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Production of oleoresin from ginger (*Zingiber officinale*) peels and evaluation of its antimicrobial and antioxidative properties

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Dried ginger (ginger peel, peeled and unpeeled ginger) extracts were produced using acetone, and subjected to antimicrobial and anti-oxidative properties. In the antimicrobial assay using agar well diffusion technique the extracts were inoculated with five different organisms (*Escherichia coli*, *Staphylococcus aureus, Salmonella typhimurium, Aspergillus niger* and *Bacillus subtilis*) at different concentrations of 0.5, 1.0 and 1.5 ml, respectively, which were compared with the zero (0.00 ml) concentration of the extracts (control). The results showed that the three extracts (ginger peel, peeled and unpeeled ginger extracts) were effective against *B. subtilis* and *A. niger* at the different concentrations but were ineffective against *E. coli, S. aureus*, and *S. typhimurium*. The antioxidant activities of the three ginger (peel, peeled and unpeeled) extracts were found to be 75.50±0.70%, 73.01±0.00% and 51.01±0.41% for ginger peel, unpeeled ginger and peeled ginger respectively with the peel extract having highest antioxidant activity. These were compared with that of the control-synthetic antioxidant- butylated hydroxytoluene (BHT) which had activity of 98.5±0 0.70%. This showed that ginger peel extract could be used as an antioxidant in the place of synthetic antioxidants in foods and related products.

Key words: Agar well diffusion, antioxidant, ginger extracts, ginger peels, oleoresin.

INTRODUCTION

Lipid oxidation remains a major concern in food processing, due to the formation of oxidation products such as fatty acid hydroperoxides and secondary degradation products (alkanes, aldehydes, alkenes) according to Dandlen et al. (2010). The later components are responsible for off -flavours and they arise from hydroperoxy radicals formed during autoxidation. The formation of these off-flavours, with characteristic rancid odours, is responsible for the decrease in both the nutritional quality and safety of foods (Donnelly and Robinson, 1995; Yanishlieva et al., 2006). Oxidation processes are also deleterious in human health, since they induce tissue damage responsible for several pathologies, including cancer, neurodegenerative and ischaemic heart diseases, malaria, arterio-sclerosis and other pathological conditions (Erdemoglu, 2006). The utilization of antioxidants can prevent food oxidation or cell damage. To prevent this degradation process of lipids, the food industry adds antioxidants of low cost and high stability, mostly synthetic ones, such as butylated hydroxyanisol (BHA), butylated hydroxytoluene (BHT), gallats and *tert*-butylhydroquinone (TBHQ), in well-defined

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concentrations.

On the other hand, antioxidants are micronutrients that have gained interest in recent years due to their ability to neutralize the actions of free radicals (Carenas and Packer, 1996). Free radicals are potentially harmful products generated during a number of natural processes in the body, and are associated with the ageing of cells and tissues. Failure to remove active oxygen compounds, over a long term, can lead to cardiovascular disease, cancer, diabetes, arthritis and various neurodegenerative disorders (Sies, 1996). The use of synthetic antioxidants is restricted in several countries, because of their possible undesirable effects on human health (Branen, 1975; Chen et al., 1992; Kahl and Kappus, 1993). This has set the stage for rising consumer demand for food products with fewer synthetic additives, increased safety, quality and shelf- life. Consequently, it is of great interest to manufacturers the idea of identification of natural preservatives for food processing that will reduce the use of artificial preservatives, and possibly replace them with alter-natives that consumers perceive as natural. This has resulted in a renewed search for preservatives from natural sources and in particular from plant extracts, including their essential oils and oleoresin, as well as the use of natural antimicrobials to preserve foods (Poojari et al., 2009). Though, there is wide range of potential antimicrobials available, only few are suitable for use. Over the years, researches carried out on ginger established that it is concentrated with active substances that have anti-bacterial, anti-flatulent, antimicrobial, anti-inflammatory, antiseptic, anti-spasm, anti-viral and antioxidative properties (Altman and Marcussen, 2001). Ginger rhizome constitutes an aromatic and pungent spice that has unique culinary, medicinal and commercial relevance to the economy of its growers in tropical and subtropical countries. The characteristic pungent odour of peeled ginger rhizome is due to its oleoresin (viscous-to-thick material) content, which is an oily liquid containing oxymethyl phenols like shagoal, zingerone and gingerol, that are probably responsible for its antioxidant property (Bode and Dong, 2004). Ginger's oleoresin is principally composed of ginger essential oil and 6-gingerol, the major pharmacologically active component, and a lesser amount of a structurally related vanilloid (Bode and Dong, 2004). Zingerone is also produced from gingerols when ginger is cooked or dried.

According to Bode and Dong (2004), a common mechanism has been offered to explain the actions and health benefits of ginger and other herbs and spices which is related to their antioxidant properties. Modern scientific research has revealed that ginger possesses numerous therapeutic properties including antioxidant effects, an ability to inhibit the formation of inflammatory compounds, and direct anti-inflammatory effects (Akoachere, 2002). The antioxidant powers of ginger have been proven in applications where ginger extract was added to meat products, and was further tested with fresh, frozen and pre-cooked pork patties. Lee et al. (1986) demonstrated that the shelf-life of some food products determined by thiobarbituric acid (TBA) value was improved by the inclusion of ginger extract. Ginger has also been shown to be effective against the growth of both Gram-negative and positive bacteria including *Escherichia coli*, *Proteus vulgaris*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Streptococcccus viridans* (Thompson et al., 1973). Orji et al. (2003) reported that different chemical substances are often obtained in members of even the same species of plants in different areas. Hence, the interest in ginger peels extract. The main objective of this research was to produce and assess extracts from ginger peels (the proximate composition, antioxidative and antimicrobial properties of ginger peels extract on test organisms).

MATERIALS AND METHODS

Procurement of raw materials and test organisms

A known weight (10 kg) of fresh ginger rhizomes (Nigerian variety-*Tafin giwa*) were purchased from Ogige market in Nsukka, Enugu State, Nigeria. Test organisms and reagents used for the antimicrobial activity were obtained from Pharmaceutical Microbiology Laboratory, Faculty of Pharmacy, University of Nigeria, Nsukka, Enugu State (Table 1). All reagents used for the antioxidant activity screening of the samples were obtained from Crop Science Laboratory, University of Nigeria, Nsukka, Enugu State, Nigeria.

Sample preparation

Ten kilograms (10 kg) fresh ginger rhizomes thoroughly were washed in tap water, 4 kg were dried without peeling, while the remaining 6 kg were peeled. The peeled and unpeeled rhizomes as well as the peels were sun dried (35±2°C) separately. The drying of the peels took four (4) days while the drying of the peeled and unpeeled ginger rhizomes took about two weeks to attain constant weight. After drying, the three samples (peels, unpeeled and peeled rhizomes) were milled into powdered form using a hammer mill (Thomas Wiley Mill model ED-5).

Extraction of oleoresin from ginger

Solvent extraction was used to extract an initial weight of 35 g of the powdered samples (peel, unpeeled and peeled ginger rhizomes) separately in a 500 ml glass column with 100 ml acetone (solvent), which was also used for washing the samples after the first extraction (Meadows et al., 2005). The solvent was allowed to percolate the separate ginger samples for 48 h each before the first extracts were collected. The glass columns containing the samples were re-soaked with additional 100 ml of acetone for another 24 h before collecting the second set of the extracts. The process was stopped when a cotton wool soaked with the extract from the glass column was devoid of ginger aroma. The extracts from each sample were pooled together and the solvent was removed using Rotavapor RE 111 with Buchi 461 water bath (Buchi, Sweden).

Table 1. Test organisms used for antimicrobial testing.

S/N	Organism	Source
1	*Escherichia coli*	Pharmaceutical Microbiology laboratory, UNN
2	*Salmonella typhimurium*	Pharmaceutical Microbiology laboratory, UNN
3	*Staphylococcus aureus*	Pharmaceutical Microbiology laboratory, UNN
4	*Aspergillus niger*	Pharmaceutical Microbiology laboratory, UNN
5	*Bacillus subtilis*	Pharmaceutical Microbiology laboratory, UNN

Table 2. Proximate composition of ginger peel.

Parameters	Composition (%)
Moisture	7.31^{c} ±0.02
Crude ash	7.46^{c} ±0.34
Crude fat	9.21^{b} ±0.00
Crude protein	9.42^{b} ±0.03
Crude fibre	7.02^{c} ±0.01
Carbohydrate	59.58^{a}±0.00

Values are means ± standard deviation of duplicate readings.

Portions (300 ml) of each extract was poured in rotary evaporators flask and evaporated at 65°C till all the solvents were expelled. The concentrated extracts (oleoresin) were collected and treated as crude ginger peel, unpeeled and peeled ginger oleoresin extracts, respectively (Lewis et al., 1972). The difference between the empty flask and flask with the separate concentrated extracts was used in obtaining the oleoresin content yield (Onyenekwe, 2000).

Sample analysis

Chemical analysis

The moisture, ash, fibre and fat contents were determined by the Association of the Official Analytical Chemists [AOAC, 2010] methods, 14004, 14009 and 14006, respectively. The Kjeldahl (Kjeltec, Tecator, Sweden) method was used to determine the nitrogen content of the samples, while protein was estimated as 6.25 N (method 7015, [AOAC, 2010]). The carbohydrate content was obtained by subtracting the above values from hundred (Pearson, 1976). The vitamins E and C content as well as the peroxide value of the ginger (peel, unpeeled and peeled) extracts were determined using the method described by Pearson (1976).

Determination of the antioxidant and antimicrobial activities of the ginger extracts

The antioxidant activities of the ginger peel, unpeeled ginger and peeled ginger extracts was determined using the Ferric Thiocyanate Method (FTC) described by Osawa and Namiki (1981) while Agar Well Diffusion method described by Wan et al. (1998) was used to determine their antimicrobial properties. The measurements (in millimeters) of the zones of inhibitions of the extracts against the test organisms (Table 1) were taken and recorded. The tests were performed in duplicates for each microorganism.

Data analysis and experimental design

The antimicrobial testing was statistically analyzed by using a 3-factorial or split-split plot in completely randomized design (CRD). While the antioxidative testing was statistically analyzed by using a 2x3 factorial or 2x3 split plot in completely randomized design (CRD). The vitamin evaluation were subjected to one-way analysis of variance and the means were separated by Duncan's multiple range test using SPSS version 17 computer statistical package. Significant levels were accepted at $p>0.05$.

RESULTS

Proximate composition of ginger peels

The result in Table 2 shows the proximate composition (% dry basis) of ginger peels. The moisture content of the ginger peels was found to be 7.31±0.02%, while that of the crude protein content was 9.42±0.03%. The crude fat, crude ash, crude fibre and carbohydrate contents were 9.21±0.01, 7.46±0.34, 7.02±0.01 and 59.58±0.00%, respectively.

Antioxidative properties of ginger sample (peel, peeled and unpeeled) extracts

Table 3 shows the result of anti-oxidant activities of crude oleoresin extracts obtained from ginger peel (A), peeled ginger (B) and unpeeled ginger (C). It revealed that the ginger peel extract displayed strong antioxidant activity as compared to the other two samples, with the peeled ginger, having the lowest antioxidant activity in the order- Sample A > Sample C > Sample B. The results also indicated that the ginger extracts significantly ($p<0.05$) inhibited linoleic acid peroxidation as compared to the control, since a high inhibition percent indicates a high antioxidant activity. Hence, the ginger peel could be said to contain all the essential components responsible for antioxidant property of ginger.

Table 3. Antioxidant activity of ginger (peel, peeled and unpeeled) crude oleoresin extracts measured after 24 h of incubation.

Sample	Absorbance at 500 nm	Presence of inhibition [k](%)
A	0.010	75.50±0.70[b]
B	0.010	51.01±0.41[d]
C	0.020	73.01±0.00[c]
Control	0.002	98.50±0.70[a]

Value are means ±standard deviation of duplicate readings. Means with different superscripts in the same column are significantly different (P<0.05)
A = Ginger peel extract; B = peeled ginger extract; C = Unpeeled ginger extract; Control = butylated hydroxytoluene (BHT)[k] Percent of inhibition = capacity of extracts to inhibit the peroxide formation in linoleic acid (a high inhibition percent indicates a high anti-oxidant activity).

Table 4. Peroxide values of ginger (peel, peeled and unpeeled) extracts.

Sample	Peroxide value (millieq/kg)
A	10.0±0.00[d]
B	15.10±0.14[b]
C	13.0±0.35[c]
Control	22.0±0.12[a]

Values are means ±standard deviation of duplicate readings. Means with different superscripts in the same column are significantly different (p<0.05).
Sample A = ginger peel; Sample B = peeled ginger; Sample C = Unpeeled ginger; Control = blank titration.

Peroxide value of ginger oleoresins

The peroxide value of the ginger (peel, peeled and unpeeled) extracts presented in Table 4 shows that the ginger peel extract (A) had a better peroxide value of 10 millieq/kg, which was found to be within the peroxide value range of fresh oils. Samples B and C had higher peroxide value which could be as a result of long storage of the oils (crude oleoresin).

Vitamin C and E contents of the ginger oleoresin extracts

The vitamin C and E contents of the ginger (peel, peeled and unpeeled) extracts presented in Table 5 shows that samples A, B and C had a vitamin C content of 14.81,11.42 and 9.61 mg/100g respectively, while the control (standard powdered ascorbic acid) had vitamin C content of 0.61 mg/100g. Thus, the samples had significantly (p<0.05) higher vitamin C contents than the control. Samples A, B and C had 0.38, 0.61 and 0.50 mg/100 g vitamin E contents respectively, while the control (standard powdered ascorbic acid) had a vitamin E content of 0.01 mg/100g. The samples differed significantly (p<0.05) from the control, with sample A having the least value, while sample B had the highest vitamin E content.

Antimicrobial properties of ginger peels, peeled and unpeeled extracts (crude oleoresin)

The result in Table 6 shows the antimicrobial properties of ginger peels, peeled and unpeeled extracts. It revealed that at a volume of 1.5 ml of the different ginger extracts, the measured zones of inhibition were 11, 8 and 14 mm for *B. subtilis* (Figure 1), for extracts A, B and C and 8, 5 and 7 mm for *A. niger* (Figure 2), for extracts A, B and C respectively. Also, *S. aureus* (Figure 3), and *S.typhimurium* (Figure 4) and *E. coli* (Figure 5), were resistant to the three ginger extracts/crude oleoresin at different doses used for the antimicrobial testing. *A. niger* (Figure 2) and *B. subtilis* (Figure 5) were inhibited at dose of 0.5, 1.0 and 1.5 ml with significant difference (p<0.05) observed between the doses and the zones of inhibition at higher dose of the ginger (peel, peeled and unpeeled) crude oleoresin/extracts.

DISCUSSION

Proximate composition (%) of ginger peel on dry basis

The peels moisture content (7.31%) when compared with that of whole ginger which is 9.38% as reported by Remadevi et al. (2004) showing that whole ginger rhizome contains more moisture than the peels. The crude protein when compared with that of whole ginger which is 9.12% as reported by Remadevi et al. (2004),

Table 5. Vitamins C and E content of ginger (peel, peeled and unpeeled) extracts.

Sample	Vitamin C (mg/100 g)	Vitamin E (mg/100 g)
A	14.81±0.01[a]	0.38±0.00[c]
B	11.42±0.03[b]	0.61±0.01[a]
C	9.61±0.01[c]	0.50±0.00[b]
Control	0.61±0.01[d]	0.01±0.00[d]

Values are means ± standard deviation of duplicate readings. Means with different superscripts in the same column are significantly different (p<0.05). Sample A = ginger peel; Sample B = peeled ginger ; Sample C = unpeeled ginger Controls = powdered ascorbic acid (standard) and encapsulated commercial vitamin e (standard).

Table 6. Antimicrobial activity of ginger (peel, peeled and unpeeled) extracts (crude oleoresin) using the agar well diffusion technique after incubation for 24, 48 and 72 h.

Sample	Doses of oleoresin (ml)	Organism zones of inhibition (mm)					
		I	II	III	IV	V	VI
	0.5	4.0±0.41[c]	+	+	+	3.0±0.41[c]	+
A	1.0	6.0±0.00[b]	+	+	+	5.0±0.00[b]	+
	1.5	11.0±0.41[a]	+	+	+	8.0±0.00[a]	+
	0.5	3.0±0.41[c]	+	+	+	0.00±0.00[c]	+
B	1.0	5.0±0.00[b]	+	+	+	3.0±0.00[b]	+
	1.5	8.0±0.00[a]	+	+	+	5.0±0.00[a]	+
	0.5	2.0±0.00[c]	+	+	+	2.0±0.00[c]	+
C	1.0	8.0±0.00[b]	+	+	+	4.0±0.00[b]	+
	1.5	14.0±0.00[a]	+	+	+	7.0±0.00[a]	+

Values are means ± standard deviation of duplicate readings. Means with different superscripts in the same column are significantly different (p<0.05). Sample A = ginger peel; Sample B = peeled ginger; Sample C = unpeeled ginger; I = *Bacillus subtilis*; II = *Staphylococcus aureus*; III = *Salmonella typhimurium*; IV = *Escherichia coli;* V = *Aspergillus niger*; VI = Control; + = Profuse growth of organisms.

indicated that there was no difference in the protein content of the whole ginger and that of the peel. The crude fat compared with that of whole ginger (5.95%) of Remadevi et al. (2004), implies that ginger peels have higher fat deposits than whole ginger. However, the crude ash when compared with that of whole ginger given as 4.77% by Remadevi et al. (2004) revealed that the peels was 32% higher, while the peels crude fiber compared with that of whole ginger which is 7.17% by Remadevi et al. (2004) was found to be 3.8% higher.

Antioxidative properties and peroxide value of ginger (peel, peeled and unpeeled) extracts

The observation of the antioxidative properties could be attributed to the fact that the peeled ginger may not contain much of the essential constituents required like shogoal, zingerone and gingerol which are probably responsible for ginger's antioxidant property as reported by Fuhrman (2000). The peroxide values of the ginger (peel, peeled and unpeeled) extracts were compared with other researchers. The peroxide value of sample A was probably due to the fact that the rancid tastes of oils become noticeable when the peroxide value is higher than 10 millieq/kg of a sample (Pearson, 1976). Expectedly in sample B and C, this showed that the longer the oils stayed un-used, the lesser their degree of freshness. Therefore, a higher peroxide value indicates a higher susceptibility of fats or oils to rancidity. In general, the greater the degree of unsaturation, the greater is the liability of fats/oils to oxidative rancidity.

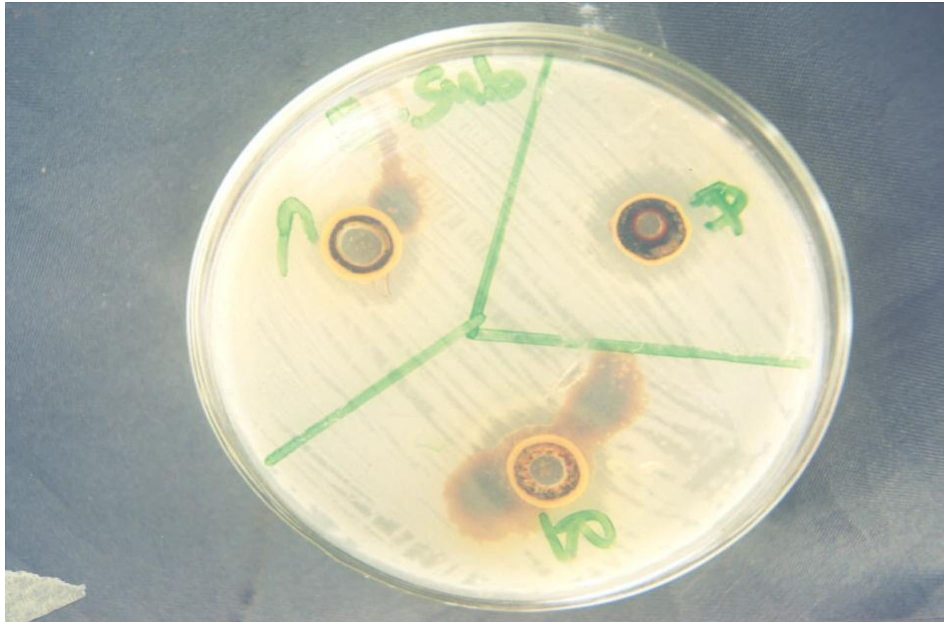

Figure 1. Petri dish showing zones of inhibition of *B. subtilis* with ginger peel, unpeeled ginger and peeled ginger crude oleoresin.

Figure 2. Petri dish showing zones of inhibition of *A. niger with* ginger peeled, unpeeled ginger and peeled ginger crude oleoresin.

Antimicrobial properties determination of ginger (peel, peeled, unpeeled) extracts

The zones of inhibition (mm) increased with increase in doses of oleoresin of the ginger samples used for the antimicrobial testing. This was in agreement with a report published on well diffusion assay protocols for phenolic compound and some plant extracts by Rauha et al. (1994), which showed that there was wide variation in a reported volume of antimicrobial used in well diffusion

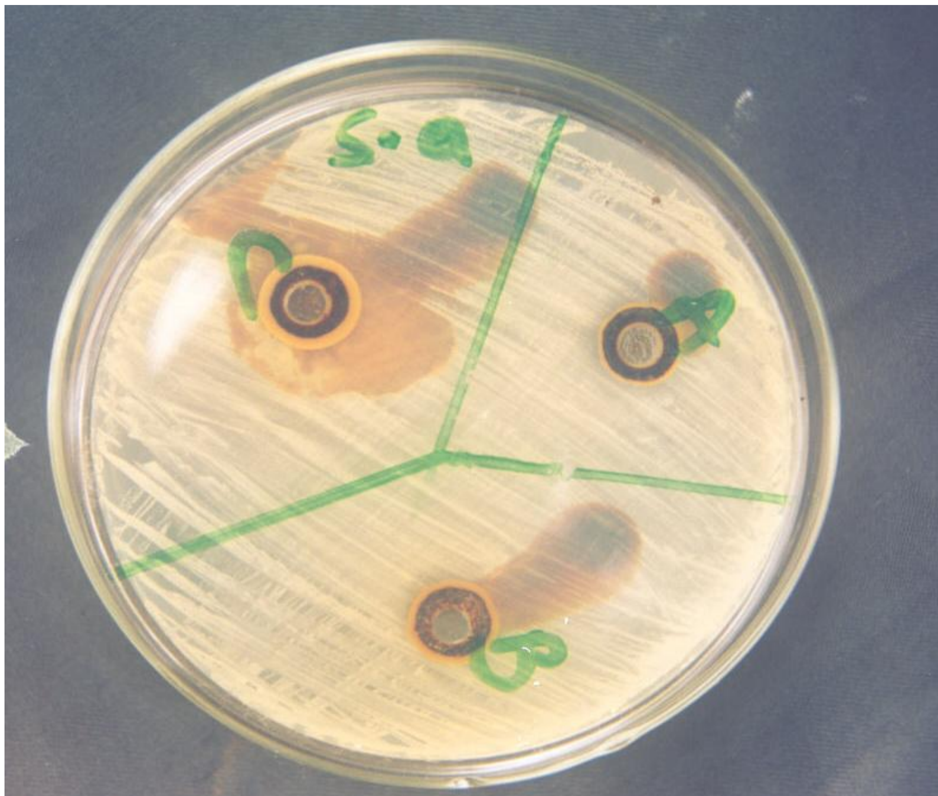

Figure 3. Petri dish showing zones of inhibition of *S. aureus* with ginger peel, unpeeled ginger and peeled ginger crude oleoresin.

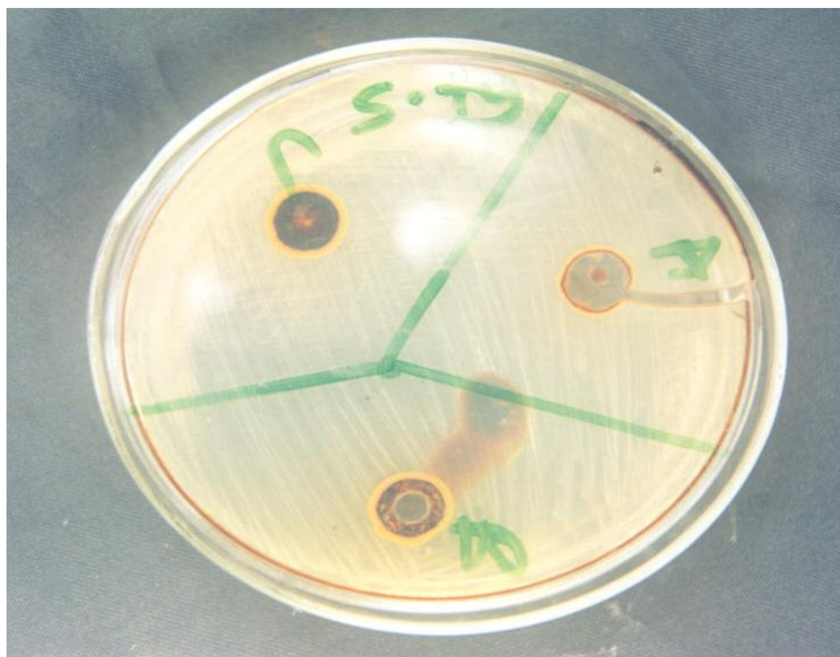

Figure 4. Petri dish showing zones of inhibition of *S. typhimurium* with ginger peel, unpeeled ginger and peeled ginger crude oleoresin.

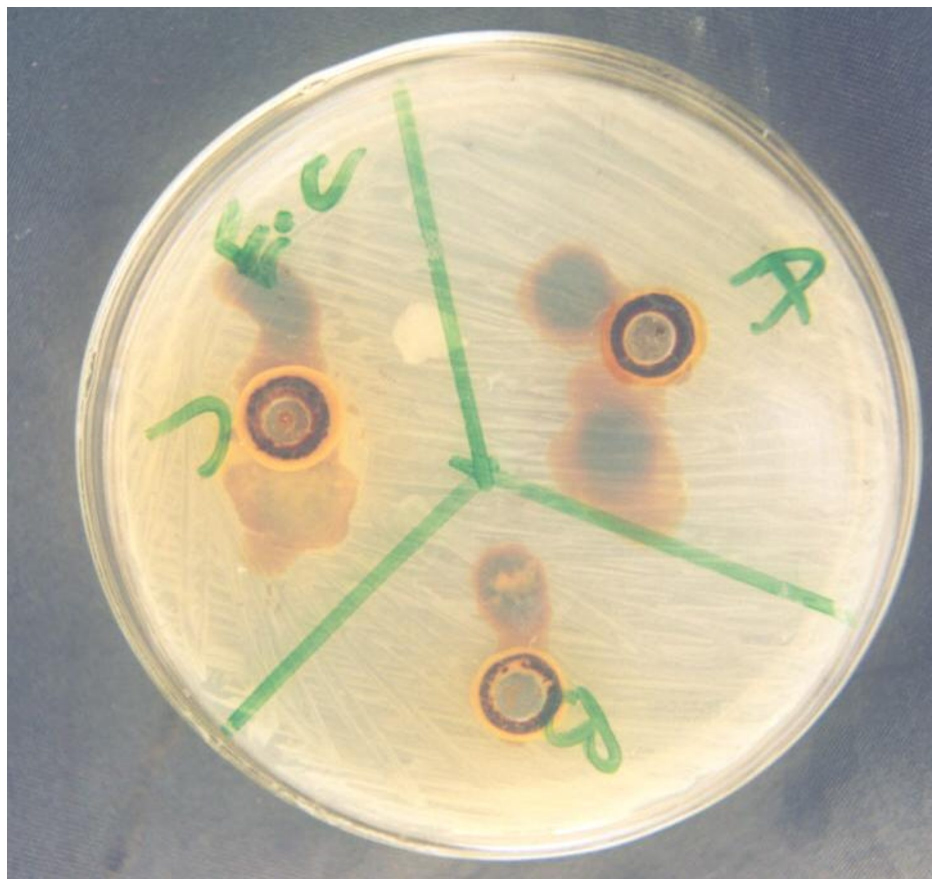

Figure 5. Petri dish showing zones of inhibition of *E.coli* with ginger peel, unpeeled ginger and peeled ginger crude oleoresin.

assay and in the well size used. There had been reports that ginger is effective against the growth of both Gram-positive and negative bacteria including *E. coli*, *S. typhimurium* and *S. aureus* (Lewis, 1972). However, in the present work, the ginger (peel, peeled and unpeeled) oleoresin/extracts were observed to be inactive at 0.5, 1.0 and 1.5ml doses against *E. coli, S. aureus* and *S. typhimurium* even after being incubated for 72 h.

This observation could be attributed to the un-even diffusion of the extracts through the agar medium because the extracts were not initially in direct contact with the organisms and must first diffuse into the agar to exert any antimicrobial effect or it could be due to low level of doses of the ginger extracts used (Yamada et al., 1992). The ginger (peel, peeled and unpeeled) crude oleoresin/extracts however, were effective against *B. subtilis* as reported by Yamada et al. (1992), who reported that *B. subtilis* and *S. aureus* are more susceptible to antimicrobial agents than *E. coli* bacteria. The ginger, extracts were also effective against *A. niger* as observed by Singh (2001) that the essential oil of ginger show antifungal activity against *Aspergillus* species. However, the two organisms that exhibited antimicrobial activity (*B. subtilis* and *A. niger*) did not show any visible activity after 24 and 48 h but after 72 h of incubation. However, lack of antimicrobial activities exhibited by all the extracts of *Cassia occidentalis* and generally at concentrations between 500 - 1000 mg on *P. multocida, S. typhi, S. typhimurium, S. pyogenes, S. pneumoniae and K. pneumoniae* is suggestive of limited antimicrobial activity of the plant. This was not pointed out by Gasquet (1993), Percez (1994) and Saraf (1994). Moreso, uniformity of antimicrobial activity exhibited by hexane, chloroform, methanol and aqueous extracts of *C. occidentalis* leaf on only *E. coli* may confirm the limited antibacterial activity of the plant even *in vivo*. Nonetheless, there is need to separate the chemical constituents of the plant leaf and then test each component on the microorganisms.

Conclusion

It is evident from this research work that ginger peels

possess both antioxidative and antimicrobial properties, an indication that no part of the ginger plant can actually be regarded as a waste. In this context, the antioxidant property of ginger peels merits special consideration since ginger is a widely used food spice that can be consumed by all. Ginger could also be said to have added advantage of being a natural antioxidant over standard oral synthetic antioxidant vitamin supplements such as vitamin E and C since it contains both of them as shown in this present work.

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