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ANTIBACTERIAL ACTIVITY AND TOXICOLOGICAL EVALUATION OF *Anogeissusleiocarpus* AND *Psidium guajava* ON *Escherichia coli* and *Staphylococcus aureus*

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ABSTRACT

The study was carried out to determine the antibacterial activities and toxicological evaluation of Anogeissusleiocarpus and Psidiumguajava on Escherichia coli and Staphylococcus aureus isolated from clinical samples. The plants leaves were extracted using Hexane, Methanol, Ethanol and Water. Various concentrations (50, 25, 12.5 and 6.25mg/ml) of the crude extract of the plants were prepared and their antibacterial activity against Staphylococcus aureus and E. coli was determined using Agar well (Diffusion) method. Toxicity of the plants was evaluated, acute toxicity test, kidney and liver function tests. The result revealed that at 50mg/ml concentration, the leaf extract of Psidiumguajava was active against Staphylococcus aureus and E. coliexhibiting the highest zones of inhibition of 19mm and 9mm respectively. Whereas The leaf extract of Anogeissusleiocarpus only inhibited the growth of Staphylococcus aureus recording highest zone of inhibition of 15mm at a concentration of 50mg/ml. The plant extracts were found to be non-toxic as the LD_{50} was above 5000mg/kg and the biochemical parameters evaluated for both liver and kidney function tests revealed values that are within normal range. Hence the study established that consumption of the leaves of P. guajava for medicinal purpose can be said to be innocuous, as such the plant could be regarded as a potential candidate in the search of potent and harmless plants of therapeutic value.

Keywords: Anogeissusleiocarpus, Escherichia coli, Psidiumguajava and Staphylococcus aureus

INTRODUCTION

In recent years there is increasing incidence of multiple antibiotic resistances in human pathogenic microorganisms, largely due to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases (Parekh and Chanda, 2007). The implication is that many antibiotics have failed in the treatment of some infectious diseases. This has forced scientist to search for new antimicrobial substances from various sources like medicinal plants. Search for new antibacterial agents is on the increase by the screening of many plant families (Parekh and Chanda, 2007).

Psidium guajava called guava is an evergreen small tree. The leaves are 2-6inches long and 1-2 inches wide, aromatic when crushed, and appeared dull-green with stiff but coriaceous with pronounced veins (Garode and Waghode, 2014). *Psidium guajava* (guava) is commonly known for its food and nutritional values, the medicinal properties of the leaves are also well known in traditional system of medicine (Garode and Waghode, 2014).

Anogeissus leiocarpus is a deciduous tree growing up to 30m in height, typically 15-18m with light green foliage, Leaves are alternate to sub-opposite, elliptic to ovate-lanceolate in shape, and 2-8cm long and 1.5-3.5cm across (Klaus *et al.,* 2004). The stem are finely pubescent, the bark is grey to beige in colour, becoming blackish with age, and fibrous with thin scales. *Anogeissus leiocarpus* contains about 40 wind dispersed seeds of 10g each (Klaus *et al.,* 2004).

Resistance to antimicrobial agents is a major global public health problem. Synthetic drugs are expensive, not always available; the good ones are not available and affordable especially in rural areas and are also associated with adverse side effect.

Increase development of resistance to current antibiotics has strengthened scientific research for discovery of new drugs that are potent, cheap, readily available, and affordable and with no or less side effects. *Anogeissus leiocarpus* and *Psidium guajava* ranked top in the list of medicinal plants in Hausa -Fulani land known for their therapeutic benefits. However, not much research has been done to scientifically evaluate and reconcile the antibacterial potentials of these plants against the traditional claims. This study was aimed at evaluating antibacterial activities and toxicological studies of *Anogeissus leiocarpus* and *Psidium guajava* against*Escherichia coli* and *Staphylococcus aureus.*

MATERIALS AND METHODS

Collection, Identification and Extraction of plant materials

Fresh samples (leaves) of *Anogeissus leiocarpus* and *Psidium guajava* plant were collected from the Usmanu Danfodiyo University, Sokoto Biological Garden. Voucher specimens were kept at the herbarium of Botany unit in the department of Biological Sciences. The leaves of the plants *Anogeissus leiocarpus* and *Psidium guajava* were rinsed with clean water and air dried under shed at room temperature for two weeks. Each of the air dried samples were pounded manually with a clean pestle and mortar, the powdered leaves were sieved, weighed and stored in a clean sterile containers ready for use according to the methods of Oyeleke and Manga (2008).

Extraction of the plants materials

The soxhlet method of extraction was employed in extracting the plants materials using Hexane, Methanol, Ethanol, and Water. A weight of 500g of each of the powdered plants samples were placed in the upper chamber in a thimble, and 500ml of the solvent (hexane, methanol, ethanol, and water each) in the bottom flask. The pulverized leaves of both *A. leiocarpus* and *P. guajava* were defatted exhaustively with the above solvents by successive soxhlet extraction according to the method of National Institute for Pharmaceutical Research Development protocol (2004). The extracted solutions were concentrated in a rotary evaporator according to the method of Oyeleke and Manga (2008). The extracts were weighed and kept in well labelled sterile sample bottles for further analysis.

Preparation of Concentrations of the crude Plants Extracts

A test stock concentration of 500mg/ml for methanol, ethanol, and water extract of *A. leiocarpus and P. guajava* (leaves) were prepared by dissolving 1g of each extract in 2mls of sterile distilled water in separate test tubes. Non-polar hexane extract was first homogenised in 0.1ml dimethyl sulfoxide (DMSO) and then added to 1.9ml of distilled water. 0.1ml of the stock concentration equivalent to 50mg was used to prepare different concentrations of the plants extract by doubling dilution (50, 25, 12.5 and 6.25mg/ml) (Oyeleke and Manga, 2008). The different concentrations of the crude plant extracts were assayed for their antibacterial activity against the test bacteria. The positive control drugs used was Tetracycline (0.5mg/ml).

Test Bacteria

The isolates (*Staphylococcus aureus* and *Escherichia Coli*) were isolated from urine and wound samples following standard procedures for sample collection, isolation and identification (Gram staining, biochemical test and serological test) (Koneman *et al*, 2005).

Determination of Antibacterial Activity of the crude extract

Bacteria identified and characterized by standard biochemical methods (*Staphylococcus aureus* and *Escherichia coli*) were tested for their sensitivity to the crude plant extracts using the agar well diffusion method described by Hugo and Russel (1992).

The suspension of the bacterial inoculums that matched McFarland scale 0.5, were spread on the surface of a Muller Hinton agar plate with sterile bent glass rod. A standard cork borer of 6mm in diameter was used to cut 6 wells at the centre of each inoculated plate and the agar removed from the well. A 0.1ml of each extracts (at concentrations of 50, 25, 12.5 and 6.25mg/ml) were introduced into the wells. A positive control (0.5ml/mg) of Tetracycline was placed in one of the wells. Sterilized distilled water was used as negative control. The plates were incubated at 37^{o}C for 24hrs, and observed for the zone of inhibition of the growth. The zones were measured with a transparent ruler and the result recorded in millimetres. The screening was done in triplicates.

Toxicity studies

Evaluation of the toxicity of the plant extract with the highest antibacterial activity against the test bacteria was carried out using acute toxicity studies. Adult albino rats weighing 190-200g of the same age group born on the same date were used after being certified healthy by a veterinary doctor. The animals were kept at animal house in a cage at Usmanu Danfodiyo University Sokoto Biological Garden where they were maintained under veterinary supervision and were fed well with standard growers "vital feed" and have access to water for 3days to

acclimatize before commencement of the The animals were housed and cared for in accordance with good laboratory practice (GLP) regulation of WHO (2008).

The acute toxicity test was carried out according to the method described by Aboudoulatif *et al.* (2010) with slight modification using thirty (30) albino rats. The limit test dose of 2000mg/kg and 5000mg/kg body weight as stipulated in the Organization for Economic Corporation Development (OECD) guidelines were used. The rats were grouped into three (3) groups, containing ten (10) rats each, and then 2000mg/kg and 5000mg/kg body weight of methanolic leave extract of *P. guajava* were administered orally with cannula attached to a graduated syringe to the first two groups, while the last group received only distilled water and served as control. The animals were observed individually for the first eight (8) hours for acute toxicity signs, behavioural changes, and at least once daily for 14 days, the first day was taken as D0 whereas the day of sacrifice was designated as D14.

The rats were sacrificed after 14days and some biochemical parameters for liver and kidney function were estimated and recorded. The function of liver was evaluated using key parameters such as alkaline phosphate (ALP), aspartate transaminase (AST), alanine transaminase (ALT), albumin, total bilirubin and divided bilirubin. The kidney function was evaluated using parameters such as urea, creatinine, sodium, potassium and chlorine ions.

experiment.

On day 14 after administration, all the albino rats were sacrificed (by slaughtering). Blood samples were immediately collected from each sacrificed rat into well labelled clean plastic test tubes and allowed to stand to ensure complete clotting. The clotted blood samples were centrifuged at 3000rpm for 10mins; clear serum samples were aspirated and stored frozen. The effect of the crude extracts on the biochemical parameters were determined using the procedures and reagents of agappe diagnostics Ltd.(2013). At Usmanu Danfodiyo University Teaching Hospital Sokoto (chemical pathology lab).

RESULTS

The results of the study revealed that the *Anogeissus leiocarpus* leaves had antibacterial activity only against *Staphylococcus aureus* while *Psidium guajava* was active against both *Staphylococcus aureus* and *E. coli*. Table 1 shows thatat a concentration of 50mg/ml, methanol leave crude extract and aqueous leave crude extract inhibited the growth of *Staphylococcus aureus* producing a zone of inhibition of 15mm and 9mm respectively. At 25mg/ml concentration ~~also~~ the methanol and aqueous extract inhibited the growth of *Staphylococcus aureus* producing an inhibition zone of 13mm and 7mm respectively (Table 1). However, at 12.5mg/ml concentration only the methanol leaves crude extract inhibited the growth of *Staphylococcus aureus* producing 11mm inhibition zone (Table 1).

Table 1: Antibacterial activity of the crude extracts of *Anogeissus leiocarpus* leaves

Conc. (mg/ml)	Mean of the zone of inhibition (mm)/Conc. of extracts(mg/ml)							
	Hexane		**Methanol**		**Ethanol**		**Aqueous**	
	S. aureus	***E. coli***	***S. aureus***	***E. coli***	***S. aureus***	***E. coli***	***S. aureus***	***E. coli***
6.25	-	-	-	-	-	-	-	-
12.5	-	-	11	-	-	-	-	-
25.0	-	-	13	-	-	-	7.0	-
50.0	-	-	15	-	-	-	9.0	-
(Tetracycline)	28	22	26	22	28	18	30	28
Distilled water	-	-	-	-	-	-	-	-

Key: - = No inhibition;

Table 2 shows that the highest antibacterial activity of *Psidium guajava* leaves crude extract against *Staphylococcus aureus* and *E. coli* was produced by the methanol extract at the concentration of 50mg/ml which produces a zone of inhibition of 19mm and 9mm respectively. Similarly, at50mg/ml concentration, the ethanol leaves crude extract inhibited the growth of *Staphylococcus aureus* producing an inhibition zone of 12mm. The lowest zone of inhibition of 6mm was produced by the ethanol extract at the concentration of 12.5mg/ml (Table 2).

Table 2: Antibacterial Activity of the Crude Extracts of *Psidium guajava* leaves

Conc. (mg/ml)	Mean of the zone of inhibition (mm)/Conc. of extracts(mg/ml) Hexane		Methanol		Ethanol		Aqueous	
	S. aureus	***E. coli***	***S. aureus***	***E. coli***	***S. aureus***	***E. coli***	***S. aureus***	***E. coli***
6.25	-	-	-	-	-	-	-	-
12.5	-	-	10	-	6.0	-	-	-
25.0	-	-	15	6.0	10	-	-	-
50.0	-	-	19	9.0	12	-	-	-
(Tetracycline)	30	28	28	28	28	28	28	28
Distilled water	-	-	-	-	-	-	-	-

Key: - = No inhibition;

The results of the general appearance and behavioural observation for the treated animals and control group are presented in Table 3. The results indicated that there were no any physical or behavioural changes between the treated and control groups of the albino rats following the administration of the crude methanolic leaves extract of *P. guajava* at 2000 and 5000mg/kgbw doses.

Table 3: Behavioural observations of the control and treated rats

Observation	Control group		Treated group	
Time	8hrs	14days	8hrs	14days
Activity	Active	Active	Active	Active
Breathing	Normal	Normal	Normal	Normal
Convulsions	Absent	Absent	Absent	Absent
Ears and eyes	Normal	Normal	Normal	Normal
Injury	Absent	Absent	Absent	Absent
Skin changes	Absent	Absent	Absent	Absent
Salivation	Absent	Absent	Absent	Absent
Mortality	Absent	Absent	Absent	Absent

Table 4a contain the result of acute toxicity test, it show that no death was recorded following the oral administration of the methanol extract of *Psidiumguajava* at dose limits of 2000mg/kgbw and 5000mg/kgbw.

Table 4b contain the result of the parameters use in determining the LD_{50}. As no death was recorded among all the test rats it indicated that the $LD_{50}$for methanol extract of *Psidiumguajava* is above 5000mg/kgbw.

Table 4a: Toxicity test of *Psidium guajava* in Rats.

Groups	Number per group	Number of death	Weight (g)	Dose
1	10	0	190	0
2	10	0	195	2000
3	10	0	200	5000

Table 4b: Determination of $LD_{50}$of methanol leave extract of *Psidium guajava* in rats

Dose	Number of death	Mean death	Dose difference	Mean death *dose difference
0.000mg/kg	0	0	0	0
2000mg/kg	0	0	2000mg	0
5000mg/kg	0	0	3000mg	0

The results of the liver and kidney function test of the treated and control group of the albino rats were presented in Table 5 and 6 respectively. Table 4 revealed that with the exception of ALP which was significantly higher, the values of all the biochemical parameters were similar for the control and treated groups. Table 5 revealed that, for the kidney function test the values of all the biochemical parameters were similar for the control and treated groups.

Table 5: Effect of crude methanolic leaves extract of *P. guajava* on liver function

Parameters	Control group 0.000mg/kgbw	Group treated with 2000mg/kgbw	Group treated with 5000mg/kgbw
AST	70.6±2.79	68.1±5.23	64.6±15.2
ALP	273±1.30	836±5.03	923±9.91
ALT	110±0.26	139±11.2	139±9.50
Albumin	2.80±2.10	2.67±0.24	2.88±0.12
Total bilirubin	0.73±1.55	0.86±0.12	0.80±0.10
Divided bilirubin	0.13±1.24	0.08±1.27	0.06±1.45

Key: Mg/kgbw = Milligram per Kilogram body weight; AST = Aspartate transaminase, ALP = Alkaline phosphate, ALT = Alanine transaminase. Note: Values are mean± S.E.M for n=10

Table 6: Effect of the methanolic leaf extract of *P. guajava* on the kidney function

Parameters	Control group 0.000mg/kgbw	Group treated with 2000mg/kgbw	Group treated with 5000mg/kgbw
Urea	6.79±0.44	6.96±1.16	7.0±0.73
Creatinine	0.73±0.17	0.72±0..22	0.78±0.17
Sodium ion (Na^+)	135.5±0.84	134.2±0.78	135.8±1.47
Potassium ion (K^+)	4.51±0.12	4.41±0.26	4.44±0.06
Chlorine ion (CL^-)	96.3±0.94	95.9±1.91	97.3±1.25

Key: Mg/kgbw = Milligram per Kilogram body weight. Note: Values are mean ± S.E.M for n=10

DISCUSSION

The findings of the study indicated that the crude extracts of *Anogeissus leiocarpus* and *Psidium guajava* leaves possess an antibacterial activity against the test organisms. The study showed that the antibacterial activities of both the two plants decreases with decrease in the concentration of the extracts producing lower inhibition zones against the test isolates at the lowest concentration of 6.25mg/ml and higher inhibition zones at the highest concentration of 50mg/ml.. This implies that the higher the concentration of the extract, the higher the antibacterial activity, thus, indicating a concentration dependent activity of the test extract. This is in agreement with the observation of the concentration dependent nature of antibiotics in which the rate of bacterial eradication increases with increase in concentration of the drug (El-Mahmood *et al.,* 2010).

The study illustrated that the methanol extract of *Anogeissus leiocarpus* have higher antibacterial activity than the other solvents. Similar findings have reported methanol to be more efficient in extracting substances from medicinal plants. This may have been due to the better solubility of the bioactive agents as polarity increased (El-Mahmood *et al.,* 2010). Hexane and ethanol extract did not inhibit the growth of the test bacteria.

The study reports that methanol and ethanol extract of *Psidium guajava* exhibited antibacterial activity against both the two test bacteria (*E.coli* and *S. aureus*). This is line with the work of Abdelrahim *et al* (2002) who reported that Guava leaf extract inhibited the growth of *S. aureus* in a study carried out using disc diffusion method. It also support the findings of Rabe and Van Staden (1997) which showed that greater overall antimicrobial activity was seen with methanol extract than with other extracts. However, the finding of this research is in contrast to the findings reported by Jajari *et al.* (1999) who obtained better results from aqueous extracts than from methanol extract. The difference in the extract performance suggests that there are multiple and different antimicrobial agents present in each type of extract acting in different ways on different bacterial strains.

Acute Toxicity study was conducted at doses of 2000mg.kg and 5000mg/kg body weight and it is usually carried out to determine whether or not a substance is safe for human use, in this investigation different biochemical parameters were analysed from the serum of the albino rats such as alkaline phosphate (ALP), alanine transaminase (ALT), aspartate transaminase (AST), total bilirubin, direct bilirubin, urea, creatinine, sodium, potassium and chlorine to evaluate the liver and kidney functions.

In this research there was significant elevation ($p<0.05$) in the alkaline phosphate (ALP) and alanine transaminase (ALT) levels among the rats treated with the extract as compared with the control group, the increase in ALP level may be due to increased functional activity of the liver and this finding is consistent with the previous report of John *et al.,(* 2014) which

shows that lowered level of ALP are less ALP has been the marker enzyme for plasma membrane and is required in certain amount for proper functioning of organs. Alkaline phosphate (ALP) is a hydrolase responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins and alkaloids. High ALP levels can occur if bile ducts are obstructed. ALP level increases if there is active bone formation occurring.

Alanine transaminase (ALT) is a transaminase, formerly called serum glutamate-pyruvate transaminase (SGPT). ALT is the most common in liver but may also be found in the blood. Significantly elevated level of ALT often suggest the existence of other medical problems such as liver damage, viral hepatitis, diabetes, heart failure, bile duct problems, infectious mononucleosis or myopathy. The increase in the ALT level in this work may be due to the liver damage. And this may be in of line with the findings Hydar *et al.,* (2013) which shows that liver damage and its recovery are usually assessed by measuring the level of serum enzymes-transaminases, particularly (ALT). Alanine transaminase is a more specific indicator of liver inflammation than the aspartate transaminase. All other biochemical parameters analysed there was no significant difference between the treated groups and the control.

In toxicology, the median lethal dose LD_{50} is a standard measurement of acute toxicity that is common than elevated levels. in milligrams (mg) of toxin per kilogram (kg) body weight. Thus, the value of LD_{50} for substance is the dose required to kill half the members of a tested population after specific test duration. The result obtained in the present study showed that there was no mortality at the dose limit of 2000 and 5000mg/kgbw following oral administration with methanolic leaf extract of *P. guajava* and there was no any sign of toxicity in all the treated rats. This suggest that the methanolic leaf extract of *P. guajava* is therefore relatively harmless acutely.

CONCLUSION

This research revealed that the crude leaves extract of *Anogeissus leiocarpus* and *Psidium guajava* possess antibacterial activity against the test isolates. *Anogeissus leiocarpus* was active only against *Staphylococcus aureus,* with an inhibition zone that ranges from 7mm to 15mm,whereas *Psidium guajava* was active against both *S. aureus* and *E. coli* producing higher zones of inhibition of 19 and 9mm respectively..The study demonstrated that *Psidium guajava* had no toxic effects based on the physical, behavioural and biochemical (liver and kidney function) parameters assessed as such the plants is suitable for human consumption. As such, the study identified *Psidium guajava* as the best and safe plant with promising antibacterial activity.

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