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Phytochemical and Antidiarrhoeal Evaluation of Two Medicinal Plants Growing in Maiduguri Metropolis Nigeria

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ABSTRACT

The plant wild custard apple otherwise known as Annona senegalensis belongs to the family Annonaceae. The flowers, leaves and fruits are edible and culinary; the leaves are also view to create a general health tonic and in the treatment of pneumonia; the bark is used in the synthesis of dyes and insecticide. Annona senegalensis is traditionally used as pain reliever as well as effects such as antioxidant, antimicrobial, anti-diarrhoeal, anti-inflammatory, anticonvulsant, antimalarial, anti-tripanasomal and anti-nociceptive. The bark and root are used for treating wide array of ailments including guinea worms, diarrhoea, gastroenteritis, lung infections, toothaches. Diospyros mespiliformis (Ebenaceae); it has been reported to have wide applications which include the use of leaf decoction as a remedy for fever, whooping cough and for wound. Roots and bark of Diospyros mespiliformis are used to stop purging and to enhance fertility, while the leaf decoction is used as remedy for fever, otitis and wound dressing. Bark and roots for infections such as malaria, pneumonia, syphilis, leprosy, pain, dermatomycoses and to facilitate child birth and also as a psycho-pharmacological drug. Annona senegalensis was collected from Baram karauwa village, Jere; while Diospyros mespiliformis was collected from Jimtilo Village, Konduga, Borno State in February, 2016. The air-dried extracts were ground into powdered form using mortar and pestle, extracted separately with ethanol (70%) by reflux technique for 6 h each. Phytochemical evaluation using standard methods revealed the presence secondary plant metabolite in both root and stem bark extracts of the two plants: saponins, tannins, cardiac glycosides were present in both; while alkaloids was only found in root bark of Annona senegalensis. Anthraquinones was present in most parts except in root bark of Annona senegalensis. Phlobatannins and flavonoids (Shinoda's rest) were absent in both extractives. Anti-diarrhoeal activity of both stem and root bark extract was carried out using castor oil-induced diarrhoea method in Wistar strain albino rats shows that Annona senegalensis exhibited 100% protection while a dose-dependent approach was found as 92.81, 96.46 and 100%, respectively at 300, 600 and 1200 mg/kg bd. wt. on the stem bark extract of Diospyros mespiliformis. The root barks extract presented a dosages-dependent fashion for Annona senegalensis but a non-dosages dependent pattern was observed on Diospyros mespiliformis. Therefore, these findings have supported the use of these plants against diarrhoea; but comparatively the stem bark of Annona senegalensis appeared to be the good candidate of choice.

Keywords: *Annona senegalensis*, *Diospyros mespiliformis*, Phytochemistry, Diarrhoea, Castor oil

INTRODUCTION

Human beings have used plants for the treatment of diverse ailments for thousands of years [1]. The world health organization (WHO) estimate that 80% of the population of some Asian and African countries presently use herbal medicine for some aspect of primary health care [2]. It is estimated that there about 700,000 species of tropical flowering plants that have medicinal properties [3]. Many of these medicinal plants are relatively safer and cheaper than synthetic or modern medicine [4]; thus the choice of *Annona senegalensis* and *Diospyros mespiliformis* for this study. It has been reported that the LD50 of methanol root bark extract of *A. senegalensis* in mice was 3808 mg/kg bd. wt. [5,6]. The intraperitoneal median lethal dose (LD50) was found to be more than 3000 mg/kg in rats. The methanol stem-bark extract of *Annona senegalensis* was investigated using both *in vivo* and *in vitro* models by oral application of effective dose (5000 $mg.kg^{-1}$) [7]. Swiss albino mice were used to investigate the acute oral toxicity of the extract. The extract was safe at doses up to 5000 mg/kg [8]. The stem bark of *Diospyros mespiliformis* had

median lethal dose (LD50) established as 1095.4 mg/kg bd. wt. i.p. in mice [9] and that for the root bark was also found to be 620 mg/kg bd. wt. in mice [10]. Any compound with oral LD50 of 5000 mg/kg or more in rat should be considered as practically harmless [11].

Diarrhoea and the associated fecal urgency and in continence result from an imbalance between the absorption and secretory mechanisms in the intestinal tract accompanied by hyper motility; this results in excess loss of fluid and electrolytes in feces [12]. Acute diarrhoea disease is one of the principal causes of infant deaths in developing countries [12]. Diarrhoea accounts for more than 5-8 million deaths worldwide each year in infant less than five year, especially in developing countries [13]. Herbs such as *Annona senegalensis* and *Diospyros mespiliformis* are used in traditional medicine to treat this disorder. *Diospyros mespiliformis* belong to the family Ebenaceae; it is a tall upright tree that can reach a height of 30 m with over 2 m in girth found in moist place of the Guinean and Sudanian woodland, throughout the West African region and generally wide spread in such localities across Africa except in the Congo basin [14]. The common name of *Diospyros mespiliformis* in English is Ebony, in Hausa Kanya, in Kanuri Burum, in Fulani Balchi, in Yoruba Igi dudu, in Igbo Akawayi. *Diospyros mespiliformis* has been reported to have wide application in traditional medicine which includes the use of leaf decoction as a remedy for fever, whooping cough and for wound [15]. Bark and roots are used for serious infections such as malaria, pneumonia, syphilis, leprosy, diarrhoea and dermatomycoses, as an anti-helmintic and to facilitate delivery [16]. Roots and bark of *Diospyros mespiliformis* are used to stop purging and to enhance fertility, while the leaf decoction is used as remedy for fever, otitis and wound dressing. Different parts used against diarrhoea, headache, toothache and as a psycho-pharmacological drug [17]. *Annona senegalensis* belongs to the family Annonaceae. It is a shrub or small tree 2-6 m tall but may reach 11m under favourable conditions; bark smooth to roughish, silvery grey or grey-brown, with leaf scars and roughly circular flakes exposing paler patches of under bark. Young branches with dense, brown, yellow or grey hairs that are lost later. It is called Gwanda daaji in Hausa and Dukuu-hi in Fulani [18]. It is sold by herbalists in South Benin for treatment of bleeding [19]. The primary use of this versatile plant is for food, but it has applications in numerous aspects of human endeavor and every part of the plant has unique properties and uses. Traditionally, the plant is used as stimulant, pain reliever, etc.; the plant possess beneficial effects such as anti-oxidant, antimicrobial, anti-diarrhoeal, anti-inflamatory, anti-parasitic, anticonvulsant, antimalarial, anti-tripasonal, anti-snake venom and anti-nociceptive among others [18]. Also, the bark can be processed to produce yellow-brown dye, insecticide, or medicine for treating a wide array of ailments, including worms parasitic on the intestines or flesh (notably guinea worms), diarrhoea, gastroenteritis, lung infections, toothaches, and even snakebites. Natural gum in the bark is used to close open wounds. Roots are also used medicinally in treating a gamut of conditions, from dizziness and indigestion to chest colds to venereal diseases [20].

The purpose of this study was to further evaluate the potency of these plants as both parts of the plant have been documented to possess anti-diarrhoeal effects in traditional medical practice from this part of the country and more so to select the best for isolation and purification of the bioactive compound(s).

MATERIAL AND METHODS

Plant materials

The plant sample of *Diospyros mespiliformis* (stem bark and root bark), were collected from Jimtilo village in Konduga Local Government of area of Borno state while Fresh root bark and stem bark of *Annona senegalensis* was collected in Baram karawa village in Jere Local Government of area of Borno State, Nigeria. The samples were identified by a Taxonomist in the Department of Biological Sciences, University of Maiduguri. Voucher specimen numbers of the herbarium samples were 11/CHM/011 and 13/CHM/002 respectively; deposited in the Research Laboratory, Department of Chemistry University of Maiduguri, Maiduguri.

Extraction of plant materials

The plant material was air-dried at room temperature and pulverized into a dry powder. Two Hundred and Fifty gram (250 g) of each is then macerated with 85% ethanol for 72 h with constant shaking. The extractive will be filtered and concentrated at low pressure to obtain a solid mass coded “EE” as ethanol extract. The solid mass obtained shall be monitored while drying to ensure it dries completely, percentage yield are calculated to obtain the yield. Then, extracts are subjected to phytochemical evaluation and anti-diarrhoea test.

Preliminary phytochemical screening

The crude extracts were subjected to qualitative phytochemical screening according to standard methods [21].

Experimental animals

All experiment performed on laboratory animals in this study follow standard procedure for treatment of animals. All the animals were handled according to the International Guiding Principles for Biomedical Research involving Animals [22] as certified by the Animal Ethics Committee of the Faculty of Veterinary Medicine, University of Maiduguri. Forty two (42) Wistar strain albino rats used for this study of both sexes weighing between 200-250 g were obtained from the Zoo, Maiduguri, Borno State. They were kept in well ventilated plastic cages under standard conditions of temperature (25°C) and light approximately 12/12 h (night/day cycle), humidity 65 ± 5% in the Pharmacology, Physiology and Biochemistry Laboratory, Faculty of Veterinary Medicine, University of Maiduguri for two weeks to acclimatize to laboratory condition before the commencement of the experiment. The rats were fed with growers mash (Sanders Feeds Nig. Ltd.) and water ad libitum. The rats were denied food for 24 h prior experiment but were allowed free access to water.

Castor oil induced diarrhoea

The effect of the plant extracts on diarrhoea was evaluated in rats using the castor-oil induced diarrhoea method [23]. Albino rats of both sexes (200-250 g) were divided into five groups of three rats each. They were fasted 24 h prior to the experiment, but allowed free access to water. Group 1 (controls) was treated with 0.2 ml of normal saline. Group 2 was treated with standard drug (Diphenoxylate 5 mg/kg bd. wt.). Group 3, 4 and 5 were treated with different doses of the extracts (300, 600 and 1200 mg/kg bd. wt.), respectively. After the administration of diphenoxylate and extract each rat received 2 ml of castor oil orally. After one hour of the above treatment, they were allowed for consecutively 3 h for observation of wet feacal matters on a neat basin which were easily read during the period of the experiment by counting the total feacal droppings [24,25].

The extract's efficacies were expressed as a percentage of diarrhoeal inhibition as:

Percentage of wet faeces inhibition=(T0-T1/T0) × 100

T0=number of wet faeces in control group

T1=number of wet faeces in test group

Severity of diarrhoea=(Diarrhoeal faeces/Total faeces) × 100

RESULTS

The results of phytoconstituents is shown on Table 1, while Table 2 shows the anti-diarrhoeal effects of the extracts on castor-oil induced diarrhoea in rats.

Table 1: Phytochemical contents of the stem and root barks extract of *Annona senegalensis* and *Diospyros mespiliformis*; Key: +=present, –= absent

S/No.	Phytoconstituents	*Annona senegalensis*		*Diospyros mespiliformis*	
		Stem bark	**Root bark**	**Stem bark**	**Root bark**
1	Test for Carbohydrate				
	Molisch's test	+	-	+	+
2	Test for Saponins Glycosides				
	Frothing test	+	-	+	+
	Fehling's test	-	+	-	-
3	Test for Tannins				
	10% of lead acetate	+	-	+	+
	1% ferric chloride FeCl3	-	+	+	+

	10% of HCl in methanol	-	-	-	-
4	Test for Phlobatannins	-	-	-	-
5	Flavonoids				
	Ferric chloride FeCl3	-	+	+	+
	Shinoda's Test	-	-	-	-
	Lead ethanoate	+	-	+	+

Table 2: Castor oil induced diarrhoea in rats treated with stem and root bark extracts of *Annona senegalensis* and *Diospyros mespiliformis* at different doses; Data are mean of triplicates values. Means with different superscript letters along the column differ significantly (P<0.05)

Plant species	**Extract /Drug**	**Dosage**	**Feacal dropping**	**Inhibition**	**Severity of diarrhoea**
		(mg/kg bd. wt.)	**(Mean ± SEM)**	**(%)**	**(%)**
Annona senegalensis	Stem bark	300	0.00 ± 0.00a	100	0
Diospyros mespiliformis	Stem bark	300	0.67 ± 1.15b	92.82	5.38
Annona senegalensis	Stem bark	600	0.00 ± 0.00a	100	0
Diospyros mespiliformis	Stem bark	600	0.33 ± 0.58c	96.46	2.65
Annona senegalensis	Stem bark	1200	0.00 ± 0.00a	100	0
Diospyros mespiliformis	Stem bark	1200	0.00 ± 0.00a	100	0
Annona senegalensis	Root bark	300	0.00 ± 0.00a	100	0
Diospyros mespiliformis	Root bark	300	0.00 ± 0.00a	100	0
Annona senegalensis	Root bark	600	1.13 ± 1.15d	87.89	9.07
Diospyros mespiliformis	Root bark	600	0.67 ± 1.15b	92.82	5.38
Annona senegalensis	Root bark	1200	0.33 ± 0.58c	96.46	2.65
Diospyros mespiliformis	Root bark	1200	0.00 ± 0.00a	100	0
Positive control	Diphenoxylate	5	0.00 ± 0.00a	100	0
Negative control	Normal saline	–	9.33 ± 4.93e	–	74.88

DISCUSSION

The preliminary phytochemical screening of the extracts showed the presence of tannins, cardiac glycoside, flavonoids, saponins glycoside in all the extracts while free anthraquinones and combined anthraquinones were absent in root barks extract of *Annona senegalensis* and alkaloids are presence only in the root barks extract of *Annona senegalensis* but phlobatannins were absent in all the four extracts as shown in Table 1.

The results of the mean feacal droppings and the percentage inhibition as shown in Table 2 revealed that the ethanolic Root bark and Stem bark extracts of *Annona senegalensis* and *Diospyros mespiliformis* showed anti-diarrhoeal activity due to the significant reduction in the number of wet feaces or diarrhoea induced by the castor oil. In case of Stem bark extract of *Diospyros mespiliformis*, The activity increased with increase in the doses of the extract administered (300 mg/kg bd.wt. 600 mg/kg bd.wt. and 1200 mg/kg bd.wt.) compared to that of the negative control group; while Stem bark extract of *Annona senegalensis* shows 100%, inhibition at all doses (300 mg/kg bd.wt. 600 mg/kg bd.wt. and 1200 mg/kg bd.wt.) respectively. The result corroborated with the earlier findings by Suleiman et al. [8], that *A. senegalensis* is a potent phytomedicine for diarrhoea. The root bark extract of *Diospyros mespiliformis* shows 100% inhibition at the doses of 300 and 1200 mg/kg bd.wt., while 92.81% at the dose of 600 mg/kg bd.wt. The results of the root bark extract of *Annona senegalensis* shows a % inhibition of 100%, 85.74% and 96.46% at a doses of (300 mg/kg bd.wt., 600 mg/kg bd.wt. and 1200 mg/kg bd.wt.), respectively. Moreover the anti-diarrhoeal activities of the plant extracts were comparable to the standard drug Diphenoxylate which at present is one of the

most efficacious and widely employed anti-diarrhoeal drugs. The castor oil-induced diarrhoea model in rats allows the observation of measurable changes in the number of stools [12]. The diarrhoea lasts for at least 8 h [26] and is a consequence of the action of ricinoleic acid liberated from castor oil by lipase enzymes [12]. The freed ricinoleic acid irritates the intestinal mucosa causing inflammation and release of prostaglandins and nitric oxide, which stimulate gastrointestinal secretion, motility, epithelial permeability and edema of the intestinal mucosa [27,28] thereby preventing the re-absorption of sodium, chloride and water. Active intestinal secretion is driven predominantly by net secretion of chloride or bicarbonate, inhibition of net sodium absorption, or increase in luminal osmotically-active molecules (osmotic pressure) [29] which can all give rise to diarrhoea where the secretory component predominates. Although we do not know the precise mechanism of hyper-secretion affected by extracts, the reduction in fecal wetness strongly suggests it may inhibit gastrointestinal hyper-secretion. The anti-diarrhoeal properties of some medicinal plants have been attributed to their phytochemical constituent like tannins and some flavonoids [30]. The anti-diarrhoeal properties of some medicinal plants have attributed to their phytochemical constituents [5,10,31]. Some flavonoids have been shown to possess anti-diarrhoeal activity attributed to their ability to inhibit intestinal mobility and hydro-electrolytic secretion [32]. The presence of these secondary metabolites alkaloids, saponins, sterols and terpenes are also responsible for the anti-diarrhoeal property of the extract [33]. Phytochemical screening on the ethanolic extracts of *Annona senegalensis* and *Diospyros mespiliformis* showed the presence of some anti-diarrhoeal secondary metabolites; these are tannins, flavonoids, alkaloids and cardiac glycoside. These phytochemicals could be responsible for the anti-diarrhoeal activities displayed by the extracts [7,8,18,34].

CONCLUSION

In conclusion, the present study revealed that *Annona senegalensis* and *Diospyros mespiliformis* contains pharmacologically active substances effective for management of diarrhoea. However, further chemical and pharmacological studies are required to isolate the bioactive compounds and elucidate the precise mechanisms responsible for the observed pharmacological activities of these plants.

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