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RESEARCH ARTICLE

Investigation of the Anti-Microbial and Analgesic Activities of crude Ethanolic Extract of Ginger (*Zingiber officinale*) Rhizome

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ABSTRACT

Phytochemical screening, anti-microbial and analgesic activities of the ethanolic extract of Ginger, *Zingiber officinale*, rhizome were carried out in this study. Phytochemical analysis of the plant extract revealed that the crude extract contains alkaloids, saponins, tannins, glycosides, flavonoids and terpenoids. The alkaloid was found to be morphine – type alkaloid, with melting point of 230-248^{o}C. Anti-microbial screening of the crude extract showed inhibitory activities against *Escherichia coli, Staphylococcus aureus, Enterobacter sp, Bacillus sp, and Aspergillus sp.* Analgesic activity of the crude extracts against acetic acid - treated laboratory albino mice, using the tail-flick and hot-plate (40 - 60^{o}C) methods showed that the plant leaves exhibit analgesic effects. The analgesic effect when compared with the control drug, morphine, showed that the extract dose of 26.0mg/g and 27.0 mg/g gave the same analgesia as 1.5 mg/g and 2.0mg/g respectively of morphine using the tail-flick method. Also the same quantity of the plant extract showed a similar analgesia as 2.0 mg/g and 2.5mg/g of the morphine drug using the hot-plate method. The alkaloid of the extract which was morphine-type alkaloid is supposed to be the likely active compound in the plant extract responsible for the analgesic effect.

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INTRODUCTION

Ginger (*Zingiber officinale*) of the family *Zingibiracae* is a knotted, thick hedge with underground stem (rhizome). The stems extend roughly 12 inches above the ground with long, narrow, ribbed green leaves and white flower buds that blooms into yellow flowers. The plant grows commonly in the clearings of the Southeastern parts of Nigeria as a native perennial herb with subterranean and digitally branching stem. Ginger rhizome has much culinary and medicinal uses. The young roots are juicy with a very mild taste. It can often be picked into vinegar or sherry as a snack. The matured older roots are extremely potent and are often used as spice. Ginger can also be made into candy or be used as flavorings for cookies, crackers and cakes

Medicinally, the roots extract has been found to be effective in treatment of nausea caused by motion sickness (Stewart, 1991). Ginger has also been used in the treatment of inflammation and has blood thinning and cholesterol lowering properties, making it effective in treating heart diseases (Awang, 1992). It has also been found to exert anti-inflammatory effect in the treatment of sore or aching muscles (Langnerd and Rampton, 2001).

Among the Southeastern part of Nigeria, particularly the Igbo communities, the rhizome extract of ginger are applied as pain killers to soothe aches, pains and feverish conditions. The rhizomes are mashed and the extract squeezed out and applied on the area. Other plants whose analgesic activity has been investigated include *Scoparia dulcis* (Umerie *et al.*, 2007). Though modern pain relievers are available in hospitals and Pharmaceutical shops, the people still believe in those herbs that their forefathers used before them. Poverty is also another factor that made them to still rely on these ethno medicines as these modern drugs are expensive.

In order to ascertain these claims by local users, the present study is therefore carried out to investigate the

anti-microbial and the analgesic effects of the rhizome extract.

MATERIALS AND METHODS

Preparation of plant material

Ginger rhizomes were cultivated in the farm at the back of the Engineering buildings in Nnamdi Azikiwe University Awka. The ginger farm is allowed to mature and the rhizomes harvested. They are washed to remove the soil particles and their backs peeled. The peeled ginger were cut into small bits and allowed to air dry. The dried rhizomes were pulverized using a manual corona blender. Two hundred (200) grams of the powdered ginger was macerated in 3 liters of absolute ethanol for 72 hours and stirred intermittently to necessitate extraction. This was sieved through a cheese cloth and funnel plugged cotton wool to obtain a clear filtrate. A semi-solid extract was obtained in vacuo using a rotary evaporator.

Experimental Animals

Adult male albino mice (18 – 22 g) were obtained from the animal house of the University of Nigeria Nsukka. The mice were housed and fed in the laboratory for 5 days before the experiment.

Microbial stock cultures

Pure cultures of *Escherichia coli, Staphylococcus aureus, Enterobacter sp, Bacillus sp, Salmonella sp, Rhizopus sp and Aspergillus sp* were obtained from the Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka, Nigeria.

Screening of the Extract

Phytochemical screening of the crude extract was carried out using the methods of Evans (2002).

Reaction of different reagents on the alkaloid extracted

The colour change of morphine with other reagents was employed to confirm the type of alkaloid present in the extract using the method of Umerie *et al*. (2007).

Drug preparation

The plant extract was prepared in normal saline in the ratio 1:2 w/v. Morphine sulphate (Ms), which served as a control drug, was also prepared in the ratio 1:10 w/v. A solution of 0.6% acetic acid was prepared as recommended by Umerie *et al*. (2007).

Effective dose (ED) and Acute toxicity assays

The method of Takemori and Portoghese (1987) as used by Umerie *et al*. (2007) was used with slight modifications. A total of ten mice were used. The mice were injected intraperitoneally each with 0.3ml of 0.6% acetic acid. Ten minutes after, graded doses of the crude extract preparations (5, 10, 15, 20, 25, 30, 35, 40, 45and 50mg/g) were then administered subcutaneously. After twenty-four hours, the mice were checked for dead, morbid or healthy conditions. The effective dose ED was determined as the mean dose administered to the surviving viable mice after the test period. The lethal dose (LD_{50}) of the extract was calculated as the geometric mean of the maximum dose that caused 0 % death and the minimum dose that caused 100 % death (Lorke, 1993; Umerie *et al*., 2007).

Analgesic assay

The hot-plate method and the tail-flick methods were used to check the analgesic activity of the extract. A total of 30 mice were used. Fifteen mice were used for the hot - plate assay and the other fifteen mice were used for the tail-flick assay. The 15 mice in each method were divided into 3 groups of 5 mice each. Two groups received treatment while the other group served as control. All the 30 mice were first injected with 0.3 ml of the 0.6 % acetic acid solution intra peritoneally (ip).

Hot-plate assay

The method described by Twycross (1984) was used. After one hour of administering the graded concentrations of the extract solution, the mice were placed on a hot-plate whose temperature was maintained at 40 – 60^{o}C, the time taken for the mice to withdraw its tail and jump out of the hot - plate was noted.

The tail-flick assay

The tail-flick method as described by Umerie *et al*. (2007) was used. Ten minutes after the administering of the graded concentrations of the extract solution in duplicate, the mean time taken for the mice to voluntarily flick their tail was observed.

Control

From the two groups, the ten mice that were reserved as control were given increasing doses of Ms solution, five mice were examined under hot -plate assay and five others for tail-flick assay.

Anti-microbial screening

The anti-microbial screening of the extract was done using the method of Ajiwe *et al*. (2008).

RESULTS

Phytochemical analysis of the crude extracts of Ginger rhizome showed the presence of saponin, alkaloid, tannins, flavonoids, glycosides, terpenoides and carbohydrates as shown in Table 1. Reaction of the different reagents with the alkaloid of the crude extract revealed the alkaloid to be morphine-type (Table 2). Melting point of the alkaloidal salt was found to be between 230 – 248^{o}C. This was very close to the value for pure alkaloid (254^{o}C) as found by Finar (2002).

Table 1: Phytochemical results of the crude ethanolic extract of *Zingiber officinale* rhizome

Parameter	Level
Alkaloids	+ +
Saponins	+ +
Tannins	+
Resins	-
Glycosides	+ +
Flavonoids	+ +
Terpenoids	+
Carbohydrates	+ +
Steroids	-

Key: + + high concentration; + moderate concentration; - Absent

Table 2: Reaction of different reagents on the alkaloid of Ginger

Reagents	Colour on reaction	Remarks
Conc. H_2SO_4	Pink, violet then brown on warming	Morphine suspected
Conc. H_2SO_4 + little Powdered K_2CrO_7	Greenish brown	Morphine or Codeine
Erdmann's reagent Conc. H_2SO_4+ 0.5% HNO_3)	Pink	Morphine present
Powdered cane sugar moistened with conc. H_2SO_4	Red	Morphine confirmed

Table 3: Tail – flick assay of Morphine and the crude ginger extract on acetic acid treated mice

Morphine solution		Ginger extract	
Dose (mg/g)	Reaction time (hr)	Dose (mg/g)	Reaction time (hr)
1.0	4	23.0	8
1.5	2	24.0	7
2.0	1	25.0	5
2.5	40 mins	26.0	2
3.0	died	27.0	1

Table 4: Hot – plate assay of Morphine and the crude extract on acetic acid treated mice

Morphine solution		Ginger extract	
Dose (mg/g)	Reaction time (hr)	Dose (mg/g)	Reaction time (hr)
1.0	6	23.0	10
1.5	4	24.0	8
2.0	2	25.0	5
2.5	1	26.0	2
3.0	died	27.0	1

Table 5: Anti-microbial activity of the rhizome extract

Organisms	Activity
Escherichia coli	+ +
Staphylococcus aureus	+
Enterobacter sp	+ +
Salmonella sp	-
Bacillus sp	+ +
Rhizopus sp	-
Aspergillus sp	+

Key: + + highly positive; + moderately positive; - negative

Effective dose assay

The intraperitoneal administration of the acetic acid solution to the mice resulted in a writhing activity of the abdominal muscles together with the stretching of the hind limbs. After twenty-four hours of subcutaneous administration of the crude ginger rhizome extract, those that received 5, 10, 45 and 50 mg/ml died of under dose and over dose respectively. Those that received 15, 40mg/ml died after 48 hr, those given 20, 30 and 35mg/ml remained morbid while those given 25mg/ml remained healthy. Effective dose of 22.5mg/g and LD_{50} of 32.5mg/g body weight were obtained.

DISCUSSION

The study has successfully looked into the phytochemical analysis, anti-microbial and analgesic activities of ginger, *Zingiber officinale* a *Zingibiracae* used in homeopathy for the treatment of several ailments like pain, fever etc. The phytochemical screening of the crude extract showed the presence of alkaloids, saponin, tannins, flavonoids, glycosides, terpenoids and carbohydrates (Table 1). Further characterization of the alkaloid showed the alkaloid as morphine type (Table 2). The phytochemical compounds identified in the plant extract like flavonoids and saponins been generally found to be haemolytic, anticancer and anti-inflammatory compounds and not analgesic (Evans, 2002; Ajali, 2004). Steroidal saponins have the ability of drastic reduction in cholesterol levels and raises high density lipoprotein (HDL).

The extract also contains glycosides known to posses anti-neoplastic properties (Kar, 2007). Cyanogenic glycosides are known to be active against slugs and snails (Harbone, 1998; Umerie *et al*., 2007), also cardiac glycosides show cardiotonic effect and have the potential for management and control of cardiac arrest. However, none of these phytocompounds except the morphine- like alkaloid have been implicated in its analgesic activities. Morphine and morphine-type alkaloids are classed as narcotic or opiate analgesics and are strong agonists (Way *et al*., 2001). These substances mediate their action by binding to opiate receptors in the central nervous system, causing inhibition of ascending pain pathways and altering the perception of and response to pain, thus producing generalized central nervous system depression (Takemori *et al*., 1999; Umerie *et al*., 2007). They are the main ingredients used in the preparation of analgesic drugs.

Writhing of the abdominal muscle of the mice, together with stretching of the hind limbs resulted after the intraperitoneal administration of 0.3 ml of 0.6% acetic acid solution, indicating painful reaction. The group of mice that received 3.0 mg/g of Ms died due to overdose. Those given 2.5mg/g recovered after 40 minutes and 1 hr respectively in both methods while 2.0 mg/g gave analgesia in 1 hr and 2 hr respectively for both methods (Table 3 and 4). The group of mice that received extracts doses of 5, 10, 45 and 50 mg/g died due to under and over dose after 24hr. Those given 15 and 40 mg/g died after 48 hr while those given 20, 30 and 35 mg/g remained morbid. The extract dose of 26.0 mg/g gave analgesia in 2 hr in both methods and their analgesia was comparable with 1.5 and 2.0 mg/g of morphine sulphate. Also the extract dose of 27.0 mg/g of the extract gave the same analgesia as 2.0 mg/g and 2.5 mg/g of morphine in tail flick and hot plate assays respectively (Table 3 and 4). The effective dose (ED) of the plant extract was then obtained as 22.2 mg/g body weight. The lethal dose (LD_{50}) of the extract was obtained as 32.5 mg/g. In both the tail – flick and hot – plate assays, analgesia were achieved with ginger extract. The wide range between the LD_{50} (32.5 mg/g) and the ED (22.5 mg/g) suggests the wide margin of safety. This is in keeping with the fact that the morphine drug and most of its relatives have high abuse liability due to its high effectivity and that the persistent administration of doses as low as 10 mg would result in addiction and physical dependence (Way *et al*., 2001; Umerie *et al*., 2007). Overdose of morphine drug results in death in man and most other species (Goldstein *et al*., 1974) as seen with 3.0 mg in laboratory mice.

In conclusion, the results of this research confirm that the rhizome extract has high analgesic activity and should be effective in the treatment and relief of fever and pain.

REFERENCES

Ajali U, 2004. Saponins: In: Chemistry of Bio-compounds. 1st ed Rhyce Karex Publishers, Enugu, Nigeria, pp: 167-175.

Ajiwe VIE, CI Agupugo, SO Umeh and HO Nnabuenyi, 2008. The Preliminary study of the Pharmaceutical constituents of Pterocarpus soyauxi (Oha-ocha) leaf. J Chem Society Nigeria (Anachem) 3: 501-511.

Awang DC, 1992. Ginger, Pharmacology Journal, 3: 309-311.

Evans WC, 2002. Trease and Evans Pharmacognosy. 15th ed Saunders Company Ltd, Edinburgh, p56.

Finar IL, 2002. Alkaloids. In :Organic Chemistry Vol 2. Stereochemistry and the Chemistry of Natural Products, 5th ed Pearson Education Ltd, Delhi, India, pp: 696-768.

Goldstein A, L Aronow and S Kalman, 1974. Drug Tolerance and Physical Dependence. In: Principles of drug action: the Basis of Pharmacognosy, 2nd ed. John Wiley and sons Inc, New York, pp: 569-621.

Harbone JB, 1998. Phytochemical methods: A guide to modern Techniques of plant analysis. 3rd ed, Chapman and Hall, London UK.

Kar A, 2007. Narcotic Analgesics (Opiate Analgesics). In: Medicinal Chemistry, 4th Ed. New Age International publishers, Ltd, New Delhi, pp: 304-341.

Langnead L and D Rampton, 2001. Herbal Treatment in Gastrointestinal and Liver disease. Benefits and Dangers, Pharmacol J, 57: 1221-1227.

Lorke, 1983. A new approach to Practical Acute Toxicity Testing. Arch of Toxicology, 53: 273-289.

Stewart J, 1991. Effects of Ginger on motion sickness and susceptibility and Gastric function, Pharmacol J, 42: 111-120.

Takemori CK, JH Hodding and DM Kraus, 1999. Mophine pediatric dosage, Handbook, 6th Ed Lexi-Comp inc, Hudson (Cleveland) p6.

Twycross RG, 1984. Use of Analgesics to achieve analgesia, Journal of the Royal College of Physicians of London, 18: 32-35.

Umerie SC, SO Umeh and SC Nwobi, 2007. Phytochemical analysis and Analgesic studies On *Scoparia dulcis* (*Scrophulariaceae*), Natur Appl Sci J, 8: 66-72.

Way WL, HL Fields and MA Schumacher, 2001. Opoid Analgesics and Antagonists. In: Basic and Clinical Pharmacology. 8th Ed, Katzung, BG McGraw-Hill Companies, inc. New York, pp: 512-531.