.

International Journal of Biochemistry Research & Review
1(1): 24-30, 2011

SCIENCEDOMAIN *international*
www.sciencedomain.org

Studies on Anti Typhoid Properties of Aqueous Methanol Leaves Extract of *Albizia ferruginea* (Musase)

S. Sarkiyayi[1*], J. Karago[2] and R. Hassan[3]

[1]Biochemistry/Chemistry Section, Department of Applied Science, Kaduna Polytechnic, Kaduna, Nigeria.
[2]Biology/Microbiology Section, Department of Applied Science, Kaduna Polytechnic, Kaduna, Nigeria.
[3]44 Reference Hospital, Kaduna, Nigeria.

Research Article

Received 6th January 2011
Accepted 10th January 2011
Online Ready 8th April 2011

ABSTRACT

Anti typhoid properties of aqueous methanol leaves extract of *Albizia ferruginea* (musase) was investigated in the present study. The phytochemical screening of the aqueous methanol leave extract of *Albizia ferruginea* revealed the presence of alkaloid, anthraquinines, carbohydrates, cardiac glycosides, flavonoid, saponin, tannin and terpens. The leave extract of *Albizia ferruginea* does not possess acute toxicity effect on animal (mice) with a dose of (LD_{50}) 5000mg/kg. The plant extract produced inhibitory activities against *Salmonella typhi* with a minimum inhibitory concentration of 1000µg/ml. The plant extract is effective as anti typhoid agent against *Salmonella typhi* on mice infected with typhoid parasites as shown by widal test.

Keywords: Albizia ferruginea, Salmonella typhi, plant, Anti typhoid;

* *Corresponding author: Email: sarkiyayi_shehu@yahoo.com*

1. INTRODUCTION

Typhoid fever is an infectious disease caused by bacterial of *Salmonella group-salmonella typhi* and S*almonella paratyphi* A, B or C. the organisms are gram-negative, flagellated, non encapsulated, non-sporulating and facultative anaerobic bacillus. The strain differs from other *salmonella,* it has only one phase antigen and process of capsular antigen called V_1 (Wanoff et al., 1997). *Salmonella typhi* causes typhoid (enteric) fever, the bacteria pass from the small intestine into blood through the lymphatic system. The reticular endothelial system become infected as well as the gall bladder and kidneys. From the gall bladder, the organisms invade the intestine causing inflammation and ulceration (Cook, 1995). According to Wanoff et al., (1997) symptoms of infection includes fever with low pulse rate, headache, toxemia, enlargement of the spleen and partly or mental confusion. A rash (rose sport) may be seen on light coloured skin. Epistasis, intestinal haemorrhage and perforation may also occur. In uncompleted (asymptomatic) typhoid, the total white cell count is low with a relative lymphocytosis and there may also be anaemia. *Salmonella typhi* also causes neutrotyphoid in those with urinary schistomiasis. The condition is an immune complex disorder of the kidney and is characterized by fever oedema, marked albuminuria and haematuria. It also causes osteomyetitis (inflammation of the bone marrow) especially in children with sickle cell disease and thalassaemia typhoid nodules can be found in the bone marrow. Inflamation of the joints (typhoid arthritis) may also occur. It causes abscesses of the spleen, meningitis and rarely pneumonia and endocarditis (Hornick,, et al., 1999). *Salmonella paratyphi* A and B causes paratyphoid (enteric) fever. The disease is generally mild with *Salmonella paratyphi* A and B being less invasive than *Salmonella typhi*. These are usually characterized with diarrhea and especially in S*almonella paratyphi B* infection. In tropical and other developing countries paratyphoid is more commonly caused by *Salmonella paratyphi* A than *Salmonella paratyphi* B (WHO, 2000). Before the early 20th century, typhoid fever was a common disease that occurred in large epidemics everywhere. In countries where modern method of sanitation and sewage disposal have been instituted, epidemics no longer occur and the disease is only rarely encountered, but in parts of the world lacking good sanitary facilities, it continues to represent a serious health problem. The incidence of typhoid fever perforation is high in West Africa in contrast to other countries (Butter et al., 2000). Typhoid fever presents one of the classical examples of water born infection. It has a worldwide distribution although, it is endemic only in communities where the standard of sanitation and personnel hygiene are low (Week et al., 2003). All ages and both sexes are susceptible through contamination of water, the cause of major out breaks can occur through cross-connection of a man with polluted water supply, faecal contamination of wells or poultry purification.

Microbial infections pose a health problem throughout the world, and plants are possible source of antimicrobial agents (Burapadaja and Bunchoo, 1995). Adenisa et al. (2000) reported that medicinal plants contain active principles which can be used as an alternative for cheap and effective herbal drugs against common bacterial infections. The plants are used by the local herbalists for treatment of a number of diseases, both bacterial and non bacterial type, there is need therefore to assess the antimicrobial activities of these plants, scientific proof and clinical validation of herbal formulations can be achieved by various methods: chemical standardization, biological assays, animal models and clinical trials. Wasswa et al., 2006) reported that activities have been used for validation of plant extracts. However, validation should go hand in hand with regulation and evaluation of herbal treatments to avoid the administration of dangerous concoctions.

Albizia ferniginen (locally known as musase) is a species of plant in the Fabaceae family. It is found in Angola, Benin, Cameroon, central Africa Republic, the Republic of the Congo, the Democratic Republic of the congo, Ivory Coast, Gabon, Gambia, Ghana, Guinea, Guinea-Bissan, Nigeria, Senegal, Sierraleone, Togo and Uganda. It is threatened by deforestation. *Albizia ferruginea* has been found to be very efficient in the treatment of malaria, stomach pain,

dysentery, skin infection (Kareru et al., 2007). In view of this, this research work was therefore aimed at extracting the active ingredients which are present in *Albizia ferruginea* and to test the efficacy of the leaves extract on mice infected with typhoid fever.

2. MATERIALS AND METHODS

2.1 COLLECTION OF SAMPLES

Fresh samples of *Albizia ferruginea* leaves were collected from Kaduna polytechnic main campus Kaduna. The Albino mice animals were obtained and bred in the animal house of the Department of pharmacology and clinical pharmacy Ahmadu, Bello University Zaria, were used in this study. They were kept under standard conditions of temperature, relative humidity and fed on normal animal feeds.

2.2 PROCESSING AND EXTRACTION OF PLANT MATERIAL

The plant leaves sample obtained, was dried for 14 days under a mild atmospheric condition so as to avoid possible reaction under sunlight. After drying, the leaves were grounded into powder using a mortar and a pestle to powdered then sieved and weighed.

The solvent used was aqueous methanol 60/40 v/v. Briefly 250cm^3 of aqueous methanol was measured and poured into the flask. Then 30g of the *Albizia ferruginea* powdered leaves was accurately weighed and wrapped in cotton cloth which was then placed into the thimble. The thimble was placed into the inner part of the soxhlet extractor . The extraction process was carried out for a period of eight hours to ensure that most of the organic substances were extracted. After the extraction, the *Albizia ferruginea* leaves extract was dried and weighted. The aqueous methanol extract was phytochemically screened using standard procedures described by Sofowara (2003) to test for the presence of Alkaloid, flavonoids, tannins, saponins, carbohydrates, cardianc glycosides, anthraquinones, steroid and terpenoid.

2.3 ACUTE TOXICITY TEST

2.3.1 FIRST STAGE OF LORKE

A pilot study was carried out on the possible toxic effects of aqueous methanol leave extract of *Albizia ferruginea* on mice by method described by Lorke (1983). Briefly,the maximum dose that did not produce death in mice and the minimal dose that was lethal to the exprimentals. This gave us clue on the quantity of the plant extract administered for determination of $LD_{50.}$ Finally ,the LD_{50} was carried out in two stages (Lorke, 1983).

Three groups I, II and III of albino-mice for each of extract used weighed separately and placed cages. The groups were given 10mg/kg, 100mg/kg and 1000mg/kg of doses of the extract, respectively. The mice were observed for 24 hours and all symptoms of intoxication and number of death were recorded.

2.3.2 SECOND STAGE OF LORKE

Base on the result obtained from the first stage of pilot studies, specific doses of the extracts were chosen and further experiments were carried out. Briefly, three groups I, II and III of four mice each were weighed and placed in separate cages; the mice were given the extracts at the dose of 5000mg/kg, and 1900mg/kg respectively for the aqueous methanol extract. They were then observed closely for 24 hours for signs and symptoms of intoxication and death.

2.4 TEST FOR THE EFFECT OF PLANT EXTRACT ON *SALMONELLA*

2.4.1 TEST PROCEDURE

A confirmed pure culture of *Samonella typhi* isolate was sub- cultured on a differential media (S.S.A). It was then allowed to grow for 24-48 hours in incubator and stored in the refrigerator at 4°C.

Twelve mice were distributed into 3 groups with four mice in each group. These were kept in separate apartments. The mice in each group I, II and III were injected intravenously with typhoid fever bacterial causative organism *(Salmonella typhi).* After which a widal test was carried out on them to confirm if they were infected with typhoid fever.

Group I and II were however placed on oral treatment with 375mg/kg and 750mg/kg of the extract for 3days, while group III was left out with no treatment in order to serve as control. Their respective behavioral changes (movement, activeness) was noted and recorded. The blood of the surviving specimen after 3days were obtained from the tail using capillary tube and a widal test was carried out on the blood serum.

2.4.2 WIDAL TEST

Two drops (0.08ml) of serum to be tested was placed on a white tile. The antigen suspension was shaken and 1 drop of the antigen sample was added. It was then mixed over an area of 3cm, rocked gently and examined for agglutination after 1 minute.

2.5 DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION

2.5.1 PREPARATION OF THE INOCULA

In preparation of the inocula, the previously prepared overnight nutrient broth cultures of the bacterial isolates were used by diluting with sterile saline solution.

The sterile saline solution was prepared by dissolving 4.25g sodium chloride in 500ml distilled water to give a stock solution. Then,10ml of the solution was separately transferred into four glass vials using 10ml syringe. The preparations were autoclaved at 21^0C for 15 minutes subsequently, 10µl (0.01ml) of each nutrient broth culture suspension was placed into 10ml sterile saline in the glass vials. These served as the inocula.

2.5.2 TEST PROCEDURE

The tube dilution technique was used as described by David (1989). The minimum inhibitory concentration of an extract that is able to completely prevent the growth of the test organism (David, 1989) in the following concentrations: 1000µg/ml and 100µg/ml were prepared from the extract using stock solutions.

To 9ml of nutrient broth inoculated with sensitive bacteria (test organism), 1ml of the prepared concentration of the plant extract was added to make up to 10ml. The test tube was plugged with sterile cotton wool and incubated at 37^0C for 18hours. This method was carried out at all the concentrations (1000µg/ml and 100µg/ml) for each plant extract using the sensitive bacteria test.

3. RESULTS AND DISCUSSION

3.1 THE PHYTOCHEMICAL SCREENING RESULTS

The phytochemical screening of the aqueous methanol leave extract of *Albizia ferruginea* indicated the presence of Alkaloid, anthraquinines, carbohydrates, cardiac glycosides, flavonoid, saponin, tannin and terpens. These were found to exhibit some pharmacological importance in the treatment of many aliments such as anti diarrhea drugs (Foster, 1990) for raising low blood pressure, stop hemorrhage and as stimulants. In many instances these may be regarded as indispensable, the main constituent of some alkaloids is guanine which has been known through research as an effective anti malaria drug (Isaac et al., 2002).

3.2 ACUTE TOXICITY (LETHAL DOSE) OF AQUEOUS METHANOL EXTRACTS OF *Albizia ferruginea*

The leave extract of *Albizia ferruginea* does not possess acute toxicity effect on animal (mice) with a dose of (LD_{50}) 5000mg/kg, this means that the plant was not toxic with large dose of 5000mg/kg over a short period of time, suggesting that the plant extract is relatively safe for oral medication.

3.3 INHIBITORY EFFECT OF PLANT EXTRACT ON *Salmonella typhi*

Group I and II were however given oral treatment with 375mg/kg and 750mg/kg of the extract for 3days, while group III was left out with no treatment in order to serve as control. The results in table 1 of the widal test showed that oral treatment with 375mg/kg and 750mg/kg of the extract for 3days was effective against *Salmonella typhi* growth in groups I and II with H (antibody titre value) and O (antibody titre value) less than 1/40 which was not significant. While group III has high values for H and O due to the presence of *Salmonella typhi* growth in the body. The plant extract can be used as an effective anti typhoid agent on humans infected with typhoid fever.

Table 1. Results for Widal test

Groups	Three days inhibitory effect of plant extract on *salmonella typhi* growth		
	Day I	Day II	Day III
Group I (375mg/kg)	H= $\frac{1}{40}$,O= $\frac{1}{40}$	H= $\frac{1}{20}$, O = $\frac{1}{40}$	H= $\frac{1}{20}$, O= $\frac{1}{20}$
Group II (750mg/kg)	H= $\frac{1}{40}$,O= $\frac{1}{20}$	H= $\frac{1}{40}$,O= $\frac{1}{40}$	H= $\frac{1}{20}$, O = $\frac{1}{40}$
Group III (no extract given)	H= $\frac{1}{160}$,O= $\frac{1}{160}$	Mice died	Mice died

Titre of 1/180 and above are significant; The O and H are alphabets used to represent the Salmonella antigens; O or (somatic) antigen located on the external wall of the organism antibody titre value; H or (flagella) antigen located on the flagella of the organism antibody titre value

3.4 MINIMUM INHIBITORY CONCENTRATION (MIC)

The minimum inhibitory concentration of the aqueous methanol leave extract of *Albizia ferruginea* was tested against *Salmonella typhi* was 1000µg/ml.
The plant extracts produced inhibitory activities against *Salmonella typhi* with a minimum inhibitory concentration of 1000µg/ml. It is also administered orally on mice infected with typhoid fever and served as a support in the therapy of the typhoid fever. Although sluggish behavior, lost in weight and death was observed in the mice in group I, II and III but much more death was observed in group III. The disease symptoms were observed in all the groups and latter disappeared in group I and II mice. This observation was due to the effect of the plant extract on the mice in group I and II after continued treatment for three days. This suggests that the plant extract has some anti typhoid activities. Similar studies were carried out by Doughari et al. (2007) on extract of *Balanites aegyptiaca* and demonstrated higher activity of 16 mm zone of inhibition at 100 mg/ml against *Salmonella typhi.*

4. CONCLUSION

From the result obtained, the aqueous methanol plant extract was effective as anti typhoid agent against *salmonella typh* The demonstration of anti typhoid activity of *Albizia ferruginea* is indeed a promising development that will help to discover new chemical classes of antibiotics that could serve for treating the infection that otherwise has become highly refractive to most of the conventional antibiotics used for its treatment

5. RECOMMENDATION

Further studies should be carried out on the plant extract and the in vivo studies would also required to isolate the bioactive constituents present in *Albizia ferruginea* for the purpose of developing anti typhoid drug in orthodox medicine.

ACKNOWLEDGMENTS

This is to acknowledge the technical assistance provided by Aminu Ado and Amblabo E..

REFERENCES

Aliuri *et al.,* V.K., S., Taji, V.N.R., (2005). Assessment of Bioactive of Indian Plants using Brine Shrinp (Artemia Sauna) Lehtality Assay: Int. J. Appl. Sci., 2(3), 125 - 134.

Cook, G.C., (1998). Communicable and tropical diseases, mainstream medicine Heinmann Medical Book, London.

Doughari, J.H., Pukuma, M.S., De, N. (2007) Antibacterial effects of Balanites a. and Moringa o. on Salmonella typhi. Afr. J. Biotechnol., 6, 18.

Isaac et al. (2002). Screening of Plants from Biological Active Substance in Africa Medical Plant. Pp 119-127.

Ivanoff, B.N., Levinc, M.M. (1997). Typhoid Fever Contineuing Challenge from a Resilient foe. Bull Lust. Pasteur., 95, 129-142.

Kareru, P.G., Kenji, G.M., Gachanja, A.N., Keriko, J.M., Mungai, G. (2007). Traditional Medicines among the Embu and Mbeere peoples of Kenya Africa. 4(1), 75-86.

Moses, N.N., James, A., M., Pierre, T., Vincent, P.K.T., (2006). Antibacterial Effects of some Cameroonian Medicinal Plant Against Common Pathogenic Bacteria. 3(2), 84 - 93.

Sofowara, A.E. (2003). Phytochemical Screening of Nigerian Medicinal Plants Part 11. Lyodia, 44, 234 - 246.

Wasswa et al. (2006). In-vitro Ascaridal Activity of Selected Indigenous Medicinal Plants Used in Ethno veterinary Practises in Uganda. Afr. J., 3(2), 94 - 108.

WHO. (2003). Manual for the Laboratory Identification and Antimicrobial Susceptibility Testing of Bacterial Pathogens of Public Health Importance in Developing World. Pp. 103 - 162.
