Medicinal and Aromatic Plant Science and Biotechnology ©2008 Global Science Books

Chemical Analysis and Antibacterial Activity of *Acacia nilotica* and *Tapinanthus dodoneifolius* Growing in Nigeria

Muluh E. Khan[1] • Stephen S. Hati[1*] • Kabiru B. Abdu[2] • Aliyu Babale[3] • Micah I. Achi[2]

[1] Department of Chemistry, Gombe State University, Gombe, Nigeria
[2] Department of Chemistry, Bayero University Kano, Nigeria
[3] Department of Biological Sciences, Gombe State University, Gombe Nigeria

Corresponding author: * **stevehati@yahoo.com**

ABSTRACT

Pods of *Acacia nilotica* and leaves of *Tapinanthus dodoneifolius* were spectrophotometrically analysed for their mineral constituents (Ca, Cu, Fe, K, Mg, Mn, Na, P and Zn). Phytochemical analysis was conducted on successive Soxhlet extracts of ethanolic, *n*-hexane and ethyl acetate of the pods and leaves, respectively. Furthermore, the antibacterial activities of the extracts were investigated. Results showed high Ca and K with low Cu and Zn concentrations in both plants. The concentration of P in the pods of *A. nilotica* was highest. Phytochemical analysis revealed the presence of steroids, tannins and saponins in both ethanol and ethyl acetate extracts respectively, for both *A. nilotica* and *T. dodoneifolius.* Flavonoids were present only in the ethanol extract of *A. nilotica.* The ethanol extract generally had more phytochemical agents in both plants studied. All solvent extracts of *T. dodoneifolius* showed activity against *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella aerogenes* and *Proteus mirabilis* showed varying activities only for *A. nilotica* solvent extracts, and only ethanol extract recorded activity against *S. aureus*, while the *n*-hexane extract showed no activity against any pathogen. The ethyl acetate extract of *A. nilotica* showed significant activity against *K. aerogenes* and *P. mirabilis*. The results in this work tend to agree with the ethno-medical claims.

Keywords: ethno-medical, mineral constituents, phytochemical analysis

INTRODUCTION

Human disease management in Nigerian history addresses in one accord evidence of the relationship of plants and medicine as elsewhere the world over (Bannerman *et al*. 1986; Hammer 1999; Raghavendra *et al.* 2006; Ayandele and Adebiyi 2007). In Kano, northern Nigeria, tuberculosis is a major health challenge, Family Health International (FHI) 2001; Nwankwo *et al.* 2005; Emokpae *et al.* 2006) and it is locally believed that the pods of *Acacia nilotica* and the leaves of *Tapinanthus dodoneifolius* possess medicinal properties that are effective in the management of tuberculosis as well as other ailments (stomach ache, diarrhea, dysentery, wound and cancer) (Deeni and Sadiq 2002). The pods of *A. nilotica* are strongly constricted, hairy, white-grey, thick and softly tomentose. The pods without seeds are known to have a high tannin content of about 50%. Generally, the *A. nilotica* tree is 5-20 m high with a dense spheric crown, stems and branches usually dark to black coloured, fissured bark, grey-pinkish slash, exuding a reddish low quality gum. In Ayurvedic medicine, *A. nilotica* is considered a remedy that is helpful for treating premature ejaculation. And in ancient Ethiopian traditional medicine, *A. nilotica* in certain mixtures have been effective in the treatment of rabies (Clement 1998; Murthy *et al.* 2003). *T. dodoneifolius* is a hemiplant parasite usually growing on *Vitellaria paradoxa* trees with large galls, commonly known as African mistletoe and 'Kauchi' in Hausa (vernacular). In northern Nigeria, the Hausa and the Fulani tribes utilize it ethnomedicinally as a remedy for several human and animal ailments (Deeni and Sadiq 2002). According to Nacoulma (1996), the aqueous extract is taken against gynecologic disturbances, digestives disorders and nervous confusions. More recently, the cardiovascular properties of *T. dodoneifolius* have also been described by Ouédraogo *et al*. (2005). Traoré (2000) analysed different extracts from *T. dodoneifolius* which showed the presence of triterpenes, sterols, carotenoids, saponosides, anthracenosides, anthocyanosides and tannins.

Both *A. nilotica* and *T. dodoneifolius* plants are found in abundance and used extensively in forms of decoction and concoctions for the management of tuberculosis in Niabawa, Kumbotso Local Government Area (LGA) of Kano State, Nigeria. Hence the need to investigate these plants originated from this locality. Though, Deeni and Sadiq (2002) have investigated the antimicrobial constituents and phytochemical constituents of *T. dodoneifolius,* but did not include samples from Kumbotso and other organic solvent extracts such as ethyl acetate. In this study, we provide basic information concerning the levels of some mineral constituents of both plants. These minerals are essential to human health. Further, phytochemical screening and analyses of extracts against important pathogen, will indicated concordance with previous works and show results of other solvent extracts not indicated earlier.

MATERIALS AND METHODS

Collection of plant material and preparation

Composite samples of healthy mature pods of *A. nilotica* (Family: Mimosaceae) from an about 8 years old tree, and leaves of *T. dodoneifolius* (Family: Loranthaceae), during the flowering stage respectively, were both collected at the peak of the dry season (April, 2007) at Naibawa in Kumbotso LGA of Kano State, Nigeria. The samples were identified by an expert botanist, Prof. S. Sanusi, Department of Biological sciences, Bayero University Kano. A voucher specimen of the samples with numbers GSU 042 for *A. nilotica* and GSU 103 for *T. dodoneifolius* has been deposited in the herbarium of the Department. Samples were thoroughly washed, shade dried away from sunlight at room temperature. Air-dried samples were ground into fine powder.

Received: 14 May, 2008. Accepted: 27 July, 2008.

Short Communication

Mineral determination

Analyses of mineral constituents (Ca, Cu, Fe, K, Mg, Mn, Na, P and Zn) were carried out on about 2 g of each sample of the pods of *A. nilotica* and the leaves of *T. dodoneifolius.* This was ashed in the oven at about 400° C for 2 h. The elements in the ash were quantitatively taken up with 20 ml concentrated HNO_3, and filtered into 50 ml volumetric flasks. The volumes were made up to the mark with distilled water. Minerals were determined by atomic absorption spectrophotometry using Pye Unicam SP9 spectrometer (Pye Unicam Ltd., Cambridge, England). Phosphorus was determined by UV spectrophotometry using Spectronic 20 (Thermo Electron Scientific Instruments LLC, USA) (Ogugbuaja *et al.* 1997). The standard calibrations method was used as described by Vogel (2000).

Solvent extractions

The powdered samples (50 g each) of the pods of *A. nilotica* and the leaves of *T. dodoneifolius* were separately charged in thimbles and extracted successively with 400 ml each of 98% ethanol, *n*-hexane and ethyl acetate using a Soxhlet extractor for 48 h. All the extracts were concentrated using a rotary flash evaporator and preserved at 5°C in airtight, well labeled bottles until further use (Raghavendra *et al.* 2006). All the extracts were subjected to phytochemical analysis and antibacterial activity assay.

Phytochemical screening

Phytochemical analysis of all the evaporated solvent extracts was conducted in accordance with a standard procedure (Harborne 1998). Tests for alkaloids, steroids, saponins, tannins, flavonoids and terpenoids were carried out in all the fractions.

Antibacterial activity assay

Five bacteria species: *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella aerogenes*, *Psuedomonas aeroginosa* and *Proteus mirabilis* stock cultures were collected from Murtala Mohammed Specialist Hospital Kano, Nigeria. The antibacterial assay was by the cup diffusion method on nutrient agar medium according to the British Society for Antimicrobial Chemotherapy (BSAC 1997). Cups were made in nutrient agar plate using a cork borer (5 mm) and inoculums containing 10^6 CFU/ml of bacteria were spread on the solid plates with a sterile swab moistened with the bacterial suspension. Each solvent extract (50 µl) of the two plants were placed in the cups made in inoculated plates. Treatments also included 50 µl Gentamicin (2 µg/ml) on separate plate, to serve as a control for comparism. All the plates were incubated for 24 h at 37°C and the zone of inhibition around the wells was measured in mm. Each treatment was conducted in triplicate.

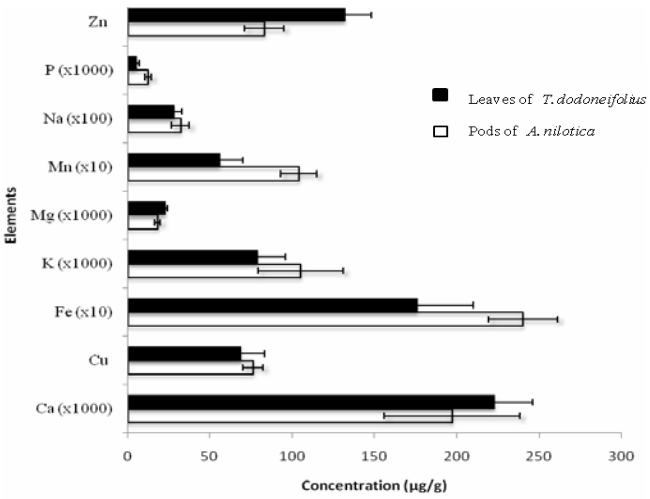

Fig. 1 Concentration of elements in *A. nilotica* pods and *T. dodoneifolius* leaves.

Data obtained were subjected to statistical analysis using coupled MSExcel-Analyse-it® (2006). Independent Student's *t*-test at $p<0.05$ was considered significant in results of both mineral contents and antibacterial assay.

RESULTS

Mineral constituents

Fig. 1 shows the results of mineral constituents in the pods of *A. nilotica* and the leaves of *T. dodoneifolius*. Cu and Zn were present in the least concentration in both plants. Ca followed by K showed highest concentrations in both plants. The concentration of P in the pods of *A. nilotica* was highest (12.3×10^3 µg/g) and least (5.6×10^3 µg/g) in the leaves of *T. dodoneifolius.* In general the concentrations of mineral constituents were higher in the pods of *A. nilotica* than in the leaves of *T. dodoneifolius*.

Phytochemical analysis

Results of phytochemical analysis (**Table 1**) revealed the presence of steroids, tannins and saponins in both ethanol and ethyl acetate extracts respectively, for both *A. nilotica* and *T. dodoneifolius.* Flavonoids were present only in the ethanol extract of *A. nilotica.* The ethanol extract generally presented more phytochemical agents in both plants studied.

Table 1 Results of phytochemical analysis of extracts from pods of *A. nilotica* and leaves of *T. dodoneifolius*.

Test for	Ethanol		Hexane		Ethyl acetate	
	A. nilotica	*T. dodoneifolius*	*A. nilotica*	*T. dodoneifolius*	*A. nilotica*	*T. dodoneifolius*
Alkaloids	-	+	-	+	-	+
Steroids	+	+	-	-	+	+
Tannins	+	+	+	+	+	+
Saponins	+	+	-	-	+	+
Flavonoids	+	-	-	-	-	-
Terpenoids	-	-	+	-	-	-

Key: Absent (-); Present (+)

Table 2 Results of antibacterial activity assay of the extracts from pods of *A. nilotica* and leaves of *T. dodoneifolius*. (Inhibition zones measured in mm)

Plant	Fraction	Microorganism				
		S. aureus	*E. coli*	*K. aerogenes*	*P. mirabilis*	*P. aeroginosa*
A. nilotica	ethanol	11.50 ± 0.10*	-	21.65 ± 0.10*	8.65 ± 0.15	-
	n-hexane	-	-	-	-	-
	ethyl acetate	-	-	9.65 ± 0.10*	5.15 ± 0.65*	-
T. dodoneifolius	ethanol	-	10.60 ± 0.10*	-	-	-
	n-hexane	-	14.00 ± 0.15	-	-	-
	ethyl acetate	-	9.55 ± 0.05*	-	-	-
Control	Gentamicin	21.65 ± 0.10	13.85 ± 0.15	14.65 ± 0.15	9.50 ± 0.10	13.10 ± 0.05

Data represented as mean of three trials ± standard error

Antibacterial activity assay

Table 2 shows the results of the antibacterial activity assay; activity was not recorded for *P. aeroginosa* for all fractions from both plants. All solvent extracts of *T. dodoneifolius* showed activity against *E. coli* and showed the only significant variations for the ethyl acetate extract when compared to the standard Gentamicin control. *S. aureas*, *K. aerogenes* and *P. mirabilis* showed varying activities only for *A. nilotica* solvent extracts, and only the ethanol extract showed activity against *S. aureas*, while the *n*-hexane extract showed no activity against any pathogen. Thus the ethyl acetate extract of *A. nilotica* showed significant activity against *K. aerogenes* and *P. mirabilis*.

DISCUSSION

The mineral composition of the pods of *A. nilotica* and the leaves of *T. dodoneifolius* indicated a higher likelihood of mineral enrichment by the use of these plants. The structural and functional roles of mineral composition of some medicinal plants to biosystems have been well established (O'Dell and Campbell 1971; Ogugbuaja *et al.* 1997). For instance the presence of P has been exploited for synthesizing numerous phytochemical compounds in plants (Habibovic *et al.* 2002). However the results of this work are in accordance with those presented earlier for some medicinal plants of northern Nigerian origin with regards to the accumulation of certain minerals (Fe, K and Mn) in fruity parts of plants than in the stems and leaves (Ogugbuaja *et al.* 1997).

Generally, the result of phytochemical analysis revealed a greater likelihood of tannins being the active phytochemical agents in both plants. This may likely be due to the presence of phenolic compounds (Harborne 1998). Terpenoids were only detected in the ethanol and hexane fractions of *A. nilotica*; this portrays a general trend of the components in medicinal plants, but *T. dodoneifolius* showed a wide spectrum of antimicrobial activities and shows great similarity with the results of Deeni and Sadiq (2002), in terms of phytochemical and the antibacterial activity, especially against *E. coli*. These give credence to the ethnomedicinal usage of the plant.

Pseudomonas species, showed resistance to all extracts, but the ethanol and the ethyl acetate fractions of *A. nilotica* were moderately promising on *K. aerogenes* and *P. mirabilis*. The *n*-hexane fraction of *A. nilotica* showed no inhibition against all the bacteria investigated in this study. This perhaps may be due to very low concentration or absence of active agents in this fraction, which indicates ineffectiveness of *n*-hexane in the extraction of the requisite inhibitory agents against the bacteria tested. Worthy to note is also the fact that biological actions are primarily due to these components in a very complicated concert of synergistic or antagonistic activities. Mixtures of such chemicals show a broad spectrum of biological effects and pharmacological properties (Albert 1952; Mayo 1994). To a large extent, the phonological age of the plant, the percentage humidity of the harvested material, place and time of harvest, and the method of extraction are possible sources of variation in the chemical composition, toxicity and bioactivity of the extracts (Felix 1982). It is very difficult though, to make connections between the present findings and the acclaimed efficacy of these plants on the management of tuberculosis. However studies have indicated that certain biflavonoids have inhibitory activity against *Mycobacterium* that causes tuberculosis (Lin *et al.* 2001) and other components that acts in similar way as the Rifampin and Pyrazinamide, antituberculosis drugs (Sadoff 2006) towards tuberculosis patients utilizing these plants studied in this work.

CONCLUDING REMARKS

Pods of *A. nilotica* and the leaves of *T. dodoneifolius* contain sufficient mineral constituents and their extracts indicate a relatively moderate number of phytochemical agents that showed significant activity on some bacteria. Hence their ethno-medical claims in the management of some human diseases are justified. It is suggested that more research be conducted that will further elucidate the effective components and possible mode of actions involved in the use of these plants in ethno-medical practices.

REFERENCES

Albert FH (1952) *Economic Botany: A Text Book of Plants and Plant Products*, McGraw Hill, UK, 244 pp

Analyse-it® (2006) General and Clinical Laboratory Analyses Software (Version 1.73), Analyse-it Software Ltd., Leeds, England

Ayandele AA, Adebiyi AO (2007) The phytochemical analysis and antimicrobial screening of extracts of *Olax subscorpioidea*. *African Journal of Biotechnology* **6**, 868-870

Bannerman PC, Mirsky R, Jessen KR, Timpl R, Duance VC (1986) Light microscopic immunolocalization of laminin, type IV collagen, nidogen, heaprin sulfate proteoglycan and fibronectin in the enteric nervous system of rat and guinea pig. *Journal of Neurocytology* **15**, 432-443

BSAC (British Society for Antimicrobial Chemotherapy) (1997) A Guide to Sensitivity Testing: Report of working party on antibiotic sensitivity testing of the British Society for Antimicrobial Chemotherapy. *Journal of Antimicrobial Chemotherapy* **27**, 1-50

Clement BA, Goff CM, Forbes TDA (1998) Toxic amines and alkaloids from *Acacia nilotica*. *Phytochemistry* **49**, 1377

Deeni YY, Sadiq NM (2002) Antimicrobial properties and phytochemical constituents of the leaves of African mistletoe (*Tapinanthus dodoneifolius* (DC) Danser) (Loranthaceae): an ethnomedicinal plant of Hausaland, Northern Nigeria. *Journal of Ethnopharmacology* **83**, 235-240

Ekpe P (2005) *Tapinanthus dodoneifolius*. Available online: http://zipcodezoo.com/Plants/T/Tapinanthus_dodoneifolius.asp

Emokpae MA, Nwokedi EE, Dutse AI (2006) Biochemical changes in adult Nigerians with pulmonary tuberculosis in Kano-Nigeria. *Highland Medical Research Journal* **4 (1)**, 15-21

Felix MT (1982) *Medicinal Microbiology*, Churchill Livingstone, London, pp 445-459

FHI: Family Health International (2001) Kano State, Nigeria. Report of the In-Depth Assessment of the HIV/AIDS Situation Institute for HIV/AIDS, Arlington, VA, USA

Habibovic P, Barrere F, Van Blitterswijk CA, De Groot K, Layrolle P (2002) Biomimeitic hydroxyapatite coating on metal implants. *Journal of American Ceramics Society* **85**, 517-522

Hammer K (1999) Antimicrobial activity of essential oils and other plant extracts. *Journal of applied Microbiology* **86**, 985-990

Harborne JB (1998) *Phytochemical Methods*, Chapman and Hall, London, pp 7-8

Lin YM, Flavin MT, Cassidy CS, Mar A, Chen FC (2001) Biflavonoids as novel antituberculosis agents. *Bioorganic and Medicinal Chemistry Letters* **11 (16)**, 2101-2104

Mayo D (1994) *Microscale Organic Laboratory Techniques* (3rd Edn), John Wiley and Sons Inc., New York, 184 pp

Murthy KNC, Vanitha A, Swami MM, Ravishankar GA (2003) Antioxidant and antimicrobial activity of *Cissus quadrangularis* L. *Journal of Medicine and Food* **6**, 99-105

Nacoulma OOG (1996) Plantes médicinales et pratiques médicinales traditionnelles au Burkina Faso: cas du plateau central. Thèse de doctorat ès-sciences. Université de Ouagadougou, Tomes II, pp 508-515

Nwankwo EK, Kwaru A, Ofulu A, Babashani M (2005) Haematological changes in tuberculosis in Kano, Nigeria. *Journal of Medical Laboratory Sciences* **14 (2)**, 35-39

O'Dell BL, Campbell BJ (1971) *Comprehensive Biochemistry*, Elsevier, Amsterdam, pp 179-266

Ouédraogo S, Traore A, Some N, Lompo M, Guissou IP, Schott C, Bucher B, Andriantsithohaina R (2005) Cardiovascular properties of *Tapinanthus dodoneifolius* (DC Danser). *African Journal of Traditional Medicine* **2 (1)**, 25-30

Ogugbuaja VO, Akinniyi JA, Ogarawu VC, Abdulrahman F (1997) *Elemental Contents of Medicinal Plants: A Monograph*, Faculty of Science Monograph Series, University of Maiduguri, **1**, pp 3-23

Raghavendra MP, Satish S, Raveesha KA (2006) Phytochemical analysis and antibacterial activity of *Oxalis corniculata*; a known medicinal plant. *mySCIENCE* **1 (1)**, 72-78

Sadoff J (2006) Advances in tuberculosis vaccine strategies. *Nature Reviews Microbiology* **4**, 2-8

Traoré R (2000) Etude pharmacologique chez l'animal de l'extrait aqueux de *Tapinanthus dodoneifolius* DC. danser (Loranthaceae) utilisée en tradithérapie antiasthmatique au Burkina Faso. Thèse de pharmacie. FSS. Université de Ouagadougou, Burkina Faso, 94 pp

Vogel AI (2000) *Vogel's Textbook of Quantitative Chemical Analysis* (6th Edn) Revised by Mendham J, Denney RC, Barnes JD, Thomas MJK, Pearson Edu. Ltd., India, pp 104-107, 602-607

Wikipedia (2007) *Acacia nilotica* subsp. *nilotica*. Available online: http://en.wikipedia.org/wiki/Acacia_nilotica