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In Vitro Antibacterial Activity and Phytochemical Analysis of *Zingiber officinale* L. Rhizome Extracts

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Abstract

The aim of this research was to determine antibacterial activity and phyto-chemical screening of ethanolic extracts of *Zingiber officinale*. Fresh rhizomes of *Zingiber officinale* (Ginger) were collected from Kachia Local Government Area, Kaduna (Kaduna south) state, Nigeria. The antibacterial activity was determined using agar well diffusion method whereas phytochemical screening was carried out to determine the phytochemical composition of the ginger rhizomes. The result of the Phytochemical screening revealed absence of alkaloids and saponins in all the extracts but showed presence of flavonoids, tannins and steroids. The result for the bioassay presented variation in the biological activity of the extracts. All the extracts exhibited antibacterial activity against all the tested bacteria with zones of inhibition diameter ranging from 7.31-18.67 mm. The highest zone was observed in chloroform extracts on Staphylococcus epidermidis at 100 mg/ml while the least zone of inhibition was observed in n-hexane extracts on *Pseudomonas aeruginosa* at 25 mg/ml. However, both *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were resistant to n-hexane extracts at 25 mg/ml.

Keywords: *Zingiber officinale*; Antibacterial activities; Phyto-chemical screening; Resistant; Agar well diffusion

Introduction

The use of natural products together with their therapeutic properties is as ancient as human civilization [1]. There is no doubt that plants are among the most perfect "natural laboratories" for the synthesis of various molecules ranging from simple skeleton to highly complex chemical structures as main sources of drugs [2]. The WHO estimated that about 65% of the World's populations are mainly relying on natural products derived from plants for their primary health care systems and most of them are from developing countries, the remaining 35% are mostly from developed countries that also used natural products indirectly to maintain a good health [3]. Spices are important natural products, which have been used since ancient times and until now. The use of spices is not restricted to food flavoring only, but also used as food preservatives and colorants, extend shelf-life of food, prevent food spoilage, food-borne diseases and frequently prescribed in traditional medicine [4]. Many plants and their components have been extensively investigated for their health-promoting benefits, including antioxidant, cardio-protective, anticancer, antimicrobial and immune-modulatory activities [5-7].

Ginger, botanically known as *Zingiber officinale*, is a perennial herbaceous plant belonging to Monocot family Zingiberaceae and order Scitamineae [8,9]. It is grown in many tropical and subtropical area including India, Africa, China, the west India and Australia, with annual world production estimated at 100,000 tons in 2000 [10]. Ginger has been widely used all over the world in ayurvedic medicine, for a wide array of unrelated ailments including arthritis, cramps, rheumatism, sprains, sore throats, muscular aches, pains, constipation, vomiting, hypertension, indigestion, dementia, fever and infectious diseases [11]. It has direct anti-microbial activity and thus can be used in treatment of bacterial infections [12]. Ginger is relatively inexpensive due to their easy availability, universally acceptable and well tolerated by the most people. It has also been "Generally Recognized as Safe" (GRAS) by the US FDA [13].

Ginger is regarded as valued spice in many parts of the world since the 4th century BC. It is an essential ingredient in many traditional Chinese medicines. Ginger has been made in to candy and is often used as flavorant in food and cakes as well as sweets, non-alcoholic beverages, ginger-bread, ginger-snacks and biscuits [14]. Africa and West India as well as being used in folklore medicine for the treatment of many diseases. The aim of this research is to determine antibacterial activity and Phyto-chemical screening of ethanolic extracts of *Zingiber officinale* L. (ginger).

Materials and Methods

Sample collection

Fresh rhizomes of *Zingiber officinale* (Ginger) were collected from Kachia Local Government Area, Kaduna (Kaduna south) state, Nigeria and were identified by a Mr Zaakariya Sani (a Botanist), in the Department of Biological Sciences, Sule Lamido University Kafin Hausa. A voucher number, numbered 0506 was given to the specimen. The ginger rhizomes after collection was washed thoroughly with running tap water, cut in to pieces and allowed to air dry at room temperature and eventually ground into powder using clean mortar and pestle [15].

Extraction and fractionation

Of the powdered sample 200 g was soaked in 800 ml of ethanol for two weeks with frequent agitation. The solution was filtered using Whatman No. 1 filter paper, evaporated under reduced pressure using rotary evaporator and the filtrate stored at refrigerator for subsequent use. The crude extract was further fractionated between chloroform,

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n-hexane and methanol respectively. To the crude extracts 100 ml of chloroform was added to obtained chloroform fraction. The chloroform fraction was further partitioned with 100 ml of n-Hexane and Methanol respectively to afford methanol and n-hexane fractions. These three fractions were collected in a separate beaker and evaporated using rotary evaporator [16].

Phytochemical analysis

Phytochemical screening was carried out to evaluate the presence of alkaloids, tannins, flavonoids, saponins, glycosides and steroids. The test was conducted following the procedure described by the Indian Pharmacopoeia (1995).

Antibacterial Screening

Preparation of stock solution

Four different concentration (12.5, 25, 50 and 100 mg/ml) of ethanolic extracts, chloroform, n-hexane and methanolic fractions were prepared in sterile sample bottles by dissolving 1 g of each extracts and fractions in 10 ml of Dimethyl sulfoxide (DMSO) [17].

Antibacterial assay

The antibacterial activities were assessed by using agar well diffusion method. The prepared Mueller Hinton ager media was poured on to well labeled Petri dishes under aseptic condition and allowed to solidify. A sterile cork borer was used to make four holes on the ager plates at fairly equidistant position with another hole at middle as control. The plates were inoculated with standardized inoculum containing the test bacteria. Each of the four concentrations was dispensed in their respective holes while the control (Ciprofloxacin) at the middle of the plate [18]. After 24 hours incubation period, zones of inhibition were observed by clear zones of inhibition, measured and recorded in millimeter using transparent plastic ruler [19].

Determination of Minimum Inhibitory Concentration (MIC)

Minimal inhibitory concentration (MIC) is the lowest concentration of the compounds that inhibit growth of the microorganism. 6.5 g of nutrient broth was prepared by dissolution in 500 ml of distilled water. 5 ml of the agar were subjected in to different test tubes and covered with cotton wool. Agar was autoclaved at 121°C for 15 minutes. For each test organism one control isolate and control extracts is used against four different concentration (12.5, 25, 50 and 100 mg/ml) to make serial dilution by adding 1 ml of the extracts to each test tubes and 0.5 ml of control isolate is diluted with 3 ml of distilled water and dispensed to each test tubes. The same procedure was applied for chloroform extracts, n-hexane extracts and methanol extracts and incubated at 37°C for 24 hours [20].

Results and Discussion

The result of the phytochemical screening of both extracts is shown in Table 1. The result revealed absence of alkaloids and saponins in all the extracts and variation of some phytochemicals in some extracts. This may be due to the fact that same solvents used for extraction were unable to dissolve appropriate amount of metabolite to be detected by phytochemical screening procedure employed. The result however showed presence of flavonoids, tannins and steroids in the rhizome of ginger both of which were reported by various researches for the possession of antimicrobial activity. Notably, Tannins have shown antibacterial activity [21]. Flavonoids possess antioxidant properties and ensure healthy circulation of blood. It helps to strengthen capillaries wall [22]. The compound is sometimes referred to as phytoestrogens. Phytoestrogens are associated with relief of menopausal systems, reduction of osteoporosis, improvement of blood cholesterol levels, and lowering the risk of certain hormone-related cancers and coronary heart disease). Tannins are polyphenols obtained from various parts of plants [23]. *Z. officinale* possesses essential oils, phenolic compounds, flavonoids, carbohydrates, proteins, alkaloids, glycosides, saponins, steroids, terpenoids and tannin as the major phytochemical groups [24].

The result for the bioassay is presented in Tables 2 and 3. There is variation in the biological activity of the extracts. All the extracts from the rhizomes of ginger exhibited antibacterial activity against all the tested bacteria with zones of inhibition diameter that ranges from 7.31-18.67 mm. The highest zone was observed in chloroform extracts on *Staphylococcus epidermidis* at 100 mg/ml while the least zone of inhibition was observed in n-hexane extracts on *Pseudomonas aeruginosa* at 25 mg/ml [25]. However, both *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* showed resistance to n-hexane extracts at 25 mg/ml concentration. All tested bacteria showed resistance at 12.5 mg/ml and this may be attributed to the concentration as clear inhibition was observed at higher concentration. The general trend observed was increase in zones of inhibition with increase in concentration of the extracts. The methanolic extracts of *Z. officinale* rhizomes possess significant antibacterial activity against *Escherichia coli*, *Salmonella enteriditis* and *Staphylococcus aureus*. While *Escherichia coli* induce diarrhea which happens to be among the major causes of death of human, zingerone exerted protective effect on this notorious diarrhea causing bacteria [26].

The result of Minimum Inhibition Concentration (MIC) indicated that ginger methanolic extracts showed most significant inhibition than the other extracts as it inhibited *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* at 50 mg/ml while ethanolic extracts inhibited *Streptococcus pneumoniae* while chloroform inhibited *Staphylococcus epidermidis* at 50 mg/ml whereas *Kelbsiella pneumoniae* was only inhibited at 100 mg/ml for both extracts [27,28].

Extracts	Alkaloids	Flavonoids	Steroids	Tannins	Saponins	Glycosides
Ethanol	-	+	-	+	-	-
Chloroform	-	+	+	+	-	+
N-hexane	-	+	+	+	-	-
Methanol	-	+	+	+	-	+

Key: Positive (+)=Presence of compounds; Negative (-)=Absence of compounds

Table 1: Phytochemical Screening Result from Ginger Rhizome Extracts.

Extracts	Concentration	Zones of inhibition in (mm) Organisms			
		S. epidermidis	*S. pneumoniae*	*P. aeruginosa*	*K. pneumoniae*
Ethanol	100 mg/ml	15.33	15.50	14.87	13.18
	50 mg/ml	12.60	13.00	11.92	10.34
	25 mg/ml	8 .67	9.67	8.31	8.12
	12.5 mg/ml	-	-	-	-
Chloroform	100 mg/ml	18.67	12.72	12.11	13.97
	50 mg/ml	14.33	10.17	10.31	11.17
	25 mg/ml	10.19	8.48	7.86	7.94
	12.5 mg/ml	-	-	-	-
N-hexane	100 mg/ml	13.66	11.35	8.54	10.11
	50 mg/ml	10.66	9.56	7.31	8.31
	25 mg/ml	8.05	7.61	-	-
	12.5 mg/ml	-	-	-	-
Methanol	100 mg/ml	17.21	13.42	14.92	12.78
	50 mg/ml	13.83	11.03	12.34	10.85
	25 mg/ml	9.98	9.21	8.98	8.52
	12.5 mg/ml	-	-	-	-
Ciproflaxacin	250 mg/ml	26.52	24.23	23.78	21.86

Table 2: Results for Degree of Sensitivity of Bacteria against Ginger.

Extracts	Concentration	Test Organism			
		S. epidermidis	*S. pneumoniae*	*P. aeruginosa*	*K. pneumoniae*
Ethanol	100 mg/ml	-	-	-	-
	50 mg/ml	+	-	+	+
	25 mg/ml	+	+	+	+
	12.5 mg/ml	+	-	+	+
Chloroform	100 mg/ml	-	-	-	-
	50 mg/ml	-	+	+	+
	25 mg/ml	+	+	+	+
	12.5 mg/ml	+	+	+	+
N-hexane	100 mg/ml	-	+	+	+
	50 mg/ml	+	+	+	+
	25 mg/ml	+	-	+	+
	12.5 mg/ml	+	-	+	+
Methanol	100 mg/ml	-	-	-	-
	50 mg/ml	-	+	-	+
	25mg/ml	+	+	+	+
	12.5 mg/ml	+	+	+	+

Table 3: Results for Minimum of Inhibition Concentration for Ginger.

Conclusion

Zingiber officinale rhizome from this study showed that Ginger extract has some importance number of secondary metabolites such as flavonoids, tannins and steroids and the bioassay revealed that all the extracts exhibited antibacterial activity against all the tested bacteria. Owing to these facts therefore *Z. officinale* can be used as potential source of folklore medicine. This study has been extended to used other extract not only ethanol to ascertain the antibacterial activity of the Ginger in various solvents. Therefore effort should be made to isolate the pure components responsible for the bioactivity in all the extracts.

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