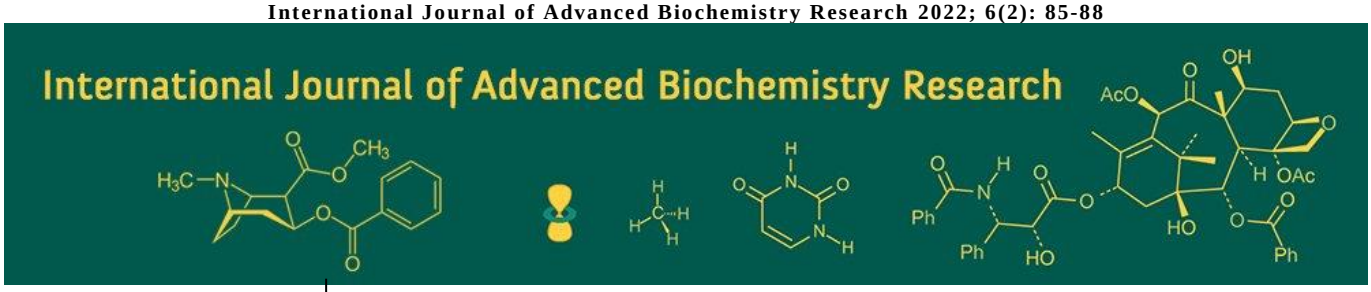

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2022; 6(2): 85-88
www.biochemjournal.com
Received: 01-04-2022
Accepted: 05-05-2022

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Phytochemical constituent and anti-diarrhoea potential of the stem bark of *Anthocleista vogelii* aqueous extract in albino rats

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DOI: https://doi.org/10.33545/26174693.2022.v6.i2b.139

Abstract
Anthocleista vogelii also known as cabbage tree is shrub-like tropical plants, reported to be useful in treating different diseases like hypertension, ulcer and fertility. This study was aimed at evaluating the phytochemical constituent of *A. vogelii* and its antidiarrhoea potentials. Aqueous extract of the stem bark of *Anthocleista vogelii* was used to treat castor oil induced diarrhoea in albino rats. The degree of gastrointestinal movement and wet fecal counts were measured. Phytochemical result of the plant revealed the presence of bioactive components such as flavonoids, alkaloids, tannin, oxalate, steroid, saponin, phytate and cyanogenic glycoside. The percentage values of these components were 79.84, 54.53, 25.39, 22.82, 20.32, 2.26, 1.80 and 1.44 µg/ml respectively. *A. vogelii* extract (100, 300 and 500) mg/kg administered to the animals showed decrease in the gastrointestinal motility. The charcoal meal motility was inhibited and there was decrease in intestinal fluid electrolyte (Na^+, K^+, Cl^- and HCO_3^-). The result obtained showed that aqueous extract of *A. vogelii* contains some pharmacologically active substance with anti-diarrhoea properties.

Keywords: Phytochemical, anti-diarrhoea, *Anthocleista vogelii*, electrolyte

Introduction
Medicinal plants have been known for many years and are widely used in treating different diseases especially in ruler areas due to their therapeutic property (Ajay, 2018) [1]. They contain some bioactive components that serve as precursors for the synthesis of new drugs (Ajay, 2018) [1]. *Anthocleista vogelii* (commonly known as cabbage tree) is shrub-like tropical plants which grow from 6 to 20 metres tall and it is native to Africa (Gabriel *et al.,* 2015) [3]. It belongs to the kingdom of plantae, phylum of tracheophytes, class of Angiosperms, order of Gentianales, family of Gentianaceae and genus of Anthocleista (Gabriel *et al.,* 2015) [3]. *Anthocleista vogelii* is usually found in wet environment and the leaves can be harvested by climbing the mature trees while the bark can be harvested by using cutlass to peel it (Ateufak *et al.,* 2006) [2]. *Anthocleista vogelii* could be used singly or in combination with other plants in the treatment and management ailments in humans (Oladijemi *et al.,* 2014) [8].
Gabriel *et al.* (2015) [3] reported that *Anthocleista vogelii* is useful in treating diseases like diabetes, cancer, malaria, obesity, hypertension, ulcer, fertility problems and typhoid fever. They added that, phytochemical screening of the plant showed the presence of alkaloids, flavonoids, saponins, terpenes and phenols.
Diarrhoea is a term use to describe health condition characterised with frequent passage of watery stool that occur at least three to four times in a day accompanied by stomach cramp, is derived from two Greek words *dia* and *rrhoea*. The former means 'through' and the latter means 'flow', thus literally means 'flowing through' (Nwachoko and Jack, 2015) [6]. It is caused by increased secretion of intestinal fluid, reduction in the absorption of fluid into the intestine (Lawrence, 2000) [5]. The description excludes babies passing out loose stool because they are feed on only breast milk (WHO, 2005) [11]. It can easily spread from one person to another due to unclean environment and through contaminated food or drinking water (Kenneth, 2003) [4]. Pathogenic organisms such as Virus, Bacteria (*Escherichia coli*) and Parasite (Giardia) and intestinal tract disorders could cause diarrhoea (Peter *et al.,* 2008) [9].

WHO, (2011) [12] noted that diarrhoea is one of the major causes of deaths in countries that are undeveloped and has encouraged research on traditional plants as a way to cure or manage these diseases since they are readily available and cheap in comparison with synthetic drugs. Thus this work examines the anti-diarrhoea potential of aqueous extract of *Anthocleista vogelii* stem bark in albino rats.

Materials and Methods

Sample Collection

The fresh stem bark of *Anthocleista vogelii* was collected between September and October, 2021 from Imaakue forest of Eeken Community in Khana Local Government Area of Rivers State, Nigeria. The cordinates of Eeken Community is 4.6582 °N and 7.3779 °E. The plant was identified and registered as RSUb025.

Sample Preparation

The collected fresh stem bark of *Anthocleista vogelii* was cut into tiny pieces and sun-dried. The dried sample was ground into fine powder. Two hundred grams of powdered *Anthocleista vogelii* was weighed and soaked in 500ml of water in a container. The solution was shaken three times a day for 3 days (72 hours), and then it was filtered using Whatmann filter paper. The resulting filtrate was evaporated using a rotary evaporator and was condensed to powder form using water bath at a temperature of 60 °C. The yield was stored in a refrigerator till when needed.

Preparation of phytochemical analysis

One gram of plant sample was weighed using a weighing balance and transferred to a test tube and 15ml of 50% m/v potassium hydroxide (KOH) was added. The test tube was allowed to react in a water bath at 60 °C for 60 minutes and the product was transferred to a separatory funnel, and 3ml of hexane was transferred to the funnel. The extracts were combined and washed three times with 10ml of 10% u/v ethanol aqueous solution. The solution was dried with dehydrated sodium sulfate and the solvent was evaporated. The sample was solubilized in 1000µl of pyridine of which 200µl was transferred to a vial for analysis.

Animal Collection

Adult female albino rats were obtained from the animal house of Rivers State University, Port Harcourt. Acclimatization of the animals took place for a period of seven (7) days prior to the commencement of experiment. The animals were grouped into five different groups of five (5) animals in each group.

Drugs

Castor oil, loperamide hydrochloride and activated charcoal.

Experimental procedure for castor oil induced diarrhoea and Fecal Count

The method of Awouter *et al.* (1978) cited in Nwachoko and Jack (2015) [6] was adopted in determining the effect of *A. vogelii* on castor oil induced diarrhoea. Albino rats weighing 100 – 150 g fasted for 18 hours and were grouped into 5. Group 1 was control, group 2 received 100mg/kg of *A. vogelii* extract, group 3 received 300mg/kg of *A. vogelii* extract, group 4 received 500mg/kg of *A. vogelii* extract while group 5 received 5mg/kg of standard drug (loperamide hydrochloride). One hour after the administration of the extract and standard drug, oral administration of 1ml of castor oil was given to all the experiment animals. The time taken for onset of diarrhoea and fecal dropping were recorded. The percentage inhibition was calculated according to Izzu *et al.* (1992) cited in Nwachoko *et al.* (2016) [7].

Castor Oil Induced and Gastrointestinal Transit

Castor oil-induced diarrhoea and gastrointestinal transit was determined by the method of Awouter *et al.* (1978) cited in Nwachoko and Jack, (2015) [6]. The method for administration of extract and drug is similar with the above for fecal count. Administration of castor oil was done and after one hour, 1ml of charcoal meal was given to each of the animals. After 1hr of the meal administration, the animals were sacrificed and the distance travelled by the charcoal meal from the pylorus to the caecum were measured and expressed as a percentage of the total length of the intestine from the pylorus to the caecum of each animal. The peristaltic index was calculated using the formula, PI = LM/LSI X 100 where PI=Peristaltic index, LM = distance travelled by charcoal meal and LSI = Length of small intestine, % inhibition = (Control-test)/control X 100.

Results

Table 1: Quantitative Phytochemical components of *Anthocleista vogelii*

Components	Sub-Class	Concentration µg/ml
Alkaloids	Epehidrine	12.5691
	Dihydrocytisine	24.9603
	Aphyllidine	1.3134
	Sparteine	6.8775
	Ribalinidine	7.0401
	Ammodendrine	1.7691
Total =		54.5295
Flavonoids	Kaempferol	30.5797
	Flavonones	10.9202
	Narigenin	10.2793
	Flavone	5.8668
	Catechin	4.3937
	Proanthocyanidin	17.7989
Total =		79.8386
Saponin	Sapogenin	2.2575
Cyanogenic Glycoside		1.4387
Steroid		20.3205
Tannin		25.3877
Oxalate		22.8219
Phytate		1.7960

Table 2: Effect of aqueous extract of *Anthocleista vogelii* on the faecal count of castor oil induced diarrhoea in albino rats

Group	Treatment	OD (MIN)	MWF	% I
1	Control	120	2.57	-
2	100 mg/kg	120	2.57	0
3	300 mg/kg	120	1.29	50
4	500 mg/kg	120	1.14	55
5	5 mg/kg	120	1.14	55

Key: OD = Onset of diarrhoea, MWF = Mean wet faeces after 8 hours, I= Inhibition.

Table 2 above shows the result of percentage inhibition of aqueous extract of *A. vogelii* against the mean wet fecal count of castor oil-induced diarrhoea in albino rats.

Table 3: Effect of aqueous extract of *Anthocleista vogelii* castor oil induced diarrhoea in albino rats

Groups	Treatment	IL (cm)	CML (cm)	PI (%)	I (%)
1	Control	77.25	30.0	38.8	-
2	100mg/kg	84.0	19.25	22.9	41.0
3	300mg/kg	87.75	15.0	17.1	50.0
4	500mg/kg	79.0	17.75	22.5	40.8
5	5mg/kg	72.0	17.25	24.0	42.5

Key: IL= Intestinal length, CML= Charcoal meal length, PI = Peristaltic Index, I= Inhibition.

The table 3 showed the result of percentage inhibition of aqueous extract of *A. vogelii* against charcoal meal motility and electrolytes concentration in castor oil-induced diarrhea albino rats.

Table 4: Effect of aqueous extract of *Anthocleista vogelii* castor oil induced diarrhea in albino rats

Groups	Na^+(mmol/L)	K^+(mmol/L)	Cl^-(mmol/L)	HCO_3^-(mmol/L)
1	6.93 ± 1.11^{bdeh}	195.3 ± 28.37^{bdfh}	64.33 ± 2.52^{bdfh}	30.0 ± 1.0^{bdfh}
2	$3.77\pm1.12^{*aceg}$	$133.3\pm20.20^{*aceg}$	$47.0\pm1.0^{*adeh}$	$22.67\pm1.15^{*adfh}$
3	$2.73\pm0.15^{*acch}$	$119.67\pm2.08^{*aceg}$	$39.0\pm4.58^{*bceh}$	$25.0\pm1.0^{*bceg}$
4	$4.73\pm0.47^{*aceg}$	$143.0\pm12.76^{*aceg}$	$43.33\pm1.53^{*aceh}$	$25.67\pm1.53^{*bceg}$
5	$5.17\pm0.74^{*adeg}$	$143.67\pm4.04^{*aceg}$	$53.33\pm2.89^{*bdfg}$	$25.33\pm1.53^{*bceg}$

Values in the table above are expressed as mean ± standard deviation of n=5. Values with superscript * differs significantly when comparing control with other groups. Values with superscript alphabet ab differs significantly when comparing group 2 with other groups. Values with superscript cd differs significantly when comparing group 3 with other groups. Values with superscript ef differs significantly when comparing group 4 with other groups. Values with superscript, GH differs significantly when comparing group 5 with other groups.

Discussion

Anthocleista vogelli is aplant commonly used by herbalists either singly or in combination with other plant materials. Table1 showed the phytochemical constituent of *A. vogelli*. The result showed that flavonoid had the highest value followed by alkaloids, tannin, oxalate, steroid, saponin, phytate and cyanogenic glycoside had the least value. The result revealed Kaempferol, flavonones, narigerin, catechin, proanthocyanidin and flavone as sub-class of flavonoids. Epehidrine, dihydrocytisine, aphyllidine, sparteine, ribalinidine and ammodendrine as sub-class of alkaloids. Sapogenin as sub-class of saponin.

Table 2 showed the inhibition potential of *Anthocleista vogelii* aqueous extract on the mean wet fecal count of albino rats. Group 4 animals which received 500 mg/kg of the extract and group 5 which received 5mg/kg of the standard drug showed the highest percentage inhibition followed by group 3 which received 300 mg/kg of the extract. There was no inhibition in group 2 which received 100 mg/kg of the extract. The onset of diarrhea in all the groups was after 120 minutes.

Table 3 showed the inhibitory effect of aqueous extract of *Anthocleista vogelii* bark on castor oil induced gastrointestinal transit in albino rats. The result showed percentage inhibition of 41.0, 50.0 and 40.8 for groups 2, 3 and 4 respectively. Group 5 had percentage inhibition of 42.5.Comparing the values of the groups treated with *Anthocleista vogelii* and standard drug, *A. vogelii* showed inhibitory activity to castor oil induced diarrhoea. Group 3 which received 300mg/kg showed the highest percentage inhibition followed by group 5 which received standard drug. The above result showed that both *Anthocleista vogelii* extract and standard drug treatment slowed down the movement of charcoal meal through the gastrointestinal tract. Also, the charcoal meal length (CML) of animals in group 1 was the highest followed by the meal CML of animals in group 2, 4 and 5. Animals in group 3 had the lowest peristaltic index and the highest percentage inhibition.

Table 4 showed the effect of aqueous extract of *Anthocleista vogelii* bark on castor oil induced electrolyte concentration in albino rats. The result showed that there was lowered intestinal fluid electrolyte (K^+, Na^+, Cl^- and HCO_3^-) concentration as a result of treatment with *A. vogelii* aqueous extract. From the result obtained, it could be said that the phytochemicals especially those found in high concentration such as flavonoid and alkaloid may be responsible for the anti-diarrhoea potential of *A.vogelii.*

Conclusion

The study revealed that the stem bark of *A. vogelii* possesses significant anti-diarrhoea potential due to its inhibitory effect on wet faecal count, inhibition of distance travelled by charcoal meal and inhibition of intestinal fluid electrolytes. The positive result supports the use of the plant for the treatment of diarrhoea.

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