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Full Length Research Paper

Phytochemical screening and antidiarrhoea activities of *Tetracarpidium conophorum* induce in albino rats

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Tetracarpidium conophorum commonly known as Walnut is used in traditional medicine for the treatment of various ailments including diarrhoea. Thus, this study was designed to investigate the phytochemical component of *T. conophorum* and its antidiarrhoea activities. The hot aqueous extract was studied for antidiarrhoea activities; castor oil was used to induce diarrhoea in albino rats. 500 mg/kg, 1000 mg/kg and 2000 mg/kg extract of *T. conophorum* were used to protect the rats against castor oil induced diarrhoea. The quantitative phytochemical screening revealed the presence of bioactive components (phenol, flavonoid, alkaloid, tannin, saponin, oxalate and phytate). The percentage values of these components were 31.78, 30.63, 22.56, 11.55, 3.39, 0.28 and 0.07 respectively. The result of the antidiarrhoea activity of *T. conophorum* showed that pretreatment with the extract inhibited diarrhoea. The inhibition of diarrhoea by the plant extract as shown in the result could be attributed to the presence of these phytochemicals.

Key words: Antidiarrhoea, *Tetracarpidium conophorum,* phytochemical.

INTRODUCTION

Plants parts are known worldwide to be sources of large amount of different groups of drugs. Owing to the outbreak of different diseases and microbial resistance to some available drugs, proper identification and evaluation of plants has been encouraged. Harnessing thoroughly the usefulness of any plant, involves the determination of proximate and phytochemical composition of such plant (Nwachoko et al., 2015).

Walnut (*Tetracarpidium conophorum*) belongs to a *Euphorhiacea* family. It is a climbing Shrub of about 10 - 20 feet long, found in the wet parts of Eastern, Western Nigeria and West Africa. In the Eastern part of Nigeria, it is known as Ukpa (Igbo's), in the West as Asala (Yoruba's) and in the Western Cameroon as Kaso. Walnuts are usually planted under an indigenous tree and in cases where they cannot be harvested manually, they are left for full maturation after which the pod falls off by itself and is picked, removed from the rotten pods, washed and sold in the market. The fruits are four winged ridge between wings and up to 3 inches in diameter with four round seeds (usually brown) in each fruits (Nuhu et al., 2000).

Diarrhoea from a Greek word dia (through) and rrhoea (flow), meaning flowing through (Ahlquist, 2001; www.medterm.com, 2012). It is defined as the passage of abnormal liquid or unformed stool at an increased frequency (Ahlquist, 2001). It is a condition of having three or more loose or liquid bowel movement per day. It has long been recognized as one of the most important health problem in developing countries. It is also associated with increased frequency, fluidity or volume of bowel movements and is characterized by increased frequency of bowel sound movement, wet stool and abdominal pain (WHO, 1999; WHO, 2005). The passage of loose or watery stools is usually at least three times in 24 h period. However, it is the consistency of the stools rather than the number that is most important. Frequent passage of formed stools is not diarrhoea. Babies fed with only breast milk often pass loose, pasty stool and is not diarrhoea (WHO, 1999; WHO, 2005). Causes of diarrhoea include infectious agents, certain medications, plants and animals toxins, gastrointestinal tract (GIT)

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disorders and substances that increase GIT secretions. It is also caused by the ingestion of poorly absorbable materials or inflammatory and dysmotility problems of the GIT (Ahlquist, 2001). In Nigeria, diarrhea resulting from infection is one of the known killer diseases among children under 5 years (Audu et al., 2000). This may not be unconnected to the fact that toddlers are at times left to play in an unhygienic environment. Such exposure can lead to bacterial and viral infections, which can cause diarrhoea. Diarrhoea is also associated with some terminal illness like AIDs and Ebola virus infection and contributes to the quick death of such patient. Diarrhoea accounts for about 4 - 5 million deaths annually, of these, 8% have been reported to be from developing countries, putting a heavy burden on the country's health budget (Syder and Merson, 1982; WHO, 2011). WHO has encouraged studies for treatment and prevention of diarrhoea with plants, since patients who cannot afford the cost of treatment with orthodox medicines, resort to the use of herbs for management and possible cure (WHO, 1978, 1999, Cynthia et al., 2008).

MATERIALS AND METHODS

The aim is to determine the phytochemical components and antidiarrhoea activities of *T. conophorum* in albino rats.

Sample collection

Fresh seeds of *T. conophorum* were bought from fruit garden market, D-line, Port Harcourt, Rivers State, Nigeria.

Sample preparation

T. conophorum were sorted out from their shelves, washed, cut into small pieces to facilitate dryness and air-dried. The dried sample was crushed into fine powder and stored in an air tight bottle prior to analysis.

Extraction of *T. Conophorum*

Plant extract was obtained using filtration method. 300g of the powdered sample was weighed into a beaker and 1500 ml of hot water was added and shakened vigorously. The solution was filtered using filter paper, and a milky filtrate was obtained which was concentrated using water bath at a temperature of 60°C to remove water from the extract for 3 weeks and aqueous concentrated *T. conophorum* was obtained and used for inducement.

Preparation for phytochemical analysis

The dried powder (20 g) was soaked sequentially for 72 h with ethyl acetate and filtered. The filtrate was concentrated under reduced pressure by using rotary evaporator at a maximum temperature of 45°C to yield 1 g crude extract. The ethylacetate extract (1 g) was subjected to Gas chromatographic analysis for phytochemical determination.

Animal collection

Albino rats were brought from the animal farm of the Department of Biochemistry University of Port Harcourt, Choba and were brought to the animal room of the Department of Chemistry, Rivers State University of Science and Technology. All the rats were kept under laboratory condition for an acclimatization period and were given normal feed and water.

Experimental procedure on castor oil induced diarrhoea

The method of Awouter et al. (1978) was adopted in assaying for the effect of *T. conophorum* extract on castor oil induced diarrhoea. Albino rats were weighed (150 -200 g), fasted for 18 h and grouped into 5 groups, 3 per group.

Group 1 (control) received 1 ml of distilled water
Group 2 received 500 mg/kg of plant extract
Group 3 received 1000 mg/kg of plant extract
Group 4 received 2000mg/kg of plant extract
Group 5 received 5mg/kg of loparamide HCl.

After 1 h, each animal was administered with 1 ml of castor oil orally. The rats were placed according to their group in a metabolic cage on a clean and dried floor for faecal count. The metabolic cages were changed from their position each hour.

Assessing of diarrhoea

Castor oil-induced diarrhoea was determined by the method of Awoutes et al. (1978). Rats weighing between 150 – 200 g and fasted for 18 h were randomly distributed into five groups. Group I which served as control, was administered with distilled water (10 ml/kg) orally, group II, III and IV were administered with 500, 1000 and 2000 mg/kg of extract and group V was administered with loperamide hydrochloride (5 mg/kg). 1 h after the administration, all the animals were given 1ml of castor oil and monitored for six hours. The time taken for onset of diarrhoea and faecal droppings were recorded. Percentage inhibition was calculated (Izzo et al., 1992; Mukherjee et al., 1995; Karim et al., 2010).

% inhibition: (control – test)/control x 100.

Table 1. Quantitative Phytochemical Analysis.

Component	Subclass	% concentration
Phenol		31.78
Flavonoids	*Epicatechin*	0.67
	Rutin	0.14
	Kaempferol	0.03
	Anthrocyanin	29.77
Total		30.63
Alkaloids	*Lunamarine*	22.02
	Ribalinidine	0.28
	Aspartein	0.02
Total		22.55
Tannin		11.55
Saponin	*Sapogenin*	3.37
Oxalate		0.28
Phytate		0.07
Grand Total		100%

Table 2:Effect of *Tetracarpidium conophorum* on castor oil Induced diarrhoea.

Group	Dose (mg/kg)	Onset of diarrhoea (mins.)	Mean Wet faeces	Percentage Inhibition
1.	Distilled H_20	300	2.0 ± 1.0	-
2.	Plant extract 500 mg/kg	300	1.7 ± 0.6	15
3.	Plant extract 1000 mg/kg	300	0.3 ± 0.6	85
4.	Plant extract 2000 mg/kg	300	0.7 ± 0.6	65
5.	Loparamide HCl 5 mg/kg	320	0.7 ± 0.6	65

RESULTS

Table 1 shows the results of the quantitative phytochemical analysis of *T. conophorum*.

Table 2 shows the percentage inhibition of diarrhoea frequency.

Discussion

T. conophorum which is generally known as 'Walnut' belongs to an *Euphorhiacea* family. Walnuts are edible nuts and give a bitter taste with stimulating effect like kola and can be cooked, roasted or sundried and the roasted could be ground like melon seed and used as a thickener in soup preparation (Malu et al., 2009). Extracts of the leaves and seeds of walnut serves as beverages which relieves abdominal pain and fever.

Table 1 showed the concentration of the phytochemcials of *Tetracarpidium conophorum* in this order *phenol* > *flavonoids* > *Alkaloid* > *Tannin* > *Saponin* > *Oxalate* > *phytate*. The phytochemcial result revealed *epicatechin*, *rutin*, *kaempferol* and *anthrocyanin* as subclass of *Flavonoid*. *lunamarine*, *ribalinidine*, *aspartein* as subclass of *Alkaloids* and *sapogenin* which is a subclass of *Saponin*. This result is similar to that obtained by Ayoola et al. (2011). This quantitative phytochemical analysis result of *T. conophorum* which reveals the presence of phytate, oxalate, sapnonin, tannin, alkaloids, flavoniod and phenol has provided the needed information on the phytochemical composition of the lesser known member of *T. conophorum*.

Castor oil produces diarrhoea due to its most active component ricinoleic acid which causes irritation and inflammation of the intestinal mucosa. This leads to the release of prostaglandins that results in stimulation of secretion by increasing the volume of intestinal contents and prevention of re-absorption of water (Gaginella et al., 1975).

Table 2 showed the inhibitory effect of aqueous extract of *T. conophorum* on the wet faecal count of castor oil induced diarrhoea in Wistar albino rats. The percentage inhibition of castor oil induced diarrhoea in group 2 was 15, group 3 had percentage inhibition of 85, while group 4 was 65. Comparing the results of groups 3 and 4 with the group pretreated with standard drugs (group 5), the plant extract significantly inhibits diarrhoea. Reports have shown that plants phytochemical components (tannins and flavonoids) are capable of evoking antidiarrhoea effect since these components may precipitate proteins of the electrolytes and reduce peristaltic movement and intestinal secretion (Okudo et al., 1989; Galvez et al., 1993; Mukherjee et al., 1998). Thus, the presence of flavonoid and tannin at high concentration as shown in

Table 1, may be responsible for the antidiarrhoea activities of *T. conophorum.*

Conclusion

This study revealed that *T. Conophorum* possesses significant antidiarrhoea activities due to its inhibitory effect on wet faecal count. This positive result could explain why some people use the plant for diarrhoea treatment.

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