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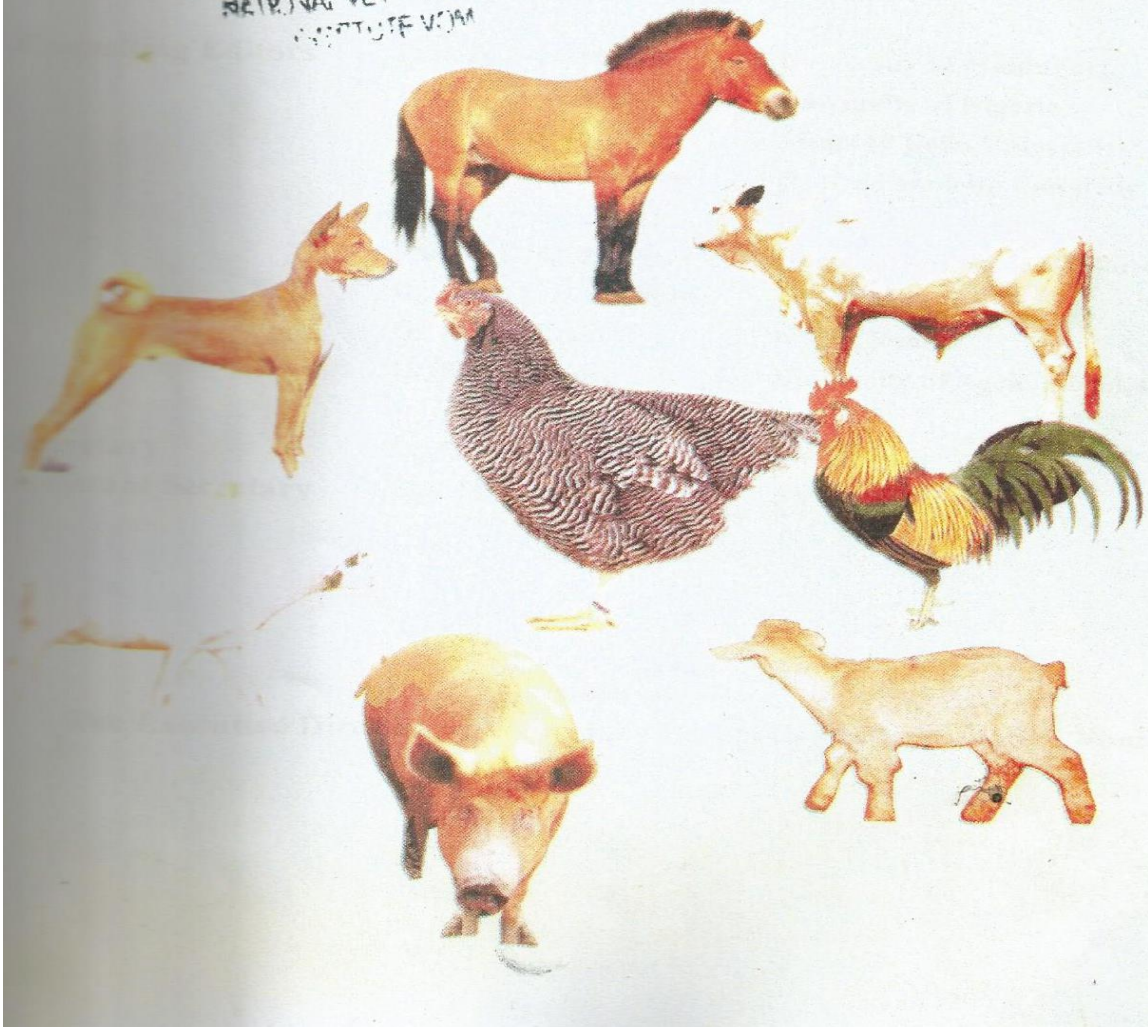

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CONTENT

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ANTIBACTERIAL EFFECTS OF *XIMENIA AMERICANA* (LINN), *PIPER GUINEENSE* (SCHUM ET THONN) AND *DISSOTIS ROTUNDIFOLIA TRIANA* (PIER)

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SUMMARY

Overcoming antibiotic resistance is one of the major issues of Health authorities in this millennium. The current study was undertaken to explore the antibacterial activities of some traditionally acclaimed medicinal plants (*Ximenia americana, Piper guineense* and *Dissotis rotundifolia triana).* Aqueous extracts of each plant material was subjected to phytochemical screening. Antibiogram assay was also carried out against four bacteria organisms, *Staphylococcus aureus, Salmonella gallinarum, Pasteurella multocida* and *Klebsiella aerogenes.* Results of phytochemical screening revealed the presence of tannins, carbohydrate, steroids and cardiac glycosides in all of the extracts. Alkaloids and cyanogenetic glycosides were only detected in *Ximenia americana* and *Dissotis rotundifolia triana.* The extracts displayed varied antibiogram activities against the four organisms with varied minimum inhibitory concentrations (MIC) and zones of inhibition. *Ximenia americana* root extract showed the highest inhibition on *Pasteurella mutocida, Klebsiella aerogenes* and *Salmonella gallinarum* and had the least MIC when compared to the other extract. However, *Dissotis rotundifolia triana* whole twigs exhibited the highest activity against *Salmonella aureus* and the lowest MIC against *Salmonella gallinarum.*

The presence of these phytochemicals may form the basis for their antimicrobial activities. Our findings also substantiate the use of these medicinal plants/herbs by traditional healers in the treatment of common bacterial infections and other ailment in humans and animals.

Keywords: *Ximenia americana, Piper guineense, Dissotis rotundifolia triana,* Antibacterial activity, Phytochemistry

INTRODUCTION

Despite the enormous advances in health care made during the last century, infectious diseases still account for 25% of mortality world wide and about 45% in low income food deficient countries (LIFDC) (15). According to the World Health Organization Report on infectious diseases, overcoming antibiotic resistance is one of the major issues of World Health Organisation (WHO) for this millennium (16).

In Africa, traditional medicine is practiced and plants have been exploited for the treatment of many infections and diseases. Many plant extracts owe their potency in antibacterial activities to the presence of substances such as tannins, phenolic compounds, glycosides, saponins, etc. Many plants have therefore become sources of important drugs.

Ximenia americana (TsadaHausa) also known as wild lime, wild olive, seaside plum or spiny mountain is traditionally indicated for the treatment of several infectious diseases of animals (7), and humans (14) such as fever, conjuctivitis, tooth decay, dysmenorrheea, mouth ulcers, rheumatism, infant diarrhoea, dysentery and abdominal pains (14). It is also used to treat febrile headache and bloody diarrhoea in calves (7).

Piper guineense (MasoroHausa, Uzizalbo, Iyere-Yoruba) commonly known as climbing black pepper or Guinea pepper is another plant of medicinal importance. Its fruit are employed in combination with other plants for rheumatism and malaria (4). The seeds are also used in the treatment of *Mycobacterium smegmatis, Candida albicans* and *Klebsiella pneumoniae* infections (1, 8). The oil from the seeds has insecticidal action and when distilled, it is used in perfumery and special soap making (8).

Dissotis rotundifolia triana (Edigibata-Nupe) is used traditionally for the treatment of sleeping sickness amongst the Nupe tribe Niger State of Nigeria.

This work was designed to verify the antibacterial properties of these medicinal plants as claimed by traditional healers.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

The plants for this study were obtained from different locations. *Ximenia americana* (XA) roots were collected from Kwall in Bassa Local Government Area of Plateau State in the month of July with the help of a traditional healer/Herdsman. The plant was identified by Prof. Husaini of Botany Department, University of Jos and Mr. Ohaeri of National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja.

Piper guineense (PG) dried seeds were purchased from traditional healers in Jos Central market. They were identified by Mr. Okonkwo of Federal College of Forestry, Jos, Plateau State.

Dissotis rotundifolia triana (DR) was collected from Bida in Niger State of Nigeria in the month of August. It was identified by Mr. Ohaeri of NIPRD, Idu Abuja.

Dried plant materials were ground to coarse powder in a wooden mortar and extracted in distilled water following the method of the Traditional healers.

Aqueous extraction

Fifty grammes (50g) of each powdered plant material was soaked in 500 millilitres (ml) of boiled distilled water in a covered glass jar for 24 hours at room temperature. The contents of the jar were filtered through a clean white muslin cloth. The filtrate was filtered through Whatman filter papers numbers 1 and 2 using a funnel under suction pressure. The filtrate was concentrated and dried over a steam-water bath or dried in the oven in a weighed crucible at 40°C. The completely dried extracts were weighed and stored in a refrigerator at 4°C in glass sample bottles with corks until needed.

Antibiogram Assay

The aqueous extract of each plant material was used for the antibiogram studies. Each extract was weighed to obtain 50mg, 75mg and 100mg. These were dissolved in sterile distilled water overnight at 4°C. Chloramphenicol and Streptomycin (Himedia-Octodiscs - OD253, Himedia Laboratories Pvt. Ltd) were used as standard antibiotics.

Preparation of Bacterial Organisms

Each of bacterial isolates used (*Staphylococcus aureus, Salmonella gallinarum, Pasteurella multocida* and *Klebsiella aerogenes* - ISB 88 Standard stock cultures, NVRI Vom, Nigeria) was re-identified by the standard method of Collins (6). A 10^3/ml suspension of each isolate was made in sterile phosphate buffered saline (PBS).

Medium

Nutrient Agar (NA) plates were prepared and dried. One millilitre (1ml) of each bacterial suspension (10^3/ml) was used to flood the prepared sterile plates. Excess medium was tipped off into a discard jar containing disinfectant and the seeded plates dried. Wells measuring about 5mm diameter were punched off to accommodate the different concentrations of the different extracts and the negative control. The plates were then labelled properly and the wells filled with 0.15ml of each of the different concentrations (50mg, 75mg and 100 mg diluted in 10 ml distilled water) of the extracts at pH of 4.98, 7.4 and 5.03 for XA, PG and DR respectively. The minimum inhibitory concentration (MIC) for each extract on the test micro-organisms was determined by the method of Jawetz *et al.*, (10). The plates were incubated for 18 hours at 37°C after which the zones of inhibition, if any were measured. Streptomycin and Chloramphenicol were used as standard antibiotics.

Phytochemical screening of extracts

Various phytochemicals were screened for according to the standard methods of Trease and Evans (13) and Sofowora (12).

RESULTS

Antibacterial Studies

Aqueous extract of *Ximenia americana* roots inhibited the growth of *Salmonella gallinarum* at a minimum inhibitory concentration (MIC) of 3.13 mg/ml with 24mm zone of inhibition. It had a moderate activity against *Staphylococcus aureus* with MIC of 12.5mg/ml and 11mm inhibition zone. Its activity against *Pasteurella multocida* was much lower than the other two micro organisms with MIC of 25 mg/ml and 12mm zone of inhibition. The lowest activity was on

Klebsiella aereogenes with MIC of 50 mg/ml and zone of inhibition of 12mm.
Aqueous *Piper guineense* seed extracts showed very low activity against the micro organisms. Its MIC was 50 mg/ml for all the organisms while zones of inhibition were 7mm, 7mm, 7mm and 8 mm for *Pasteurella multocida, Klebsiella aereogenes, Staphylococcus aureus* and *Salmonella gallinarum* respectively.
Aqueous *Dissotis rotundifolia triana* extract recorded its highest activity against *Salmonella gallinarum* with an MIC of 1.32 mg/ml and zone of inhibition of 14mm. For the other micro-organisms, its MIC was 50 mg/ml while zones of inhibition were 8mm, 7mm and 15mm for *Pasteurella multocida, Klebsiella aereogenes* and *Staphylococcus aureus* respectively. Streptomycin and Chloramphenicol at the lowest concentrations of 1mg/ml for all the organisms had very clear and higher zones of inhibition. Results are presented in Table 1 and Figures 1 (a-d).

TABLE 1: Antibacterial activities of different aqueous plant extracts on selected microorganism

Drug/extract	Zones of inhibition (mm)	Minimum inhibitory concentration (MIC mg/ml)
Pasteurella multocida		
Ximenia root	12	25
Piper seed	7	50
Dissotis whole twigs	8	50
Streptomycin	25	1
Chlorampenicol	21	1
Klebsiella aerogenes		
Ximenia root	12	50
Piper seed	7	50
Dissotis whole twigs	7	50
Streptomycin	15	1
Chlorampenicol	18	1
Staphylococcus aureus		
Ximenia root	11	12.5
Piper seed	7	50
Dissotis whole twigs	15	50
Streptomycin	24	1
Chlorampenicol	19	1
Salmonella gallinarum		
Ximenia root	24	3.13
Piper seed	8	50
Dissotis whole twigs	14	1.32
Streptomycin	15	1
Chlorampenicol	12	1

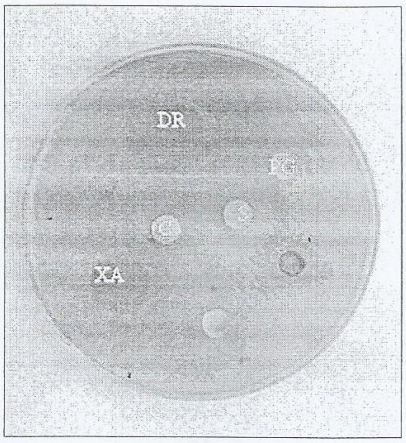

Figure 1a. Antibacterial activity of aqueous extracts of *Ximenia* roots (XA), *Piper* seed (PG), and *Dissotis* twigs (DR) on *Staphylococcus aureus* showing zones of inhibition with streptomycin and chloramphenicol as standards.

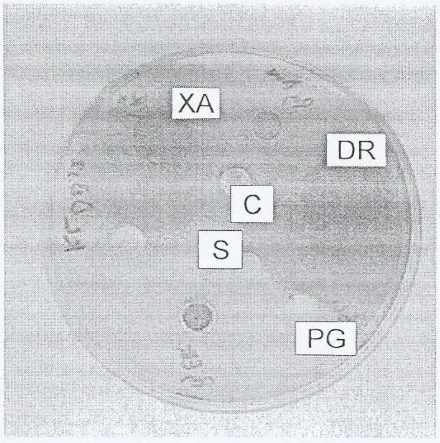

Figure 1b. Antibacterial activity of aqueous extracts of *Ximenia* roots (XA), *Piper* seed (PG), and *Dissotis* twigs (DR) on *Klebsiella aerogenes* showing zones of inhibition with streptomycin and chloramphenicol as standards.

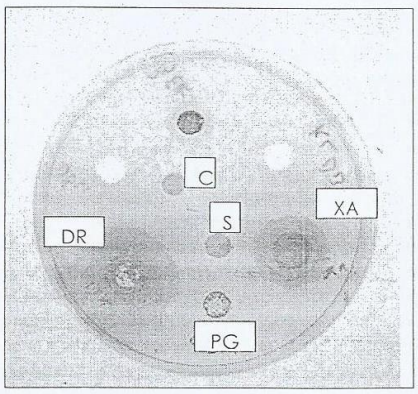

Figure 1c. Antibacterial activity of aqueous extracts of *Ximenia* roots (XA), *Piper* seed (PG), and *Dissotis* twigs (DR) on *Pasteurella multocida* showing zones of inhibition with streptomycin and chloramphenicol as standards.

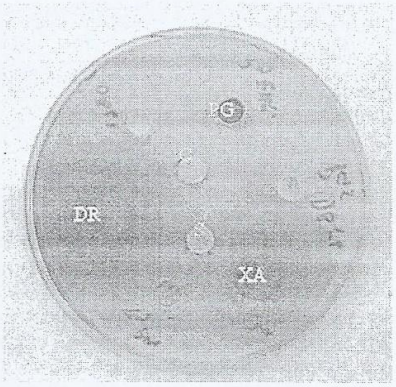

Figure 1d. Antibacterial activity of aqueous extracts of *Ximenia* roots (XA), *Piper* seed (PG), and *Dissotis* twigs (DR) on *Salmonella gallinarum* showing zones of inhibition with streptomycin and chloramphenicol as standards.

Phytochemical screening

The results of the phytochemical screening of the different extracts showed that *Ximenia americana* root extract had saponins, tannins ,flavonoids, carbohydrates, cyanogenetic glycosides, steroids, alkaloids, and reducing sugars. *Piper guineense* seed extract had cyanogenetic glycosides, steroids, cardiac glycosides and alkaloids. *Dissotis rotundifolia triana* extract had saponins, tannins, flavonoids, carbohydrates, steroids, cardiac glycosides, and reducing sugars. The results are presented in Table 2.

TABLE 2: Results of Phytochemical Screening of Extracted Plant Materials

Constituents	*Ximenia americana* roots	*Piper guineense* seeds	*Dissotis rotundifolia triana* twigs
Saponin	+	-	+
Tannins	+	Trace +	+
Flavonoids	Trace + (yellowish pink)	-	+
Carbohydrate	+	+	+
Cyanogenetic glycosides	+	+	-
Steroids (Salkowski & Liebermann tests)	+	+	+
Cardiac glycosides	Trace +	+	+
Anthraquinones (free & combined)	-	-	-
Alkaloids (Dragendorf's, Meyer, Wagner & Hager's tests)	+	+	-
Reducing sugars (free & monosaccharides)	+	-	+

DISCUSSION

The presence of some phytochemicals in the aqueous extracts from the three plants is an indication that they possess medicinal properties. Saponins are known to have antiseptic, emulsifying, detergent, analgesic and anti-inflammatory properties (11, 13). Tannins also possess antiseptic properties while reducing sugars have hypopglycaemic effects. Anthraquinones have anti-viral and laxative effects (13), while glycosides are used as laxatives, cathartics and to increase the force of contraction of the heart muscle in congestive heart failure. Alkaloids have varied medicinal properties which include anti-protozoal, (2) anticancer, anti-inflammatory, analgesic and central nervous system effects (6). Previous workers earlier reported on the use of *Ximenia americana* root extract in the treatment of bacterial infections and rheumatism (3, 7). Similarly, Dwuma-Badu *et al.*, (8), Addea Mensah *et al.*, (1) and Akinniyi *et al.* (4) Stated that *Piper guinneense* seed extract can be used in the treatment of fungal and bacterial infections. Our findings support these reports since these extracts have activities against the bacterial organisms studied. *Staphylococcus aureus, Pasteurella multocida, Salmonella gallinarum* and *Klebsiella auroegenes* are common bacteria that cause infections in humans and animals, therefore the use of extracts from these plants by traditional healers may be beneficial and justifiable to the rural populace in the treatment of common bacterial infections of humans and animals.

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