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COMPARATIVE STUDIES OF PHYTOCHEMICAL AND ANTIMICROBIAL PROPERTIES OF GINGER (*Zingiber officinale*) FROM NIGERIA AND INDIA

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ABSTRACT

Ginger (*Zingiber officinale*) is a well known and widely used herb, especially in Asia, which contains several interesting bioactive constituents and possesses health promoting properties. In this study, phytochemical content as well as antimicrobial properties of Nigerian and Indian ginger were assessed in an effort to compare and validate the medicinal potential of the plant. Aqueous, hydromethanolic and hydroethanolic extracts of Indian and Nigerian ginger were prepared by extraction process. Both varieties were found to contain high amount of secondary metabolites. The comparison, carried out between different extracts of the two varieties using t-test at p=0.05, have shown no any significant difference in terms of antimicrobial activity while none of the samples show any activity against a fungus spp: *Candida spp*. This study validated the medicinal potential of *Zingiber officinale.*

KEYWORDS: Ginger, *Zingiber officinale*, Phytochemical content, antimicrobial properties.

I. INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Medicinal plants are of great importance to the health of individuals and communities. Herbal medicines serve the health needs of about 80% of the world's population (Gosh, *et al*., 2011). From 250 to 500 thousand plant species are estimated to exist on the planet, and only between 1 and 10% are used as food by humans and other animals (Cowan, 1999). According to World Health Organization (WHO), traditional medicine is estimated to be used by 80% of the population of most developing countries (WHO Bulletin, 2002). The medicinal value of plants lies in some chemical substances or group of compounds that produce a definite physiological action in the human body. These chemical substances are called secondary metabolites. The most important of these bioactive groups of compounds are alkaloids, terpenoids, steroids, flavonoids, tannins and phenolic compounds (Hill, 1952).

Ginger (*Zingiber officinale*) consists of the fresh or dried roots of *Zingiber officinale*. The English botanist William Roscoe (1753-1831) gave the plant the name *Zingiber officinale* in an 1807 publication (Ghosh *et al.*, 2011). The name of the genus, *Zingiber*, derives from a Sanskrit word denoting "**horn-shaped,**" in reference to the protrusions on the rhizome (Awang, 1992; Bisset and Wichtl, 1994). Ginger is a sterile, reed-like plant with a pungent and aromatic rhizome on which it relies for vegetative propagation (Mabberley, 1997; Vaughan and Geissler, 1997).

Ginger is one of the most popular spices worldwide and is widely used in food, medicines, drinks and toiletries around the globe (Kokate 1999; Ali, 2009; Shah and Seth, 2010). Its rhizome is valued as a spice for its combination of pungent and aromatic qualities, which arise from its content of phenolic compounds and essential oil, respectively. It is used as flavouring agent in a vast array of foods, including savoury dishes such as curries, and sweets such as cakes and biscuits (Wohlmuth, 2008). Ginger rhizome is also used in several traditional systems of medicine, including Traditional Chinese Medicine, Ayurveda and Western herbal medicine (Williamson, 2002). The rizhome is also found to inhibit colon cancer and suppression of the

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transformation, hyperproliferation of cells, and inflammation that initiate and promote carcinogenesis, angiogenesis and metastasis (Lee *et al*., 2008).

The aim of this study is to compare and determine the difference, in terms of total phytochemical content and antimicrobial properties, between Indian ginger and Nigerian ginger. It is planned to be achieved by carrying out the following processes.

- To prepare Aqueous, Hydroethanolic and Hydromethanolic extracts of the plant of both varieties.
- To carry out both qualitative and quantitative phytochemical screening on the two varieties.
- To determine the antimicrobial effect of both varieties on different strains of bacteria and fungi.
- To carry out statistical analysis on the generated result so as to come up with useful information about the two varieties.

II. MATERIALS AND METHODS

SAMPLE COLLECTION

The Indian ginger was collected from Spencer store in Ansal Plaza, Greater Noida, India. It was Utterkand variety from Utterkand State of northern India as gathered from the store's staff. It was dried fine-powder that is well packaged and stored at good environmental conditions with temperature of about 25ºC. The fresh Nigerian ginger was collected from Rimi Market in Kano State of Nigeria. It was brought, as explained by the trader, from Kachia town in Kaduna state of northern Nigeria. Both varieties were identified at the department of Biotechnology, Sharda University. The fresh Nigerian ginger was air-dried and ground to fine-powder and both were stored at laboratory standard conditions.

The bacterial and fungal samples were collected from the microbiology laboratory of Sharda University hospital and were stored under standard laboratory conditions.

Chemical Reagents

All chemicals used in this study are of analytical grade and were obtained from laboratories of departments of biotechnology and chemistry, Sharda University, Greater Noida, India.

Preparations of Extracts

Aqueous Extract

50g of both Indian and Nigerian ginger powder were dissolved in 200 ml of distilled water then placed on hot plate at 50-55^{0}C for 2 hours. The mixtures were regularly shaken at intervals for 2-3 days after which they were filtered using filter paper. The filtrates were dried in a hot oven at 50^{0}C. After drying, the extracts were stored in an air tight container at 4˚C for further use.

Hydroethanolic and Hydromethanolic Extracts

The hydroethanolic and hydromethanolic extracts of the samples (both Indian and Nigerian ginger) were prepared in a ratio of 20:80 respectively (i.e water:ethanol/methanol is 20:80). 50g of the sample was weighed using electric balance and dissolved to make 200ml solution. The mixtures were regularly shaken at intervals for 2-3 days after which they were filtered using filter paper. The filtrates were then dried in a hot oven at 50^{0}C. After drying, the extracts were stored in air tight container at 4˚C for further use.

PHYTOCHEMICAL SCREENING

Primary Metabolites

Test for Carbohydrates and Glycosides

Fehling's Test: 1ml each of the six extracts and Fehling's solutions A and B were boiled on water bath (Amin *et al*., 2013).

Borfoed's Test: To 1ml of the six extracts, 1ml of Borfoed's reagent was added and heated on a boiling water bath for 2mins (Amin *et al*., 2013).

Test for Proteins and Amino Acids

Ninhydrin Test: Two drops of ninhydrin solution were added to 1ml of the all the sample extracts (Amin *et al*., 2013).

Test of Fixed Oils and Fats

Spot Test: A small quantity of each of the extracts were pressed between two filter papers to observe oil stain on the filter papers (Amin *et al.*, 2013).

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Secondary Metabolites

Test for alkaloids

Mayer's Test: To a few ml of each of the extracts, a drop of Mayer's reagent was added by the side of the test tube (Evans, 1997).

Test for Flavonoids

To 5ml of dilute ammonia solution, a portion of each of the extracts was added, followed by addition of concentrated sulphuric acid (Sofowora, 1993; Khan *et al*., 2011).

Test for Terpenoids (Salkowski Test)

5ml of each of the extracts were mixed with 2ml of chloroform and concentrated sulphuric acid to form a layer (Khan *et al*., 2011).

Test for Ascorbic Acid

To each of the sample extracts, 2ml of water were added followed by addition of Sodium bicarbonate and ferrous sulphate and shaken well (Sharma and Paliwal, 2013).

Test for Saponins

Small portions of each of the solid extracts were diluted with distilled water and made up to 20ml. The suspensions were then be shaken in a test tube for 15 min (Kokate, 1999).

Test for Phytosterols

Libermann-Burchard's Test: Portions of each of the extracts were mixed in test tubes with 2ml of acetic anhydride. And to this, 2drops of concentrated sulphuric acid were added slowly along the sides of the test tubes (Finar, 1986).

Test for Phenolic Compounds

Ferric Chloride Test: To 5ml of each of the extracts, few drops of neutral 5% ferric chloride solution were added (Mace, 1963).

Test for Tannins

2ml of each of the sample extracts were diluted with distilled water and then followed by 2-3 drops of 5% ferric chloride ($FeCl_2$) solutions (Ciulci, 1994).

Test for Phlobatannins

10ml of each of the six different extracts were boiled with 1% aqueous HCl acid in a test tube (Krishnaiah *et al.*, 2009).

Test for Steroids

To 0.5 ml of each of the extracts, 2ml of glacial acetic acid and 2ml of Sulphuric acid were added (Venkatesan *et al*., 2009).

Test for Cardiac Glycoside

To 2ml of each of the ginger extracts, 1ml of glacial acetic acid, 2ml ferric chloride and 2ml of conc. sulphuric acid were added (Krishnaiah *et al.*, 2009).

ANTIMICROBIAL SUSCEPTIBILITY TEST

Disc Diffusion Techniques

Nutrient agar was prepared using standard microbiological procedure and carefully poured in to sterile Petri dishes to solidify. After solidification, they were then streaked with clinical isolates (test organisms) as described by Bauer *et al.*,(1996). One disc with appropriate potency [(100, 200, 400, 600, 800, 1000)ug/disc] of each of the extracts was picked for each concentration and aseptically placed on the plates, both positive and negative controls were prepared. The plates were then incubated aerobically at 35^0C for 16-18 hours after which the zones of inhibition in diameter (mm) were measured.

Determination of the Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentrations (MIC) of all the six ginger extracts were determined by microdilution techniques in Mueller Hilton broth according to Sanches *et al., (*2005). The inocula were prepared at a density adjusted to 0.5 Mcfarland turbidity standard [10^8 colony- forming units (CFU/ml)] and were diluted 1:10 for the broth microdilution procedure. Microtiter plates were incubated at 37ºC, and the MICs were recorded after 24 hours of incubation. Two susceptibility endpoints were recorded for each isolate. The MIC is defined as the lowest concentration of extract at which the microorganism tested does not demonstrate viable growth.

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III. RESULTS AND DISCUSSION

Percentage yield and phyto-profile of the Extracts from successive extraction of *Zingiber officinale* of Nigeria and India

The yield of successive extracts of *Zingiber officinale* (in grams) and their corresponding percentage yields are shown on table 1 and phyto-profile on table 2. Total weight of ginger powder taken is 50g.

Table 1: Weights (in grams) and percentage yield of different extracts of *Zingiber Officinale* (50g).

S.No	Extract	Weight (gram)	% Yield (w/w)
1	Aqueous (Nigeria)	3.045	6.09%
2	Hydromethanolic (Nigeria)	5.070	10.14%
3	Hydroethanolic (Nigeria)	5.402	10.80%
4	Aqueous (India)	2.142	4.28%
5	Hydromethanolic (India)	4.120	8.24%
6	Hydroethanolic (India)	3.605	7.21%

Table 1 and figure 1 presents the values of extracts (in percentage) in different solvents from highly-polar to less-polar i.e. Aqueous, hydroethanolic and hydromethanolic. The values were high in hydroethanolic and hydromethanolic extracts of Nigerian ginger; 10.14% and 10.80% respectively. But minimum values were obtained in aqueous extract of Indian ginger 4.28%.

This indicates that most of the phytochemicals present in ginger are less-polar in nature and could be best extracted using less-polar solvents.

Figure 2: Graphical representation of percentage yield of different extracts of *Zingiber Officinale* (50g)

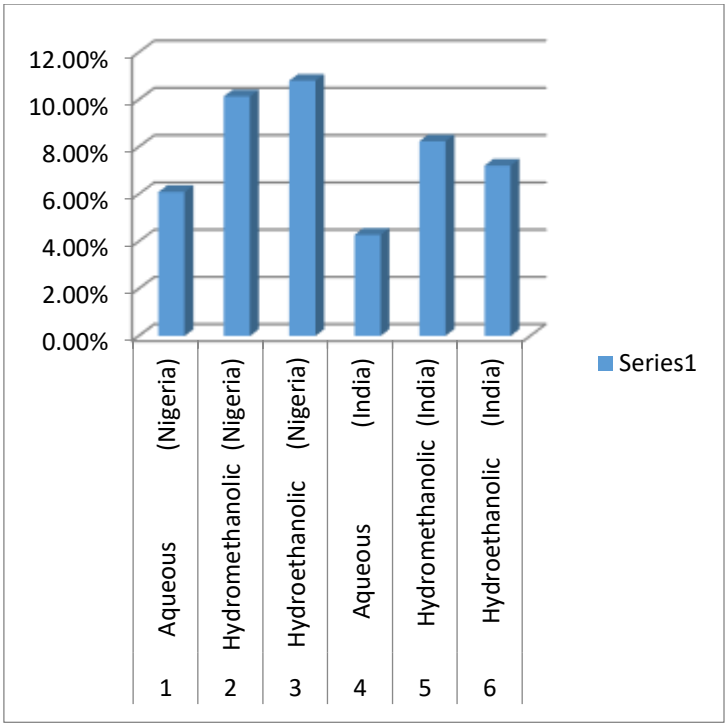

Table 2: Phyto-profile and percentage yield of different extracts of *Zingiber officinale*

S.No	Extract	Nature	Consistency	Colour	Odour	% Yield (w/w)
1	Aqueous (Nigeria)	Solid	Dry	Light brown	Pungent	6.09%
2	Hydromethanolic (Nigeria)	Solid	Sticky	Blackish brown	Pungent	10.14%
3	Hydroethanolic (Nigeria)	Solid	Sticky	Yellow brown	Pungent	10.80%
4	Aqueous (India)	Crispy solid	Dry	Dark brown	Pungent	4.28%
5	Hydromethanolic (India)	Solid	sticky	Orange brown	Pungent	8.24%
6	Hydroethanolic (India)	Solid	sticky	Yellow brown	Pungent	7.21%

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Fluorescence analysis of *Zingiber officinale*

Fluorescence is an important phenomena exhibited by various chemical constituents present in plant material. Many phytochemicals florescence when suitably illuminated. The Fluorescence characteristics of different extracts of *Zingiber officinale* is shown on table 3. The Fluorescence was observed under UV light at 280nm.

Table 3: Fluorescence characteristics of different extracts of *Zingiber officinale*

S.No	Extract	Under ordinary light	Under UV light (280nm)
1	Aqueous (Nigeria)	Light brown	Colourless
2	Hydromethanolic (Nigeria)	Blackish brown	Brownish yellow
3	Hydroethanolic (Nigeria)	Yellow brown	Pale yellow
4	Aqueous (India)	Dark brown	Pale brown
5	Hydromethanolic (India)	Orange brown	Pale yellow
6	Hydroethanolic (India)	Yellow brown	Yellow

The fluorescence colour observed is specific for each compound. A non-fluorescence compound may fluorescence if mixed with impurities that are fluorescent. Some constituents show florescence in the visible range in a day light. The UV produces florescence in many natural products. If the substance themselves are not fluorescent, they may often be converted into florescence derivatives after reacting with different reagents hence some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmaceutical evaluation (Paliwal, 2015).

Phytochemical screening of different extracts of *Zingiber officinale*

Phytochemical components are responsible for both pharmacological and toxic activities in plants. These metabolites are said to be useful to a plant itself but can be toxic to animal, including man. The presence of these chemical compounds in this plant is an indication that the plant, if properly screened, could yield drugs of pharmaceutical importance (Paliwal, 2015). Phytochemical constituents of *Zingiber officinale* are shown on table 4.

Table 4: Qualitative phytochemical screening of different extracts of *Zingiber officinale*

S.No	Plant Constituents	Test Performed	Nigeria			India		
			Aq	HM	HE	Aq	HM	HE
1	Carbohydrates	Fehling's Test Barfoed's test	_ _	_ _	_ _	_ _	_ _	_ _
2	Proteins and amino acids	Ninhydrin	_	_	_	_	_	_
3	Fats and Oil	Stain test	_	++	++	_	++	+
4	Alkaloids	Mayer's test	_	_	_	_	_	_
5	Flavonoids	Ammonia test	++	++	+	+	+++	+++
6	Terpenoids	Yellow brown	++	+++	+	+++	+	++
7	Steroids	Libermann-Burchard's Test	_	+	+	_	+	+
8	Saponins	Frothing test	++	++	++	++	+++	+++
9	Tannins	Ferric chloride test	_	_	_	_	_	_
10	Phenolics	Ferric chloride test	++	+++	+++	++	+	++
11	Phytesterol	Acid test	+	+	+	+	+	+
12	Ascorbic acid	Ferrous sulphate test	+++	+++	+++	+++	+++	+++
13	Phlobotannins	Hydrochloric acid test	+	_	_	+	_	_
14	Cardiac glycosides	Killer killani test	++	+	+++	+	++	++

WHERE **(-):** absent, **(+):** Weak , **(++):** Moderate: **(+++):** Strong
Aq: Aqueous, **HM:** Hydromethanolic, **HE:** Hydroethanolic

The results showed the presence of flavonoids, phenolics, saponins tannins, terpenoids, phytesterol, steroids, fats and oil, and cardiac glycosides and high amount of ascorbic acid in different extracts of *Zingiber*

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officinale of both Nigeria and India.

Crude flavonoids and saponins were present in high amount while phenolics were relatively low. Indian ginger was found to contain relatively high amount of flavonoids, phonolics and saponins than Nigerian one. This could be attributed due to different geographical location.

Flavonoids showed a wide range of biological activities such as inhibition of cell-proliferation, induction of apoptosis, inhibition of enzymes and other antibacterial and antioxidant effects (Paliwal, 2015). Saponins helps in boosting the immune system by lowering the cholesterol levels in the blood and reducing risk of getting intestinal cancer. Various reports have shown that phenolic compounds contribute to the quality and nutritional value of ginger in terms of modifying colour, taste, aroma and flavour and also in providing beneficial health effect. Phenolics also provide plants with defence mechanism to neutralize reactive oxygen species (ROS) in order to survive and prevent molecular damage and damage by microorganisms, insects and herbivores (Paliwal, 2015).

All these phytochemicals posses good antioxidant activities and has been reported to exhibit multiple biological effects including anti-inflammatory and antitumor activities.

Antibacterial activity of successive extract of *Zingiber officinale* against *E.coli* ATCC 25922

The antibacterial activity of the extract against *E.coli* was studied by measuring, (in mm), the zone of inhibition of each extract at different concentrations of 250, 500, 750 and 1000 µg/ml. The bacterial growth majorly inhibited by hydroethanolic extract and hydromethanolic extracts of Nigerian ginger at 500, 750 and 1000 µg/ml in comparison to standard (Ampicilin). While hydromethanolic extract of Indian ginger showed the highest antibacterial activity in comparison to standard.

Table 5: Results of antibacterial activity of *Zingiber officinale* extract against ***E.Coli*** at different concentrations of 250, 500, 750 and 1000 µg/ml

Nigeria	**Zone of Inhibition**			
Concentration(µg/ml)	**STD**	**AQ**	**HM**	**HE**
250	8.67 ± 0.33	0.00 ± 0.00	9.33 ± 0.33	8.67 ±0.33
500	9.00 ± 0.00	8.33 ± 0.33	10.00 ± 0.00	10.00 ± 0.00
750	9.00 ± 0.00	7.67 ± 0.67	10.00 ± 1.00	10.33 ± 0.33
1000	10.67 ± 0.33	10.33 ± 0.33	12.00 ± 0.00	11.67 ± 0.67

India	**Zone of Inhibition**			
Concentration(µg/ml)	**STD**	**AQ**	**HM**	**HE**
250	9.00 ± 0.00	11.33 ± 0.33	10.33 ± 0.33	9.67 ± 0.33
500	13.67 ± 0.33	7.33 ± 0.33	8.67 ± 0.33	8.67 ± 0.33
750	13.67 ± 0.33	8.33 ± 0.33	11.67 ± 0.33	9.33 ± 0.33
1000	13.33 ± 0.33	10.33 ± 0.33	15.67 ± 0.33	11.33 ± 0.33

Results were expressed as: mean ± standard error
n = number of replicates (n=3)

Antibacterial activity of successive extract of *Zingiber officinale* against *Klebsiella spp* ATCC 15/7

Table 6: Results of antibacterial activity of *Zingiber officinale* extracts against ***Klebsiella spp*** at different concentrations of 250, 500, 750 and 1000 µg/ml

Nigeria	**Zone of Inhibition**			
Concentration(µg/ml)	**STD**	**AQ**	**HM**	**HE**
250	8.67 ± 0.33	11.67 ± 0.33	7.33 ± 0.33	8.67 ±0.33
500	9.67 ± 0.33	11.33 ± 0.33	8.33 ± 0.33	11.33 ± 1.33
750	9.67 ± 0.33	10.33 ± 0.33	10.33 ± 0.33	10.33 ± 0.33
1000	10.33 ± 0.33	12.33 ± 0.33	12.33 ± 0.33	10.67 ± 1.67

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India	Zone of Inhibition			
Concentration(µg/ml)	STD	AQ	HM	HE
250	9.67 ± 0.33	7.33 ± 0.33	10.33 ± 0.33	0.00 ± 0.00
500	11.33 ± 0.33	9.67 ± 0.67	9.33 ± 0.67	10.67 ± 0.67
750	9.33 ± 0.33	8.67 ± 0.33	10.33 ± 0.33	12.33 ± 0.33
1000	11.33 ± 0.33	11.67 ± 0.33	10.67 ± 0.33	12.67 ± 0.67

Antibacterial activity of successive extract of *Zingiber officinale* against *Staphylococcus aureus* ATCC 2592

Table 7: Results of antibacterial activity of *Zingiber officinale* extracts against ***Staphylococcus aureus*** at different concentrations of 250, 500, 750 and 1000 µg/ml

Nigeria	Zone of Inhibition			
Concentration(µg/ml)	STD	AQ	HM	HE
250	8.67 ± 0.33	8.67 ± 0.33	7.33 ± 0.33	8.67 ±0.33
500	8.67 ± 0.33	9.33 ± 0.67	8.33 ± 0.33	11.33 ± 1.33
750	9.67 ± 0.33	10.33 ± 0.33	10.33 ± 0.33	11.33 ± 0.67
1000	12.33 ± 0.67	12.33 ± 0.33	12.33 ± 0.67	13.67 ± 1.67

India	Zone of Inhibition			
Concentration(µg/ml)	STD	AQ	HM	HE
250	9.67 ± 0.33	7.33 ± 0.33	9.33 ± 0.33	9.00 ± 0.33
500	11.33 ± 0.67	9.67 ± 0.67	10.33 ± 0.67	10.67 ± 0.67
750	10.33 ± 0.33	10.67 ± 0.33	10.33 ± 0.33	12.33 ± 0.33
1000	11.33 ± 0.67	11.67 ± 0.33	10.67 ± 0.33	12.67 ± 0.67

Standard positive amoxicillin=11mm
Negative water =0mm

From table 5, 6 and 7, it could be observed that all the extracts from both samples exert antimicrobial action against the three selected bacteria for this study. *E. Coli* is greatly inhibited followed by *Staphylococcus spp* and then *Klebsiella spp*. There is no any clear difference observed after comparison between the two samples in terms of inhibiting the bacterial growth.

The inhibition effect might be associated with the presence of some phenolics in the extracts which serves as the main active antimicrobial agent that cause disruption of microbial cell membranes.

There was no any observed inhibitory activity by all the different extracts against the fungus: *Candida spp* strain after it was incubated at 27ºC for 48Hrs.

IV. CONCLUSION

The results of this study indicated that *Zingiber officinale* from both India and Nigeria is very rich in phytochemicals that are very active in exerting antimicrobial action against some pathogens. This could be attributed to the fact that high amount of phenolic and flavonoid compounds were recorded in both samples.

Further work is required to establish the components in phenolics and flavonoids that might have contributed to the high antimicrobial activities so far observed.

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