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In vitro Antimicrobial Effect of Crude Tannins Isolated from the Leaf of *Annona senegalensis*

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Authors' contributions

This work was carried out in collaboration between all authors. Author MSJ designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author WAU managed the analyses of the study. Author YA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This study was aimed at evaluating the antimicrobial effect of tannin isolated from the leaf of *Annona senegalensis* on *Shigella dysenteriae, Escherichia coli* and *Salmonella typhi*. The study was carried out in the laboratory of Biochemistry department, Modibbo Adama University of Technology, Yola, Adamawa State, Nigeria, between July and October, 2013.The antimicrobial activity was carried out using agar disc diffusion method. The phytochemical screening of the leaf of the plant revealed the presence of tannins, alkaloids, saponins, flavonoids, terpenoids, cardiac glycosides and anthraquinones while

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phenols and steroids were absent. Quantitatively, tannins were found to be 24% in the leaf of the plant. Isolated tannin exhibited antibacterial activities against all the tested bacteria; *Shigella dysenteriae* gave the zone of inhibition (18.6mm), *Escherichia coli* (13.9mm) and *Salmonella typhi* (10.6mm). Minimum Inhibitory concentration of the crude tannins on the test organisms ranged from 6.25mg/ml to 12.5mg/ml. These findings confirmed the basis of traditional use of the leaf of *Annona senegalensis* in the treatment of diseases such as dysentery and diarrhea.

Keywords: Antimicrobial; phytochemicals; zone of inhibition; crude tannins.

1. INTRODUCTION

Medicinal plants are cheap starting materials for the synthesis of new drugs and the chances of the body accepting preparations from plants are more than that prepared from laboratory [1]. Also, Fansoworth [2] has reported that aqueous decoction of drug has a greater bioavailability to the body than synthetic formulation today in the market. This is due to the fact that presentation of the active constituent of the plant material in the solubilized form brings about increase absorption of the extract by the body.

Clark and Hufford [3] reported that, even in years back there was growing interest in evaluating the plants that possesses antibacterial activity for various diseases. Herbal Medicine is still the main stream of about 75 to 80% of the whole population and the major part of traditional therapy involves the use of plant extract and there active constituents [4]. Some plant has also been reported to exhibit antimicrobial activities against various pathogens [5].

Annona senegalensis commonly known as African custard apple is an important plant that is used in traditional medicine to cure some diseases like toothache and diarrhea. The leaf of the plant is used for treatment of cancerous tumor, intestinal trouble and venereal disease. It is used for astringent and for treating dysentery and malaria [6]. Locally, the plant is known as “Gwandar daaji” in Hausa, “Uburu Ocha” in Igbo and Abo in Yoruba [7]. The part of the plant that is used for therapeutic purposes include root, bark, leaf, flower and seed.

Tannins are polyphenols that are obtained from various parts of different plants [8]. In addition to use in leather processing industries, tannin have shown potential antiviral [9], anti bacterial [10,11] and anti parasitic effects [12]. In the past few years tannins have been studied for this effect against cancer through different mechanisms [13].

This work is designed to extract crude tannin from the leaf of *Annona senegalensis* and to evaluate its efficacy or otherwise on causative organisms of the dysentery.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

Fresh leaves of *Annona senegalensis* was collected in Sangere under Girei Local government Area of Adamawa state, Nigeria, in the month of July, 2013.It was identified and authenticated By Mr.Briston Pola of the department of Plant Science, Modibbo Adama University of Technology, Yola, Adamawa State, Nigeria.

2.2 Sources of Test Organisms

The microorganisms *Shigella dysenteriae*, *Salmonella typhi* and *Escherichia coli* were obtained from the Laboratory of Microbiology Department, ModibboAdama University of Technology, Yola, Adamawa State, Nigeria. All the microorganisms are clinical isolates identified in the mentioned laboratory.

2.3 Preparation of the Extracts for Phytochemical Analysis

Exactly 50g of the powdered sample was weighed and transferred into 500ml of distilled water in a conical flask. This was allowed to stand for 24 hours at room temperature. The suspension was filtered using sterile what man No. 1 filter paper to obtain crude extract. A rotary evaporator was used to concentrate the extract. The concentrated extract obtained was stored in a refrigerator in a screw-cap bottle until when it was required.

2.4 Phytochemical Analysis

Chemical tests were carried out on the aqueous extracts to identify the constituents as described by Sofowora and Adewumi [14], Trease and Evans [15].

2.5 Extraction and Quantitative Determination of Tannins

The dry leaf was ground and 100mg of the fine powdered portion was dissolved with 1ml of concentrated HCl in methanol (1:100, v/v) in a water bath at 30ºC for 30mins and was shaken for every 5 minutes. The sample was centrifuged at 1200g and the supernatant was collected. Extraction was repeated twice; the combined supernatant was then filtered, evaporated to dryness and the weight was taken. The extract was kept for the antimicrobial test [16].

2.6 Preparation of Culture Medium

Nutrient agar used for the study was manufactured by Titan Biotech. Ltd, Rajastan, India and was prepared according to manufacturer's instructions.

2.7 Antimicrobial Activity Testing

The method described by Emeruwa [17] was used for the study. Wells were made using a cork borer on the surface of 20ml nutrient agar plates that was previously streaked with test organisms using 1.0McFarland standard (3×10^8 organisms). Extracted tannin of0.2ml was aseptically introduced into the wells made; 100mg of ciprofloxacin was used as positive control which was added into a separate well. The plates were allowed to stand on the working bench for 30minutes and were incubated for 24hours at 37ºC. The presence of zone of inhibition was regarded as the presence of antimicrobial activity and this was expressed as the length of the diameter of the zones of inhibition.

2.8 Determination of Minimum Inhibitory Concentration (MIC)

Different concentrations of tannins extracted from the leaf of *Annon asenegalensis* ranging between 1.5 and 50 mg/ml were introduced into different test tubes; each tube was inoculated with an overnight culture of *Salmonella typhi, Shigella dysenteriae* and

Escherichia coli. The tubes were incubated at 37ºC for 24 hours. The least concentration of tannin that did not permit any visible growth of the incubated test organism in broth culture was regarded as the Minimum Inhibitory Concentration (MIC) in each case [18].

3. RESULTS

The leaf of *Annona senegalensis* was found to contain tannins, alkaloids, saponins, flavonoids, terpenoids, anthraquinone and cardiac glycosides. Phenols and steroids were absent (Table 1).Quantitative determination of tannins yielded 24% in the fresh leaf of the plant.

Table 1. Phytochemical screening of leaf of *Annona senegalensis*

Phytochemicals	Results
Tannins	+ +
Alkaloids	+
Saponins	+
Terpenoids	+
Phenols	-
Steroids	-
Anthraquinones	+
Flavonoids	+ +
Cardiac glycosides	+ +

Key: + = present, - = absent and ++ = highly present

The crude tannin exhibited antibacterial activities against all the tested bacteria; *Shigella dysenteriae* gave the zone of inhibition (18.6mm), *Escherichia coli* (13.9mm) and *Salmonella typhi* (10.6mm) as shown in Table 2.

Table 2. Antimicrobial activity of tannin isolated from the leaf of *Annona senegalensis*

Test Organisms	Zones of inhibition (mm)	
	Crude Tanins	Ciprofloxacin
E. coli	13.90	34.00
S. typhi	10.60	21.00
S. dysentriae	18.60	26.00

Determination Minimum Inhibitory concentration (MIC) of the crude tannins gave 6.25mg/ml on *Shigella dysenteriae* and 12.5mg/ml on both *Escherichia coli* and *Salmonella typhi.* This is presented in Table 3.

Table 3. Minimum Inhibitory Concentration of Tannins Isolated from the Leaf of *Annona senegalensis*

Organisms	Concentrations (mg/ml)					
	50	25	12.5	6.25	3.0	1.5
E. coli	-	-	-	+	+	+
S. typhi	-	-	-	+	+	+
S. dysentriae	-	-	-	-	+	+

Keys: + = indicates growth and - = indicates no growth

4. DISCUSSION

Phytochemical analysis of the extract of leaf of *Annona senegalensis* revealed the presence of chemical constituents namely tannins, alkaloid, saponin, flavonoid, terpenoid, cardiac glycoside and anthraquinone. However, the presence of alkaloid and anthraquinone was in contrast with the work of Tijjani et al. [19], who reported that alkaloid and anthraquinone were absent in the leaf of the plant. The observed differences may be due to the different solvent used or where the plant is harvested or could be due to storage.

The various phytochemical compound detected are known to have beneficial medicinal value. There are established records that showed the benefits of these compounds detected in *Annona senegalensis,* for instance, tannins are reported to exhibit antiviral, antibacterial, antitumor activities. It was also reported that certain tannins are able to inhibit HIV replication selectively and is also used as diuretic [20].

Saponin in medicine, it is used in hyperchlolestrolaemia, hyperglycemia, antioxidant, anti-cancer, anti-inflammatory and weight loss etc. it is also known to have anti-fungi properties [21].

Flavonoids have been referred to as nature's biological response modifiers because of strong experimental evidence of their inherent ability to modify the body's reaction to allergic and carcinogens. They show anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activity [22]. Anthraquinones are generally used as dyes and are also known as antibacterial agent [23].

Cardiac glycosides are used in the treatment of congestive heart failure. They are also used to strengthen a weakened heart and allow it to function more efficiently though the dosage must be controlled carefully, since the therapeutic is close to toxic dose [21]. Terpenoid are active against bacteria, fungi and viruses [24] and protozoa.

Moreover, these chemical constituent were reported to exhibit prominent effect on human and other animal system through different mechanisms of action which make them useful for medicinal purposes. For instance anthraquinone are known to complex irreversibly with nucleophilic amino acid in protein [25]. Often leading to inactivation of the protein and loss of function. For that reason, the potential range of quinone antimicrobial effect is great. Probable targets in the microbial cell are surface – exposed adhesions, cell wall polypeptides and membrane bound enzymes. Quinone may also render substrate unavailable to the microorganism [25].

Mechanism of action of alkaloids such as berberine and harmane is attributed to their ability to intercalate with DNA / cell wall of an organism thereby disrupting the activity of the microorganism [26].

However, the mechanism of action of terpenoids is not fully understood but is speculated to involve in membrane disruption by the lipophilic compound. According to Mendez et al. [27] found that increasing the hydrophilicity of kaurene diterpenoids by addition of a methyl group drastically reduced their antimicrobial activity [21].

Cardiac glycosides are known to work by inhibiting the Na^+/K^+ pump. This causes an increase in the level of sodium ion in the myocyte which then lead to a rise in the level of

Ca^{+}. This inhibition increases the amount of Ca^{+} ion available for contraction of the heart, muscles which improve cardiac output and reduce distension of the heart [21].

Crude tannins from the leaf of the plant were found to be 24% and this is in agreement with the earlier study carried out by Simon [28] who obtained the same value. However, Bakar [29] reported that tannin is very rich in fruit of *Annona senegalensis* than in the leaf even though he has not mentioned the values.

The antimicrobial activity of tannins isolated from the leaf showed highest activity against *Shigella dysenteriae* and moderate in *Escherichia coli* and *Salmonella typhi* and ciprofloxacin was used as standard antibiotic as shown in Table 2 .

The sensitivity observed in this study is in agreement with earlier investigation by Doss et al. [30] and Akiyama et al. [10]. It was reported by Jone et al. [31] that tannins has the ability to interfere with the bacterial cell wall synthesis where they formed complex with polysaccharide through so called non-specific forces such as hydrogen bonding and hydrogen effects as well as by covalent bond formation. Thus, tannin irreversibly inhibits the enzyme trans peptidase by reacting with serine residue in the trans peptidase. This reaction is irreversible and so the growth of the bacteria cell is inhibited. Also reported by Viljoen et al. [32], the ability for the tannin compounds to cause the bacterial colonies to disintegrate probably results from their interference with the bacterial cell wall, thereby inhibiting the microbial growth.

The antimicrobial effect exhibited by ciprofloxacin gave higher zones of inhibition on the test organisms than the extracted tannin and this may be attributed to the concentration of the drug used.

The minimum inhibitory concentration (MIC) of tannin isolated in this study against the test organism ranged between 1.5 and 50mg/ml as shown in Table 3. Antimicrobial agent with low activity against an organism had a high MIC while a highly active antimicrobial agent gave low MIC. For instance *Shigella dysenteriae* has the highest zone of inhibition at the same concentration with other organism, but gave the low MIC. The antibacterial effectiveness with the increasing concentration of tannins observed in the present study is in agreement with the earlier investigation as reported by Kurosaki and Nishi [33] and Banso and Adeyemo [34]. However, a minimum inhibitory concentration (MIC) is generally regarded as the most basic laboratory measurement of activity of an antimicrobial agent against an organism.

The clinical significance of Minimum Inhibitory Concentration (MIC) are used not only to determine the amount of antibiotic that the patient will receive but also the type of antibiotic used, which in turn lowers the opportunity for microbial agents. Applying MIC testing to number of bacterial strains in the same species provides an estimate of the concentration that inhibits 50% (MIC_{50}) and 90% (MIC_{90}) of bacterial isolates and can indicate shifts in the susceptibility of bacterial population to antibiotics [35].

5. CONCLUSION

In conclusion, the result of this study supports the traditional use of the *Annona senegalensis* for the treatment of diseases such as dysentery, diarrhea and typhoid. It also suggests that the tannin isolated from the test plant could serve as a potential source of antibiotics.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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