

Full Length Research Paper

Comparative phytochemical screening and biological evaluation of n-hexane and water extracts of *Acacia tortilis*

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N-Hexane and water extracts of the leaves, stem-bark and roots of the indigenous plant *Acacia tortilis* locally reputed for the treatment of cough, malaria, asthma and stomach pain in Girei Local Government Area (LGA), Adamawa State were phytochemically screened and biologically tested against *Escherichia coli*, *Shigella* sp., *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Salmonella typhi*. Disc diffusion method was used for the analysis and both fractions were compared. The water extract was quite active on most of the microbes while some of the micro-organisms developed resistance against the N-hexane extract. The comparison of their activity indicated that there is a statistical difference, which is statistically significant. This confirms the fact that *Acacia tortilis* is used by the indigenes in forms of concoctions and decoction using water as the extracting solvent. It equally ascertains the bioactive components in the plant, thus, agreeing with the potential therapeutic significance of the plant as a natural source of drug development

Key words: N-Hexane, *Acacia tortilis*, Michika, phytochemically, therapeutic.

INTRODUCTION

The medicinal flora in the tropical eco-region has a preponderance of plants that provide raw material for addressing a range of medical disorders and pharmaceutical requirements. Nigeria is long recognized for her grass land savanna vegetation with a very rich botanical diversity (Jones et al., 2001; Hepper and Keay, 2000). Ethnic diversity in this temperate zone reflects an untapped wealth of indigenous uses of medicinal plants for the treatment of various health problems (Conuteix, 1961). Their secondary metabolites (Active ingredients) are a source of our sustenance (Ghani, 1990; Dobeis, 1993; Fatope, 2001; Kubmarawa et al., 2007) and are due to the presence of some valuable phytochemicals that combat most of the human maladies (Chidambara et al., 2003).

For a long period, plants have been a valuable source of natural products for maintaining human health and according to the World Health Organization (WHO); medicinal plants would be the best source to obtain a variety of drugs.

The success story of chemotherapy lies in the continuous search for new drugs to counter the challenges posed by resistant strains of microorganisms. The investigation of certain indigenous plants for their antimicrobial properties may yield useful results. A large number of plants are used to combat different diseases and are known to possess antimicrobial activity (Arora and Kaur, 1999).

Though the pharmaceutical industries have tried producing some new antibiotics in the last three decades, the resistance to them by microorganisms has instead increased (Cohen, 1992). In general, bacteria have the genetic ability to transmit and acquire resistance to drugs which are utilized as therapeutic agents. Such a fact is a cause for concern because of the number of patients in

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hospitals who have suppressed immunity due to new bacterial strains, which are resistant (Bisset, 1994). There is now growing evidence that indicates a strong relationship between ethnic knowledge and sustainable use of biodiversity (Sullivan and Shealy, 1997). The time-tested ethnic knowledge when supplemented with the latest scientific insights can offer new models of economic development, that are both eco-friendly and socially acceptable (Croom, 1983). For-instance, the Nomadic Fulani's of Adamawa highlands, Nigeria, choose faith healing first, traditional herbal medicine next and modern medicine only when the first two have failed (Shariff, 2001; Sudhakar et al., 2007).

Thus, the use of traditional medicine and medicinal plants in most developing countries as a normative basis for the maintenance of good health has been widely observed (UNESCO, 1996). Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural herbal remedies (UNESCO, 1998). Isolation of medicinal agents less susceptible to regular antibiotics and recovery of increasing resistant isolates during antibacterial therapy is on the rise throughout the world (Gerding, 1991; Gold and Moellering, 1996; Archibald et al., 1997; O'Brien et al., 1999; Cookson, 2000; WHO, 2001; Cohen, 2002; Fridkin et al., 2002). One of the measures to minimize the increasing rate of resistance in the long-run is to have continuous in-depth investigation for new safe and effective antimicrobials as alternative agents to substitute the non effective ones. Natural resources, especially plants and microorganisms are potent candidates for such purposes.

Umbrella thorn, *Acacia tortilis* also called umbrella thorn or Israeli Babool, is a medium to large canopied tree native primarily to the Savannah and Sahel of Africa but also occurring in the Middle East. Locally, it is reputed for its numerous medicinal and social uses as a cure for malaria, asthma, cough (concoction and decoction) and the pods used by natives for decoration, furniture, wagon wheels, fences, cages and pens.

The plant, *Acacia tortilis* (forsk) (mimosaceae), is called, umbrella thorn acacia (in English), Samar, sammar, smor, samra, sayyal (in Arabic), haak-en-steek (in Afrikaans), Kindil (in Kanuri), Gabaruwa (in Hausa), and Chilluki (in Fulani).

This research work is aimed at filling the knowledge gaps in this important sub-area of cultural biodiversity, directly relevant to the livelihood of the tribal communities, since the need for the integration of local knowledge for a sustainable management and conservation of natural resources receives attention on a daily bases (Posey, 1992). Also in mind is the constitution of compendium of all the medicinal plants, their cures and the parts used and where they are found, the statistical comparison

of the reactivity of the active ingredient(s) in the plant parts in various solvents. It was also prompted by the strong push in the chemical industries to move away from the use of large amounts of organic solvents and when possible, to perform chemical reactions in water so that there is less organic waste, a paradigm of green chemistry (Eric and Denis, 2006) and consequently to produce drugs from plants after clinical studies. This will reduce the distance of the patient to the drug and equally render the drugs affordable.

MATERIALS AND METHODS

Collection of plant materials

Fresh samples of the leaves, stem-bark and roots of the indigenous plant *Chilluki* were collected in Girei LGA, Adamawa State and were identified in the Biological Sciences Department Federal University of Technology Yola. The FHI number is 0796 and a specimen of the plant was deposited in the herbarium. The samples (1.00 kg) each were air dried in the laboratory before pounding to a fine powder using pestle and mortar to about 70 mesh sizes and then stored in dry containers.

Extraction

The powdered sample (150 g each) was accurately weighed and percolated with 2.0 L each of water and distilled ethanol for 72 h. After which there was decantation, filtration, and concentration using rotary evaporator (R110) at 35°C to obtain water and N-hexane soluble fractions, (F_W^1), and (F_H^1) labeled, F_W^L (11 g), F_W^S (09 g) F_W^R (7.6 g) and F_H^L (07 g), F_H^S (5.8 g), F_H^R (4.3 g) for water and N-Hexane fractions, respectively. The various fractions were divided into two portions each for phytochemical screening and the biological evaluation.

Qualitative chemical test

Standard methods described by Evans and Abulude (Sofowara, 1993; Evans, 2000; Abulude et al., 2001, 2007) were used to test for the presence of phytochemical compound(s) (saponins, tannins, volatile oils, alkaloids, phenols and flavonoids) in the fractions.

Microorganisms

Organisms used for this study were, gram-negative (*Pseudomonas auroginosa* SHY3005, *Escherichia coli* SHY 3007, *Shigella dysenteriae* SHY 3001, *Salmonella typhi* SHY 3002) and gram-positive (*Staphylococcus aureus* SHY 3004, *Streptococcus pyogenes* SHY3001) bacteria. These organisms were clinical isolates obtained from Yola Specialist Hospital, Adamawa State, Nigeria.

Determination of antibacterial activity

The antibacterial activity of the extracts was determined using the agar well diffusion technique (Adeniyi and Ayepola, 2008). Sensitivity test agar plates were seeded with 0.1 ml of an overnight culture of each bacterial isolate (equivalent to 10^7 to 10^8 cfu ml⁻¹).

Table 1. Phytochemical constituents of the water extract of *A. tortilis*.

| Phytochemical component | Plant extract | | |
|-------------------------|---------------|-----------|------|
| | Leaf | Stem-bark | Root |
| Saponin | + | + | + |
| Tannin | + | - | + |
| Volatile oil | + | + | - |
| Alkaloid | + | + | + |
| Phenol | + | + | + |
| Flavonoid | + | + | - |

+ = present; - = absent.

Table 2. Phytochemical constituents of the N -Hexane extract of *A. tortilis*.

| Phytochemical component | Plant extract | | |
|-------------------------|---------------|-----------|------|
| | Leaf | Stem-bark | Root |
| Saponin | - | + | + |
| Tannin | + | - | + |
| Volatile oil | + | + | - |
| Alkaloid | + | + | + |
| Phenol | + | + | + |
| Flavonoid | - | - | - |

+ = present; - = absent.

The seeded plates were allowed to set and a standard cork borer of 8 mm diameter was used to cut uniform wells on the surface of the agar. The wells were then filled with 0.1 ml of each extract at a concentration of 0.025 mg / ml. The antibiotic Ampicillin at concentration of 0.01g /ml was used as positive control and distilled water as negative control. The plates were incubated at 37°C for 24 h after which the diameter of the zones of inhibition were measured (Fereshteh et al., 2005).

Statistical analysis

The one way ANOVA test (using coupled MS-Excel-Analyse-it® (Analyse-it®, 2010)) was used to analyze and compare the water and N-hexane results at a 95% confident level. Values of $P \geq 0.05$ were considered significant. Results were expressed as Mean \pm SE of mean.

RESULTS

Phytochemical investigation

The phytochemical analysis of the water and N-hexane extracts from the leaves, stem bark and roots of the indigenous plant, *A. tortilis* are shown in Tables 1 and 2; the antimicrobial activities of the fractions against some gram-positive and gram-negative bacteria are shown in Tables 3 and 4.

DISCUSSION

The data obtained were subjected to statistical analysis using coupled MS-Excel-Analyse-it® (Analyse-it®, 2010). Independent Student's *t*-test at $p \geq 0.05$ was considered significant for the comparison of the antimicrobial activities of the water and N-hexane extracts.

Tables 1 and 2 indicate the presence of most of the pharmacologically useful classes of compounds (saponins, tannins, volatile oil, alkaloids, phenols and flavonoids) tested for, except that in the N-hexane fraction; flavonoids were completely absent.

These secondary metabolites have been shown to have therapeutic activities in plants and function in a synergistic or antagonistic fashion for the treatment of diseases (Trease and Evans, 1996). Saponins, a special class of glycosides, have expectorant action which is very useful in the management of upper respiratory tract inflammation; saponins present in plants are cardiotoxic in nature and are reported to have anti-diabetic and anti-fungal properties (Finar, 1989; Trease and Evans, 1989; Kamel, 1991). Tannins are reported to possess physiological astringent and haemostatic properties, which hasten wound healing and ameliorate inflamed mucus membrane and also inhibit the growth of microorganisms by precipitating microbial proteins and making nutritional

Table 3. Antimicrobial activity of the water extracts of *A. tortilis* on some (clinical isolates) gram-positive and gram-negative microorganisms.

| Microorganism | Zone of growth inhibition (mm) | | | | |
|----------------------|--------------------------------|-----------|------|------------|-----------------|
| | Leaf | Stem-bark | Root | Ampicillin | Distilled water |
| <i>S. typhi</i> | 20 | 16 | NA | 26 | - |
| <i>E. coli</i> | 24 | 17 | 12 | 25 | - |
| <i>S. pyogene</i> | 20 | 15 | 6 | 25 | - |
| <i>S. dysentryae</i> | 21 | 15 | 6 | 24 | - |
| <i>P. auroginosa</i> | 15 | 17 | 13 | 21 | - |
| <i>S. aureus</i> | NA | 9 | 13 | 20 | - |

NA = Non active; Concentration of the extract used = 0.025 mg/ml. Concentration of the ampicillin = 0.01 mg/ml.

Table 4. Antimicrobial activity of the N-Hexane extracts of *A. tortilis* on some (clinical isolates) gram-positive and gram-negative microorganisms.

| Microorganism | Zone of growth inhibition (mm) | | | | |
|----------------------|--------------------------------|-----------|------|------------|-----------------|
| | Leaf | Stem-bark | Root | Ampicillin | Distilled water |
| <i>S. typhi</i> | 4 | 6 | 10 | 26 | - |
| <i>E. coli</i> | 2 | NA | 11 | 25 | - |
| <i>S. pyogene</i> | NA | 17 | 14 | 25 | - |
| <i>S. dysentryae</i> | NA | NA | 16 | 24 | - |
| <i>P. auroginosa</i> | NA | 11 | 10 | 21 | - |
| <i>S. aureus</i> | NA | NA | 5 | 20 | - |

NA = Non active; Concentration of the extract used = 0.025 mg/ml. Concentration of the ampicillin = 0.01 mg/ml.

proteins unavailable for them; they form irreversible complexes with proline rich proteins, resulting in the inhibition of the cell protein synthesis. They have important roles such as stable and potent anti-oxidants (Trease and Evans, 1983; Tyler et al., 1988; Awosika, 1991; Ogunleye and Ibitoye, 2003). They act as binders and for treatment of diarrhea and dysentery (Dharmananda, 2003). Alkaloids are reported to have analgesic, anti-inflammatory and adrogenic activities which help to alleviate pains, develop resistance against diseases and endurance against stress (Gupta, 1994). Plant phenolic compounds especially flavonoids are currently of growing interest owing to their supposed properties in promoting health (anti-oxidants) (Rauha et al., 2000).

Thus, the present investigation clearly reveals the antibacterial nature of this plant and portrays it as a potential source of useful drug thereby suggesting that it could be exploited in the management of diseases caused by these bacteria in human and plant systems (Raghavendra et al., 2006).

Antimicrobial activities of the extract

The biological activities of the plant extracts and the

measured diameters of zone of inhibition in (mm) against the microorganisms are shown in Tables 3 and 4. Zones of inhibition indicate the effect of the extracts on the microorganisms. The result showed that the water extract of the plant parts have more antimicrobial activity on the microbe than the N-hexane extract. The roots and the stem-bark are the most effective, though there is no activity on *S. typhi* and *S. aureus*.

Comparative classical statistical analysis of variance and comparing values of the Kruskal Wallis (post hoc) test was carried. From the analysis, it was realized that in comparing the variables of the control (Ampicilline) with the variables water and N-hexane, the results were all statistically significant ($p \geq 0.0004$). This shows that water is the best proposed solvent of extraction - the much advocated "green chemistry paradigm" (Eric and Denis, 2006).

This is a confirmation of the usage of *A. tortilis* by the indigenes in forms of concoctions and decoction using the universal solvent, water as the medium of extraction. N-hexane on the other hand is a good solvent (but water better) for extraction but not as good as ordinary water.

The aforementioned is a clear indication that the plant can treat cough, malaria, asthma, stomach pain and other diseases. This is inconsonance with the activity of

plant secondary metabolites of medicinal plants as they play a significant role in the anti-microbial, bacteriostatic and bactericidal activities (Lin et al., 2001). This thus, supports the use of the plant by herbalists and Girei LGAs indigenes for the treatment of their human ailments. Ampicillin (positive control) is higher in performance, with greater zones of inhibition than the extract(s) probably because impurities are interfering with extract's activity. When active compounds are isolated from the extracts, they may give higher activity than ampicillin.

Conclusion

Knowledge of the healing systems of plants is transferred orally from generation to generation without any written documentation and many of the traditional methods have a superstitious element. More so, lack of documentation of traditional healing methods has resulted in confusion amongst users. Thus, this piece of work strongly recommends the necessity of proper documentation of the actual healing methods, along with the main characteristic feature of the medicinal plants. It strongly suggests that the indigenous plant, *Gabaruwa* because of its phytochemical content and biological activity, could be a good source for alternative drugs specially when extracted with the universal solvent water.

REFERENCES

- Abulude FO (2007). Phytochemical Screening and mineral contents of Leaves of Some Nigerian Woody plants. Res. J. Phytochem. 1:33 - 39
- Abulude FO, Akajagbor, Dafiehwere (2001). Distribution of trace minerals, phosphorus and phytate in some varieties of mushrooms found in Nigeria. Adv. Food Sci. 23:113-116
- Adeniyi BA, Ayepola OO (2008). The phytochemical and Antimicrobial Activity of Leave Extracts of *Eucalyptus camaldulensis* and *Eucalyptus torrelliana* (Myrtaceae). Res. J. Med. Plants 2(1):34 – 38.
- Analyse-it® (2010). General and Clinical Laboratory Analyses Software (Version 1.73), Analyse-it Software Ltd., Leeds, England.
- Archibald L, Phillips L, Mnet D (1997). Antimicrobial resistance in isolates from inpatients and outpatients in the United States: increasing importance of the intensive care unit. Clin. Infect. Dis. 24:211 -215.
- Arora D, Kaur J (1999). Antimicrobial activity of species. Int. J. Antimicrob. Agents 12:257 -266
- Awosika, F, (1991). Local Medicinal plants and health of consumers. Clin. Pharm. Herbal Med. 9:28 -29.
- Bisset NM (1994). Herbal Drugs and Phytopharmaceuticals. CRC Press, London. pp. 106 -108.
- Chidambara K, Vanitha A, Mahadeva M, Ravishankar G (2003). Antioxidant and Antimicrobial activity of *Cissus quadrangularis* L. J. Med. Food 6:2.
- Cohen ML (1992). Epidemiology of drug resistance: Implications of a post-microbial era. Science 257:1050-1055.
- Cohen ML (2002). Changing patterns of infectious diseases. Nature 406:762-767.
- Conuteix PJ (1961). The Major Significance of Minor Forest Products. In: Kappel RSC (Ed.), Local Value and Use of Forest in West Africa Humid Forest Zones. FAO, Rome. pp. 81-86.
- Cookson BD (2000). Methicillin-resistant *Staphylococcus aureus* in Community. New battlefronts or are the battles lost? Infect. Control Hosp. Epidemiol. 2:398-403.
- Croom EM (1983). Documenting and evaluating herbal remedies. Ecol. Bot. 37:13 -27.
- Dharmananda S (2003). Gallnuts and the uses of tannins in Chinese medicine. A paper Delivered at the Institute for Traditional Medicine, Portland, Oregon.
- Dobelis IN (1993). Magic and medicine of plants. The Readers Digest Association Inc. Pleasant, New York. pp. 8-48.4(1):15-21.
- Eric VV, Denis AD (2006). Modern Physical Organic Chemistry. University Science Books, Sausalito, California. p 151.
- Evans WC (2000). Trease and Evans Pharmacology, 4th Edn. WB Saunders Company Ltd. pp. 224- 239.
- Fatope MO (2001). "Natural Products Science: Looking Back and Looking Forward". A Professorial Inaugural Lecture, Bayero University Kano, Nigeria.
- Fereshteh E, Morteza Y, Tafakori V (2005). Antimicrobial activity of *Datura innoxia* and *Datura stramonium*. Fitoterapia 76:118 -119.
- Finar IL (1989). Chemistry; Stereochemistry and the Chemistry of Natural products, 5th Edn, Vol. 2. Longman Group, UK. pp. 517-605.
- Fridkin S K, Hill HA, Volkava NV, Edwards JR, Lawton RM, Gaynes RP (2002). Temporal changes in prevalence of antimicrobial resistance in 23 U. S Hospitals. Emerg. Infect. Dis. 8:697-700.
- Gerding DN, Larson TA, Hughes RA (1991). Aminoglycoside resistance and aminoglycoside usage: ten years of experience in one hospital. Antimicrob. Agents Chemther. 35:1284-1290.
- Ghani A (1990). Introduction to Pharmacognosy. Ahmadu Bello University Press, Ltd. Zaria, Nigeria. pp. 45-47, 187-197.
- Gold HS, Moellering RC (1996). Antimicrobial drug resistance. New Engl. J. Med. 335:1445-1453.
- Gupta SS (1994). Prospects and prospective of natural products in medicine. Indian J. Pharmacol. 26:1-12.
- Hepper FN, Keay R (2000). History of the flora of West Tropical Africa. Niger. Field 65:141-148.
- Jones AM, Govan JR, Doherty CJ, Doherty CJ, Dodd ME, Isalska BJ, Stanbridge T N, Webb AK (2001). Spread of a multiresistant strain of *Pseudomonas aeruginosa* in an adult cystic fibrosis clinic. Lancet 358:557-558
- Kamel JM (1991). An extract of the mesocarps of fruits of *Balanite aegyptiaca* exhibited a prominent anti-diabetic properties in Mice. Chem. Pharmacol. Bull. 39:1229 -1233.
- Kubmarawa D, Ajoku A, Enwerem M, Okorie A (2007). Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria. Afr. J. Biotechnol. 6(14):1690-1696.
- Lin YM, Flavin MT, Cassidy CS, Mar A, Chen FC (2001). Bioflavonoid as novel anti-tuberculosis agents. Bioorg. Med. Chem. Lett. 11(16):2101-2104.
- O'Brien FG, Pearman JW, Gracy M (1999). Community strain of methicillin-resistant *Staphylococcus aureus* involved in a hospital outbreak. J. Clin. Microbiol. 37:2858-2862.
- Ogunleye DS, Ibitoye SF (2003). Studies of antimicrobial activity and chemical constituents of *Ximenia Americana*. Trop. J. Pharm. Res. 2:239-241.
- Posey D (1992). Traditional knowledge, Conservation and the Rain Forest Harvest In: Potkin M, Famolare L (Eds.), Sustainable Harvest and Marketing of Rain Forest Products. Island Press, Washington DC. pp. 46 -50.
- Raghavendra MP, Satish S, Raveesha KA (2006). Phytochemical analysis and antibacterial activity of *Oxalis corniculata*; a known medicinal plant. Agriculture Microbiology Laboratory, Department of Studies in Botany, University of Mysore, Manasagangotri, Mysore, 570 006, India. (mySCIENCE 1(1), 2006, 72–78. <http://myscience.uni-mysore.ac.in>
- Rauha JP, Remes S, Herinonen W, Hopia M, Kgjala T, Pitinlaja K, Vaorela H, Vaorela P (2000). Antimicrobial effects of finished plant extract containing flavanoids and other phenolic compounds. Int. J. Food Microbiol. 56:3 -12
- Shariff ZU (2001). Modern Herbal Therapy for Common Ailments.

- Nature pharmacy Series, Spectrum Books Limited, Ibadan, Nigeria in Association with Safari Books (Export) Limited, United Kingdom. pp. 9-84.
- Sofowara A (1993). Medicinal plants and Traditional medicine in Africa. Spectrum Books Ltd. Ibadan, Nigeria. p 289.
- Sudhakar RC, Reddy KN, Thulsi RK, Chiranjibi P (2007). Ethnobotanical Studies on Medicinal plants Used by the Chenchus of Nallamalais in Kunool District, Andhra Pradesh, India. Res. J. Med. Plants (4):128-133.
- Sullivan K, Shealy CN (1997). Complete Natural Home Remedies. Element Books Limited, Shaftesbury, UK. pp. 3-4.
- Trease GE, Evans MD (1989). A text book of Pharmacognosy, 13th Edn. Baillier, Tindal and Causse, London. pp. 144-148.
- Trease GE, Evans WC (1983). Textbook of Pharmacognosy, 12th Edn. Balliere, Tindall, London.
- Trease GE, Evans WC (1996). Pharmacognosy. Alden Press, Oxford. pp. 213-232.
- Tyler VE, Brady LR, Roberts JE (1988). Pharmacology. Lea and Ferbigier, Philadelphia. pp. 85-90.
- UNESCO (1996). Culture and Health Orientation texts- World Decade for Cultural Development (1988-1997). Document CLT/DEC/PRO-1996, Paris, France. 129 p.
- UNESCO (1998). FIT/504-RAF-48 Terminal Report: Promotion of Ethnobotany and Sustainable Use of Plant Resources in Africa. 60 p.
- WHO (2001). WHO Global Strategy for Containment of Antimicrobial Resistance. Accessed at: www.who.int/emc-documents/antimicrobial_resistance/docs/Global_Start.Pdt