PRELIMINARY PHYTOCHEMICAL AND ANTIBACTERIAL SCREENING OF *Acanthospermum hispidium*

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ABSTRACT

Acantospermum hispidium is a medicinal plant commonly known as ‘‘*Kashin yawo’’* in northern Nigeria. The leaves are used locally for the treatment of acute fever, cough, diarrhea, dysentery, typhoid and pneumonia. The present research was conducted to investigate the preliminary phytochemical compounds and antibacterial effect of *Acantospermum hispidum* plant on clinical bacterial isolates of *Escherichia coli* and *Staphylococcus aureus*. The plant was subjected to series of phytochemical screening tests and its potency was tested against *E. coli* and *S. aureus* using paper - disc diffusion and broth dilution techniques. Aqueous extract had activity only at its highest concentration (80 mg/ml), while the ethanolic extract had more pronounced activity for both bacterial isolates compared to N- hexane, petroleum ether, chloroform and aqueous extracts. Phytochemical analysis of *Acantospermum hispidium* plant extracts indicated the presence of tannins, saponins, flavonoids, and alkaloids which contain chemical constituents of pharmacological importance. Glycoside was absent in all the extracts.

Keywords: Phytochemicals, antibacterial, *Acantospermum hispidum*

1.0 INTRODUCTION

Staphylococcus aureus and *Escherichia coli* are amongst the most commonly known disease-causing bacteria in present time. There is need for potent curative agents which can be safely used against these and even other pathogens. Many people in developing countries live below the poverty line and so, cannot afford the ‘‘expensive” modern antibiotics (Sale *et al*., 2015). Medicinal plants have served through ages as a constant source of medicament for treatment of a variety of diseases (Okoli *et al.,* 2007). The history of herbal medicine is almost as old as human civilization (Choudhary *et al*., 2015). The plants are known to be used as antihelmintics, antibacterials and antifungals (Kala, 2004). Despite the extensive use of synthetic chemicals in modern clinical practice all over the world, interest in exploiting potential use of plant as a source of drugs are under study (Ayurvedic Pharmacopoeia, 2004). The study of phytochemistry is important and relevant because it helps to impact knowledge of various plant constituents which can be tested for their pharmacological activity (Elof, 1998).

Acanthospermum hispidium belongs to a family of Asteraceae. It is called Kashin yawo (Hausa) in Northern Nigeria. The plant commonly known as Bristly Starbur is an upright dichotomous plant with Y shape branching which gives it one of its common names, Slingshot weed. The

scientific name of the genus, Acanthospermum, is from the greek word *acantha* (thorn) and sperma (seed) which refers to prickly fruit.

Ethnomedicinally, *A. hispidium* is used in the treatment of yellow fever, malaria and stomach disorder (Mann *et al*., 2003). It is also used in some parts of South America as sudorific and diuretic. The plant has being scientifically investigated for its antibacterial and antiviral (Summerfeild *et al* 1997; Anani *et al*., 2000; Kamanzi *et al*., 2002; Fleischer *et al*., 2003; Hoffman *et al*., 2004), antimalaria (Sanon *et al*., 2003; Gafon *et al*., 2012), antitrpanosomal, and antileishmanian (Kamanzi *et al*., 2004; Gafon *et al*., 2012) activity.

In this research, phytochemical screening and antibacterial activity of *A. hispidium* was carried out with the view to further substantiate the earlier claims by various researchers on its potential use in traditional medicine.

2.0 MATERIALS AND METHODS

In this research, leaves of *Acantospermum hispidum* were utilized. The plant was collected from an uncultivated farmland located at the southern geographical area of Usman Danfodio University Sokoto state, Nigeria. It was identified and authenticated (accession number: BUKHAN 136) by Malam Baha'uddeen Sa'eed Adam of the herbarium curator, department of Plant Biology, Bayero University, Kano Nigeria.

2.1 Preparation of Treatment Samples

The leaves and stem of *A. hispidium* were thoroughly washed under running tap, rinsed with distilled water and air dried under shed at an ambient temperature. This was pounded into powder by using a mortar and pestle. The powder was sieved to get a fine powder as described by Fatope *et al* (1993). The content was then stored in a clean container until required for use.

2.2 Extraction Procedure

A quantity of 100g of the fine powder of the plant was weighed and suspended into a 1000 cm^3 capacity conical flask. This was percolated with 500cm^3 of 95% ethanol. This procedure was repeated using distilled water, N- hexane, chloroform and petroleum ether. The mixtures were allowed to stand for two weeks with constant agitation (Sale *et al*., 2015). The percolates were then filtered and the solvents (ethanol, water, N-hexane, chloroform, petroleum- ether) evaporated using rotary evaporator (R110) to obtain extract of the plant in each solvent; this serves as stock solutions. They were stored in a refrigerator at 4^oC until required for use.

2.3 Phytochemical Screening

Phytochemical screening of the extract were carried out to identify the constituents, using standard Phytochemical method as described by Sale *et al*. (2015), Poongothai *et al*. (2011) and Akintobi *et al*. (2013). The screening involves detection of saponin, flavonoid, tannin, alkaloid and glycoside.

2.3.1 Preparation of Extract for Phytochemical Screening

Ethanol, N- hexane, chloroform, petroleum ether and aqueous extracts of *Acantospermum hispidium* leaves were prepared in 20g/200ml and dried at 60^OC protected from light. The residue was weighed and dissolved in 10% known volume of *dimethyl sulphoxide* (DMSO). The extracts were used for the detection of qualitative phytochemical analyses. (Poongothai *et al*., 2011)

2.3.2 Screening Procedure

Simple standard chemical tests were carried out for the qualitative phytochemical screening of *Acantospermum hispidium*. These tests were used to detect the presence of bioactive agents such as alkaloids, tannins, saponins, cardiac glycosides, amino acid, reducing sugar, steroids,

triterpanoids, anthraquinones, etc. The phyto-constituents were assayed for, using standard method as described by (Akintobi *et al*., 2013).

2.3.2.1 Alkaloid Test

Five grams of the *Acantospermum hispidium* extract and 5ml of honey was added to 5ml of 1% aqueous hydrochloric acid (HCl) at 60^{o}C for 5min. The sample was filtered a 3 layered muslin cloth. One milliliter of the filtrate was treated with few drops draggendoff's reagent. Blue black turbidity serves as preliminary evidence of alkaloids. (Akintobi *et al.*, 2013).

2.3.2.2 Saponins Test

Five grams each of the extract and 5ml of honey were shaken separately with distilled water in a test tube. Frothing which persists on warming was taken as an indication for the presence of saponins. (Akintobi *et al.*, 2013).

2.3.2.3 Tannins Test

Five gram each of the extract and 5ml of honey were stirred separately with 100ml of distilled water and filtered. 1ml of ferric chloride reagent was added to the filtrate. A blue black or blue green precipitate was an indication of presence of tannins. (Akintobi *et al.*, 2013).

2.3.2.4 Flavonoids Test

Five milliliters of dilute ammonia solution was added to aqueous filtrate of the test sample followed by the addition of 1ml concentrated H_2SO_4. Yellow coloration indicates the presence of flavanoids (Akintobi *et al* 2013).

2.3.2.5 Glycosides (Keller-Killiani Test)

Five grams of each of the extract and 5ml of honey were dissolved separately in 2ml of glacial acetic acid containing a drop of ferric chloride solution. This was underplayed with 1ml of concentrated H_2SO_4. A brown ring at the interface indicates a deoxy-sugar characteristic of

cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a green ring may form which just gradually spreads throughout the layer (Akintobi *et al.*, 2013).

2.4 Test for Organism

Clinical isolates of bacteria were used for the antibacterial screening. The isolates were *Escherichia coli* and *Staphylococcus aureus*. They were obtained from the laboratory unit of the Department of Microbiology, Usman Danfodio University Sokoto Nigeria and were authenticated using standard biochemical tests as described by Cheesbrough (2002). The isolates were maintained on a freshly prepared nutrient agar slant and kept refrigerated at 4 ^{o}C until required for use.

2.4.1 Preparation of Sensitivity Disc

The sensitivity discs were prepared using sterile Whatman's grade one filter paper Cheesbrough (2002). A paper puncher was used to obtain disc 6.0mm in diameter. These were then placed in sterile screw-capped bottles and sterilized in an oven using dry heat at 140^{o}C for an hour. The discs were allowed to cool until use. Stock solution of the plant extract was prepared: One gram of each fraction was weighed and dissolved in 10 cm^3 of dimethyl sulphoxide to arrive at 100 mg/ml concentration.

2.4.2 Preparation of Inoculum

An overnight broth of the test bacterium was used to prepare standard inoculum of 3.30×10^6 µg/ml. This value was arrived at by approximate dilution of the broth culture in 0.85% sodium chloride solution to which standard turbidity of 1% barium sulphate suspension was compared (Mukhtar and Okafor, 2002).

2.4.3 Sensitivity Test

Agar diffusion method was employed (Kirby *et al*., 1996). The freshly prepared nutrient agar plates were dried in a drier for about 10 minutes to remove surface moisture. The plates were aseptically inoculated uniformly with the test organism by streaking method. With the aid of a sterile forceps, paper discs that have been impregnated with the leaf extracts of *A. hipidium* at different concentrations were arranged radially and pressed firmly onto the inoculated surface with each disc sufficiently spaced out. Positive control discs containing standard antibiotic (Ciprofloxacin) and negative control discs containing neither an antibiotic no the extract were used. The plates were incubated at 37^{O}C for 18 hours in aerobic condition. Diameter of zone of inhibition was measured using a meter rule and was recorded in millimeter (Mukhtar and Okafor, 2002).

3.0 RESULT

Four (4) phytochemical constituents were found in the plant and have demonstrated exciting potentials for the expansion of wide range of modern chemotherapies against a wide spectrum of microorganisms. The phytochemical compounds recovered from extracts of the plant screened were presented in Table 1 below. The Phytochemical compounds found were tannin, flavonoid, alkaloid and saponin. Glycoside was absent in all the extracts.

Antibacterial activity of the various extracts of *Acanthospermum hispidium* was summarized in Table 2 below.

It was also recorded that ethanol extract of *A. hispidium* had a high antibacterial activity on *E. coli* and *S. aureus*. However, the antibacterial activity was seen to be slightly higher in *S. aureus*. While with the aqueous extract, the antibacterial activity was found only at its highest concentration (80mg/ml).

TABLE 1: Phytochemical characteristics of the extracts of Acanthospermum hispidium

Ingredient	N-Hexane extract	Petroleum ether extract	Chloroform extract	Ethanol extract	Aqueous extract
Tannin	+	+	+	+	+
Saponin	+	+	+	+	+
Alkaloid	+	+	-	++	++
Glycoside	-	-	-	-	-
Flavonoid	+	+	+	++	+

Key: - Indicates absent, + indicates present

Table 2: Antibacterial activity of the various extracts of *Acanthospermum hispidium (kashin yawo)*

Plant extract	Concentration of Extract	Inhibition Zone (mm) On *Escherichia coli*	Inhibition Zone (mm) On *Staphylococcus aureus*
N-Hexane	30mg/ml	0.00	0.00
	60mg/ml	16.00	15.00
	80mg/ml	19.00	16.00
Petroleum ether	30mg/ml	19.00	16.00
	60mg/ml	19.00	16.00
	80mg/ml	20.00	16.00
Chloroform	30mg/ml	19.00	19.00
	60mg/ml	19.00	18.00
	80mg/ml	20.00	19.00
Ethanol	30mg/ml	21.00	20.00
	60mg/ml	22.00	22.00
	80mg/ml	22.00	23.00
Aqueous	30mg/ml	0.00	0.00
	60mg/ml	0.00	0.00
	80mg/ml	18.00	16.00

Key: Zone of inhibition in mm, Values greater than 12mm indicate activity

DISCUSSION

The antibacterial activities observed could be due to the presence of secondary metabolites (Table1), which have been reported as active constituents responsible for antimicrobial activities

(Sofowora, 1993; Adoum *et al*., 1997; Oyi *et al*., 2002 and Salihu and Garba, 2008). The observation in this study contradicts (Nair *et al*., 1976; Edewor and Olajire, 2011) who reported that glycoside was present in the plant extract. The chloroform and petroleum ether extracts also showed antibacterial activities though slightly lower than ethanolic extract. N- Hexane had the lowest activity especially against *S. aureus*. N-hexane and aqueous extract only showed antibacterial activities at their highest concentrations and no antibacterial activities was found at low concentrations. This could be due to more dissolved Phytochemicals in the ethanolic extract and hence responsible for the antibacterial activity observed in this study.

The resistance of test bacteria against these extracts at low concentration may be as a result of transfer of resistance plasmids or indiscriminate and sub-therapeutic use of the plant. The non-susceptibility of test bacteria at low concentration of this study may (aqueous and N-hexane) be partly explained by some previous studies of variation in antibacterial activities of different extracts (Bibitha *et al*., 2002). While the antibacterial activities of the plant extract against the test bacteria agree with (Yusha'u *et al*., 2008) who reported that antibacterial activity may vary from one plant to another.

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REFERENCES

Adoum, O. A., Akinniyi, J. A., and Omar. A. (1997). The effect of geographical location on the antimicrobial activities and trace element concentration in the root of Calotropis procera (Ait.) R. Br. *Annals of Brono* 13(14), 199-207.

Akintobi, O. A., Onoh C. C., Ogele J. O., Idowu, A. A., Ojo O. V. and Okonko, I. O. (2013). Antimicrobial activity of Zingiber officinale (Ginger) extract against some selected pathogenic bacteria, *Nature of science*, 11(1).

Anani, K. Hudson, J. B., de Souze, C., Akpagana K., Tower, GHN. Amason J. T. Gbeassor, M. (2000). Investigation of medicinal plant of Togo for Antiviral and Antimicrobial activity. *Pharm. Biol.*38 (1). 40-45.

Bibitha, B., Jisha, V. K., Salitha, C. V., Mohan, S. and Valsa, A. K. (2002). Antibacterial activity of different plant extracts: A short communication *Indian Journal of Microbiology*. 42: 361-363.

Cheesbrough, M. (2002). Distribution laboratory practice in tropical countries. Cambridge University Press, London. 2: 137-140.

Choudhary, M., Kumar, V., Malhotra, H. and Singh, S. (2015). Medicinal plants with potential anti-arthritic activity, *J Intercult Ethnopharmacol*, 4(2): 147-179.

Edewor, T., Olajira, A. (2011).Two flavones from Acantospermum hispidium D.C and their antibacterial activity. *Int. J. Org. Chem*.1 (3): 132-141.

Ellof, J. N. (1998). Which extract should be used for the screening and isolation of antimicrobial components from plants? *Ethanopharmacologia* 60: 1-6.

Fatope, M. O, Ibrahim H. and Takeda, Y. (1993). Screening of higher plant reputed as pesticides using the brine shrimp lethality assay. *International journal of pharmacognosy* 31:250-254.

Fleischer,T. C., Ameade, E. P., Sawer I. K. (2003). Antimicrobial activity of leaves and flowering top of *Acanthospermum hispidium Fitoteerapia.* 74 (1-2): 130-132.

Gafon, H., Bero, J.,Alembert, T., Tchinda A. T., Fernard, G., Gbenou, J., Moudachirou M., Michel, F. M. and Quetin-Leclercq, J. (2012). Antiparasitic activities of two sesquiterpenic lactones isolated from Acanthospermum hispidium D. C. J. *Ethnopharmacol.* 141: 411-417.

Hoffman, B. R., Delasalas, H., Blanco, K. Wiederhold, N., Lewis, R. E. and Williams, L. (2004). Screening of Antibacteria and Antifungal activities of ten Medicinal plants from Ghana. *Pharm. Biol.* 42 (1): 13-17.

Kala C. P., Farooquee N. A. and Dhar U. (2004). Prioritization of medicinal plants on the basis of available knowledge, existing practices and use value status in Uttaranchal, India. *Biodiversity and conversation*. 13:453- 469.

Kamanzi, A. K., schamid C., Brun R., Kone M. W. and Traore, D. (2004). Antitrypanosomal and Antiplasmodial activities of Medicinal plants from Cote D'ivoire *J. Ethnopharmacol*. 90 (2-3): 221-227.

Kamanzi, A. K.,Kone, M., Terreaux, C., Traore, D., Hostettmann K., Dosso M. (2002). Evaluation of the Antimicrobial potential of medicinal plant from the Ivory Coast *Phytother. Res.* 16: 497-502.

Kirby, W. M., Bauer, A. W., Sherris, J. C. and Tutcch, M. C. (1966). Antibiotic susceptibility testing by a standardized single disc method. *American journal of clinical pathology* 45:493-496.

Lutterodt, G. D., Ismail, A., Bashier, R. H. and Baharudin, H. M. (1994): Antimicrobial effects of Psidium guajava extract as one mechanism of its anti-diarrhoeal action. *Malaysian Journal of Medical sciences* 6, 17-20.

Mann, A. Gabate M. Umar, N. A. (2003). Medicinal and Economical plants in Nupeland Jube-Evans books and publication, Bida, Niger State.

Mukhtar M.D and Okafor, M. C. (2002). Antibacteria activity of Gueria senegalensis *jour. Emperic Research*: 1 (2), 166-72.

Nair, A.G., Subramanan, S.S., Bohlmann, F., Schoneweiss, S., Mabry, T. J. (1976). New diterpene galactoside from Acanthospermun hispidium. *Phytochemistry*.15 (11), 1776-1778.

Okoli R.I, Aigbe O., J.O, Mensah J.K, (2007), Medicinal Herbs Used for Managing Some Common Ailments among Esan People of Edo State, Nigeria, Pakistan Journal of Nutrition 6 (5): 490-496.

Oyi, A. R., Onaolapo, J. A., and Adigun, J. O. (2002): Phytochemical and antimicrobial screening of the Latex of Jatropha curcas Linn (Euphorbiaceae). *Journal of phytomedicine and therapeutics* 1(1-2), 63-74.

Oyi, R. A., Onaolapo, J. A., Haruna, A. K. and Morah, C.O. (2007). Antimicrobial screening and stability studies of crude extracts of Jatropha curcas Linn Latex (Euphorbiaceae). *Nigeria Journal of pharmaceutical sciences* 6(2), 14.

Poongothai, A., Sreena, K.P., Sreejith, K., Uthiralingam M. and Annapoorani, S. (2011). Preliminary phytochemicals screening of ficus racemosa linn back, *International Journal of Pharmaceutical and Biological Sciences* vol. 2 issue 2, ISSN 0975-6299

Salihu, L. and Garba, S. (2008). Preliminary investigation of the bioactive constituents of some medicinal plants. *Biological and Environmental sciences Journal for the Tropics*. 5(1), 164-168.

Saleh, S. B., Ibrahim, T., Salisu, A. R., Shaida, S. S., Jibril, H., Tela, I. H., Abdullahi, S. S., Adekemi, E. A., Kabir, H. M., Bala, I. and Suleiman A. (2015) Antitrypanosomal Activity of Leaf Extracts of *Acalypha Wilkesiana*. International Journal of Life Science and Engineering Vol. 1, No. 3, 2015, pp. 114-119.

Sanon, S., Azas, N., Gasquest, M., Olivier, E.,Mahrou, V., Baro, N., Cuzin-Quattara, N., Traore, A. S., Esposito, F., Balasard, G., Timon-David, P. (2003). Antiplasmodium activities of alkaloid

extract from pavetta crassipe and Acanthospermum hispidium, two plants use in traditional Medicine in Bukinsfaso. *Paracitol.Res.* 90 (4), 314-317.

Sofowora, A. (1993). Medicinal plant and traditional mecicine in Africa. Spectrum books Limited, Ibadan, Nigeria. 220-224.

Summerfield A. Keil G. M. Menttenlerter T. C., Rziha, J. H. Saalmuller,A. (1997). Antiviral activity of an extract from leaves of the Tropical plant Acanthospermum hispidium *Antivi. Res.* 36: 55-62.

The Ayurvedic Pharmacopoeia of India. (2004). Government of India Ministry of Health and Family Welfare. Part 1, vol. 1.

Trease, G. E. and Evans, W. C. (1989). Pharmacognosy 1st edition. Amadu Bello University press Limited, Zaria, Nigeria. 188- 198.

Yusha'u, M., Bukar, A. and Balarabe, A. I. (2008). Prevalence and sensitivity of enterobacterial isolates from patients with urinary tract infections to *Acalypha wilkisenia* extracts. *Biological and environmental sciences Journal for the tropics.* 5(3), 72-76.