

Full Length Research Paper

Antimicrobial activity and phytochemical screening of crude water extract of the stem bark of *Ficus glumosa*.

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Water and methanol extracts of the stem bark of *Ficus glumosa* were screened for their antimicrobial properties against *Salmonella* species. Preliminary results showed that the active compound was in the water extract. Therefore, the water extracts (hot and cold) were subjected to column chromatographic fractions and antibacterial screening. The most active fraction was subjected to phytochemical screening and finally Gas Chromatography Mass Spectrography (GC/MS) analysis. The result indicated that the most active fraction was the 3rd hot water extract and its GC/MS analysis was narrowed down to 3,3'-(4-methyl-1,3-phenylene)bis(1-heptylurea), isothujol and Terbutyl azine.

Key words: Phytochemical screening, *Ficus glumosa*, column chromatography and antimicrobial activity.

INTRODUCTION

Due to the prevalent claims of typhoid fever infections around Sokoto, Nigeria and high patronage of traditional medicines, different mixtures are being used to treat typhoid fever. Plants like *Cassia occidentalis* are very popular for the treatment of typhoid fever. However scientific investigation could not establish its *in vitro* activity against the causative organism of typhoid fever (Faruq et al., 2006). Therefore, other plants commonly used for the treatment of typhoid fever were collected and

screened to verify their efficacy. Many drugs commonly in use today are of herbal origin. Higher plants as source of medicinal compound have been playing a dominant role in the maintenance of human health since the antiquities (Suffnes and Dowos, 1982). Medicinal plants are the best source for obtaining a variety of newer herbal drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy (Doss and Rangasamydhanabalan, 2008).

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The stem bark of *Ficus sycomorus* is used traditionally to treat fungal diseases, jaundice and dysentery (Hassan et al., 2007). *Ficus glumosa* can be used to prepare numerous traditional medicines. In Cote d'Ivoire, Central African Republic and Zimbabwe, the latex is applied to alleviate pain from sprains, treat diarrhoea and sore eyes. In Central Africa, Senegal (Casamanca), East Africa and Tanzania, the bark is used as mouth wash against toothache; it is used to prevent conjunctivitis, stomach disorders and wash sores daily until recovery. In Senegal and Cote d'Ivoire, the roots and fruits are used to cure female sterility (Jansen, 2005). The gums of *F. glumosa* also have pharmaceutical and industrial applications as demulcents, adhesives in pill manufacturing and lithography, paints, ink, corrosion inhibitors and as emulsifying agents (Ameh, 2013). On the other hand, the methanolic extract of the stem bark of *F. glumosa* has demonstrated *in vivo* anti-diabetic and *in vitro* antioxidant activities (Nana et al., 2012). Additionally, the methanolic leaf extract of *F. glumosa* significantly lowers blood glucose level and has anti-lipidemic and hepatotoxic effects (Zayyanu, 2010). *F. glumosa* leaves and stem bark are used for treating skin disease (Olaokun et al., 2013) and also the methanol leaf extract exhibits pharmacological activity against diarrhoea (Tanko et al., 2012). The stem bark of *F. glumosa* is particularly used for treating typhoid fever among traditional healers in Sokoto, Nigeria (Discussion). The bark is used either as cold or hot water decoction.

This study was designed to evaluate the antimicrobial efficacy of *F. glumosa* and to determine the active component in the plant extract.

MATERIALS AND METHODS

Collection and treatment of sample

Dried stem bark of *F. glumosa* was obtained from the Traditional Medicine Department of Usmanu Danfodiyo University, Sokoto. The sample stem bark was pulverized into moderately coarse powder and subjected to aqueous extractions.

Test organism

The bacterial organisms used were *Salmonella paratyphi* and *Salmonella typhi*. They were all isolates obtained from Microbiology Department of Usmanu Danfodiyo University, Sokoto, Nigeria and cultured in appropriate media.

Extraction of plant material

The extraction was carried out by soaking 50 g of the powder in 250 cm³ of methanol and distilled water for 24 h at room temperature. The extract was decanted and concentrated by evaporating on water bath at 100°C for 12 h to obtain crude

extract.

Initial anti-salmonella screening of crude extract

The antimicrobial activity of the extracts was tested on the test isolate, using the agar well diffusion inhibition test. The organism was aseptically introduced and evenly spread on the solidified media in a Petri dish as described by Opara and Ansa (1993). Four wells were made in each solidified agar plate with a sterile 12 mm diameter cork borer allowing at least 30 mm between adjacent wells and between peripheral wells and the edge of the Petri dish. On each of the four wells created in the nutrient agar plate, the mixture of the plain agar and extracts were aseptically filled. Tetracycline of 3.30 mg/cm³ was used as a positive control. The plates were left on the work bench for 20 min to allow the extract to solidify. The plate was then inverted and incubated at 37°C for 24 h. Zones of inhibition around the wells indicated antibacterial activity against the bacteria. The diameter of these zones was measured by the use of transparent plastic ruler and then recorded.

Chromatographic separation of crude extract

A piece of glass wool was inserted at the bottom of a glass column, and then 40 g silica gel powder (80 to 120 µm mesh) was packed into the column; and glass wool was inserted just above the silica gel surface. The column was washed with 35 cm³ distilled water. The sample (5 cm³ of water extract) was transferred onto the column and sequentially eluted with 35 cm³ of distilled water, water/methanol mixture (1/1 v x v) and 100% ethanol. Flow rate was observed and fractions were collected at 10 cm³/fraction.

Antisalmonella screening of the chromatographic fraction

The chromatographic fractions were subjected to anti-*Salmonella* activity as described above.

Phytochemical screening of the active fraction

Each of the active fractions was subjected to phytochemical analysis as described by Trease and Evan (1989), Oloyede (2005) and Sumitra et al. (2006).

Gas Chromatography Mass Spectrography (GC/MS) analysis of the active fraction

The active chromatographic fraction was subjected to GC-MS analysis using SHIMADZU QP2010 GC-MS.

RESULTS AND DISCUSSION

Crude water extract of the bark of the plant was subjected to antibacterial activity test on *Salmonella typhi* and *Salmonella paratyphi* pathogens. The result of that test (Table 1) revealed that the extract is active on both *Salmonella* species; it was even more active on *S. typhi* than *S. paratyphi* pathogens. Kitzberger et al. (2006) reported that the antibacterial activity can be considerable when the diameter of

Table 1. Result of the anti- *Samonella* activity of crude water extracts.

Test organisms	Diameter of inhibition zones (mm)			
	Methanol extract		Water extract	
	30 mg/cm ³	60 mg/cm ³	30 mg/cm ³	60 mg/cm ³
<i>Salmonella paratyphi</i>	0.0	0.0	0.0	15.3
<i>Samonella typhi</i>	0.0	0.0	0.0	16.5

Diameter of cork borer used= 12 mm.

inhibition zone observed is 9 mm or more around the paper disc. Our findings of 15.3 and 16.5 mm are consistent with the above findings. However, activity was only observed in the water extract, which indicates that the active compound was extractible by water as against methanol (Figure 1). Therefore, subsequent investigations were narrowed down to the water extract only.

In the subsequent work, two water extracts were employed, (that is, hot and cold water extracts). The essence was to identify the best extraction method of the active compound. In a similar research, Indranil et al. (2006) and Gideon et al. (2012) indicated a promising potential for hot water extract compared to cold water extract. Onuh et al. (2008) also prove this in their findings for *Jatropha curcus* leaves in the management of plant diseases. On the contrary, the methanol extract showed no activity. It could be the nature of biological active components whose activity cannot be enhanced in the presence of methanol. Although, Talko et al. (2012) reported that methanol extract of *F. glumosa* exhibited a significant anti-diarrheal activity. In addition, the stronger extraction capacity of water could have produced greater number of active constituents responsible for antimicrobial activity. Each of the two water extracts was subjected to column chromatographic separations, where nine fractions were obtained for each (Figures 2 and 3). During the chromatographic separation, there were no clearly separated coloured bands; therefore, the fractions were collected in aliquots of 10 cm³ each. Each of the chromatographic fractions was subjected to anti-bacterial activity tests against the *S. typhi*. The result (Tables 2 and 3) revealed that the third fraction of hot water extract (H3) as the most active has relatively high antibacterial activity. This indicates that the infusion will be more effective in infectious diseases than the cold water decoction. The result of the phytochemical screening of the active chromatographic fractions (Table 4) revealed the presence of phenolic compound(s), saponins and steroids. Tanko et al., (2012), in their study, reported the presence of flavonoids, tannins, saponins, cardiac glycosides and triterpenes in their phytochemical analysis of *F. Glumosa*, which is

similar to our study in terms of phenol (tannins) and saponins. Phenolic compounds were found to have antioxidant and antibacterial effect (Ouattara et al., 2011). Some mechanisms of antimicrobial activity of phenolic compounds include their ability to denature microbial proteins as surface active agents.

In order to ascertain the identity of the active fraction, it was subjected to GC/MS analysis. The GC chromatogram gave eleven peaks (Table 5 and Figure 4). Each of the eleven compounds represented by the peaks was fragmented by the MS and the mass spectra compared with those in the e-library (NIST library) to come up with compound matching. The matching revealed that two of the compounds (RT; 47.65, and 55.99) were esters of phthalic acid, three of the fractions (RT; 52.167, 51.77 and 51.37) correspond to 3,3-(4-methyl-1,3-phenylene)bis(1-heptylurea), one of the fractions (RT; 53.62) was isothujol, still another one (RT; 50.32) was Terbutyl azine and the rest were fatty acid derivatives and hydrocarbons (Figure 4). The phthalic acids derivatives are not plant based compounds but are rather components of plastic materials; therefore, they must have found their way into the sample through dissolution of some plastic materials used in the process by organic solvents. Fatty acids and hydrocarbons are not known to possess pharmacological activities. Therefore, the observed antibacterial activity must have been from either of the remaining three identified compounds (mentioned above) or combination of two or more of them. However, this work did go further to isolate each of these compounds and study them individually. That will be reported in the follow-up work.

Conclusion

The study has shown that the observed anti-*Salmonella* effects of *F. glumosa* stem bark, though *in vitro*, appear interesting and promising. This work has successfully narrowed down the observed effect to three compounds but falls short of isolating them individually for further studies.

Conflict of Interest

The authors have not declared any conflict of interest.

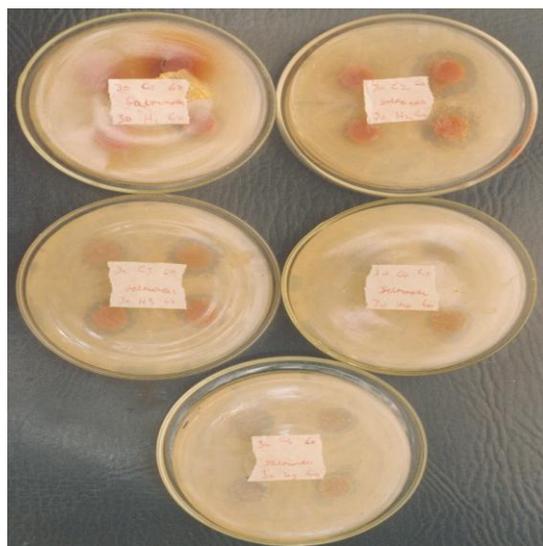
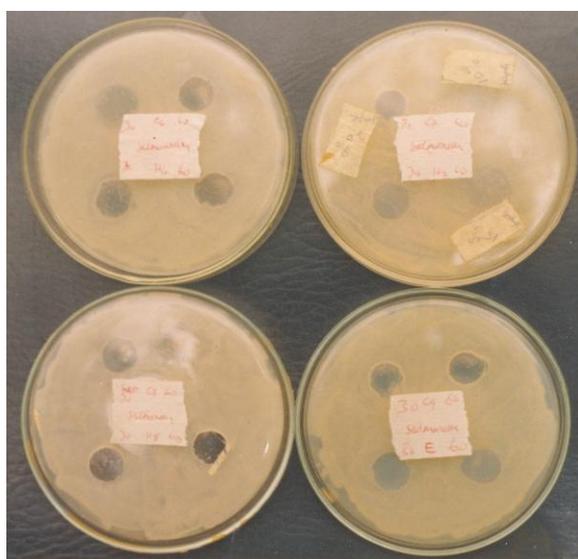


Figure 1. Anti-bacterial activities of methanol and Water extract of *F. glumosa* stem back extract.

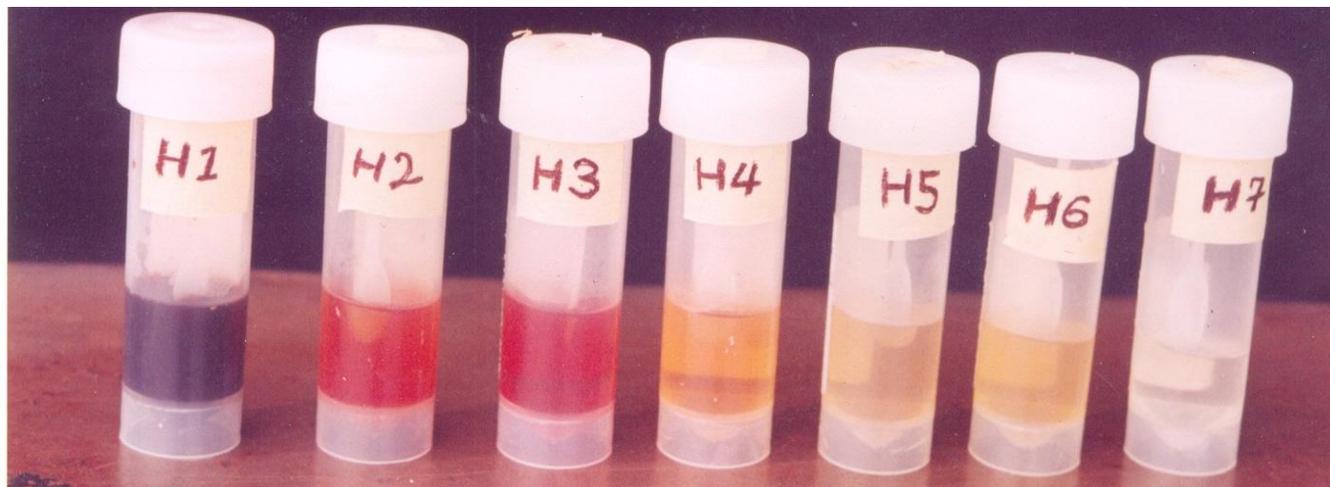


Figure 2. Column chromatographic fractions of boiled water extract of *F. glumosa*.

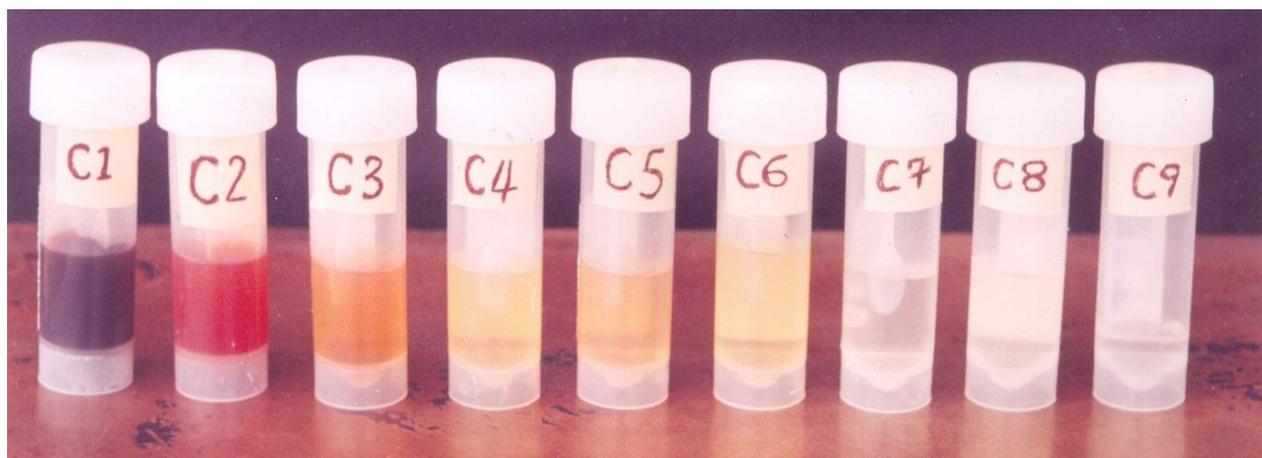


Figure 3. Column chromatographic fractions of cold water extract of *F. glumosa*.

Table 2. Result of antibacterial activity test on the column chromatographic fractions of cold water extract against *S. typhi*.

Concentration in mg/cm ³	C1	C2	C3	C4	C5	C6	C7	C8	C9
30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
60	0.0	16.0	17.5	0.0	0.0	0.0	0.0	0.0	0.0

C- Means cold water extract.

Table 3. Result of Antibacterial Activity Tests on the Chromatographic Fractions of Hot Water Extract against *Salmonella typhi*.

Concentration in mg/cm ³	H1	H2	H3	H4	H5	H6	H7	H8	H9
30	0.0	0.0	18.0	0.0	0.0	0.0	0.0	0.0	0.0
60	16.5	17.5	19.0	0.0	0.0	0.0	0.0	0.0	0.0

H- Hot water extract.

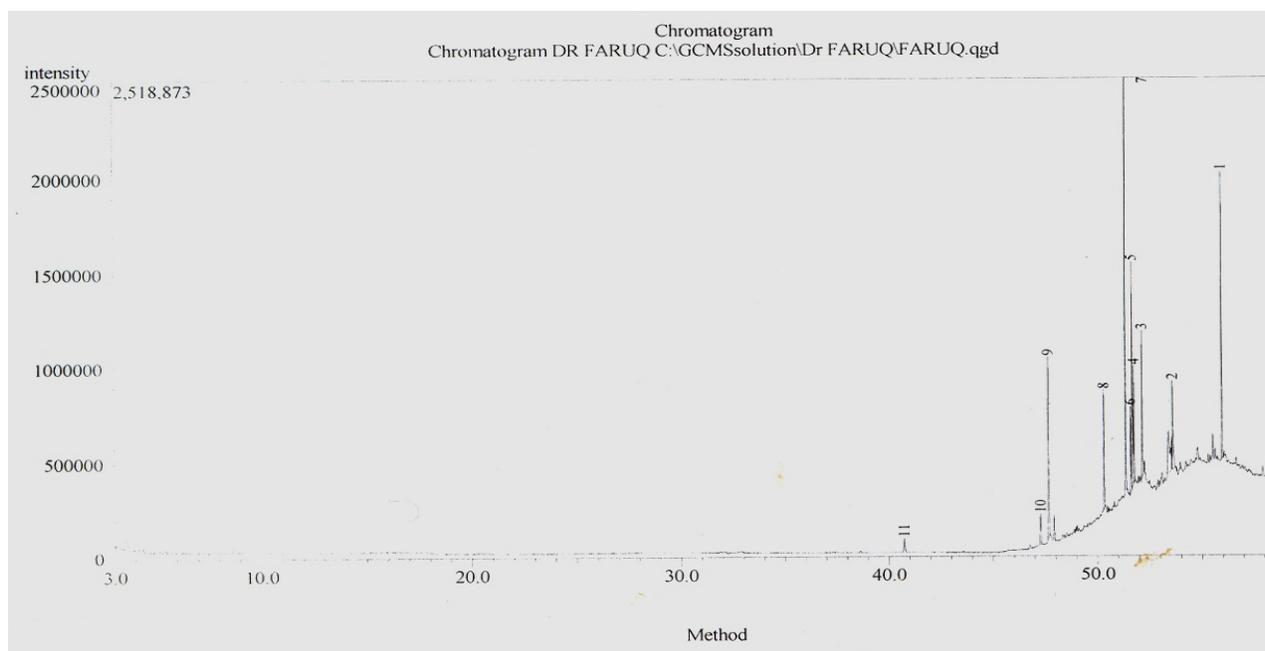
Table 4. Result of phytochemical analysis on the active chromatographic fraction.

Components	H3
Phenolics	+
Saponins	+
Alkaloids	?
Cardiac glycosides	?
Steroids	+
Flavonoids	?
Anthraquinone glycosides	?

Key: + Means present, ? Means absent.

Table 5. GC/MS result of the most active fractions.

GC/MS peak number	Retention time (Indexes) (Min)	Base peak	Molecular ion peak	Identified compounds
1	56.0	57	354	Esters of phthalic acid
2	53.6	93	354	Isothujol
3	52.2	148	354	3,3-(4-methyl-1,3-phenylene)bis(1-heptylurea)
4	51.8	148	280	3,3-(4-methyl-1,3-phenylene)bis(1-heptylurea)
5	51.7	81	280	
6	51.6	81	280	
7	51.4	148	342	3,3-(4-methyl-1,3-phenylene)bis(1-heptylurea)
8	50.3	199	326	Terbutyl azine
9	47.7	148	374	Esters of phthalic acid
10	47.3	79	300	
11	40.7	93	300	

**Figure 4.** GCMS result.

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REFERENCES

- Ameh PO (2013). Physicochemical properties and rheological behaviour of *Ficus glumosa* gum in aqueous solution. *Afr. J. Pure Appl. Chem.* 7(1):35-43.
- Doss AR (2008). Preliminary phytochemical screening and antibacterial studies of leaf extracts of *Solanum trilobatum* Linn. *Ethnobotanical leaflets* 6(12):42.
- Gideon IO, Williams OT, Perpetua UN, Bright EI (2012). Antimicrobial and phytochemical evaluation of the leaf, stem bark and root extracts of *Cyathulaprostrata* (L) *blume* against some human pathogens. *J. Intercult. Ethnopharmacol.* 1(1):35-43.
- Faruq UZ, Hassan LG, Manga BS, Uba A, Olatunji MK, Tal MT, Isiak FS (2006). Anti-bacterial activity of *Cassia occidentalis* on *Salmonella typhi*. Proceedings of the 28th Annual International Conference Chemicals Society of Nigeria. pp. 177-181.
- Hassan SW, Lawal M, Muhammad BY, Umar RA, Bilbis LS, Faruq UZ, Ebbo AA (2007). Antifungal activity and phytochemical analysis of column chromatographic fractions of stem bark extracts of *Ficus sycomonus* L. (*Moraceae*). *J. Plant Sci.* ISSN 1816-4951. Academic Journals Inc U SA.
- Indranil B, Soroj KC, Soumendrananth C, Goutam C (2006). Antibacterial potentiality of *Argemone maxicana* solvent extracts against some pathogenic bacteria. *Mem. Inst. Oswaldo Cruz* 101(6) Riode Janeiro.
- Jansen PCM (2005). *Ficus glumosa* Delile. In: Jansen PCM, Cardon, D (Eds.), *PROTA 3: Dyes and tannins/Colorants et tanins*. PROTA, Wageningen, Netherlands.
- Kitzberger CSG, Smania A, Pedrosa RC, Ferreira SRS (2006). Antioxidant and antimicrobial activities of shiitake (*Lentinula edoles*) extracts obtained by organic solvents and supercritical fluids. *J. Food Eng.* 80:631-638.
- Nana F, Louis PS, Keumedjio F, Pantaléon A, Rizwana M, Victor K, Vincent R, Muhammad IC, Bonaventure TN (2012). Ceramides and cytotoxic constituents from *Ficus glumosa* Del. (*Moraceae*). *J. Brazilian Chem. Soc.* 23(3):482-487.
- Oloyede OI (2005). Chemical profile of unripe pulp of *Carica papaya*, pak. *J. Nurtr.* 4:379-381.
- Onuh MO, Ohazurike NC, Emeribe EO (2008). Efficacy of *Jatropha curcus* leaf extract in the control of brown blotch disease of cowpea (*Vigna unguiculata*). *Biological Agriculture and Horticulture: An Int. J. Sustain. Prod. Syst.* 25(3):201-207.
- Opara AA, Ansa MA (1993). The antibacterial activity of tea and coffee on selected organism. *J. Med. Lad. Sci.* 3:45-48.
- Olaokun OO, Lyndy JM, Jacobus NE, Vinny N (2013). Evaluation of the inhibition of carbohydrate hydrolysing enzymes, antioxidant activity and polyphenolic content of extracts of ten African *Ficus* species (*Moraceae*) used traditionally to treat diabetes. *BMC Complementary and Alternative medicine*, 13:94.
- Ouattara L, Koudou J, Zongo C, Barro N, Savadogo A, Bassole IHN, Ouattara AS, Alfred ST (2011). Antioxidant and Antibacterial Activities of Three Species of *Lannea* from Burkina Faso. *J. Appl. Sci.* 11:157-162.
- Suffnes M, Dowos J (1982). Current stating of the NCI plant and animal product programme. *J. Natural Prod.* 45:1-14.
- Sumitra CP, Jigna, Nehal K (2006). Evaluation of Antibacterial Activity and phytochemical Analysis of *Bauhinia variegata* L. bark. *Afr. J. Biomed. Res.* 9:53-56.
- Tanko Y, Alladey O, Ahmed MK, Mohammed A, Musa KY (2012). The effect of methanol leaves extract of *Ficus glumosa* on gastrointestinal motility and on castor oil induced diarrhea in laboratory animals. *J. Nat. Prod. Plant* 2(3):360-367.
- Trease C, Evan CW (1989). *A text book of pharmacology*. Thirteenth edition Brilliens, Tindall limited London.40-58:224-323.
- Zayyanu UU (2010). The effect of ethanolic leaves extract of *Ficus glumosa* on blood glucose levels and some physiological parameters in diabetic rats (Unpublished). A thesis submitted to the post graduate school, Ahmadu Bello University Zaria Nigeria.