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ANTI-DIARRHEA EFFICACY OF *ANNONA MURICATA* L. ON ANIMAL MODEL

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ABSTRACT : *Diarrhea is known for its severe health threat associated with individuals. The treatment is associated with the possible uses of Annona muricata ethnomedicinal report. This study evaluates the efficacy of A. muricata ethanol and hexane leaves extracts on diarrhea. The pulverized sample was macerated using ethanol and hexane solvent. The acute toxicity report was experimented on mice model. Effects of A. muricata on gastro-intestinal tract disorders were assessed in castor oil- induced diarrhea and charcoal meal model in experimental animals at graded doses of 30-120 mg/kg of ethanol and hexane leaves extracts, when compared with standard drug and untreated control. The animals were able to withstand ethanol and hexane extract at highest toxicity dose of 5 g/kg with no visible toxicity sign. Significant peristalsis inhibitions were observed in the treated groups at 30, 60 and 120 mg/kg of A. muricata extracts which triggered a definite reduction in the distance travelled by charcoal when compared with the distance of the small intestinal with dose dependent. Also, it showed reduction in the weight and number of diarrhea stool, with prolong onset of action in castor oil induced diarrhea. The results acquire validate the ethnomedicinal uses of A. muricata.*

KEYWORDS: Annona Muricata**,** Anti-Diarrhea, Castor Oil, Therapeutic, Charcoal Meals.

INTRODUCTION

Natural substance emerged from plants materials showed their therapeutic effect from since time of antiquity for animal and human primary health care (Idu *et al*., 2007). Bioconstituents properties in plants make them useful to herbal practioners due to the present of several compounds found in them. Plants potential has been established as medicine used in the management of diverse disorders or some disease. Traditional practitioners tender the claims being validated by scientific studies (Idu *et al*., 2007; Aigbokhan, 2014). Plants comprise immense support to man either in the provision of shelter and food or serves as medication to manage several diseases (Ramakrishnan and Sivaranjani, 2013). Several natural products are applicable to scientific sustenance to promote their diverse biological efficacies (Idu *et al*., 2007). The main benefit in plant usage is consequential to medicine secured than synthetic product and it proffer an improved and affordable action. Also, it should be observed that some medicinal plants are toxic for safe consumption in their crude form.

Plants are significant basis for natural products that promotes maximum protection against several diseases causing human cells indemnity (Abdel-lateif *et al*., 2016; Sanders et al., 2012). Natural products enhance proper health care owing to the present of their bioactive constituents in relation to pharmacology drug development and research finding by absolute accessibility of various chemical components (Lahlou, 2013; Dhawale and Ghyare, 2016). The recompense of natural plants products with several therapeutics properties is made up of feasible convenience, partial cultural trait, individual affinity, increase categorize organic and natural products, readily validated with synergic secondary metabolites effects (Carmona *et al*., 2013; McQuaid, 2012).

Annona muricata commonly known as soursop is native to Central America and Caribbean but of recent, it is extensively cultivated in certain regions, becoming accessible in tropical climates universally. It is from the family Annonaceae in the genus *Annona*. Being an evergreen tree, it uses have been scientifically made known to diverse diseases (Kossouoh *et al*., 2007). Other local Nigeria names include: Shawshopu by the Igbo's, factually thorny custard apple. The ethnomedicinal benefits include; anti-oxidant, anti-arthritic, gastroprotective, anti-cancer, anti-diabetic, anti-inflammatory, anti-convulsant, anti-nociceptive, ant-microbial, anti-plasmodial, anti-hypertensive, hepatoprotective and wound healing (Adewole and Ojewole, (2009).

Diarrhoea is one of the gastrointestinal disorders typify with increased stooling occurrence (Farthing, 2002). It is known with its regular channel of slack, runny, and soft stools usually with or lacking of abdominal cramps known as gas. Diarrhea is described clinically with augmented stool liquidity usually linked with increased stool occurrence and weight. It is distinguished with increased dynamic luminal secretion and decreased water and electrolyte reabsorption in the lumen. The resultant net discharge triggers increase associated with stool weight and volume. Decrease in abnormal motor and transit time effects take place in patients prone to diarrhea (Akah *et al.*, 1999). Dehydration is recurrent. Diarrhea stimulated by colonic association with frequently occurring small stools volume, traces of blood, and sensational urgency (Schiller *et al.*, 2000). The vital factors in assessing acute diarrhea is such as animal exposure and travel history, water sources, current food ingestion, history of copious diarrheal occurrences, history of topical antibiotic management, fever, hematochezia, dehydration, vomiting, abdominal pain and nausea. Imperative clinical character includes: sudden versus regular symptoms, symptom interval, such as bowel mobility occurrence, stool sizes, dysentery with fever, hematochezia or secretion in stool, tenesmus, volume depletion such as; tachycardia, orthostasis and thirst, reduction in urine output lethargy or mystification and skin turgor (Fine *et al*., 1998).

MATERIALS AND METHODS

Collection of Plant Material

Annona muricata leaves was collected from Oreodo Local Government Area Benin City Edo State, Nigeria and was identified and authenticated by Dr. Akinnibosun of the Department of Plant Biology and Biotechnology, University of Benin, Edo State, Nigeria.

Preparation of Plant Material

Leaves were rinsed and air dried for 21 days, afterwards oven dry at 40 ^{0}C for 12 hours. Crispy leaves were powdered using electrical mechanical engine. Pulverized leaves sample was properly stored in moisture free container for further use. The plant sample of 800 grams and 900 grams was macerated using ethanol 1.5 litre and n-Hexane 2 litre solvent system separately for 72 hours afterwards, it was filtered and the filtrate in their respective sample bottles were concentrated using crucibles and water bath into semi-solid forms.

Experimental Animals

For the experiment 50 adult wistar rats and 50 adult wistar mice weighing 190-220 and 30-40 g were used. Animals were housed in wooden cages (five per cage) and maintained under controlled room temperature (25 ± 1^0) with relative humidity of 45-55 % under 12: 12 hr light and dark cycle for one week with free access to food and water ad libitum. Every procedure with the use of animals obtained approval from the Institutional Animal Ethical Committee, and the experiment was carried out in conformity with Guidelines for CPCSEA.

Acute Toxicity Study

Ethanol and Hexane extract was used for pharmacological screening on male Wistar mice". Acute toxicity test was carried out according to Lorke's method (1983). This method has two phases, 1 and 2 respectively.

Phase 1

The mice were allotted into three groups (three rats each). The rats were administered oral doses of 10, 100 and 1000 mg/kg of the extract. The rats were kept under close observation for 24 hours to monitor their behavior as well as occurrence of mortality.

Phase 2

Three Wistar mice were allotted into three groups (one animal each). The mice were administered higher doses (1600, 2900 and 5000 mg/kg) of the extracts and were observed for 24 h (special attention given to the first 4 h) and once daily for a period of 14 days for signs of toxicity which include paw/licking, change in skin color, changes in fur, eye lacrimation, nostril discharge, salivation, diarrhea, tremor, convulsion and death. The LD_{50} was calculated using the formula:

Castor Oil-Induced Diarrhea in Mice

Fifty (50) mice were fasted for 18 hours and randomly divided into five groups (n=5). *Annona muricata* ethanol and hexane leaves extract at (30, 60, and 120 mg/kg) were orally given to the treated groups. The control group received 0.2 ml/kg body weight of distilled water, and reference group received 3 mg/kg body weight loperamide. An hour later, the whole animals were predisposed to 0.2 ml/rat of castor oil orally via gavage. They were kept in a separate transparent plastic container with plain filter paper at the base (Awouters *et al*., 1987). The onset and severity of diarrhea was evaluated for 4 hours. Total number of faeces (diarrheal and non-diarrheal) expelled were compared with that of the control group. Total score of diarrheal faeces for control group was measured as 100 %. Results were presented as percentage inhibition of diarrhea.

Gastrointestinal Motility Test

Albino rats were randomly divided into five groups (5 per group) and fasted prior to the study for 18 h with freely access to water. Control group received distilled water orally (0.2 ml/kg body weight); treated groups were given ethanol and n-Hexane leaves of *Annona muricata* extracts at graded doses (30, 50, and 100 mg/kg) orally. The reference group received standard drug (05 mg/kg body weight of atropine sulphate) i.p. An hour later, each animal was administered with 1 ml castor oil. Thereafter charcoal meal (10 % activated charcoal in 5 % gum acacia) at 1ml via oral route was giving an hour later. All animals were sacrificed an hour thereafter, and distance travelled by charcoal meal in the intestine, from the pylorus to the caecum was measured and evaluated as percentage of distance moved (Pazhani *et al*., 2001).

RESULTS

From the table below, the acute toxicity of ethanol and hexane *Annona murica* leaves extracts showed no visible sign or death associated with the extracts rather it can be ascertained safe for this first line of toxicity study. No mortality was recorded at the maximum dose of 5 g/kg after14 days of observation. No visible sign of toxicity was observed throughout the 14 days observable period

The table below showed the effect of *Annona murica* of the various anti-diarrhea models across ethanol and hexane which showed significant different at lowest doses when compared with the control groups in the castor oil induced model and charcoal transit time model. This is an indicator that the leaves extract of the various solvent systems showed their therapeutic effects against an increase in git motility which in return has anti-diarrhea properties.

Table 1: Acute Toxicity Effect of Ethanol Leaves Extract of *Annona murica*

Doses (mg/kg)	Number of Animals Used	Number of Deaths	Toxicity signs
10	4	0/4	No visible toxic signs
100	4	0/4	No visible toxic signs
1000	4	0/4	No visible toxic signs
1600	4	0/4	No visible toxic signs
2900	4	0/4	No visible toxic signs
5000	4	0/4	No visible toxic signs

Table 2: Acute Toxicity Effect of Hexane Leaves Extract of *Annona murica*

Doses (mg/kg)	Number of Animals Used	Number of Deaths	Toxicity Signs
10	4	0/4	No visible toxic signs
100	4	0/4	No visible toxic signs
1000	4	0/4	No visible toxic signs
1600	4	0/4	No visible toxic signs
2900	4	0/4	No visible toxic signs
5000	4	0/4	No visible toxic signs

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

D_0 = Highest dose that gave no mortality,
D_{100} = Lowest dose that produced mortality.

Table 3: Anti-Diarrhea Effect of *Annona murica* Ethanol Extract in Charcoal Meal Induced Diarrhea

Treatment	Dose mg/kg	Total length of Intestine (cm)	Length travel by charcoal meal (cm)	% Peristalsis index diarrhea	% inhibition
Control	DW	84.17±0.88	33.17±4.14	39.40±4.21	-
Atropine	5	95.15±6.41	21.23±2.54	22.31±2.03	43.4
Annona murica	30	80.65±4.84	6.67±4.00**	8.27±3.01**	79
Annona murica	60	78.61±9.33	15.02±3.06*	19.10±1.11*	51.5
Annona murica	120	83.47±4.06	20.83±1.42	24.96±1.84	36.7

To calculate for % peristalsis index (PI) = LM/LSI LM----length of charcoal meal, LSI----length of small intestine. P-value < 0.05.

Table 4: Anti-Diarrhea Effect of *Annona murica* Hexane Extract in Charcoal Meal Induced Diarrhea

Treatment	Dose mg/kg	Total length of Intestine (cm)	Length travel by charcoal meal (cm)	% Peristalsis index	% inhibition
Control	DW	84.17±0.88	33.17±4.14	39.40±4.21	-
Atropine	5	95.15±6.41	21.23±2.54	22.31±2.03	43.4
Annona murica	30	90.32±2.33	2.29±2.83***	2.54±3.13***	93.6
Annona murica	60	74.12±1.88	21.06±2.01	28.41±4.97	27.9
Annona murica	120	91.0±1.26	18.31±3.52	20.12±4.91	48.9

To calculate for % peristalsis index (PI) = LM/LSI LM----length of charcoal meal, LSI----length of small intestine. P-value < 0.05.

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Table 5: Anti-Diarrhoea Effect of *Annona murica Ethanol* Extract in Castor Oil Induced Diarrhea

Treatment	Dose mg/kg	Onset of stool (sec) Mean±SEM	Total number of stool Mean±SEM	Number of diarrhea Mean±SEM	Weight of stool (g) Mean±SEM	% inhibition of diarrhoea
Control	DW	2460±993.8	5.90±2.00	4.31±2.40	0.54±0.10	-
Loperamide	3	2590±14.15	3.51±0.67	1.03±1.02**	0.43±0.45	76
Annona murica	25	3660±2601	1.59±0.88	0.63±0.53***	0.03±0.09	85.4
Annona murica	50	4049±2590	3.55±1.86	2.33±1.30	0.11±0.16	45.9
Annona murica	100	4077±2726	2.45±2.06	2.20±1.60*	0.03±0.00	50

P-value < 0.05.

Table 6: Anti-Diarrhoea Effect of *Annona murica* - Hexane Extract in Castor Oil Induced Diarrhea

Treatment	Dose mg/kg	Onset of stool (sec) Mean±SEM	Total number of stool Mean±SEM	Number of diarrhea Mean±SEM	Weight of stool (g) Mean±SEM	% inhibition of diarrhoea
Control	DW	2460±993.8	5.90±2.00	4.31±2.40	0.54±0.10	-
Loperamide	3	2590±14.15	3.51±0.67	1.03±1.02**	0.43±0.45	76
Annona murica	25	1120±141.9	5.40±1.02	3.00±0.58	0.43±0.32	28.7
Annona murica	50	4270±2163	5.00±2.13	2.54±2.27	0.41±0.16	39.7
Annona murica	100	4160±2016	1.49±0.52	0.53±0.43***	0.19±0.11	87.4

P-value < 0.05.

DISCUSSION

The therapeutic property of *Annona muricata* is as a result of the different secondary metabolites found naturally in plant, such as alkaloids, tannins, flavonoids and saponins (Adeloye *et al*., 2007: Harborne and Williams, 2000). The biological property of several medicinal plant displays direct function due to the chemical constituents in them. The anti-diarrhea activities of *Annona muricata* could be solely responsible for the various plant constituents (Hajhashemi *et al*., 2000).

Annona muricata ethanol and hexane leaves extract at 5000 mg/kg showed no toxicity effect with any trace of visible toxicity signs as shown in Table 1 and 2. Intestinal transit time occurs with lack of any induced conditions. The extract at graded doses in normal intestinal transit model showed significant decrease to distance travelled by charcoal meal when

compared with control, this is in line with earlier report of (Rang *et al*., 2007). At lower doses of ethanol and hexane extracts of 30 and 60 mg/kg curiously reduced the percentage peristalsis movement. Peristalsis inhibition was distinct on the treated group (*Annona muricata*) at 30 and 60 mg/kg in line with total length of small intestine. Percentage peristalsis was therefore inhibited up to 8.27 % at a dose of 30 mg/kg of ethanol extract and 2.54 % at a dose of 30 mg/kg of hexane extract when compared with control (39.40 %) as shown in Table 3 and 4 supporting the earlier report of (Adeyemi and Akindele, 2008).

Ethanol and hexane extracts at graded doses used in castor oil induced diarrhea model significantly reduces castor oil induced diarrhea confirming the authenticity of (Onoagbe *et al*., 2010). For the sack of the mechanisms of action elicited by ethanol and hexane extracts on castor oil-induced diarrhea α_2- adrenergic receptor antagonist of the extracts (Gabriel *et al*., 2005). At 30 and 60 mg/kg of ethanol and hexane extracts, diarrhea was assuaged and extremely reduced (Table 5 and 6). This propounded that ethanol and hexane extracts possibly had efficacies on alpha receptor as its mode of actions. Other inhibitory property showed by the extract was validated with the use of Castor oil induced diarrhoea. While Loperamide showed similar effect when compared with the control. It was noted that possible stooling onset and numbers of diarrhea excreted were decreased within 30 and 60 mg/kg of ethanol and hexane extracts of *A. muricata*, thereby it is action could still be associated with inhibitory or relaxation of intestinal spasm triggered by ricinoleic acid found in castor oil (Karamenderes and Apaydin, 2003). Furthermore, due to substantiated potential anti-secretory property previously observed in 30 and 60 mg/kg of *A. muricata* extracts formed definite decrease in the volume and weight of the stool (Schiller, 2000; Quignot *et al.*, 2014). This is a clearer indication that the plant extracts in their respective doses validated their legend report.

CONCLUSION

Annona muricata leaves extracts was confirmed to elicited anti-diarrhea property charcoal meal model and castor oil induced diarrhea validated with the earlier report from its ethnomedicinal investigations.

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