ANTIBACTERIAL PROPERTIES OF RAIN TREE (*ALBIZIA SAMAN*) AND MEXICAN SUNFLOWER (*Tithonia diversifolia*) USED AS FODDER IN RUMINANT NUTRITION

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Abstract

Browse plants are important fodder resources for ruminant. The secondary metabolites in them are potential source of antimicrobial drugs with reference to antibacterial agent. This study was designed to determine the phytochemical constituents and antibacterial activity of Albizia saman leaves (ASL) and Tithonia diversifolia leaves (TDL) against four bacterial species using Agar well diffusion method. Concentration of alkaloids (14.85 and 12.45mg/g) were higher in both plants. Acetone and ethanol extracts of ASL and TDL exhibited maximum zone of inhibition against Staphylococcus aureus (19.00mm and 14.50mm; 23.00mm and 21.50mm) and Pseudomonas aeruginosa (15.50mm and 12.00mm; 18.50mm and 17.00mm) compared with the reference antibiotics, Gentamycin (9.97mm). Albizia saman and Tithonia diversifolia leaves exhibited a broad spectrum antibacterial properties against pathogenic bacterial of ruminants.

Keywords: *Albizia saman, Tithonia diversifolia*, Antibacterial properties, Secondary metabolites

Introduction

In Nigeria, ruminants depend mainly on natural grasses and legumes to supply them the daily nutrients requirement for production or maintenance. Their grazing or browsing nature and preference for herbaceous plants affords them opportunity to varieties of leaves from trees, shrubs and weeds. However, virtually every part of these forages (leaves, stem, roots, barks, fruits and seeds) contain secondary metabolites such as phenolic compounds, essential oils and sarasaponins which have been reported to exhibit some medicinal properties (Savithramma *et al.*, 2011).

In an attempt to proffer solutions to the challenges of disease infections, the use of synthetic antibiotics then becomes a routine practice for treatment of infected animals; prolong useage has resulted to development of resistance to pathogenic microbial strains (Franca *et al.*, 2012). Also, the fast growing health consciousness among consumers regarding antibiotic residues in animal products are issues of public health concern (Virdis *et al.*, 2010). Such negative effects of antibiotics coupled with the changing patterns of antimicrobial resistance in livestock necessitated an investigative study for antibacterial agent from fodder plants as alternative to conventional antimicrobial agents against some pathogenic bacterial causing diseases in ruminants.

Tithonia divertisifolia (Mexican sunflower) is a member of the family *Asteracaea*. It is an annual shrub found growing aggressively on road sides, cultivated farmlands, fallow land and river banks in Nigeria while *Albizia saman*, formerly called *Samanea saman* (family: *Leguminosae*) and commonly called rain tree is a large size tree widely distributed in the humid and sub-humid tropics.They are well relished by ruminants. Phytochemical screening of plants bioactive compounds with medicinal properties have been documented (Savithramma *et al.*, 2011). This study was therefore, designed to evaluate the phytochemical constituents and antibacterial properties of leaf extracts of *A. saman* and *T. divertisifolia* against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Salmonella typhii,*

Materials and Methods

Collection of plant materials

Foliages of *Albizia saman* tree and *Tithonia diversifolia* were collected within the paddocks of the Institute of Agricultural Research and Training, Moor Plantation, Ibadan. The leaves were plucked from a healthy and uninfected plant at 8- 10 weeks of regrowth and washed thoroughly under a running tap water to remove dust and other foreign particles. Leaves were finally rinsed with sterile distilled water and thereafter, air dried under shade.

Phytochemical Analysis and preparation of Leaf Extracts

100 grams of fresh leaves (*A. saman and T. diversifolia*) were boiled with 200 ml of solvents (n-hexane) for 1 hour. The extracts obtained were filtered through Whatman filter paper No.1. The filtrates were vacuum dried (on water bath at 40 – 50°C) using a rotary evaporator. Evaporation of solvent affords a crude extract of the soluble

components. The extracts were stored at $0-4^0$ C in air-tight containers for further use. The Residues (extracts) were later re-dissolved with the appropriate solvents for the quantitative phytochemical analysis according to prescribed methods (Mc Donald et al., 2001; Edeoga *et. al.*, 2005). Ethanol and acetone extracts of the plants were obtained according to Puri *et al.*, (1999).

Source of microorganisms and Standardization of microorganisms

The test organisms were obtained from the stock culture of Microbiology Laboratory, Institute of Agricultural Research and Training, Moor Plantation Ibadan, Nigeria. Culture was standardized as described (NCCLS, 2002).

Antibacterial activity (Agar well diffusion method)

The antibacterial activity of extracts was determined by the standard agar well diffusion method against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Salmonella typhii.* s reported (Hassan et al., 2011) while the control was gentamycin.

Statistical Analysis

Data obtained for phytochemical constituents were subjected to t- test at ($P<0.05$) and Descriptive graphical statistics for antibacterial activities of the leaf extracts on all the test organisms.

Results and Discussion

The quantitative phytochemical constituents (Mg/100g) of *A. saman* and *T. diversifolia* is presented in Table 1. The values for alkaloids, tannin, saponin, phenol, flavonoids, and glycosides contents obtained in leaves of *A. saman* and *T. diversifolia* were 14.85 and 12.46mg/g, 0.29 and 0.34mg/g, 0.75 and 0.59mg/g, 0.25 and 0.22mg/g, 0.26 and 0.32mg/g, 0.39 and 0.35mg/g respectively. However, higher content of alkaloids (14.85 and 12.45mg/g) were obtained in both plants. Alkaloids, even in small quantity have been reported to produce strong antimicrobial effects (Mariital *et al.*, 2011). Although, Tannin give rise to astringent sensation in the mouth which tends to reduce feed intake, this level (0.29 and 0.34 mg/100g) of tannin is of no adverse effect on digestibility in ruminant nutrition. Saponin, though a phytochemical with bitter principles, reduces palatability of livestock feeds but exhibits hypocholesterolaemic activity. The levels of saponin (0.75 and 0.59 mg/100g) obtained were low to cause any detrimental effect on ruminant nutrition. Hence, phytochemical constituents of *A. saman* and *T. diversifolia* in this study conform to the report (Edeoga, *et al.*, 2005) for the bioactive compounds in plants that play significant roles as antimicrobial agents. The antibacterial activity of acetone and ethanol extracts of *A. saman* and *T. diversifolia* leaves against *E. coli, S. aureus P. aeruginosa* and *S. typhii* as recorded from the zone of inhibition is shown in Figures 1 – 4. The extracts (ethanol and acetone) of the two plants exhibited high antibacterial activity against the test organisms as measured from the zone of inhibition of microbial growth as reported (Jegasser *et al.*, 2011; Odeyemi *et al.*, 2014) for leaf extracts of *A. saman* and *T. diversifolia* respectively. Ethanol and acetone extracts of *A. saman* and *T. diversifolia* exhibited a large zone of inhibition (Figures 2 and 3) against *S. aureus* and *P. aeruginosa* compared with the control (Gentamycin) except, *E. coli* and *S. typhii.* (Figures 1 and 4). The high zone of inhibition of these extracts against *S. aureus* and *P. aeruginosa* could be attributed to the high concentration and activities of alkaloids combined with other phytochemicals in the plant extracts as reported (Marjorie, 1996). The strong antibacterial activity of the fodder plants on *S. aureus* and *P. aeruginosa* in this study conformed to the study (Mariital *et al.*, 2011) for *Scaboxus multiflorus* and *Acacia nilotica* leaf extracts.

Conclusion

Inhibition of microbial growth is a demonstration of the sensitivities of *S. aureus, P. aeruginosa, S. typhii* and *E. coli.* to *A. saman* and *T. diversifolia* foliages. This implied good antibacterial potentials of the fodder plants, against udder health problems in dairy ruminants caused by specific bacteria such as *Staphylococcus species* and *Pseudomonas species*; an opportunistic pathogens which causes diseases like mastitis, bovine diarrhea, fever-like disease, abortion and other food borne related pathogens in farm environment.

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Table 1: Quantitative Phytochemical constituents of leaf extracts of *Albizia saman* and *Tithonia diversifolia*.

Phytochemical Constituents (mg/100g)	*Albizia saman*	*Tithonia diversifolia*	Probability P<0.05	Remark
Tannin	0.29	0.34	0.0006	*
Phenol	0.26	0.22	0.0023	*
Alkaloid	14.85	12.46	0.0001	*
Saponin	0.75	0.59	0.0001	*
Trypsin inhibitor(u/mg)	0.17	0.16	0.0082	*
Oxalate	0.17	0.14	0.0025	*
Phytate	0.11	0.12	0.0041	*
Flavonoids	0.26	0.32	0.0003	*
Glycoside	0.39	0.35	0.0008	*
Steroids	0.13	0.13	0.0389	*
Terpene	0.12	0.11	0.0061	*

Note * means significant

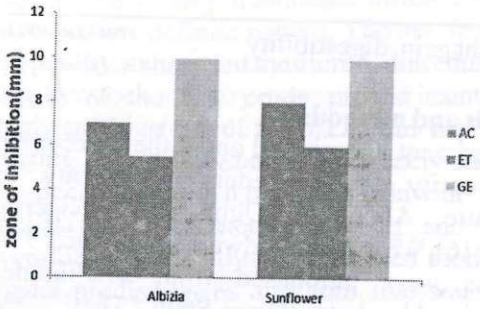


Figure 1: Antibacterial activity of leave against *Escherichia coli*

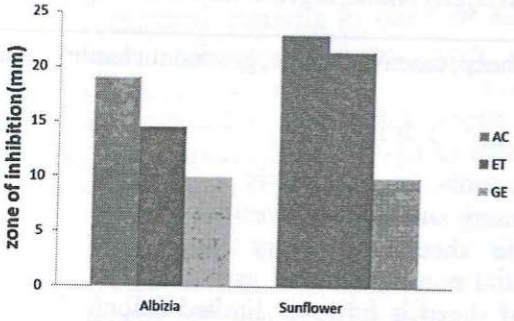


Figure 2: Antibacterial activity of leave against *Staphylococcus aureus*

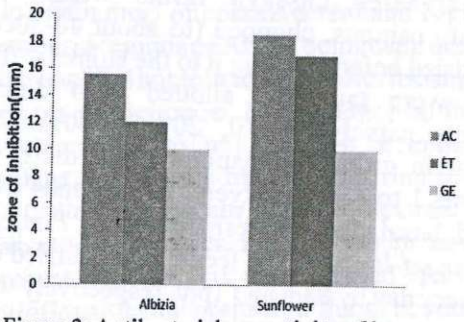


Figure 3: Antibacterial activity of leave against *Pseudomonas aeruginosa*

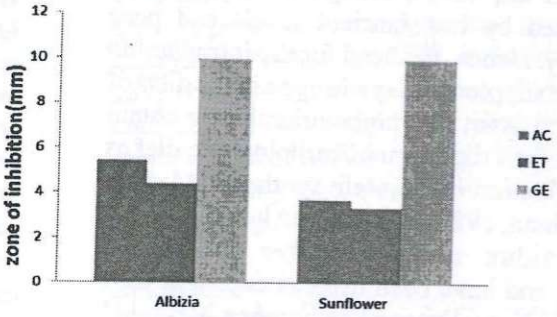


Figure 4: Antibacterial activity of leave against *Salmonella typhii*

AC = Acetone; ET = Ethanol; GE = Gentamycin