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Evaluation of Phytochemical Content and Antibacterial Activity of *Zingiber officinale* and *Curcuma longa* Synergistic Combinations (Peeled and Unpeeled)

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Abstract

This study compared the phytochemical contents and the antibacterial activities of peeled and unpeeled blended mix juice of Zingiber officinale and Curcuma longa rhizome against Bacillus subtilis and Staphylococcus aureus (Gram positive bacteria).The results of the phytochemical screening indicated the presence of phytochemicals including flavonoid, tannins, phenol, reducing sugar, saponin, steroids and cardiac glycosides, most of which have been linked to antibacterial activity, in both combinations (peeled and unpeeled). However, terpenoid, which is also a powerful antibacterial compound, was present only in the unpeeled blend mix whereas the result for the quantitative analysis showed that the quantity of phytochemicals in peeled and unpeeled juice blend were: tannin (23.54 ± 0.20 mg/100 g and 22.6 ± 0.48 mg/100 g), flavonoid (40.84 ± 0.14 mg/100 g and 41.78 ± 0.26 mg/100 g), and phenol (32.83±0.28 mg/100 g and 31.07 ±0.67 mg/100 g), reducing sugar (29.54 ± 0.23 mg/100 g and 31.54 ±0.47 mg/100 g), saponin (40.48 ± 0.14 mg/100 g and 41.78 ±0.26 mg/100 g),steroids (27.43 ± 0.16 mg/100 g and 28.42 ± 0.35 mg/100 g) and cardiac glycosides (29.54 ± 0.21 mg/100 g and 30.11± 0.67 mg/100 g) respectively. The result of the antibacterial activity revealed that the peeled combination had antibacterial activity against B. subtilis with a zone of inhibition of 20 mm and none against S. aureus, while the unpeeled combination had antibacterial effect on S. aureus with a zone of inhibition of 20 mm and none against B. subtilis. In comparison to Levofloxacin, which showed zones of inhibition of 20 mm and 17 mm against S. aureus and B. subtilis, the peeled and unpeeled blended extracts of Z. officinale and C. longa rhizome combination showed antibacterial effectiveness. This result demonstrated that the quality and quantity of phytochemicals present in the combined rhizomes of ginger and turmeric can be affected by the presence or absence of peels and consequently on their therapeutic action.

Keywords: *Synergestic combination, Zingiber officinale, Curcuma longa, Peels, Phytochemical content, Antibacterial activity*

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Introduction

Antimicrobial medications have altered not just the management of infectious diseases but also the course of human history. They are being developed to eradicate disease-causing germs, the most well-known being antibiotics (Ike *et al*., 2019). As consumer preferences shift to natural products, the use of spices and natural aromatic plants as antimicrobials instead of synthetic food additives has been back on the agenda recently. This is because of the increased side effects of synthetic drugs and the emergence of resistance to these synthetic treatments by bacteria (Avci et al., 2020; Alkhathlan et al., 2020). The UN has predicted an estimated 10 million deaths from

antibiotic resistance by 2050, and it may be the leading cause of death even more than number cancer mortality (Carro, 2019). Bacteria uses a variety of mechanisms to evolve antibiotic resistance including active drug efflux, target site alteration and enzyme degradation (Gupta & Birdi, 2017). Because *Zingiberaceae* plants generate a range of chemicals that can be utilized as spices, herbs, and seasonings in food, as well as in the pharmaceutical and beauty sectors, they have drawn a lot of interest (Ghasemzadeh et al., 2016; Panpatil *et al*., 2013). These compounds especially the secondary metabolites are being advocated as potential antibacterial and resistance-modifying agents (Khameneh et al., 2019). Both ginger (*Z. officinale*) and turmeric (*C. longa*) belongs to Zingiberaceae family and are utilized historically as colors and preservatives in food. All tropical and subtropical areas of the planet are home to *Z. officinale* and *C. longa*. They are a common home cure in traditional medicine for a number of illnesses such as the common cold, arthritis and inflammatory bowel disease (Amer & Rizk, 2022). *Bacillus subtilis* has been reported to cause serious neonatal infections such as sepsis (Lampropoulois et al., 2021), whereas *S. aureus* is a major contributor to osteoarticular, bacteremia, infective endocarditis, pleuropulmonary and infections related to soft tissue, skin, and devices (Rasheed and Hussein, 2021). Sadly, the WHO has listed these microorganisms, particularly *S. aureus*, as priority pathogens for which innovative therapies are desperately required because of antibiotic resistance (Carro, 2019; WHO, 2021). One of the strategies in the development of new antibiotic therapies with effective treatment modalities to combat pathogens resistant to conventional drugs is the use of plant extract in synergetic combinations (Cheesman *et al*., 2019). In addition, peels have been reported to have antimicrobial properties (Das, 2017). According to Ogori et al., 2021 and Samaniego-Sánchez, 2011, after being blended, the juice extract of ginger or turmeric can be utilized wholly or added to drinks and tea. Therefore, this work evaluated the phytochemical content as well as antibacterial property of different blends of ginger and turmeric synergistic combinations (peeled and unpeeled). To the best of our knowledge, the synergistic combination of ginger and turmeric different blends (peeled and unpeeled) has not been studied.

Materials and Methods

Ginger and turmeric, the major materials utilized in this study were obtained from the market and verified by a botanist at Covenant University. Analytical grade chemicals and reagents were used.

Overview of Experimental Design

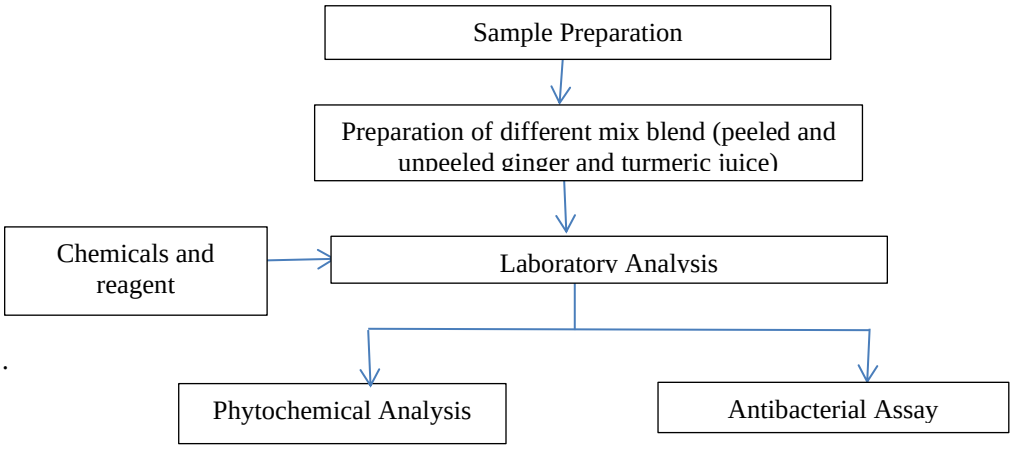

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Figure 1: Experimental Design

Extract Preparation

For the preparation of the ginger and turmeric mix (i.e peeled and unpeeled combinations)., the Top 10 Home Remedies Team's recommended procedure was adhered to as described by Ogori *et al*., (2021).

For the peeled mix juice, the rhizomes of ginger and turmeric (50 g each) were peeled, chopped into bits, and added to a blender. Supplemental small amounts of distilled water was added to reduce friction as the ingredients blended. The juice, designated as PBE (peeled blended mix extract/Juice), was then obtained by filtering the juice pulp through a sterile muslin cloth.

The same process was followed for the preparation of unpeeled mix juice. Fresh ginger and turmeric rhizomes weighing 50 g each were washed under running water under ideal conditions before being sliced into smaller pieces. They were then blended while also being given a little amount of distilled water to reduce friction. The juice, designated as part UPBE (unpeeled blended mix extract/Juice), was then obtained by filtering the juice pulp through a sterile muslin cloth. The juice mixtures (PBE and UPBE) were then refrigerated at 4°C until needed.

Phytochemical Analysis

The phytochemical content in PBE and UPBE were identified and quantified using standard methods described by (Adewolu et al., 2021; Kebede, Gadisa, and Tufa, 2021; Akinseye, Morayo, and Olawumi, 2017; Panchal and Charuben, 2017) with little modifications..

Qualitative analysis

a) Detection of Steroids

2 mL each of PBE and UPBE separately and 2 mL of H_2SO_4 were combined with acetic anhydride (2 mL). The presence of steroids in the test samples is indicated by a change in color to blue or green from violet.

b) Detection of Terpenoids

Salkowski s test: After separately combining 5 mL of PBE and UPBE with chloroform (2 mL), a layer was formed by adding 3 mL of concentrated H_2SO_4 carefully to the mixture. The emergence of brownish red colour indicates terpenoids.

c) Detection of reducing sugar

Benedict's test- Benedict's solution and the different blend mix (PBE and UPBE) were combined in an equal amount (2 mL each) and heated in a boiled for 10 minutes. The change in the color mixtures to either yellow, green, and red would indicate the presence of reducing sugar.

d) Detection of Tannins

Both blend mix (PBE and UPBE), 5 mL each were diluted with distilled water (10 mL) separately, then the mixture was heated in a water bath. They were then mixed with Iron (III) chloride. Tannins' presence is implied by the formation of a dark green colour.

e) Detection of Saponins

Distilled water (5 mL) and 1 mL each of PBE and UPBE were mixed separately followed by ferocious shaking to produce a stable, long-lasting froth. The mixtures were shaken again after olive oil (3 drops) was added to the foam. Formation of an emulsion indicates saponins.

f) Detection of Flavonoids

T**est with lead acetate:** Lead acetate solution (3 drops) was added separately to PBE and UPBE. Development of a yellowish precipitate indicates flavonoids.

g) Detection of cardiac glycosides

Killiani test: PBE and UPBE (5 mL each) were treated with 2 mL of glacial acetic acid and one drop of ferric chloride solution. To downplay this, H_2SO_4 (1 mL) was utilized. At the interface, the deoxysugar property of cardenolides is represented by a brown ring. Below the brown ring, a violet ring may form, and in the thin acetic acid layer, a greenish ring could progressively form.

h) Detection of Phlobotannins

Ammonia solution (10%, just few drops) was added to 1 mL of each of the test samples. Phlobatannins

are present in samples as evidenced by the formation of pink precipitates.

i) Detection of Phenols

Ferric chloride test: Ferric chloride solution (few drops) was added separately to 5 mL of PBE and UPBE. Blueish black coloration is a sign of the presence of phenol present.

Quantitative determination

a) Estimation of Steroids

In order to determine the total steroid, 0.5 mL of 0.5% w/v Potassium hexacyanoferrate (III) solution was added to two different flasks containing iron (III) chloride, 4N sulphuric acid, and 1 mL of test extract of (PBE and UPBE) respectively. The mixtures were heated at 70°C for 30 minutes with periodic shaking before being diluted with sterile water to the proper concentration. At 780 nm, the absorbance was calculated against a blank for the reagent. The amount of cholesterol equivalents (mg of CHO/100 g of extract) was used to express the total amount of steroids in samples.

b) Estimation of Flavonoids

The flavonoid content was calculated via the aluminum chloride colorimetric technique. PBE and UPBE were dissolved in 1 mL of methanol each, together with 0.5 mL of 1.2% aluminum chloride and potassium acetate (120 mM, 0.5 mL). After 30 minutes at room temperature, this mixture's absorbance was assessed at a wavelength of 415 nm. The amount of rutin equivalent (mg/100 g of extracted compound) was used to express the flavonoid content in the samples.

c) Estimation of Total Phenols

To assess the phenol content, the Follins method was employed. The test samples (PBE or UPBE, 1 mL each) was added to different tubes along with 2.5 mL of a 10% dissolved Folin-reagent Ciocalteu's in water as well as 2.5 mL of a 7.5% aqueous solution of $NaHCO_3$. The samples were then kept in an incubator for 45 minutes at 45°C. At a wavelength of 765 nm, a spectrophotometer was used to measure the absorbance. The samples were set up for each experiment in triplicate, and the mean absorbance was computed. The calibration line was created by repeating the procedure using the gallic acid standard solution. Using the obtained absorbance, the equivalent of gallic acid concentration (mg of GA/100 g of extract) was calculated.

d) Estimation of Tannins

The Folin-Ciocaltau reagent (0.5 mL) was added to different flasks holding 9 mL in total of (distilled water, test sample; either PBE or UPBE and saturated sodium carbonate solution). 30 minutes were given for the reaction mixture to rest at room temperature. The supernatant was centrifuged and absorbance at 725 nm was measured with an ultraviolet-visible spectrophotometer. The absorbance of different tannic acid concentrations was plotted as the acid was produced at gradually larger concentrations. The tannic acid equivalent (TAE) (mg/100 g) was used to calculate the amount of tannin in the sample.

e) Estimation of Saponins

A total of 250 liters of Distilled water (250 µL) was added to 50 µL of test samples. After which vanillin reagent (250 µL) was added and then sulphuric acid (72%, 2.5 mL). The entire mixture was then shaken together for uniformity and maintained for ten minutes at a temperature of 60 °C. The absorbance was measured at 544 nm after colling in ice. Diosgenin equivalents (mg DE/100 g extract), which were calculated from a standard curve, were used to express the results.

g) Estimation of terpenoids

Five milliliters of each of the test samples (5 mL each) were added to a chloroform-methanol solution (20 mL, 2:1), rapidly agitated, and left to stand for 15 minutes before being centrifuged. The resultant precipitate was dissolved in Sodium Dodecyl Sulphate (SDS) solution (10%, 40 mL) after the supernatant was discarded. 1 ml of the 0.01 m ferric chloride solution was added at 30-second intervals, and the mixture was thoroughly agitated before being allowed to stand for 30 minutes.

Using a stock triterpene solution of 100 mg/L, standard triterpenes were produced. At 510 nm, the absorbances of the sample and typical triterpene concentrations were measured.

h) Estimation of glycosides

To 10 mL each of test samples, 50 mL of chloroform was added and vortexed together. Then sodium nitroprusside (2%, 2 mL) and pyridine (10 mL) was added after which it it was shaken vigorously again for 10 min. A brownish yellow tint was later created by adding 3 mL of 20% NaOH. A 100 mg/mL stock glycoside standard was used to generate glycoside standards. The absorbance of test sample and standards were then read at a 510 nm wavelength.

Assessment of Antibacterial Property

Antibacterial activity of PBE and UPBE were tested against *B. subtilis* and *S. aureus.*

Test Pathogens

The study's test organisms: *S. aureus* and *B. subtilis*, were clinical isolates collected from the University of Lagos' Microbiology Laboratory.

Antibacterial Assay

Agar well diffusion was used to assess the antibacterial effectiveness of PBE and UPBE as described by Abhay and Rupa, 2016. Briefly stated, warm agar was seeded with 1 mL of calibrated test organisms (*S. aureus* and *B. subtilis*) individually, and the roll-palm method was used to fully mix the mixture before pour plating. Then 10 mm-diameter wells were drilled with a cork borer after the nutritional agar plates had solidified. PBE and UPBE in the exact amount of 150 µL, along with 6.25 g/mL of Levofloxacin, were poured into the wells and left to stand for several hours to allow for sufficient diffusion. Afterward, the plates were checked for zones of inhibition after 24 hours of incubation at 37 ºC. A comparison was made between the zone of inhibition formed by these test samples (PBE and UPBE) and the levofloxacin-formed zone.

Result

Phytochemical Screening

The result of the qualitative and quantitative phytochemical screening of peeled and unpeeled blended extracts of ginger and turmeric rhizome combination is shown in Tables 1 and 2 below respectively.

Table 1: Qualitative phytochemical screening of combined blended extracts of ginger and turmeric rhizome (peeled and unpeeled) combination

Test	PBE (peeled blended extract)	UPBE (Unpeeled blended extract)
Steroid	+	+
Terpernoid	-	+
Reducing sugar	+	+
Tannin	+	+
Saponin	+	+
Flavonoid	+	+
Cardiac glycoside	+	+
Phenol	+	+
Phlobatanin	+	+

Note: + = present, - =Not present

Table 2: Quantitative phytochemical screening of combined blended extracts of ginger and turmeric rhizome (peeled and unpeeled) combination

TEST	PBE	UPBE
Steroid		
Terpenoid	27.43±0.16	28.42± 0.345
Reducing sugar	-	14.355± 0.544
Tannin	29.46 ±0.23	31.54± 0.481
Saponin	23.54±0.2	22.6± 0.481
Flavonoid	40.475±0.525	31.34± 0.467
Cardiac glycoside	40.835±0.135	41.78± 0.255
Phenol	29.535±0.21	30.105± 0.403
	32.825±0.275	31.07± 0.665

Antibacterial Activity

The figure below depicts the antibacterial activity of peeled blended, unpeeled blended combination and Levofloxacin against *S. aureus and B. subtilis.*

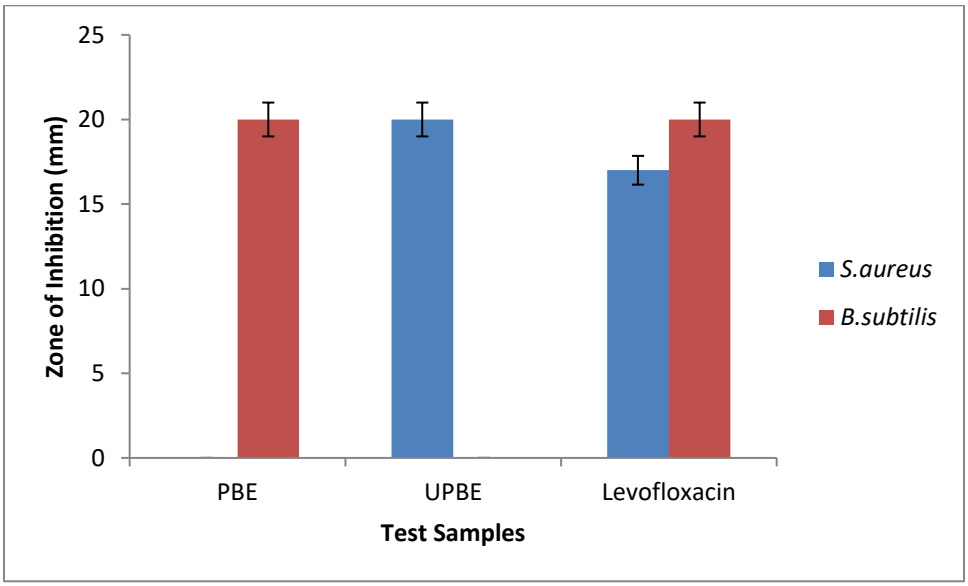

Figure 2: Inhibition zone diameter (mm) of test samples; peeled blended (PBE), unpeeled blended (UPBE) and Levofloxacin against *Staphylococcus aureus and Bacillus subtilis*

Discussion

One of the strategies in the development of new antibiotic therapies with effective treatment modalities is the use of plant extract in synergetic combinations (Cheesman *et al*., 2019). However, in order to use plant extracts as therapeutic agents, it is essential to determine their phytochemical contents since these secondary metabolites are usually responsible for the pharmocological properties exhibited by plants (Yusuf and Abdullahi, 2019). The result of the phytochemical

screening of both peeled and unpeeled blended extract of ginger and turmeric rhizome combination (Tables 1 and 2) revealed the presence of steroid, reducing sugar, tannin, saponin flavonoid, cardiac glycoside, phenol in similar quantities except for steroid that was present in commendable higher quantity in the peeled combination (40.475±0.525) than the unpeeled combination (31.34± 0.467). In addition, terpenoid was found present in the unpeeled combination but absent in the peeled combination. The phytochemical content result in this study is superior to the those previously reported for ginger and turmeric rhizome singly. For example, Arawande *et al*., (2018) reported the absence of phenol, tannin, saponin, glycosides in aqueous extract of ginger and also reported absence of phenol, tannin, reducing sugar, phlobatanin in aqueous extract of turmeric. The result of the antibacterial activity (Figure 2) revealed that PBE exhibited a zone of inhibition of 20 mm which was similar to that of the standard drug (20 mm) against *B. subtilis* while UPBE had none. This could be because saponins which have been implied to be active against *B. subtilis* (Tijani *et al.*, 2017) was present in greater quantity in PBE than in UPBE. On the other hand, PBE had a zone of inhibition of 20 mm which is greater than that exhibited by the standard drug (17 mm) against *S. aureus* while PBE had none. This could also be because terpenoids which have been reported to be active against *S. aureus* (Guimaraes *et al*., 2019) was present in UPBE and not in PBE. The result of the antibacterial activity of the combined extract of ginger and turmeric (peeled and unpeeled) against *S. aureus* and *B.subtilis* in this study are superior to those of ginger and turmeric singly (Lucky *et al*., 2017; Gul & Bakht, 2015; Islam *et al*., 2014).

Conclusion

The findings from this study confirms that plant extracts in synergetic combinations improves effectiveness in combating resistant microorganisms. This study revealed that ginger and turmeric combination (peeled and unpeeled) showed similar/comparable antibacterial activity to Levofloxacin against the test pathogens used. This activity exhibited by ginger and turmeric combination (peeled and unpeeled) can be attributed to the phytochemicals contained in them.

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