extract combinations. Compounds like terpenoids, flavonoids, tannins, phenols, coumarins and phlorotannin present in high proportions in the three-samples indicated their role as antioxidants, antimicrobials, and anti-inflammatory agents. Saponins have diverse biological activities, including anti-inflammatory and anti-cancer properties. Compared to other studies, it was found that clove oil extract has glycosides, alkaloids, tannins, terpenoids, saponins and phenol (24), while the study carried out elsewhere showed the presence of saponin, HCN, phytin, oxalate and tannin only in the ginger extract (25). Overall, the combination of cloves and lemon with ginger extracts appears to result in a synergistic enhancement of certain phytochemicals, particularly those with potential health-promoting properties such as antioxidants and anti-inflammatory agents. This synergistic study suggests that combined extracts have potential health benefits.

FTIR analysis

The results of FTIR (Fourier-transform infrared spectroscopy) analysis indicated that both extract combinations have common functional groups associated with antioxidant properties, aroma, and flavor enhancement. The presence of α, β-unsaturated ketone suggests potential bioactivities in both combinations, while each combination exhibits unique functional groups, contributing to their distinct medicinal and biological properties. Lemon, cloves, and ginger combination offers a wider variety of functional groups compared to lemon and clove alone, potentially broadening their range of applications. Previous research has

also investigated the presence of asymmetric stretching of C-H and aromatic/alkene carbon double bonds and 1621 cm^{-1} in the spectra of *C. ferruginea* and stretching of C=O, bending of C-H, stretching of C-O, stretching of C-C, and stretching of C-N aliphatic bonds, respectively (26). In summary, while both combinations share some common functional groups, the addition of ginger introduces unique compounds that may enhance the overall medicinal properties of the mixture. The results are shown in Table-2 and Table-3.

GC-MS analysis

The result of GC analysis of extract combinations (Table 4 and Table 5), exhibit some common compounds such as eugenol (from cloves) and limonene (from lemon), as these are major constituents known for their distinct aromas and biological activities. Cloves and lemon may contain terpenes and phenolic compounds, which contribute to their therapeutic properties and flavor profiles. However, current study includes ginger extract along with clove and lemon and contains compounds like gingerol which is known for its anti-inflammatory, antioxidant, and antimicrobial properties. By comparing current study with previous study by Hosseini and colleagues in 2019 found that the most important parts of lemon verbena were eugenol (14.63%), D-limonene (12.41%), caryophyllene oxide (8.78%), α-curcumene (7.91%), trans-citral (7.44%), β-spathulenol (6.92%), z-citral (5.38%), eucalyptol (5.3%), sulcatone (3.11%), and caryophyllene (3.03%). Clove oil's main components included eugenol (79.4%), β-caryophyllene (13.36%), eugenol acetate (4.49%), and α-caryophyllene (1.67%) (27). Additionally, α-copaene was also present. In addition, the synergistic effects of these compounds result in a more potent and versatile product, making the extract combinations a promising option for natural remedies and wellness formulations.

Antimicrobial activity of extract

Table 6 exhibits the results of antibacterial well diffusion tests using two combinations of extracts against four different bacterial strains: *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, and Pseudomonas aeruginosa*. For each combination of extract (methanol extraction with clove, lemon, and ginger). The zones of inhibition are measured at two different concentrations (50µl and 100µl) for each bacterial strain. The inhibition zone of extract (C and L) which has 40 mm inhibition zone on *Staphylococcus aureus* while the control agent (gentamicin) has less inhibition zone which is 21mm on the same bacteria. The table show that the 2-strain gram + (*S. aureus and B. subtilis*) were the most effect by the two extract that show high zone of inhibition. Based on the study the inhibition zone of ginger extract on gram + bacteria *(S. aureus and B. subtilis)* 7.2 mm and 5.12 mm respectively exhibition less antimicrobial activity (28) Most 100µ volume extract show highest inhibition 2 the bacterial growth which show in table highest zone for *Staphylococcus aureus is* 35 mm for methanol extraction of combination (clove, lemon and ginger) compering result of another study where the metabolic extract of clove shows Clove extract inhibit growth of bacteria *E. coli and S. aureus* by inhibition zone 24 mm and 28 mm respectively (29).

DPPH assay

Table 7 shows the results of the study's assessment of antioxidant activity in methanol extracts of lemon and cloves revealed higher effectiveness compared to a previous study (27). In the current research, the combination of lemon and cloves demonstrated antioxidant activity ranging from 97.05 % to 94.29 % at varying concentrations. However, the addition of ginger to the mixture led to a decrease in antioxidant activity, with values ranging from 94.05 % to 90.5 %. Conversely, lemon verbena essential oil exhibited stronger radical scavenging activity [19], with a DPPH value of 11.33 ± 01, compared to clove oil's value of 81.18 ± 02. Additionally, lemon verbena oil showed a higher total phenolic content of 816.07 ± 46.81, further indicating its superior antioxidant potential compared to

clove oil. Therefore, the current study's combination of lemon and cloves presents a more effective antioxidant solution compared to clove oil alone, as demonstrated by its higher DPPH values.

Antimicrobial activity of Cream and shampoo

Table 8 shows the results of the antibacterial activity assessment of both the cream and shampoo products are highly commendable and indicative of their effectiveness in promoting skin and scalp health. The substantial inhibition zones of 26 mm and 29 mm observed for the cream and shampoo, respectively, against *Escherichia coli* bacteria demonstrate their potent antibacterial properties. This suggests that these products have the capability to effectively combat harmful bacteria, thereby reducing the risk of bacterial infections and promoting overall skin and scalp hygiene. Similarly, the significant inhibition zones of 24 mm and 26 mm observed for the cream and shampoo, respectively, against *Bacillus* bacteria further highlight their impressive antibacterial efficacy. This indicates that these products possess broad-spectrum antibacterial activity, targeting a wide range of bacteria and offering comprehensive protection against microbial threats.

Conclusion:

The study's comprehensive analysis of phytochemical constituents, FTIR spectra, GC-MS profiles, antioxidant activity, glucose reduction, and antimicrobial efficacy of combined extracts and products from cloves, lemon, and ginger demonstrates their rich bioactivity and potential health benefits. The shampoo and soap prepared from the synergistic extract showed very good antimicrobial activity and hence can be recommended for proper formulation to explore its health benefits.

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