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Antibacterial Effects of Saponin From Prosopis Africana And Ziziphus Abyssinica Leaves And Stem Barks

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Abstract
This study was carried out to determine the antibacterial effects of saponins from Prosopis africana and Ziziphus abyssinica. Saponins were isolated using ethanol, diethyl ether and isopropanol as solvents, and evaluated for antimicrobial activity against medically important bacteria (S. aureus, and E. coli), using nutrient agar. Antimicrobial activity was performed using paper disc method. The saponin isolated from P. africana leaves was active and showed the highest zone of inhibition at concentration 30mg/ml against S. aureus and E. coli. The saponin isolated from Z. abyssinica leaves showed the highest zone of inhibition at concentration 10 and 30mg/ml respectively. The stem barks of both P. africana and Z. abyssinica did not show any zone of inhibition. The saponin extract show no significant difference. Saponin obtained from P. africana leaves is more effective at higher concentration while it is more effective at lower concentration from Z. abyssinica leaves. This attests to the fact that the plants' leaves contain bioactive saponin compounds of potentially therapeutic significance and could be promising sources for drug development as a cure for bacterial diseases.
Key Word: Extract; Prosopis Africana; Zizipus abyssinica; Saponins; Inhibition

I. Introduction

The extraction of bioactive ingredients from plant resources has always been a hot topic in scientific research (1;2). Saponins are the active components and the main secondary metabolites of the plants used in many ethnic medicines (1;3). The biological activities of various saponins, such as antibacterial, anti-inflammatory, antifungal and antiviral activities, are based on their chemical structures. It has been reported that a type of *Rehmannia glutinosa* saponin, denoted 3-O-glucopyranose oleanolic acid, exhibits anti-inflammatory activity in the inflammatory exudation and proliferation phase of *Randia dumetorum* Lam (3;4). Saponins exist in all types of plants in nature, such as ginseng, lentil, Cassia obtusifolia, Croton macrostachyus, and fenugreek (5;6;7). The contents of saponins vary among different plants, but higher abundances are found in quinoa and fenugreek (7).

With advances in medicinal chemistry, most modern antibacterials are semisynthetic modifications of various natural compounds (8). *Prosopis africana* (Guill., Perrott. and Rich.) Taubert (Fabaceae) is one of the plants used to stimulate wound healing in traditional medicine of south-eastern Nigeria. It is a tree with a very hard wood and easily distinguishable by its dark rough bark, pale drooping foliage with small pointed leaflets and sausage-shape fruits (9). However, Prosopis africana is also known to contain anti- nutritional substances. According to Saman *et al*. (2016) (10), anti-nutrition al factors are secondary metabolites found in plants and are known to be biologically active substances. These substances are found in fruits, seeds, and other parts of the plants (11). They occur in varying amounts depending on the kind of crop, and their mode of propagation (12). Habtamu and Negussie (2014) (13) reported that anti-n u t ri en t s are chemicals produced by plants for their defense and other biological functions.

The *Ziziphus mauritiana* leaves are eaten by cattle, camels and goats by which they found minerals, useful for their health (14). *Ziziphus mauritana* fruits have highly useful contents quantity that is useful for human health (15). Our study demonstrates *Zizipus* alcoholic extract as an important source of new therapeutic agents for pain management (16). The bark is crushed to a pulp and placed on the wound surface as a dressing, while a poultice of the boiled seeds is usually applied externally to relieve sore throat, (17). Dried ripe fruit is a mild laxative and fruits are applied on cuts and ulcers; are employed in pulmonary ailments and fevers. The leaves are helpful in liver trouble, asthma and fever. The powdered root is dusted on wounds (18). It is used to treat sore throats, alleviate stress and helps in the common colds (19;20).

Saponins are glycosides widely distributed in the plant kingdom, include a diverse group of compounds characterized by their structure containing a steroidal or triterpenoid aglycone and one or more sugar chains.

Their structural diversity is reflected in their physicochemical and biological properties, which are exploited in a number of traditional as soaps, fish poison, and molluscicides and industrial applications (21)

Escherichia coli are a gram negative motile, non-sporing bacillus, morphologically identical with salmonella. They are found in the gut of man and animal and widely distributed in the environment. Once outside become pathogenic in urinary tract infection and wounds. Infections are mostly indigenous (from patient intestinal) or exogenous (acquired from outside) (22). *E. coli* are responsible for producing infection in children. Outbreak of acute gastroenteritis occurs in children under 18 month's age. This is characterized by massive fluid use from the gut, causing acute dehydration, acidosis and hypovolacine shock. Some time toxic and fatty changes in liver may occur and the syndrome is called cholera infantum. In adults it causes travelers' diarrhea. Travelers' diarrhea is caused by entero toxigenic *Escherichia coli* (ET.E.C). There are two types of toxin produced by this organism as Heat labile and Heat stable (23).

Inhibitory activities exhibited by the extracts against the isolated fungi could be linked to diverse phytochemicals such as flavonoids, tannins, saponins that have been reported. These compounds are appraised to have bactericidal and fungicidal effects (24).

II. Materials And Methods

The leaves and stem barks of *Z. abyssinica and P. africana* were obtained from the surroundings of Kebbi State University of Science and Technology, Aliero (K.S.U.S.T.A) and authenticated by the Taxonomist, Dr. Drahmendra Singh, Head of Department Biological Science The leaves of both plants were collected by hand picking while the stem barks were obtained by using sterile sharp knife. The test organisms were collected from microbiology laboratory of KSUSTA. The samples were washed thoroughly with distilled water, then oven dried at a temperature of 50^{o}c to get rid of the moisture content. After drying, they were pounded to powder using pestle and mortar, and then sieved.

One hundred grams (100 g) of oven-dried powdered leaves and stem barks of *P. africana* and *Z. abyssinica* was weighed and poured in to a conical flask and 280 ml of ethanol was measured and added in the conical flask containing the extract and covered with cotton wool, then capped with aluminum foil for 24hrs at room temperature. The aqueous extract was filtered and allowed to evaporate on water bath. Saponin content of the crude extract of both leaves and the stem bark was isolated according to the methods modified by Hostettamnn *et al*., (1995) (25). The saponin was tested by obtaining 5 ml of saponin extract then pipetted and poured in test tube, after which 5ml of distilled water was added and shaken. The presence was confirmed by production of soapsuds.

The medium used for antimicrobial activity was nutrient Agar. The medium in the petridishes was allowed to solidify for 24hrs before being inoculated. Discs made from filter paper (whatman No1) of 5 mm diameter and sterilized by autoclave were soaked in aqueous solution at concentration 10, 20 and 30 mg/ml saponin extract for 24hrs then placed on the surface of the agar medium. The inhibition zone around the paper disc was measured.

III. Results

The results obtained showed the antibacterial effects of saponins extracted from *P. africana* and *Z. abyssinica* against *Escherichia coli* and *Staphylococcus aureus* using disc diffusion method. The means of zones of inhibition (mm) at different concentrations (mg/ml) were observed as; 1.83 mm, 1.87 mm and 2.05 mm from 10, 20 and 30 mg/ml respectively, with control having 2.77 mm against *E coli*. This was close to that of *S. aureus* that had 1.80 mm, 1.80 mm and 2.05 mm from 10, 20 and 30 mg/ml resoectively with control having 2.40 mm. (Table 1).

The antibacterial activity of Saponins from *P. africana* showed mean zones of inhibition (mm) of 1.76 mm, 1.80 mm and 1.92 mm from 10, 20 and 30 mg/ml respectively with control recording 2.23 mm against *E coli*. This was also close to that of *S. aureus* that respectively had 2.02 mm, 1.80 mm and 2.00 mm from 10, 20 and 30 mg/ml with control recording 2.55 mm. (Table 3). But the concentrations 10, 20, and 30 mg/ml from *P. africana* stem bark did not show any inhibited effect on both organisms (Table2 and 4).

Table 1 also shows the antibacterial effect of Saponin extracted from *P. africana* leaves against *E. coli* and *S. aureus*. At concentration 30 mg/ml both organisms had 2.05 mm while at 20 mg/ml they recorded 1.87 and 1.80 mm respectively, and at 10 mg/ml, 1.83 mm and 1.80 mm, whereas at 20 mg/ml and 10 mg/ml, *S. aureus* had the same zones of inhibition by saponins from leaf extract of *P. africana.* . These agree with the study of Del, (2020) (7) who reported leave extract of *P. africana* had higher antibacterial effect than the bark extract and can be used to treat head, eye, ear and tooth troubles or as bath for the treatment of migraine and vertigo, Del (2020) (7) also reported that macerated leaves of *P. africana* was traditionally created with soporific properties and were thought to ensure male fertility because of its high antibacterial effect.

Table 2 shows the antibacterial effect of saponin extracted from *P. africana* stem bark against *E. coli* and *S. aureus* and the extract shows no zone of inhibition at the lowest concentration and very weak effect at the

highest concentrations. This agrees with the study of Yao *et* al/. (2014) (3) who reported the bark of the *P. africana* trees in east Sudan contained 9-12 % Saponin and 14-16% tannins capable of giving reddish brown color to both leather and cloth. The Saponins is recognized as an antinutritive factor when fed to ruminants at concentration above 5 %, this is because the stem bark has low level of Saponins when compared with leaves. This also agrees with the study of Yao *et al*. (2014) (3), who reported that saponins in foods have traditionally been considered as antinutritional factors.

Table no 1: Antimicrobial activity of saponin from *P. Africana* leaves.

Test organism	Concentration (mg/ml): Zone of inhibition			Control
	10	20	30	
E. coli	1.83 ±0.15	1.87 ±0.12	2.05 ±0.18	2.77 ±0.76
S. aureus	1.80 ±0.06	1.80 ±0.17	2.05 ±0.24	2.40 ±0.39

Table no 3 shows the antibacterial effects of saponins from *Z. abyssinica* leaves against *E. coli* and *S. aureus*. At concentrations 10, 20 and 30 mg/ml, *E. coli* had 1.92, 1.80 and 1.76 mm respectively, while *S. aureus* had 2.00, 1.80 and 2.02 at 10 20 and 30 mg/ml respectively. The leaves of *Z. abyssinica* at 30 mg/ml showed higher zone of inhibition (1.92 mm) against *E. coli* when compared with the concentration at 20mg/ml and 10 mg/ml which showed 1.76 and 1.80 mm respectively. The leaves of *Z. abyssinica* at 10 mg/ml showed higher zone of inhibition 2.02 mm when compared with 20 mg/ml and 30 mg/ml 1.80 and 2.00 respectively. This agrees with the study of Henneh *et al*. (2020) (26) who reported that the antimicrobial activity of different species related to leaves of *Z. abyssinica* are mainly attributed to its most active ingredients. Saponins are reported as the most active ingredients to which the antimicrobial activities of many plants species at high concentrations. Henneh *et* sl., (2021) (27) demonstrate that *Zizipus* alcoholic extracts are important sources of new therapeutic agents for pain management

Table no 2: The antibacterial activity of Saponin from *P. Africana* stem bark

Test organism	Concentration (mg/ml): Zone of inhibition			
	10	20	30	Control
E. coli	-	-	-	2.10±0.65
S. aureus	-	-	-	2.36± 0.56

TABLE 3: Antibacterial activity of Saponin from *Z. abyssinica* leaves

Test organism	Concentration (mg/ml): Zone of inhibition			
	10	20	30	Control
E. coli	1.76 ±0.14	1.86 ±0.51	1.92 ±0.09	2.23 ±0.59
S. aureus	2.02 ±0.50	1.80 ±0.09	2.00 ±0.23	2.55 ±0.50

TABLE 4: The antibacterial activity of Saponin from *Z. abyssinica* stem bark

Test organism	Concentration (mg/ml): Zone of inhibition			
	10	20	30	Control
E. coli	0.00	0.67 ±0.06	1.05 ±0.08	2.735 ±0.30
S. aureus	0.00	0.70 ±0.07	0.05 ±0.14	2.06 ±0.39

Table 4 shows the antibacterial effects of Saponins extracted from *Z. abyssinica* stem bark against *E.coli* and S. *aureus* at different concentrations. No zone of inhibition at the lowest concentrations. The stem bark had higher antiviral activity than antibacterial activity, as reported by the study of Mukharjee *et al*., (2003) (28) who reported that the stem bark of *Z. abyssinica* has been found to retard the progression of HIV 1 infection, and it also helps in the treatment of common cold.

The leaves of *P.africana* have more antimicrobial activity than the bark due to the higher content of Saponins in the leaves than the bark. Saponin obtained from *P. africana* leaves is more effective at higher concentration while it is more effective at lower concentration from *Z. abyssinica* leaves. Further research should be done with other pathogens. The research should be carried out using various chemical compounds extract of either root or flower of the plants (*P. africana* and *Z. abyssinica)*.

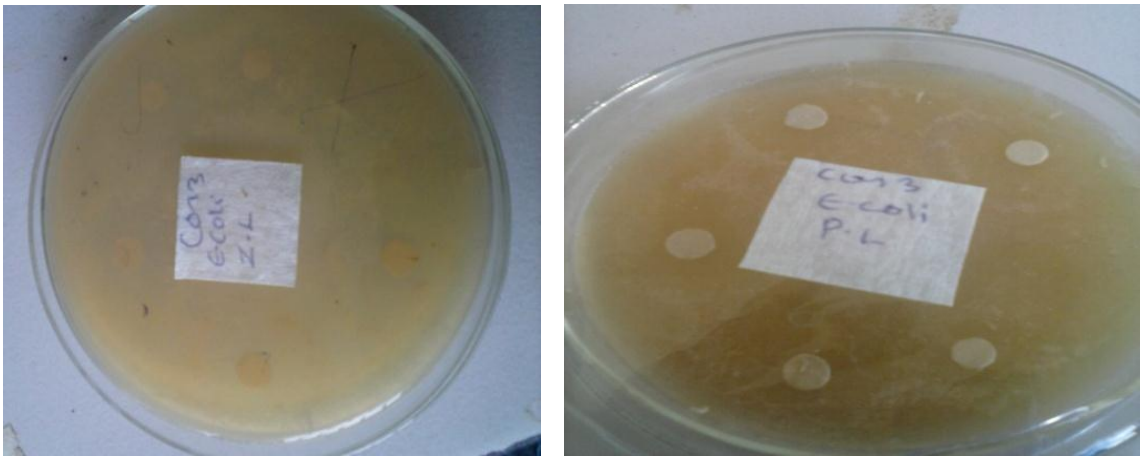

Plate 1: Zone of inhibition against *E. coli* on *Z. abyssinica* and *P. africana* leaves extracts

IV. Conclusion And Recommendations

The leaves of *P.africana* had more antimicrobial activity than the bark due to its high content of Saponins in the leaves than the bark. The research also showed that Saponins obtained from *Prosopis africana* leaves are more effective at higher concentrations more effective at lower concentration from *Ziziphus abyssinica* leaves. Further research on the stem bark of both plants is recommended

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