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Phytochemical Screening of Annona Senegalensis Stem Bark and Its Anti-Diarrheal Effect

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Abstract
The aqueous and methanolic extracts of stem bark of Annona senegalensis were screened for the presence of phytochemicals using standard methods. Alkaloids, tannins, flavonoids, terpenoids, saponin, phoblotannins and anthroquinones were found to be present while glycosides were absent. The anti-diarrheal activity of the extracts was assayed using agar well diffusion method to establish the inhibition zone diameter for selected microbes that cause diarrhea. Themethanolic extract showed high activity against the tested microbes with inhibition diameter of 26 mm and,25 mm for Escherichia Coli and Clostridium difficile respectively. The aqueous extract exhibited highest activity against Clostridium difficile with inhibition zone diameter of 27mm.However, the standard drug Ciprofloxacin used as positive control showed higher activity for all the tested microbes except for Clostridium difficile for which it had the same inhibition zone diameter as the aqueous extract. The result of this study showed that both aqueous and methanolic extracts have effects on the tested microbes so can ameliorate diarrhea thus validate the use of these extracts in treating same in the traditional settings.

Keywords: *Anti-diarrheal, Phytochemical screening, Aqueous extract, methanolic extract, Inhibition zone diameter*

1. Introduction

Plants apart from serving as sources of food, shelter, furniture and fuel also serve as a source of medicine traditionally. Traditionally, plants have been employed to treat or cure different types of ailments (Edeoga, *et al.*,2005). Plants also serve as one of the main sources of modern medicine manufactured by pharmaceutical industries (Bruice, 2011). The African continent has a wide variety of plants which the peoples have utilized in solving their different health needs. It is estimated that about 80 percent of the rural population depend herbal medicine (Dinez *et al,*2017). *Annona Senegalensis* which is one of the plants used traditionally for the treatment of many aliments belongs to *Annonaceae*family**.** *Annona Sengalensis* is commonly known as African custard apple, wild custard apple or wild sour sop. In Nigeria the plant is recognized by almost all the ethnics groups hence the plant is not strange. The plant is known as gwandar daji, okpokpo/uwu, uburu-ocha, onwu and abo among the Hausa, Idoma,Igbo, Igede, Tiv and Yoruba tribes respectively. *Annona Sengalensis* is a shrub or a small tree reaching up about 2-6 meters in height. The bark is smooth to roughish, silvery or grey brown, with leaf scars and roughly circular flakes exposing paler patches of under-bark. Young branches have dense brown, yellow hairs that are lost later. Unripe fruits are green turning yellow to orange when ripe. Flowers of the plant are added to food to spice and garnish it because of their pineapple like flavor. The leaves of the plant are eaten by humans and livestock. The stem bark can be processed to make yellowish-brown dye, insecticide for parasitic worms, treat diarrhea, gastroenteritis, lung infections, tooth aches and even snake bites.

The stem bark of plant is commonly used in Benue State, North Central Nigeria for traditional treatment of different ailments such as diarrhea, dysentery and toothaches. The natural gum in the bark is used to close open wounds. The roots are also used traditionally in treating dizziness, indigestion, cold and venereal diseases (Awa*et al*, 2012). However some of these claims have not been scientifically verified .This work therefore aims at validating the use of the stem bark of in treating diarrhea among the rural populace or disproving the claim .Diarrhea means constant passage of liquid faeces up to three times a day and it is a major health threat causing about one billion death per annum (Gong ,*et al.*,2017).Diarrhea is one of the leading causes of death especially in developing countries where a huge amount of resources are spent annually in trying to curtain the menace. Although manyeffective syntheticdrugs are available for the treatment, searching for anti-diarrheal from herbalsources is recommended by the World Health Organization (WHO) because of its cost effectiveness, benign nature and availability to the rural communities (Stark, *et al.*,2013).

Figure 1: Showing the Fruits, Leaves, Branches and Stem of Annona Senegalensis

2. Materials and Methods

2.1. Sample Collection.

The fresh stem-barks of *Annona Senegalensis* were collected from Mbayion village in Gboko Local Government Area of Benue State, Nigeria. The identification and authentication were done by Dr. Waya of Botany Department, Benue State University, Makurdi, Benue State, Nigeria and a voucher specimen were deposited with number 9121.

2.2. Sample Preparation

The stem-bark was washed and dried under shade for two weeks. The sample was pulverized to coarse powder and properly stored further use. 200g of the ground stem bark was macerated in 2litres of methanol for 48 hours with intermittent shaking. The suspension was filtered using Whatman filter paper No 42 and concentrated in a rotary evaporator maintained at 40^{o} C to obtain the methanolic extract which was stored in a clean container and kept in a freezer. In the same way, another 200g of the pulverized sample was soaked in 2 litres of distilled water for 48 hours, filtered and concentrated using a rotary evaporator to obtain the aqueous extract which was properly stored prior to use in running the phytochemical tests.

2.3. Phytochemical Screening

The chemical tests were carried out separately on the aqueous and methanolic extractsof annona *senegalensis* to determine their phytochemical constituents using standard procedures as described by Sofowora (1993).

2.3.1. Test for Terpenoids

About 0.5 g each of the extract was placed in a test tube containing 3mL of chloroform. Concentrated H_2SO_4 (3mL) was carefully added to form a layer and observed for any change in colour. A reddish brown colouration at the interface indicates the presence of terpenoids.

2.3.2. Test for Alkaloids

The extract 0.5g was measured into a test tube containing 5mL of 1% $HCl_{(aq)}$ and heatedfor about 10 minutes in a water bath. It was then divided into two portions. To the first portion, 3 drops of Dragendorf's reagent was added, immediate turbidity indicated the presence of alkaloids. To the second portion, 3 drops of Meyer reagent was added, immediate formation of precipitates indicated the presence of alkaloids.

2.3.3. Test for Saponins

To about 0.5 g of extract in a test tube was added 5 mL of distilled water which was vigorously shaken and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

2.3.4.Test for Tannins

About 0.5 g of the extract was boiled in 10 mL of water in a test tube and then filtered. A few drops of ferric chloride solution were added and observed. A brownish green or a blue-black colouration indicates the presence of tannins.

2.3.5. Test for Phlobatannins

To a test tube containing 5mL of distilled water was added 0.5g of the extract and boiled with 1% aqueous hydrochloric acid and observed formation of any precipitate. Red precipitates which was observed after 5 minutes indicated the presence of phlobatannins.

2.3.6. Test for Flavonoids

To a test tube containing 2mL chloroform was added 0.5g of the plant extract and5 drops of ammonia solution. Yellow – brown colouration in the ammonium layer indicates the presence of flavonoids.

2.3.7. Test for Anthraquinones

0.5 g of the extract was boiled with 10 mL, H_2SO_4 and filtered while hot. The filtrate was shaken with 5 mL of chloroform. The chloroform layer was pipette into another test tube and 1 mL of dilute ammonia was added. The resulting solution was observed for colour change.

2.4. Antimicrobial Assay

This was conducted using Agar Well diffusion method to determine the inhibition zone diameter for the four selected microbes which are *Clostridium difficile, Escherichia Coli, Bacillus cereus*and*Shigella.* These microbes are known to be causative agents of diarrhea. Mueller Hinton Agar (38g) was placed in one - liter capacity conical flask containing distilled water. The flask was corked with cotton wool, wrapped in aluminum foil and autoclaved at 121^{o}C for 15 minutes. The Mueller Hinton Agar was dispensed into four petri dishes and allowed to cool around 42-45^{o}C after which one milliliter (1mL) inoculum of various species was striped on the Mueller Hinton Agar in the petri dishes. A corked borer 6mm was used to sink three wells on each of the inoculated plates. The extracts were introduced into the wells with the help of micro pipette. The plates were labeled, wrapped and incubated overnight at the temperature of 35-37^{o}C after which the diameter of zone of inhibition was read with the aid of a transparent ruler.

3. Results and Discussion

The result of phytochemical screening of the aqueous and methanolic extracts of the stem bark of *annonoa senegalensis* as shown on table 1 below revealed the presence of important phytochemicals such as terpenoids, flavonoids, alkaloids and phlobatannins, tannins which have been shown to possess antimicrobial activities (Liu and Boyer,2004). The presence of these bioactive compounds in the stem bark of *annonoa senegalensis* as secondary metabolites thus help to validate its use in traditional herbal medicine to treat diseases especially diarrhea. This result is in agreement with the findings of (Ogbadoyi,*et al* (2010) which showed that the stem bark of *annonna senegalensis Pers* contained phytochemicals that experimentally suppressed African trypanosomiasis.

Phytochemical Constituent	Methanol Extracts	Aqueous Extracts
Saponins	+	+
Tannins	+	+
Flavonoids	+	+
Phlobatannins	+	+
Steroids	+	+
Terpenes	+	+
Glycosides	-	-
Alkaloids	+	+

Table 1: Result of Phytochemical Screening for the Methanolic and Aqueous of Stem Bark Extracts of Annona Senegalensis

The anti-diarrhea activity of the stem bark extracts of *anonna senegalensis* was measured by establishing the zone of inhibition diameter of the extracts for the four test microbes which are associated with diarrhea. The zone of inhibition is a qualitative means to measure the ability of chemical agent to inhibit the growth of a control organism that is under investigation. Zone of inhibition is therefore the area on an agar plate where the growth of a control organism is prevented by a chemical agent that was applied. The value of inhibition zone diameter (IZD) thus obtained helps to show whether the microbe is resistant or susceptible to the chemical agent applied. The IZD value of 10 mm or less infers that the microbe is resistant to the applied chemical agent while IZD of 16 mm and above shows that the microbe is susceptible (Johnson and Case,1995). The result is of zone of inhibition of aqueous and methanol extracts of *anonnasenegalensis* and Ciprofloxacin a standard drug used in the treatment of diarrhea is presented in table **2** below.

Isolates	Zone of Inhibition (mm)		
	Aqueous Extract	Methanol Extract	Control (+)
Shigella	21	20	28
Clostridium difficile	23	26	34
E.coli	21	25	30
Bacillus cereus	27	23	27

Table 2: The Anti-Diarrhea Activity of Stem Bark of Anonna Senegalensis

3.1. Control (+) Ciprofloxacin

The result showed that all the test microbes were susceptible to both the aqueous and methanolic extracts .The values of (IZD) obtained for the test microbes are all above 16 mm which showed that the extracts have the ability to

inhibit the growth of these microbes thus validate the use in traditional herbal medicine to treat diarrhea. The aqueous extract showed highest activity against *Bacillus cereus* having the same IZD as Ciprofloxacin a standard drug in clinical use. This makes the plant a potent phytomedicine for the treatment of diarrhea. This result obtained is also in agreement with the findings of (Awa. *et al,* 2012) which showed that the stem bark of anonna*senegalensis*contained hydroxylated phenols which are toxic to micro-organisms. It can be deduced that hydroxylated phenols were responsible for the slowing down of the spontaneous contraction of the intestine therefore slowing down the rate at which the wastes come out. The methanol extract of the stem bark of *Annona Senegalensis* showed a highest activity against Clostridium *difficile* which is one of the causative agents of diarrhea and validates its use by traditional medicine practitioner to treat such in rural areas.

4. Conclusion

The result phytochemical screening of stem bark *Annona senegalensis* showed the presence of saponins, tannins, alkaloid, flavonoids, terpenes and anthraquinones. The presence of these phytochemicals in the stem bark showed why *Annona Senegalensis* is used as an anti-diarrhea .This work has therefore established the scientific base for its use in the rural communities in Benue state Nigeria to treat diarrhea and other ailments .However more work needs to done to determine required dose and indentify these active ingredients and also elucidate their structures .

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