Nigerian Journal of Pharmaceutical Research
Vol. 9 No 2 pp. 103 – 109 (September 2011)

THE PHYTOCHEMICAL, ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF THE CRUDE EXTRACT OF SIDA ACUTA (MALVACEAE) LEAF

C. F. Anowi, A. F. Onyegbule, V. Egbe and C. C. Akwuruoha

Nnamdi Azikiwe University, Awka, Anambra State

Abstract
The phytochemical constituents and anti-microbial activity of the crude extract of *Sida acuta (malvaceae)* leaf were investigated during the wet season. The methanolic and aqueous crude extracts of *Sida acuta (malvaceae)* was obtained through standard procedure. The minimal inhibitory concentration of these crude extracts were determined using five cultures of *Candida albicans, Staphylococcus aureus, Escherichia coli, Salmonella coliform* and *Klebsiella spp*. The methanolic crude extracts of *Sida acuta (malvaceae)* was found to be active against *Staphylococcus aureus* and *Candida albicans* with a minimal inhibitory concentration of 62.50mg/ml and 31.250mg/ml respectively. Also, the aqueous crude extract of *Sida acuta (malvaceae)* was found to be active against *Staphylococcus aureus, Candida albicans* and *Escherichia coli* with a minimal inhibitory concentration of 62.5mg/ml, 31.25mg/ml and 62.5mg/ml respectively. The leaves of *Sida acuta (malvaceae)* showed good anti-bacteria and anti-fungal activity.

INTRODUCTION

Herbal medicine, a form of complimentary and alternative medicine is becoming increasingly popular in both developing and developed countries. World Health Organisation has described traditional medicine as one of the surest means to achieve total health care coverage of the world's population.

In Nigeria, tl 103 ars to be an overwhelming increase in the public awareness and usage of herbal medicinal products in the treatment and/or prevention of diseases. This may not be unconnected to the active mass media advertisement embarked upon by the producers and marketers of the herbal medicinal products (HMPs) who have taken the advantage of the relatively high cost of the conventional pharmaceutical dosage forms, inaccessibility of the orthodox medical services to a vast majority of people particularly in the rural areas and the reservations by the public due to the prevalence of fake, substandard or counterfeit drugs in the market. These have placed the HMPs as a ready alternative to conventional dosage forms in the treatment of diseases. With this increased usage, the safety, efficacy and quality of these medicines have been an important concern for health authorities and health professionals. Although herbal remedies are often perceived as being natural and therefore safe, they are not free from adverse effects which may be due to factors such as adulteration, substitution, contamination, incorrect preparation and/or dosage, inappropriate labeling and/or advertisement. In an attempt to enhance the acceptability of the HMPs by consumers, many of the products have been formulated into conventional modern dosage forms such as tablets, capsules, suspensions, solutions and powders. With the

prevalence in the Nigerian market of these products, it would be of great interest to evaluate the pharmaceutical qualities of these HMPs irrespective of the medicinal content and therapeutic claims.

This traditional medicine uses numerous plants, among them is *Sida acuta Burm F. (Malvaceae). Sida acuta* is a shrub indigenous to pantropical areas, widely distributed in these regions and widely used in traditional medicine. The aerial part of the plant is the most frequently used part. In Central America, the plant is used to treat asthma, renal inflammation cold, fever, headache, ulcers and worms (Caceres *et al*, 1987; Coee *et al*, 1996). In Colombia the plant is known to treat snake bites. It has been demonstrated that the ethanolic extract of the plant had an effective moderate activity against the venom of Bothrox athrox (Otero *et al*, 2000 a, b) In west Africa, particularly in Burkina Faso *S. acuta* often called "arbre ă balai in French and 'zon-raaga" in Moorè an indigenous language, grows around habitations in farms and in bushes. The plant is traditionally used in the treatment of malaria, diarrhea and many other diseases (Nacoulma *et al* 1966). Research focused on malaria led to the identification of alkaloids, principally cryptolepine the major alkaloids of the plant, as its anti malarial agent (Banzonzi, *et al* 2004; Karon *et al*, 2003) More recently, it was found that polyphenol extract of the plant had been shown to have a weak antioxidant activity in vitrofree radicals scavenging assays, on the other hand the extract was very active on pathogenic bacteria and this activity may be influenced by the polymerization size of the phenolic compounds (Karon, *et al* 2005).

Phytochemical screening on *S. acuta* resulted in the isolation of several alkaloids and steroidal compounds with the potential to induce quinone reductase and to inhibit 7,12-dimethylbenz – (a) anthacene – induced preneoplastic lesions in mouse mammary organ (Cao *et al*, 1993; Dinan *et al*, 2001; Jang *et al*, 2003).

Among the compounds isolated from S. *acuta*, its alkaloids appeared to be of great interest in pharmacological studies. These alkaloids belong to the family of indoquinolines. Many investigations have been done on this family of compounds and the results showed that they are new leads in the establishment of drug against many diseases. For example, cryptolepine 5-methylindolo (2-3b) – quinoline, the main alkaloid of the plant, has been well investigated for its various biological properties. First isolated from cryptolepis species (*C. triangularis* and *C. sanguinolenta* (Periplocaceae) from Africa (Clinquart, 1929; Dwuma-Badu *et al*, 1978); the compound has been also isolated from other plants such as *S. acuta* (Malvaceae) from Sri Lanka (Gunatilaka *et al*, 1980); Microphilis guyanensis (sapotaceae) and Genipa Americana (Rubiaceae) from Surina (Yang *et al*, 1999). *Sida acuta* is a shrub indigenous to pantropical regions. The plant is widely used for its various pharmacological properties. Among compounds of pharmacological interest occurring in the plant are indoloquinoline alkaloids. The aim of the present study is to investigate the antimicrobial activity of alkaloids of S. *acuta* from Madonna university environment on some Gram-positive and Gram-negative bacteria. The selected microorganism includes reference strains and fresh clinical strains isolated from pathologic products.

IMPORTANCE OF HERBS OVER SYNTHETIC PRODUCTS

- Herbal 104 nedies are compounded from natural products and therefore are greater likelihood of been

accepted by the body than synthetic products.

- Most synthetic products have severe side effects which are not associated with herbal preparation.
- It is not known whether there is resistance to herbal preparations as with orthodox drugs.
- Herbal preparation is more accessible to most of the population in developing countries. It is reported that 60-85% of the population of every country in the developing world has to rely on herbal medicine due to shortage of hospitals.

MATERIALS AND METHODS

COLLECTION, IDENTIFICATION & ANALYSIS OF PLANT MATERIALS

Fresh leaves of *Sida acuta* were collected from the premises of Madonna University Elele campus, rivers state, Nigeria in November 2009.The plant was identified by F. Osuala of the Department of Pharmacognosy, Faculty of Pharmacy, Madonna University, Elele, Rivers State Nigeria. The plant material was air dried on the laboratory bench for 5 days and later transferred into a hot air oven to ensure complete dryness. Then the plant material was grounded to powder using an electric mill. Four hundred and sixty grammes of the powdered aerial part of the plant material was obtained, and about 10g of the powder was for phytochemical analysis and 450g was macerated with 2500ml of 98% methanol for 72 hours. Preliminary phytochemical analysis was performed to investigate the presence of secondary metabolites in the leaves. These metabolites are the biological constituents of the plant part under study which may suggest the possible antimicrobial activity. These phytochemical screening were performed using standard procedures. Saponins, tanins, alkaloids, flavonoids, terpenoids, reducing sugars, resins, fat & oil, glycosides, proteins and steroids were tested for.

EXTRACTION

Methanolic and aqueous extracts were used in this project work. Each extract was prepared by macerating 500g of powdered leaf sample of *Sida acuta* in 500ml of methanol and distilled water respectively. The mixtures were allowed to stand for 24 hours after which they were filtered with What-man No 1 filter paper, to obtain filtrates (also known as extracts). The extracts were then concentrated to eliminate the extracting solvent by evaporation in the sun and water bath for the methanol and distilled water respectively. The evaporation in the water bath was done at a temperature of 50^0C.

STANDARDIZATION OF EXTRACTS

The extracts were standardized by weighing out 1gram of each concentrate and dissolving in 4ml of water which gave 250 mg/ml. Different concentrations like 125 mg/ml, 62.500 mg/ml, 31.250 mg/ml and 15.625 mg/ml were obtained from the standard by dilution (Brantner and Grein *et al.*).

TEST ORGANISM

Isolates of the test organisms used were obtained from Madonna University Teaching Hospital Elele. The isolates includes; *Staphylococcus aureus, Candida albicans, Klebsiella spp, E. coli,* and *Salmonella coliform.* They were designated as A, B, C, D and E respectively. The test organisms were standardized to obtain an appropriate cell density of the bacteria.

PREPARATION OF MEDIA

Nutrient agar was prepared by

dissolving 28g of nutrient agar powder in 1L of distilled water using 1Lconical flask which was covered with cotton wool, paper foil and masking tape. This was sterilized in the autoclave for 15 minutes at 121^0C. After autoclaving, the media was allowed to cool to 45^0C before been dispensed into Petri dishes which has been sterilized earlier.

ANTI MICROBIAL ASSAY OF THE EXTRACT

This was conducted in two phases termed A and B. **Phase A**: A sterile forceps was used to introduce a sterile filter paper in the different agar-containing plates. The soaked strip was placed on the surface of the nutrient agar at one side of the plate. A loopful of A was taken and streaked across the plate in a perpendicular angular to the paper strip of the aqueous extract and this was also done for the methanolic extract. This process was performed for organisms B, C, D, and E were labeled properly and incubated at 37^0C for 24 hours.

Phase B: paper disc agar method was used. Six-milliliter diameter paper discs were punched using Whatman No 1 filter paper. The discs were sterilized in hot air oven at 160^0C for 1 hour. The discs were soaked in the various concentrations of the two extracts and allowed to dry. This was placed in the Petri dishes containing nutrient agar on which the different organisms have been cultured. The impregnated plates were incubated at 37^0C fro 24 hours. Zones of inhibitions were obtained and recorded in millimeter as evidence of antimicrobial activity.

RESULTS AND DISCUSSION

Traditionally, medicinal plants have been used for the treatment of microbial infectious diseases during pre-historic time by native traditional people. The table (table 1) above only showed the tests in which the results were positive. The results showed that the leaves of *Sida acuta* contains tannins, steroids, terpenoids, glycosides, proteins, carbohydrates, flavonoids, alkaloids, saponin, fats and oil. The study showed that crude methanolic extract of *Sida acuta* was active against *Staphylococcus aureus* and *Candida Albicans* with a minimal inhibitory concentration of 62.5mg/ml and 31.25mg/ml respectively; while crude aqueous extract of *Sida acuta* was found to be active against *Staphylococcus aureus, Candida albicans* and *Escherichia coli* with a minimum inhibitory concentrations of 62.5mg/ml, 31.25mg/ml and 62.5mg/ml respectively. The activity recorded on the tested plant emphasised the medicinal value of the plant.

CONCLUSION

In Africa, traditional medicines are used extensively in the cure of various diseases like diarrhea, urinary tract infection etc, today. One of the most extensively used herbal plants today is *Sida acuta*, which is effective in treatment or prevention of diseases. In Eastern and Southern parts of Nigeria, this plant is employed traditionally for the treatment of diarrhea and fever. The antimicrobial activity study of the methanolic and aqueous extracts showed that *Sida acuta* was effective on some isolates, *Staphylococcus aureus, Candida albicans and Eshericia coli*, while *Klebsiella sp and, Salmonella coliform* did not show activity at all for the methanolic and aqueous extracts.. The plant leaves showed good anti-bacterial and anti-fungal activity.

RECOMMENDATION

It is recommended that more work should be done on the treatment of diseases. Investigation should be carried out on the effectiveness of both industrial and local antibiotics like

Table 1 - PHYTOCHEMICAL CONSTITUENTS OF *SIDA ACUTA* LEAF

2^0 METABOLITES	PHYTOCHEMICAL. TEST	OBSERVATION	INFERENCE
Saponins	Frothing test.	Formation of a stable froth.	Present
Tannin	Ferric chloride Test.	Appearance of greenish black precipitate.	Present
Flavonoid	Ammonium and Aluminum chloride Tests.	Appearance of yellow coloration.	Present
Carbohydrate (Reducing sugar)	Fehling's Test.	Appearance of a brick red precipitate.	Present
Alkaloids	Dragendoff's and Wagner's reagents.	Appearance of Reddish brown coloration.	Present
Proteins	Million's test.	Appearance of a white precipitate.	Present
Fats and oil	Sudan III test and Emulsion test.	Appearance of stained oil globules and milky white emulsion respectively.	Present
Glycosides	Hydrolysis test.	Appearance of brick red precipitate.	Present
Terpenoids	Sulphuric acid test.	Appearance of a grey colour.	Present
Steroids	Sulphuric acid test.	Appearance of reddish brown interface.	Present

Table 2 - SENSITIVITY PATTERN OF METHANOLIC AND AQUEOUS EXTRACTS OF *SIDA ACUTA* LEAVES ON FIVE ORGANISMS

EXTRACTS	CONCENTRATION (mg/ml)	ZONE OF INHIBITION (mm)				
		A	B	C	D	E
METHANOLIC EXTRACT	250.000	12	6	-	-	-
	125.000	10	3	-	-	-
	62.500	8	2	-	-	-
	31.250	-	1	-	-	-
	15.625	-	-	-	-	-
AQUEOUS EXTRACT	250.000	10	6	-	6	-
	125.000	8	3	-	4	-
	62.500	4	2	-	3	-
	31.250	-	1	-	-	-
	15.625	-	-	-	-	-

NB: A= *Staphylococcus aureus*,**B**= *candida albicans*, **C**= *Klebsiella spp*, ***D***= *Eshericia coli* and **E**= *Salmonella coliform*

Sida acuta used in the treatment of diseases like diarrhea. Comparison should be drawn between the two groups of antibiotics and recommendation for patient in the treatment of infectious diseases caused by micro-organism should be based on relative sensitivity of the causative organism.

REFERENCES

Banzouzi JT, Prado R, Menan H, Valentin A, Roumestan C, Mallie M, Pelissier Y, Blache Y (2004). Studies on medicinal plants of Ivory Coast: investigation of *Sida acuta* for in vitro antiplasmodial activities and identification of an active constituent. Phytomed, 11: 338-341.

Caceres A, Giron LM, Martinez AM (1987). Diuretic activity of plants used for the treatment of urinary ailments in Guatemala. J. Ethnopharmacol. 19: 233-245.

Cao JH, Qi YP (1993). Studies on the chemical constituents of the herb huanghuaren (*Sida acuta* Burm. f.). Zhongguo Zhong Yao Za Zhi. 18: 681-2, 703.

Coee FG, Anderson GJ (1996). Ethnobotany of the Garifuna of the eastern Nicaragua. Economic Botany 50: 71-107.

Clinquart E (1929). Sur la composition de *Cryptolepis triangularis*, plante congolaise. Bull. Acad. Roy. Med. Belg. 9: 627-635.

Dinan L., Bourne P, Whiting P (2001). Phytoecdysteroid profiles in seeds of *Sida* spp. (Malvaceae). Phytochem. Anal. 12: 110-119.

Dwuma-Badu D, Ayin JKS, Fiagbe NIY, Knapp JE, Shiff PL, Slatkin DJJ, 1978. Quindoline from *Cryptolepis sanguinolenta*. J. Pharm. Sci 67: 433-635.

Gunatilaka AAL, Sotheeswaran S, Balasubramaiam, Chandraseka AI, Sriyani HTB, (1980). Studies on medicinal plants of Sri Lanka III. Pharmacologically important alkaloids of some *Sida* species. Planta Medica 39: 66-72.

Jang DS, Park EJ, Kang YH, Su BN, Hawthorne ME, Vigo JS, Graham JG, Cabieses F, Fong HH, Mehta RG, Pezzuto JM, Kinghorn AD (2003). Compounds obtained from *Sida acuta* with the potential to induce quinone reductase and to inhibit 7, 12

dimethylbenz[a] - anthracene-induced preneoplastic lesions in a mouse mammary organ culture model. Arch. Pharm. Res. 26 (8): 585-590.

Karou D, Dicko MH, Sanon S, Simpore J, Traore AS (2003). Antimalarial activity of *Sida acuta* Burm f. (Malvaceae) and *Pterocarpus erinaceus* Poir. (Fabaceae). J. Ethnopharmacol. 89: 291-294.

Karou D, Dicko MH, Simpore J, Traore AS (2005). Antioxidant and antibacterial activities of polyphenols from ethnomedicinal plants of Burkina Faso. Afr. J. Biotechnol. 4: 823 – 828.

Nacoulma/Ouedraogo OG (1996). Plantes médicinales et pratiques médicales traditionnelles au Burkina Faso: cas du plateau central Mossi. Thèse d'Etat, Université d'Ouagadougou.

Otero R, Nunez V, Jimenez SL, Fonnegra R, Osorio RG, Garcia ME, Diaz A (2000a). Snakebites and ethnobotany in the Northwest region of Colombia: Part II: neutralisation of lethal and enzymatic effects of *Bothrops atrox* venom. J. Ethnopharmacol. 71: 505-11.

Otero R, Nunez V, Barona J, Fonnegra R, Jimenez SL, Osorio RG, Saldarriaga M, Diaz A (2000b). Snakebites and ethnobotany in the Northwest region of Colombia. Part III: neutralisation of the haemorrhagic effect of *Bothrops atrox* venom. J. Ethnopharmacol. 73: 233-41.

Yang SW, Abdel-Kader M, Malone S, Werkhoven MCM, Wisse JH, Bursuker I, Neddermann K, Fairchild C, Raventos-Suarez C, Menendez AT, Lane K, and Kingston DGI (1999). Synthesis and biological evaluation analogues of cryptolepine, an alkaloid isolated from the Surinam rainforest. J. Nat. Prod. 62: 976-983.