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Original Research Article

Phytochemicals Screening and Antimicrobial Activity of the Stem Bark Extracts of *Azanzagarckeana* (kola of Tula)

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Abstract

Azanzagarckeana known as Kola of Tula is one of the vulnerable medicinal plant of Tula people in Gombe state, North Eastern Nigeria and are usually collected from the wild. In the present study the photochemical analysis and the antimicrobial activity of the extracts of the steam bark of the plant were investigated. Methanol ethanol, acetone, petroleum ether and water were used as the extrants. Serial extraction using Soxhlet apparatus was used. The result of the study shows that the water extract indicate the presence of carbohydrate and flavonoid in appreciable amount, while the methanol extract showed the presence of flavonoid, cardiacglycoside and alkaloid in an appreciable amount. Tannins, phlobatannin, flavonoid, quinoline alkaloid were detected in the acetone extracts while saponnin and haemolytic activity were seen in appreciable quantities in the ethyl acetate extracts. The petroleum ether extract showed only steroid in an appreciable quantity. Antimicrobial activity was carried out using Disc diffusion method. Nine microorganisms; S. aureus, salmonella typi, E. coli, K. pneumonia, Klebsiella ssp, P. aeroginosa, Shigella dysentriae, Bacllus subtilis and Nissera gonorriea was used for the antimicrobial test. Ampiclox was used as standard reference drug. The antimicrobial activity of the extracts showed that the ethyl acetate extract had the best antimicrobial activities by inhibiting the growth of eight of the test organisms. The methanol and acetone extract inhibited the growth of four microorganisms whereas the water extract inhibited the growth of three organisms. The petroleum ether was the least active extract because it inhibited the growth of only one organism. E. coli, S. aureus and salmonella typhi were the most sensitive organisms because they were susceptible to three organisms each.

Keywords: Azanzagarckeana, stem bark, Phytochemical and antimicrobial activity.

INTRODUCTION

The local people have a long history of traditional plant usage for medicinal purposes. Plants are a source of large amount of drugs comprising of different groups such as antispasmodics, emetics, anti-cancer, antimicrobials etc. A large number of plants are claimed to possess the antibiotic properties in traditional system and are also used extensively by the tribal people worldwide. It is now believed that nature has given the cure of every disease in one way or another (Prashant *et al*., 2011). The plant kingdom represents an enormous reservoir of biologically active compounds with various chemical structures and protective/disease preventive properties (phytochemicals). (Shakeri *et al*., 2012).

The potential of higher plants as source for new drugs is still largely unexplored. Among the estimated 250,000-500,000 plant species, only a small percentage has been investigated phytochemically and the fraction submitted to biological or pharmacological screening is even smaller (Mahesh and Satish, 2008).

Many medicines including strychnine, aspirin, vincristine, and taxol are of herbal or plant origin (Farnsworth *et al*., 1985; Agyare *et al*., 2006).. The increasing use or overuse of antibiotics in the treatment of bacterial infections is bringing on an increase in pathogenic organisms that are resistant to available antibiotics (Agyare *et al*., 2006). Synthetically made therapeutic drugs have over the years developed problems such as toxicity, resistance by micro-organisms allergy, super infection or even addiction (Sofowora, 1982; Adamu *et al*. 2013:; Farnsworth, 1993).

Consequently, it is necessary to increase administered doses, combine antibiotics or provide new

antibiotics with lesser tendency for pathogenic organisms to develop resistance to.

The medicinal actions of plants are unique to a particular plant species or group. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. These biologically active compounds with various chemical structures and protective/disease preventive properties (phytochemicals) are often secondary metabolites present in smaller quantities in higher plants. The most important of these bioactive constituents of plants are alkaloids, saponins, terpenoid, tannins, flavonoids and phenolic compounds. Secondary metabolites are the classes of compounds which are known to show curative activity against several ailments in man, and therefore could explain the traditional use of medicinal plant for the treatment of some illnesses.

In continuation of our efforts to verify the efficacy of traditional medicine we have collected the stem bark of Azanzagarckeana (Kola of Tula) in order to carry out phytochemical screening and to investigate the in vitro antibacterial activity.

The plant Azanzagarckeana, common names-Goron Tula,(kola of Tula); a place in Gombe Nigeria, Burkill, (1985). Belong to the family Malvaceae, in the order, Malvales was reported to have some medicinal values; A decoction made from the roots are taken orally for painful menstruation and to treat coughs and chest pains. An infusion made from the roots and leaves is dropped into the ear to treat earache or taken orally as an antiemetic, (Alfred 2013). A decoction is made from the root for, treatment of venereal diseases and to treat coughs and chest pains. It is taken as a treatment drug for infertility and a drug that causes evacuation (purgative). A paste made from pounding its fruits is applied onto the cheek with abscess to draw it and onto boils in the mouth for relief. An infusion made from both its stem and leave is taken to treat liver problem.

MATERIALS AND METHOD

Collection of plant materials

The stem bark of Azanzagarckeana was collected in Tula Wange (Tantan) and Yiri (Bwane), kaltungo local Government Area of Gombe State in April when the leaves were green. The fresh stem bark were removed, air dried under shade in the laboratory and pulverized using motorized miller.

Extraction of plant material

80 gram of the powdered stem bark of Azanza garckeana were serially extracted with hexane, ethyl acetate, acetone, ethanol and distilled water using soxhlet extractor apparatusfor 8 hours each. The extracts were evaporated to dryness on rotary evaporator, the percentage yield of the extracts were then determined.

Phytochemicals screening of the crude extracts

Phytochemical screening were carried out on the crude extract of the stem bark of Azanzagarckeana by soxhlet extraction method using standard procedures to identify the phytochemical constituents as described by (Harborne, 1973; Trease and Evans, 1989; Sofowora, 1993; Mshelia *et al*., 2007; Victor and Chidi,2009).

Source and Maintenance of Organism

Both Gram negativeand Gram positivebacteria were obtained and confirmed at the research laboratory of the Department of medical microbiology and Parasitology, Federal Teaching Hospital, Gombe. They were maintained on Muller-Hinton agar (MHA) (Oxford, UK) where the isolated colonies were obtained.

Disc-Agar Diffusion Method

Plant extracts were tested for antibacterial activity by the disc diffusion method. A single colony was aseptically transferred with an inoculating loop to about 20 ml of the prepared nutrient agar.

Filter papers are cut out with a diameter of 10mm. The filter paper is then transferred to the oven and sterilized for one hour. Using sterilized forceps, the filter papers were then transferred to the various extracts at a concentration of 1000µg/ml. The filter papers were left in the extracts for about 20 minutes so as to soak the extracts very well. The filter papers were then transferred to a cultured agar plates, the plates are then incubated at 37ºC for 24 hours in the incubator. Standard ampiclox and negative controls with acetone were also performed.

RESULTS AND DISCUSION

The percentage recovery of the extracts showed that water gives (12.0%), Methanol (9.16%), Acetone (8.50%), Ethylacetate (6.39%) and petroleum ether (8.62%) yields.

Table-1: Phytochemical screening of the stem bark of azanzagarckeana

		Extracts				
S.N	**TEST**	**water**	**methanol**	**acetone**	**Ethyl acetate**	**Petroleum ether**
1	Carbohydrate-**Mollish's test** -Barfoed test	+++ +++	++ ++	++ ++	+ -	- -
2	Tannins –Bromine water -Ferric chloride test -formaldehyde test	- ++ -	++ + -	- +++ -	- - -	- - -
3	Phlobatanin-HCl test -Lime water tset	- -	- +	++ +++	- -	- -
4	Flavonoid – Lead acetate test -Pew test	+++ +++	++ +++	+++ +++	+ ++	- -
5	Cardiac glycoside	-	+++	++	++	+
6	Cyanogenic glycoside- sodium picrate paper test	-	+	++	++	-
7	Chlorogenic acid	-	-	-	++	+
8	Saponin-froth test	+	++	++	+++	-
9	Haemolysis	-	++	+	+++	-
10	Alkaloid General test Drangedoff -Mayers test -wagner Quinoline alkaloid Indole alkaloid	 + + + ++ -	 ++ +++ ++ + +	 ++ - - +++ ++	 ++ ++ - ++ -	
11	Anthraquinone (Borntrager test)	+	+	+	+	+
12	Terpens and steroids -Lieberna-Burchard -Salkowski test	 - ++	 - ++	 - ++	 + ++	 ++ +++

(+++): Appreciable quantity (++): Moderate quantity(+): Traces (-): Negative test (absence of turbidity, flocculation and precipitation).

Table-2: Antibacterial activity of stem bark extracts from Azanzagarckeana showing zone of inhibition in millimeter

Bacterial strain	**Zone of inhibition in millimeter.**						
	Water	Methanol	Acetone	Ethyl acetate	Petroleum ether	Ampiclox	Acetone
E. coli	25	0	15	20	0	22	0
Klebsiellaspp	20	23	0	0	0	32	0
K. pneumonia	0	0	14	20	0	18	0
S. aureus	0	15	0	24	22	30	0
P. aeruginosa	0	0	24	16	0	10	0
Salmonella typi	0	6	6	22	0	16	0
Shigelladysentriae	0	0	0	16	0	20	0
Bacillus subtilis	8	0	0	8	0	26	0
Nisseragonorrieae	0	4	0	4	0	22	0

RESULT

The yields of the crude stem extracts are as follows; Water extracted the largest quantity 9.60g representing 12.0%. This is followed by methanol stem bark extract constituting 9.16%, petroleum ether 8.62%, acetone 8.50%. The ethyl acetate extracts of stems bark; yield the smallest percentage constituting 6.39%.

Table 1 showed the result for the phytochemical screening of the stem bark of Azanzagarckeana (kola of Tula). The water extract showed appreciable amount of carbohydrate using the two methods used while the methanol and acetone extracts showed moderate amount using both method. The petroleum ether extract showed total absence of using both reagent while the ethyl acetate extract showed traces for general test for carbohydrate using Mollisch's test. Three methods were used to determine the presence of tannin in the stem bark. The acetone extract showed appreciable amount of tannins using ferric chloride test, while the water extract and the methanol extract showed moderate amount of tannin when tested with ferric chloride and bromine water respectively. The ethyl acetate and petroleum ether extracts showed total absence of tannin. Phlobatannin

was totally absence in the water, ethyl acetate and petroleum ether extracts, while the acetone extract showed moderate and appreciable amount of phlobatannin with the two reagents used, while the acetone extract showed traces with lime water test. Lead acetate and Pew test were used to determine the presence of flavonoid in the stem bark. The water and acetone extract showed appreciable amount of flavonoid with the two methods used, while methanol extract showed appreciable amount of flavonoid using Pew test. The petroleum ether extract showed total absence of flavonoid whereas the ethyl acetate showed traces and moderate amount with the respective method. Appreciable amount of cardiac glycoside was detected in the methanol extract while moderate amount was detected in the acetone and ethyl acetate extract and the water extract showed total absence of the cardiac glycoside. The cyanogenic glycoside was detected in in moderate amount in acetone and ethyl acetate with traces in methanol while the remaining extracts showed total absence. Moderate and traces of chlorogenic acid was detected in the ethyl acetate and petroleum ether extract respectively, while the remaining extracts showed total absence. The ethyl acetate extract showed appreciable amount of saponnins, while moderate amount was seen in methanol and acetone extracts and the remaining extracts showed total absence. The ethyl acetate extract showed appreciable haemolysis activity on human blood, while the water and petroleum extract does not show any effect. All the extracs showed the presences of alkaloid with at least one of the test method used. The methanol extracts showed positive test with all the method used, while water extract showed positive with four of the method while acetone and ethyl acetate showed positive test with three of the method. All the extracts showed traces of anthraquinone. The ethyl acetate and petroleum ether extracts showed traces and moderate amounts of triterpenoid respectively, while all the remaining extracts showed total absence. The presence of steroid was detected in all the extracts with appreciable amount seen in the petroleum ether extract while all the remaining showed moderate amount.

Table 2 showed the activity of the extracts on microorganisms. The result showed that ethyl acetate extract was the most active extract by inhibiting the growth of eight test organisms followed by petroleum ether extract. The methanol and acetone extracts inhibited the growth of four organisms while the least active is the water extract that inhibited the growth of three organisms. The water extract showed the largest zone of inhibition (25mm) on E.coli, followed by acetone extract on P.aeruginosa and ethyl acetate on S. aureus. The result showed that some of the extracts showed larger zone of inhibition compared to the standard ampiclox. It can be seen that the water extract showed zone of inhibition of 25mm compared to the standard ampiclox for E. coli, while the ethyl acetate extract showed zone of inhibition of 20mm compared to the standard with only 18mm on K. pneumonia. All the extracts that showed activity against P. aeruginosa had higher zone of inhibition compared to the ampiclox standard. The activity of ethyl acetate extract on salmonella typi is higher than the standard ampiclox.

DISCUSION

The biological activity of plant depends on many factors like plant parts, geographical source soil conditions and time of the harvest, moisture content and post-harvest processing method. For instance high temperature during drying, grinding or extraction may denature certain chemical constituents. Different concentrations of solvent or types of solvents are used for extractions of bioactive compounds, because different plants constitute different composition of active compounds. The qualitative phytochemical analysis for carbohydrate, tannins, phlobatannin, flavonoids, cardiac glycoside, cyanogenic glycoside, chlorogenic acid, saponnin, haemolysis, alkaloid, anthraquinoneterpenes and steroid were screened in stem bark extract of Azanzagarckeana plant. The screening of the extracts indicate the presence of carbohydrate, flavonoid, saponnin, alkaloid and steroid in the water extracts while the methanol extract showed the presence of carbohydrate, tannin, flavonoid, cardiac glycoside, saponin, alkaloid and steroid. The alkaloid, steroid, saponnin, cardiac and cyanogenic glycosides, flavonoid, phlobatannin and carbohydrate was seen in the acetone extract. The ethyl acetate shows appreciable quantity of saponnin and haemolysis and moderate amount of alkaloid, flavonoid, cardiac and cyanogenic glycoside and steroid. The petroleum ether showed appreciable amount of steroid. The result showed that more of the compounds were extracted by the polar solvents, while only the steroids were more readily extracted by the non-polar solvent.

Several phyto constituent like flavonoids (Tsuchiya *et al*., 1990; Cushinin and Lamb, 2005: De-Sausa *et al*. 2007), Phenolic and poly phenols (Mason and Lalasserman, 1987), Tannins (Rievere *et al*., 2009; Teklit and Firehiwot, 2016) terpenoids (Scorticini and Pia Rossi 1991) Sesquiterpenes (Goren *et al*., 1996) ; saponnins(Mandal *et al*. 2005; Manjunatha 2006); Steroids (Manickam and Veerabahu, 2014) etc are effective antimicrobial substances against wide range of microorganisms. The ethyl acetate extract showed best antibacterial activity which may be due to the presence of saponin, flavonoid, alkaloid or steroid which were detected in the ethyl acetate extracts. The active compounds extracted by ethyl acetate are not very polar or non-polar, because it is soluble in the ethyl acetate. The activity of the acetone extract may be due to the presence of flavonoid which was detected in appreciable amount (Korkina and Afanasiev, 1997). The activity can also be attributed to the presence of saponin (Mandal *et al*. 2005, Manjunatha, 2006). Alkaloids were present in the acetone, methanol and water extracts which may be responsible for the activity

of this extracts. The flavonoid which is referred to as natures store of bioactive compounds may also be responsible for the activity of the methanol and water extracts because they were seen in appreciable quantity.

Various workers have shown that gram positive bacteria are more susceptible toward plants as compared to gram negative bacteria as in this study (Lin *et al*., 1999; Parekh and Chanda, 2006). This difference may be attributed to the facts that the cell wall in gram positive bacteria is of a single layer, whereas the gram negative cell wall is multilayered structure (Yao *et al*., 1995). Therefore the passage of active compound through the gram negative cell may be inhibited to certain level by the double layer. The water extract had high activity against E.coli and Klebsiella species which may be due to the presence of selective active compound which is highly polar in nature and are extracted by water.

CONCLUSION

Azanzagarckeana known as Kola of Tula is one of the vulnerable medicinal plant as it was assayed for anti-bacterial activity and most of the extracts were found to be active against the selected species of microorganism. This support the use of the plants by the natives of Tula for the treatment of diseases. Water extract was found to be more effective against bacterial strain of E. coli. The result showed that some of the extracts showed larger zone of inhibition compared to the standard ampiclox. It can be seen that the water extract showed zone of inhibition of 25mm compared to the standard ampiclox for E. coli. All the extracts that showed activity against P. aeruginosa had higher zone of inhibition compared to the ampiclox standard. The activity of ethyl acetate extract on salmonella typi is higher than the standard ampiclox.

RECOMENDATION

It is recommended that further analysis on minimum bacteriocidal concentration (MBC) to be carried out to determine whether the plant extract is bacteriocidal or bacteriostatic. There is also the need to carry out the analysis on the anti-oxidant and cytotoxity of the plant to accertain its safety and efficacy for use as medicinal drug.

REFERENCES

- Adamu, H.M., Ushie, O.A., Lawal, D.S., Oga, I. A. (2013). Phytochemical Screening of Fruit of Azanzagarckeanaand Root of Acacia macrothyrsa. International Journal of Traditional and Natural Medicines, 3(1)19-25. Retrieved on 10/12/2014, available at www.modernscientificpress.com/journal/IJTNM.aspx.
- Agyare, C., Mensah, A. Y., & Osei-Asante, S. (2006). Antimicrobial activity and phytochemical studies of some medicinal plants from Ghana.
- Amabye, T. G., & Tadesse, F. M. (2016). Phytochemical and antibacterial activity of moringa oleifera available in the market of Mekelle. Journal of Analytical & Pharmaceutical Research, 2(1), 1-4. .Trease, & Evans. (1989).Text Book of Pharmacognosy 12th ed., ELBS Publications..49, 126, 132-137, 205, 248.
- Burkill, H.M. (1985). The useful plants of west tropical Africa, 4.
- Cushnie, T. T., & Lamb, A. J. (2005). Antimicrobial activity of flavonoids. International journal of antimicrobial agents, 26(5), 343-356.
- Farnsworth, N. R. (1993). Biological approaches to the screening and evaluation of natural products. Biological evaluation of plants with reference to the Malagasy flora, Madagascar, 35-43.
- Gören, N., Woerdenbag, H. J., & Bozok-Johansson, C. (1996). Cytotoxic and antibacterial activities of sesquiterpene lactones isolated from Tanacetum praeteritum subsp. praeteritum. Planta Medica, 62(05), 419-422.
- Harborne, J.B. (1973). Phytochemical methods. Chapman and Hall London, 113.
- Korkina, L.G., Afanas'ev, I.B. (1997). Antioxidant and chelating properties of flavonoids. Adv Pharmacol, 38; 151-163.29.
- Lin, J., Opoku, A. R., & Geheeb-Keller, M. (1999). HutchingsAD., Terblanche, SE., Jager, A., Van, J. J. Ethnopharmacol, 68, 267.
- Lin, J., Opoku, A.R., Geheeb-Keller, M., Hutchings, A.D., Terblanche, S.E., Jager, A.K., van Staden, J. (1999). Preliminary screening of some traditional zulu medicinal plants for anti-inflammatory and antimicrobial activities. J Ethnopharmacol. 68; 267-274.
- Mahesh, B., & Satish, S. (2008). Antimicrobial Activity of Some Important Medicinal Plant Against Plant and Human Pathogens. World Journal of Agricultural Sciences, 4(S); 839-843.
- Mandal, P., Babu, S. S., & Mandal, N. C. (2005). Antimicrobial activity of saponins from Acacia auriculiformis. Fitoterapia, 76(5), 462-465.
- Manjunatha, B. K. (2006). Antibacterial activity of Pterocarpus santalinus. Indian journal of pharmaceutical sciences, 68(1), 115.
- Mason, T. L., & Wasserman, B. P. (1987). Inactivation of red beet beta-glucan synthase by native and oxidized phenolic compounds. Phytochem. 26, 2197-2202.
- Mshelia, E. H., Zaria, L. T., Mohammed, A. H., & Jaji, N. (2008). Phytochemical Analysis and Antibacterial Screening of Asparagus Flagellaris (Kunth) Bak used in The Traditional Treatment of Sexually Transmitted Diseases and Urinary Infections. Ethiopian Journal of Environmental Studies and Management, 1(2), 44-48.
- Murugan, M., & Mohan, V. R. (2014). Phytochemical, FT-IR and antibacterial activity of

whole plant extract of Aerva lanata (L.) Juss. Ex. Schult. Journal of Medicinal Plants Studies, 4(3), 51-57.

- Njoku, V. O., & Obi, C. (2009). Phytochemical constituents of some selected medicinal plants. African Journal of Pure and Applied Chemistry, 3(11), 228-233.
- Parekh, J., & Chanda, S. (2007). In vitro antibacterial activity of the crude methanol extract of Woodfordia fruticosa Kurz. Flower (Lythraceae). Brazilian Journal of Microbiology, 38(2), 204-207.
- Rivière, C., Pieters, L., Dejaegher, B., Vander Heyden, Y., Van, M. C., & Quetin-Leclercq, J. (2009). Polyphenols isolated from antiradical extracts of Mallotus metcalfianus. Phytochemistry, 70(1), 86-94.
- Ruela de Sousa, R. R., Queiroz, K. C. S., Souza, A. C. S., Gurgueira, S. A., Augusto, A. C., Miranda, M. A., ... & Aoyama, H. (2007). Phosphoprotein levels, MAPK activities and NFκB expression are affected by fisetin. Journal of enzyme inhibition and medicinal chemistry, 22(4), 439-444.
- Scortichini, M., & Rossi, M. P. (1991). Preliminary in vitro evaluation of the antimicrobial activity of terpenes and terpenoids towards Erwinia amylovora (Burrill) Winslow *et al*. Journal of Applied Bacteriology, 71(2), 109-112.
- Shakeri, A., Nourallah, H., Jafar, V., Ali, G., Fatemeh, Z.T. (2012). Phytochemical Screening, Antimicrobial and Antioxidant Activities of Anabasis Aphylla L. Extracts. Kragujevac J. Sci. 34; 71-78.
- Sharma, D. C., Shukla, R., Ali, J., Sharma, S., Bajpai, P., & Pathak, N. (2016). Phytochemical evaluation, antioxidant assay, antibacterial activity and determination of cell viability (J774 and THP1 alpha cell lines) of P. sylvestris leaf crude and methanol purified fractions. EXCLI journal, 15, 85.
- Sofowora A. (1982). Medicinal plants and traditional medicine in West Africa. New York: Wiley,
- Sofowora, A. (1993). "Medicinal Plants and Traditional Medicine in Africa" Spectrum Books Ltd., Ibadan, Nigeria., 191-289.
- Tiwari, P., Bimlesh, K., Mandeep, K., Gurpreet, K., & Harleen, K. (2011). Phytochemical screening and Extraction: A Review. Internationale Pharmaceutica Sciencia, 1 (1), 98-106.
- Tsuchiya, H., Sato, M., Miyazaki, T., Fujiwara, S., Tanigaki, S., Ohyama, M., Tanaka, T. and Iinuma, M. (1996). Comparative study on the antibacterial activity of phytochemical flavanones against methicillin resistant Staphylococcus aureus. J. Ethnopharmacol., 50; 27-34.
- Yao, J., Moellering, R. (1995). Antibacterial agents. In: Manual of Clinical Microbiology, Murray P, Baron E, Pfaller M, Tenover F, Yolken R (Eds), ASM, Washington DC, 1281-1290.