



Anti-Diarrhoeic and antibacterial Effects of Aqueous Pod Extract of *Acacia nilotica* in Albino Rats

UMARU^{1*}, B., ONYIYILI, P.A¹ AND SAKA². S.

¹Department of Veterinary Biochemistry, Physiology and Pharmacology, University of Maiduguri, P.M.B 1069, Maiduguri, Nigeria. ² School of Health Sciences, Department of Pharmacy, Laboratory of Molecular Pharmacology, University Campus, Rio. Patra 265 04, Greece

* Correspondence : bukamar@yahoo.co.uk, +306998208873

SUMMARY

The anti-diarrhoea effect of aqueous pod extract of *Acacia nilotica* was investigated in rats. The pod extract of *A. nilotica* was obtained by Soxhlet extraction using distilled water as a solvent and subjected to phytochemical screening. The result of phytochemical screening revealed the presence of saponins, tannins, alkaloids, cardiac glycosides, flavonoids, and reducing sugars. The elemental analysis of the extract revealed high concentration of sodium followed by magnesium, iron, cadmium, zinc, manganese and potassium. The aqueous extract significantly ($P < 0.05$) reduced the number of unformed faeces in castor oil induced diarrhoea in rats. It also significantly ($P < 0.05$) reduced the gastrointestinal transit of activated charcoal. Enteropooling in the experimental rats was significantly ($P < 0.05$) reduced.

The in vitro antibacterial and fungal study showed that the extract inhibited the growth of *Staphylococcus aureus*, *Proteus vulgaris*, *Shigella dysentery*, *E. coli*, *Streptococcus pyogenes*, *Pseudomonas aerogenosa*, *Salmonella typhi*, and *Bacillus albus*, as well as *Candida albicans*. The aqueous pod extract was therefore found to possess active anti-diarrhoeic, antibacterial and mycostatic ingredients.

KEY WORDS: Anti-diarrhoea, *A. nilotica* aqueous pod extract, antibacteria, Mycostatic.

INTRODUCTION

Acacia nilotica belong to member of the Family *Fabaceae*. Its tender branches are used as fodder for goats and sheep; stem bark crude extract is used to treat toothache, while the pods and leaves are useful in management of stomach ailments, skin disorders and diarrhoea (Duke, 1983). *Acacia nilotica* inner bark contain tannin (18 – 23%) used for tanning and dyeing of leather, (National Academy of Science, 1980). Tender pods and shoots are used as vegetables and is fed

to camels, sheep and goats especially in Sudan, where it is used to improve milk yield in these animals. In South Africa, the Zulus use the stem bark extract in treating cough, and the Chipi use the root bark in managing cases of tuberculosis, while the Masai use the stem bark and root decoction, to alleviate mood. In Ayurvedic medicine, the stem bark has been used for management of premature ejaculation (Pande *et al.*, 1981). In north eastern Nigeria herbs from decoction of the pod of *Acacia nilotica* have been used in folk medicine for the control of diarrhoea, but has not been scientifically evaluated for its efficacy and toxicity. Therefore this study was design to investigate its efficacy as anti-diarrhoeic and antibacterial agent.

MATERIALS AND METHODS

Plant Collection, Identification and Extract Preparation

Fresh pods and leaves of *Acacia nilotica* were collected from Lai –Lai grazing reserve, Potiskum Local Government Area of Yobe State and identified by a Plant Taxonomist at the Department of Biology University of Maiduguri. Voucher specimen was deposited at the Department of Veterinary Physiology and Pharmacology herbarium, University of Maiduguri, Nigeria.

The pods were air dried at room temperature for three weeks. Crushing of the pods was done in the laboratory using pestle and mortar, after which it was ground into powder. About two hundred grammes (200 gm) of the powdered pod was weighed and introduced into a conical flask and 1 litre of distilled water was added thereafter. The mixtures was then shaken and allowed to stand for 30 minutes, after which it was boiled for one hour, cooled and shaken vigorously, before filtration using whatman No. 1 filter paper. The filtrate was concentrated in a

rotatory evaporator and stored at 4 °C until used, and the yield was 6.75% (w/w).

EXPERIMENTAL ANIMALS

Wister albino rats of both sexes and weight were used for this experiment. They were kept in plastic cages and allowed a 2-3 week period of acclimatization before the commencement of the experiment. The experimental rats were fed with grower mash and drinking water *ad libitum*.

Phytochemical Screening

The aqueous pod extract was subjected to phytochemical screening for the identification of various classes of chemical compounds such as sterol, triterpenes, alkaloid, flavonoid, carbohydrates, tannins, emodols, anthracenoides, saponin and polyuronides using the method of Harborne (1975), Ioan (1976), Trease and Evans (1989) and Sofowora (1993).

Determination of the Elemental Content

Acacia nilotica pod powder was screened for elemental constituents using the technique described by Bhatia, (2005).

Trace Elemental Content

About 5 gm of air dried sample in an evaporating dish was placed in an oven at 80°C and dried to a constant weight. The sample was placed in a weighed crucible and ashed at 500°C in hotspot furnace for three hours and screened for elemental constituents according to the method of Bhatia (2005).

Effect of the aqueous extract on castor oil induced diarrhoea in rats

Twenty five rats weighing between 120-190 gm were used for this study. The method of Offia and Chikwendu (1999) was used. The rats were deprived of feed 12 hours before the commencement of the experiment, but were allowed free access to water. They were divided into 5 groups of five rats each. Rats in groups A, B, C received 250 mg/kg, 500 mg/kg, and 750 mg/kg dose of aqueous pod extract of *Acacia nilotica* orally respectively. Those in group D received (2 ml) of normal saline orally, while those in group E were given diphenoxylate HCL 5 mg /kg body weight intraperitoneally (I.P). The rats were housed singly in a cage lined with white blotting paper. One hour after extract treatment, the rats were each given 1ml of castor

oil orally. The rats were observed for 6 hours for watery (wet) or unformed faeces. The unformed faeces from each rat were counted at the end of the experiment and a group mean obtained.

Effect of aqueous extract on gastrointestinal transit of activated charcoal

Twenty five rats weighing between 130-198 gm were also used for this experiment. The method of Chitme *et al.* (2004) was used. The animals were deprived of feed 18 hours to the experiment, and thereafter divided into 5 groups of 5 rats each. The rats were allowed free access to water. Group 1 received (2 ml) of normal saline orally and Group 2 received intraperitoneally (I.P) 3 mg/kg of atropine sulphate. Groups 3, 4 and 5 were treated orally with 250 mg/kg, 500 mg/kg and 750 mg/kg doses of aqueous pod extract of *Acacia nilotica* respectively. Ten minutes after drug and extract administration 1 ml of 5% activated charcoal suspension in 10% aqueous solution of Acacia powder was given orally to each rat. The rats were sacrifice 30 minutes later and the abdomen opened. The distance travelled by charcoal meal from pylorus was measured and expressed as percentage of the total length of the intestine from pylorus to the caecum (Mascolo *et al.*, 1999).

Effect of aqueous extract on castor oil induced enterpooling

Twenty five rats weighing between 110-200 gm were used. The intra-luminal fluid accumulated was determined by the method of Robert *et al.* (1976). The rats were fasted overnight and separated into five groups of 5 rats each. Group 1 rats were treated with 3 mg/kg body weight of atropine sulphate (I.P), while Group 2 rats received 2 ml of normal saline orally. Groups 3, 4 and 5 rats were given 250 mg/kg, 500 mg/kg and 750 mg/kg doses of the aqueous pods extract of *Acacia nilotica* orally respectively. After one hour, each rat was treated with 1 ml of castor oil. One hour after the castor oil treatment, the rats were sacrificed and the small intestine removed, tied on both ends with thread and weighed, the intestinal content was collected by milking and the volume measured. The intestine thereafter was re-weighed and the difference between full and empty intestine calculated.

Effect of the aqueous extract on in vitro sensitivity testing

The plates were aerobically incubated overnight at 37°C after which the individual plates were examined for zones of inhibition around the wells alongside the commercial disc preparations. The zone of inhibition present, were quantified by the direct linear measurement of their diameter (Cruickshank, R. 1973; Carter, 1975; Chesbrough, 1975).

Determination of minimum inhibitory concentration (MIC) of the aqueous pod extract.

The method of Greenwood (1989) was used to determine the MIC of the aqueous pod extract of *Acacia nilotica*. Six sterile test tubes were arranged in a test tube rack and 0.5 ml of each sterile nutrient broth pipetted in each tube. Half a millimetre of the crude extract containing 200 mg/ml was pipetted into test tube one to obtain a concentration of 100 mg/ml. Thereafter serial dilution of the extract to obtain concentrations of 50, 25, 12.5, 6.25 and 3.13 mg/ml respectively were done. The test organism 0.5 ml and extract was pipetted into each of the test tube and incubated at 37°C for 24 hours. The MIC was recorded as the least concentration of pod extracts that completely inhibit the growth of the test organisms.

Statistical analysis

All values were expressed as Mean \pm Standard Deviation, while analysis of variance (ANOVA) was used to analyse the extent of variation between groups and P values equal to or less than 0.05 were considered significant (Mead and Curnow, 1982). Graphpad instat 3.0 for windows USA[®] computer software was used to analyse the data.

RESULTS**Extraction**

The aqueous pod extract was light green and have slight bitter taste. The yield was 6.75% (w/w).

Phytochemical Tests

The result of the phytochemical analysis of the aqueous pod extract of *A. nilotica* is shown in (Table I). The result showed that the pod extract contained gallic tannins, flavone aglycone, pentoses, ketoses, reducing sugars, cardiac glycosides, alkaloids, saponins, and emodols.

Saponins and tannins occurred in very high concentrations, while alkaloids, flavone aglycone and reducing sugar occurred in moderate concentration and emodols, cardiac glycosides, pentoses and ketoses were found in low concentrations.

Elemental analysis

Potassium, sodium, zinc, manganese, cadmium, magnesium and iron were detected in the extract (Table I) of *A. nilotica* pod. Sodium had the highest concentration of 36.8ppm. The concentrations of magnesium and iron were 0.85ppm and 0.55ppm respectively.

Table I. Phytochemical composition and Concentration of trace elements in *A. nilotica* pod powder

| CHEMICAL COMPONENT | POD OF A. NILOTICA | ELEMENTS | CONCENTRATION (PPM) |
|---------------------------|--------------------|---------------|---------------------|
| Sterol and triterpenes | — | Calcium (Ca) | 0.00 |
| Emodols | + | Copper(Cu) | 0.00 |
| Saponins | +++ | Lead(Pb) | 0.00 |
| Alkaloid | ++ | Arsenic(As) | 0.00 |
| Cardiac glycosides | + | Potassium(K) | 0.01 |
| Polyuronides | — | Manganese(Mn) | 0.02 |
| Reducing sugar | ++ | Zinc(Zn) | 0.07 |
| Phlabotannins | — | Cadmium(Cd) | 0.10 |
| Cyanogenic glycosides | — | Iron(Fe) | 0.55 |
| Anthracenosides | — | Magnesium(Mg) | 0.85 |
| Pentoses | + | Sodium(Na) | 36.80 |
| Ketoses | + | | |
| Anthraquinone derivatives | — | | |
| Gallic tannins | +++ | | |
| Catechol tannins | — | | |
| Flavone aglycone | ++ | | |

WHO Standard Concentration (WHO, 1996)

(-)=absence

(+)=slightly present

(++)=moderately present

(+++)=copiously present

The effect of aqueous pod extract of *A. nilotica* on castor oil induced diarrhoea

The result of the effect of the extract on castor oil induced diarrhoea is shown (Table II), aqueous extract of the pod of *A. nilotica* (250-750 mg/kg *per os*) and diphenoxylate 3 mg/kg body weight, significantly ($P < 0.05$) protected rats against castor oil induced diarrhoea in dose dependent manner when compared with the control group. The extract and diphenoxylate provided 41.46-97.56 percent protection to the animals against diarrhoea induced by castor oil. The maximal effect of the extract was similar to that of diphenoxylate.

The effect of aqueous pod extract on castor oil induced enteropooling

The result of the effect of *A. nilotica* aqueous pod extract on castor oil induced enteropooling in rats is presented in Table III. There was no significant difference ($P > 0.05$) in fluid accumulated in the intestine of the rats in the control group and the group treated with the extract at 250 mg/kg body weight. Treatments with 500 and 750 mg/kg body weight of extract and atropine (3 mg/kg) significantly ($P < 0.05$) decreased the accumulated fluid when compared with the control group. Atropine was observed to be superior to the extract in decreasing the intestinal fluid contents.

The effect of aqueous pod extract on gastro intestinal transit of activated charcoal.

The distance travelled by the charcoal meal in the control in comparison to the entire length of the small intestine from the pyloric sphincter to the ileocaecum was used as index of gastrointestinal motility in rats. The aqueous pod extract of *A. nilotica* (250-750 mg/kg) given orally significantly ($P < 0.05$) reduced the gastrointestinal distance travelled by the charcoal meal in rats compared with control group (Table IV). The extract produced a dose dependent decrease of gastrointestinal transit in rats. The gastrointestinal transit of charcoal meal produced by atropine (3 mg/kg) was similar to that of 750 mg/kg of *A. nilotica* extract.

Table II. Effect of aqueous pod extract of *Acacia nilotica* on castor oil induced diarrhoea in wistar albino rats

| Treatment (mg/kg) | No. of rats | Mean no. of unformed faeces | Percent protection (%) |
|------------------------------|-------------|-----------------------------|------------------------|
| Control (CO + Normal saline) | 5 | 8.20 ± 4.27 | 0.000 |
| 250mg/kg + CO | 5 | 4.80 ± 2.49* | 41.46 |
| 500mg/kg + CO | 5 | 0.80 ± 1.09 | 90.20 |
| 750mg/kg + CO | 5 | 0.40 ± 0.89* | 95.12 |
| Diphenoxylate(3mg/kg) + CO | 5 | 0.20 ± 0.44* | 97.56 |

CO = Castor oil.

P0.05 means significantly different from the control
b = Mean Standard deviation based on five observations

Table III. Effect of aqueous pod extract of *Acacia nilotica* on castor oil induced enteropooling in wistar albino rats

| Extract treatment (mg/kg) | Wt. of intestine + content (gram) | Wt. of empty intestine(gram) | weight of accumulated fluid (gram) | % of fluid accumulation |
|---------------------------|-----------------------------------|------------------------------|------------------------------------|-------------------------|
| Control | 8.00 ± 1.50 | 4.88 ± 1.00 | 4.06 ± 0.59 | 50.75% |
| 250 | 9.14 ± 2.14 | 4.66 ± 0.76 | 3.48 ± 1.45 | 38.07% |
| 500 | 7.86 ± 1.99 | 3.88 ± 0.55 | 2.48 ± 1.21* | 31.55% |
| 750 | 5.60 ± 1.28 | 3.96 ± 0.64 | 1.64 ± 0.68* | 29.28% |
| Atropine(3mg/kg) | 3.70 ± 0.47 | 3.12 ± 0.30 | 0.58 ± 0.3* | 15.67% |

CO=Castor oil
P0.05 means significantly different from the control
b = Mean Standard deviation based on five observations

Table IV. Effect of the aqueous pod extract of *Acacia nilotica* on mean gastrointestinal transit of activated charcoal in rats

| Treatment | Total length of the intestine (cm) | Total movement of charcoal(cm) | Percentage(%)distance travelled by activated charcoal |
|----------------------------|------------------------------------|--------------------------------|---|
| Distilled H ₂ O | 108.70 ± 16.82 | 80.00 ± 13.6 | 73.59 |
| Atropine SO (3mg/kg) | 106.80 ± 11.24 | 41.86 ± 6.95* | 39.19 |
| Extract 250mg/kg | 127.10 ± 9.90 | 64.20 ± 4.03* | 50.50 |
| Extract 500mg/kg | 113.80 ± 3.05 | 59.0 ± 7.72* | 47.36 |
| Extract 750mg/kg | 117.70 ± 10.76 | 49.16 ± 5.13* | 41.77 |

P0.05 means significantly different from the control
b = Mean Standard deviation based on five observations

Table V. Zone of inhibition (mm) provided by the aqueous pod extract of *A. nilotica* on growth of some micro organisms

| Organisms | Extracts concentration (mg/ml). | | | | Tetracycline 25mg (control) |
|-------------------------|---------------------------------|-----|-----|-----|-----------------------------|
| | 700 | 400 | 200 | 100 | |
| Salmonella typhi (mm) | 12 | 11 | 10 | 8 | 15 |
| Shigella dysentery (mm) | 17 | 17 | 15 | 14 | 30 |
| Escherichia coli (mm) | 10 | 8 | 7 | 6 | 20 |
| P.aerogenosa (mm) | 12 | 11 | 10 | 8 | 13 |
| Staph. aureus (mm) | 18 | 17 | 15 | 12 | 22 |
| Strep. Pyogenus (mm) | 12 | 12 | 11 | 10 | 16 |
| P. vulgaris (mm) | 12 | 11 | 10 | 9 | 20 |
| Bacillus albus (mm) | 17 | 16 | 14 | 12 | 26 |
| C. albican (mm) | 17 | 15 | 14 | 14 | 30 |

KEY:
Disc measurement = 0.6cm
Inoculation Temp. = 37°C
Duration of inoculation = 24hrs
Organisms used = Lab. Isolates
Control Drug = Tetracycline 25mg.

Table VI. Minimum inhibitory concentration of the aqueous pod extract of *A. nilotica*

| Organisms | Extracts concentration (mg/ml). | | | | | |
|---------------------------|------------------------------------|----|----|------|------|------|
| | 100 | 50 | 25 | 12.5 | 6.25 | 3.12 |
| <i>Staph. aureus</i> | S | S | S | R | R | R |
| <i>Strepto. pyogenus</i> | S | S | S | R | R | R |
| <i>Salmonella typhi</i> | S | S | S | R | R | R |
| <i>Shigella dysentery</i> | S | S | S | R | R | R |
| <i>P. aeruginosa</i> | S | S | S | R | R | R |
| <i>Escherichia coli</i> | S | R | R | R | R | R |
| <i>Bacillus albus</i> | S | S | S | S | R | R |
| <i>Candida albican</i> | S | S | S | R | R | R |

KEY:

S = Sensitive

R = Resistance

DISCUSSION

The phytochemical test of the aqueous pod extract of *A. nilotica* indicated the presence of saponins and tannins in high concentration; alkaloids, reducing sugars and flavonoids occur in moderate concentration, while pentoses, ketosis, cardiac glycosides and emodols occur in low concentrations. It has been shown from scientific investigations that the potential use of extracts as therapeutic agents is due to the presence of active principles or chemical compounds in the extract (Abdulrahman, 2004). Antispasmodic and anti-diarrhoea effects of many medicinal plants have been attributed to their rich flavonoid and tannin contents (Abdullahi *et al.*, 2001; Oyewole, 2003). The concentration of the essential and non-essential elements in the aqueous pod extract of *A. nilotica*, appears to be within the safety limit (WHO, 1996). The aqueous extract of *A. nilotica* was observed to reduce diarrhoea produced by castor oil. Castor oil is triglyceride of fatty acids. It contains ricinoleic acid, which when released induces irritation of the gastrointestinal mucosa, induces inflammation, cause increased fluid secretion and enhance motility of the gastrointestinal tract resulting in diarrhoea (Ammon *et al.*, 1974). Since the extract has the ability to inhibit the castor oil induced diarrhoea, the mechanism of anti-diarrhoea effect exerted by the extract may include decreased gastrointestinal secretion and /or inhibition of gastrointestinal motility. Diphenoxylate (Standard drug) an opiate used in this study is known to inhibit gastrointestinal secretion and

motility (Ammon *et al.*, 1974). From this study, it is possible that the extract may mediate its effect through similar mechanism.

The extract was also observed to dose dependently prolonged the gastrointestinal transit period of activated charcoal. The passage of charcoal meal through the gastrointestinal tract in rats is used as a parameter to measure gastrointestinal motility and to study the laxative as well as the inhibition of intestinal motility (Abdullahi *et al.*, 2001). The results of the study on the gastrointestinal transit revealed that the extract at 750 mg/kg reduced the distance travelled to 41.77%, while Atropine (3 mg/kg) an antimuscarinic drug reduced the transit distance to 39.19%. The extract was also observed to reduce the castor oil induced enteropooling. The ability of the extract to reduce the weight of intestinal content could be by preventing fluid and electrolyte secretion into the intestinal lumen and / or allow the content enough time to be exposed to the absorptive surfaces of the intestinal tract, (Gangarosa *et al.*, 1960; Turnberg *et al.*, 1970; Schulthesis, 1998).

Some flavonoids are known to have anti-microbial properties. The inhibition of the growth of *Staphylococcus epidermidis*, *Streptococcus viridans*, and *Escherichia coli* was reported by Okerulu, and Chinwe, (2001) to be due to flavonoids. Ogundipe *et al.* (1998) reported that flavonoids, tannins and saponins have inhibitory effect on the growth of *Bacillus subtilis*, *E. coli*, *S. aureus* and *Candida albicans*. Flavonoids present in the extract of *A. nilotica* used in this study have been demonstrated to inhibit contraction induced by spasmogens (Haruna and Choudhury, 1997; Abdullahi *et al.*, 2001). The ability of the extract to inhibit gastrointestinal transit in rats suggests that the pod of this plant possess antispasmodic agents. Properties such as this (inhibition of contraction) may be responsible for the anti-diarrhoeic effect of the water extract of this plant. The results provide some justification for the use of this plant as natural anti-diarrhoeic agents and as well as remedy against colic.

CONCLUSION

The extract was found to have anti-diarrhoeic effect, since it inhibit castor oil induced diarrhoea, decreased the gastrointestinal transit

time and reduced enteropooling. It also inhibits the growth of some bacterial and fungal organism that causes diarrhoea.

ACKNOWLEDGMENTS

We thank Dr. U.S Hassan, Mr. Tanko Usman, Bitrus Wampana, Mal. Isa Gulani and Ibrahim Wiam for their technical assistance.

REFERENCES

- ABDULLAHI, A.L., AGBO, M.O., GAMANIAL, K.S. AND WAMBEBE, C. (2001): Anti-diarrhea activity of the aqueous extract of *Terminalia avicenniodes* Root. *Phytotherapy Research*, **19**:431-434.
- ABDULRAHMAN, F.I., (2004): Studies on the chemical contents and Pharmacological Activities of the root bark extract of *Vitex doniana* (Black Plum). PhD Thesis Department of Chemistry University of Maiduguri.
- AMMON, H.V. THOMAS, P.J. AND PHILIPS, S. (1974): Effect of Oleic and Ricinoleic acid on rat jejunal water and electrolyte movement. *Journal of Clinical investigations* **54**:374-379.
- BHATIA, S. C. (2005): Environmental Pollution and Control in Chemical Processes *Industries Khanna Publishers* 2-B Nath Market, Nai Sarak Delhi. 110006 772-802; 808-831
- CARTER, G.R (1975): Diagnostic Procedure in Veterinary Microbiology. 2nd ed. Springfield, Illinois, U.S.A 260-271.
- CHESBROUGH, M. (1975): Medical Laboratory Manual for Tropical Countries. Vol. II. Tropical Health Technique. Butterworth's and Co. L.t.d, Kent, England. 198-204.
- CHITME, H.R. CHANDA, B. AND KAUSHRK, S. (2004): Studies on the anti-diarrhea of *Calotropis gigantean*. V.br. in Experimental animals. *J. Pharm. Pharmaceuticals Science*. **7**:70-75.
- COUNCIL FOR INTERNATIONAL ORGANIZATION OF MEDICAL SCIENCES (C.I.O.M.O), (1985): International Guiding Principles for Biomedical Research Involving Animals WHO 1211, Geneva 27, Switzerland.
- CRUICSHANK, R. (1973): Medical Microbiology. A Guide to the Laboratory Diagnostic and Control of Infection. 12th ed. Vol. I. Churchill Livingstone, London. 601-612.
- DUKE J. A (1983): Antibiotic sensitivity testing in Microbial Chemotherapy (Greenwood, D. ed), Oxford University Press, New York 91 – 100.
- GANGAROSA, E.J., BEISEL, W.R., BENYAJATIC, C., SRINZ, H.; PIYARATAN, P (1960): The Nature in Asiatic cholera and its Relation to Pathogenesis: A Biopsy Study. *American journal of Tropical Medicine Hygiene*. **9**:125-135.
- GREENWOOD, D. (1989): Antibiotic sensitive testing. In: Antimicrobial Chemotherapy Greenwood. D. ed.), Oxford University Press, New York. 91-100.
- HARBORNE, J.B. (1975): Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis. Chapman and Hall, London. 279.
- HARUNA, A.K. AND CHOUDHURY, M.K. (1997): Antispasmodic properties of the aqueous extract of *Aristolochia albidia*. *Dutch Phytotherapy Research*, **2**:527-528.
- IOAN C. (1976): Practical Manual on the Industrial Utilization of Medicinal and Aromatic plants, Methodology for analysis of Vegetables drugs. UNIDO, Romania 128-141.
- MASCOLO, N.A., CAPASSAN, R GERMANO M. P AND CAPASSO F (1999): Inhibiting effect of cannabonoid agents on gastric emptying in the rat. *Archives of Pharmacology*, **360**: 321 – 223
- MEAD, R. AND CURNOW, R.N. (1982): A simple Experiment in Statistical Method in Agriculture Biology. Chapman Hall, London. 33-46.
- NATIONAL ACADEMY OF SCIENCE, (NAS), (1980): Firewood Crops, Shrubs and Tree specie for Energy Production. National Academy of Sciences, Washington D.C.2-10.
- OFFIAH V. N AND CHIKWENDU U. A (1999): Anti-diarrhea effects of *ocimun gratissimum Lin* extract in experimental animals *J. Ethanophorm*. **68** 327 – 330.
- OGUNDIPE, O.O.; MOODY, J.O. AND ODEOLA, H.A. (1998): Antimicrobial and Anti-inflammation activities of *Alchornea laxifora* extractives. In. Proceedings of 1st International Workshop on Herbal Medicinal Products, Ibadan, Nigeria. Nov. 22-24, 1998. 53-55.
- OKERULU, I.O. AND CHINWE, J.A. (2001): The phytochemical analysis and anti-microbial screening of extracts of *Tetracarpidium conophorum*. *Journal of Chemical Society of Nigeria*.
- OYEWOLE, J.A.O. (2003): Evaluation of the Anti-inflammatory Properties of *Sclerocarya birrea* (*Anacardiaceae*) stem-bark extract in rats. *Journal of Ethnopharmacology*, **85**:217-220.
- PANDE, M. B., TALPADA, P M PEBEL J. S., AND SHUKLA P C (1981): Note on the nutritive value of babul (*Acacia nilotica* Linn) Seeds (extracted) In: *Indian J. Ani. Sci.* **51** (1): 107 – 108.
- ROBERTS, A. NEIZAMIS, J. E LANCASTER C, HANCHAR A. JKLEPPER, M.S. (1976): Enteropooling assay a test for diarrhea produced by prostaglandins, **11**: 809 – 828
- SCHULTHEISS, P.J. (1998): Renal and Intestinal Absorptive Defects in Mice Lacking the NHE3 Na⁺/H⁺ Exchanger. *Nat. Genet.* **19**:282-285.
- SOFOWORA, E.A. (1993): Recent Trends in Research into African Medicinal plants. *J. Ethnopharmacol.* **38**: 209-214.
- TREASE G. E AND EVANS, M. D (1989): A Textbook of pharmacognosy, 13th ed. Baillier, Tindal and Causee London, 144 – 148, 687-689.
- TURNBERG, L.A.; FORDTAN, J.S.; Carter, N.W.; Rector, F.C.Jr. (1970): Mechanism of Bicarbonate Absorption and its Relationship to Sodium Transport in the Human Jejunum. *J. Clin. Invest.* **49**:548-556.
- WHO (1996): Guideline for Elemental Concentration, trace elements in Health and Human Nutrition.50-228.