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Phytochemicals Screening and in Vitro Antimicrobial Activity of the Root Bark Extracts of A*zanza garckeana* (kola of Tula)

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Abstract: *Petroleum ether, ethyl acetate, acetone, methanol and water extracts from the root bark of the plant Azanza garckeana were subjected to phytochemicals and antimicrobial screening. The results of the phytochemical screening showed that water extract showed the presence of carbohydrate and flavonoids in high concentration and alkaloid, quinoline and anthraquinone in a moderate quantity. Methanol extract indicated the presence of flavonoids, cardiac glycoside and alkaloids in appreciable quantity while carbohydrate, tannin, phlobatannin, saponin in a moderate quantity. Acetone extract showed the presence of phlobatannin, flavonoids and quinoline in appreciable quantity where as carbohydrates, tannin, cardiac glycoside, saponin, alkaloid, anthraquinone, terpenes and steroids were in moderate quantities. Ethyl acetate extract of the root bark indicated the presence of flavonoids, cyanogenic glycoside and saponin in appreciable quantity and showed moderate amount of alkaloid, anthraquinone, terpenes and steroid, cardiac glycosides and chlorogenic acid. Petroleum ether extract showed the presence of cardiac glycoside only in moderate quantity. The antimicrobial activity shows that the ethyl acetate extract inhibited the growth of eight organism followed by the acetone extract. The petroleum ether extract showed least activity by inhibiting the growth of only three organisms while the water and methanol extracts inhibited the growth of six and five organism respectively. Shigella dysentreae and Staphylococcus aureus were the most susceptible organism by being sensitive to all the test extracts at various degree, while salmonella typhi and P. aeruginosa were the most resistant organisms by being resistant to three of the test extracts.*

Key words: *Azanza garckeana, root bark, Phytochemical and antimicrobial activity*

Introduction

The use of herbal medicine for the treatment of diseases and infections is as old as mankind. The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. The earliest mention of medicinal use of plants in Hindu culture is founds in “Rigveda”, which is said to have been written between 4500 - 1600 B.C. and is supposed to

be the oldest repository of human knowledge (Joshi *et al*, 2011).

Plants are the richest resource of drugs in traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. With the increasing incidence of diseases caused by bacteria and other pathogenic microorganisms as well as the development of drug resistance organisms, there is an urgent need to search for alternatives from plants and other sources to combat these pathogens. 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)

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, terpenoids, tannins, flavonoids and phenolic compounds.

Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The harmful effect of microorganisms can be controlled with drugs and these results in the emergence of multiple antimicrobial and it has created alarming clinical situations in the treatment of infections. The increasing incidence of antibiotic resistance among bacterial pathogens necessitates medicinal plants as an alternate therapy in restricting the resistant infectious organisms.

In general, bacteria have the genetic ability to transmit and acquire resistance to synthetic drugs which are utilized as therapeutic agents. There is a wide variation in the susceptibility of organisms to toxic compounds. It is probable that a large number of plants with biological activities remain untested. Screening of plant extracts for anti-microbial activity has given interesting results, in that most plant – derived anti-bacterial and anti-fungal compounds inhibit the growth of the organisms. 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).

This work will look at the phytochemical constituent present in the root bark of Azanza garckeana and its antimicrobial activity on some selected microorganism. The plant A*zanza garckeana*, common names- Goron Tula,(kola of Tula); Burkill, (1985). The plant 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. The plant is also 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 plant sample (root bark) of *Azanza garckeana* 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 root bark were removed and the soil particles removed, it is then air dried under shade in the laboratory and pulverized using motorized miller.

Extraction of plant material The pulverized powdered root bark of Azanza garckeana were serially extracted with hexane, ethyl acetate, acetone ethanol and distilled water using soxhlet extractor for 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 root bark of Azanza garckeana obtained by soxhlet extraction method using standard procedures to identify the phytochemical constituents by characteristic of colour changes as described by (Harborne, 1973; Trease and Evans, 1989; Sofowora, 1993; Mshelia *et al,* 2008; Victor and Chidi,2009).

Source and Maintenance of Organism Gram negative and Gram positive were obtained and confirmed at the laboratory of the Department of medical microbiology and parasitology, Federal Teaching Hospital, Gombe. They were maintained on Muller-Hinton agar (MHA) (Oxoid, UK) to obtain isolated colonies.

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 1cm. the filter paper is then transferred to the oven and sterilized for one hour. Using sterilized forceps, the filter papers are then transferred to the various extracts. The filter paper is left in the extracts for about 20 minutes so as to soak the extracts very well. The filter papers are then transferred to a cultured agar plates, the plates are then incubated at 37ºC for 24 hours in the incubator. Negative controls were performed using paper discs loaded with acetone and standard loaded with ampiclox. The zone of inhibition was determined after the 24hours.

RESULTS

The percentage recovery of the extracts showed that water (10.78%), Methanol (8.90%), Acetone (9.14%), Ethylacetate (7.68%) and petroleum ether (8.50%)

TABLE 1: PHYTOCHEMICAL SCREENING OF THE ROOT BARK OF A*zanza garckeana*

S.N	TEST	Rw	RM	RA	REA	RPE

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 root extracts from *Azanza garckeana* (mg/ml)

Bacterial strain	Extracts\zone of inhibition (mm)						
	Water	**Methanol**	**Acetone**	**Ethyl acetate**	**Petroleum ether**	ampiclox	Acetone
E. coli	*18*	*0*	*20*	*23*	*0*	22	0
Klebsiella spp	*0*	*18*	*25*	*23*	*0*	32	0
Klebsiella pneumonia	*14*	*0*	*0*	*20*	*25*	18	0
Staphylococcus aureus	*28*	*20*	*24*	*22*	*22*	30	0
Salmonella typi	*0*	*10*	*6*	*0*	*0*	16	0
P. aeruginosa	*14*	*0*	*0*	*20*	*0*	20	0
Shigella dysentriae	22	23	3	22	4	26	0
Bacillus subtilis	6	0	16	20	0	22	0
Nissera gonorrieae	0	8	12	24	0	10	0

RESULTS

The phytochemical screening of the root bark showed the water extract shows appreciable amount of carbohydrate when tested for general test for carbohydrate and moderate quantity when barfoed test was used for both the water and the methanol extracts. The acetone and methanol extracts showed moderate amount of carbohydrate when tested with Mollisch's method, while the ethyl acetate and petroleum ether extracts showed total absence. The petroleum ether, ethyl acetate and water extracts showed total absence of tannins while methanol extracts showed moderate and traces of tannins with all methods used while the acetone extract showed moderate amount of tannin when ferric chloride test was conducted and traces with formaldehyde test. Phlobatannin was only detected in moderate amount in the acetone extracts while only traces were seen in the methanol extract and all the remaining extracts showed total absence of phlobatannin. The water extract showed total absence of cardiac glycoside while moderate amount was seen in acetone, ethyl acetate and petroleum ether extracts and appreciable amount was detected in the methanol extracts. Appreciable amount of cyanogenic glycoside was detected in ethyl acetate, moderate quantity in the acetone extract while traces were detected in methanol and petroleum ether extract while water extracts showed total absence. The presence of chlorogenic acid was detected only in the ethyl acetate while all the remaining extracts showed total absence of the chlorogenic acid. The ethyl acetate extract showed appreciable amount of saponnin and haemolysis activity while both methanol and acetone extracts showed moderate amount of the two phytochemicals. All the extracts with exception of petroleum ether extract showed various amount of alkaloid. Anthraquinone was detected in all the extracts just as the flavonoid. Steroid was detected only in the petroleum ether extract while all the extracts showed the presence of terpenoid in moderate or trace quantity.

All the extracts showed activity against at least one of the test organism. The ethyl acetate extract showed highest activity by inhibiting eight of the test organism followed by the acetone extract that inhibited seven organisms. The petroleum ether extract was the least active because it inhibited the growth of only three organisms followed by methanol extract that inhibited the growth of four organisms and then the water extract. The water extract showed the largest zone of inhibition against S. aureus (28mm) followed by the petroleum ether (25mm) against K. pneumonia and the acetone extracts (25mm) against Klebsiella spp. .

DISCUSSION

The presence of wide range of phytochemical constituent indicates that the plant could be used in a multitudes of ways which may be beneficiary to the population (Parekh and Chanda 2007) An important part of natural product form plants, bio molecules and secondary metabolites usually exhibits some kind of biological activities (Sofowora, 1996). They are widely used in the human therapy, vetenary, agriculture, scientific research and in countless other areas (Bhattarai *et al*. 2008). The usefulness of plant material medicinally is due the presence of bioactive constituents such as Alkaloid, tannins flavonoids and phenolic compounds (Meleses *et al,* 2016)

The antimicrobial activity is contributed by essential oil, flavonoids, triterpenoid and other natural poly phenolic compounds found in the plant (Yogesh *et al,* 2016). Plant essential oils and extracts have been used for many thousands of years in food preservation, pharmaceutical, alternative medicine and natural therapies, it is necessary to investigate those plants scientifically which have been used in traditional medicine to improve the quality of health care. Plant extracts are potential sources of novel antimicrobial compounds especially against bacterial pathogens. In vitro studies in this investigation showed that the root extracts of **A*zanza garckeana*** inhibited bacterial growth but their effectiveness varied. The antimicrobial activity of plant extracts previously reviewed are classified as strong, medium or weak.

The ethyl acetate extracts inhibited highest number of micro organisms which can be due the presence of flavonoid in the extracts. The amount of the flavonoid is also high in the water; methanol and acetone extracts which may be responsible for their high degree of activity on the test organisms. Marjorie (1999); Preeti *et al* (2014) have observed that the presence of flavonoid in a plant extract shows good antimicrobial activity. The water, methanol and acetone extracts also showed appreciable and moderate amount of alkaloid and may also be responsible for the inhibitory activity of the extracts on the test organisms (Rajani *et al,* 2016; Teklit *et al* 2016; Rhoades 1979; Akindele *et a*l 2007). The amount of saponnin is directly proportional to the heamolytic effect of the extract as shown in the result. The presence of saponin in the extract may be the responsible for the heamolysis of the red blood cell just as observed by Winter *et al,* 2011.

The petroleum ether extracts showed high zone of inhibition against K. pneumonia and S. aureus, may be due to an active constituents which are selective on the organisms. The demonstration of antimicrobial activity of the root extract of Azanza garckeana against both gram positive and gram negative bacteria may be indicative of the presence of broad spectrum of anti biotic compound.

The optimal effectiveness of the plant extracts may not be due to one main active constituent but may probably be due to the combined action of different compounds in the plant (Bhandarkar *et al*, 2003). The selective activity of the petroleum ether extract towards certain bacteria may be due to the presence of lipopolysaccaride in outer membrane of gram negative bacteria, which act as a permeability barrier and restricts diffusion of active compounds through its lipopolysaccaride covering (Niv and Yechiel, 2005; Zaria *et al*, 1995). Unlike gram negative bacteria gram positive bacteria allow the direct contact of the extract constituent with the phospholipid bilayer of the cell membrane causing either enhanced ion permeability, leakage of vital intracellular constituents or impairment of the bacterial enzyme systems (Zhao *et al,* 2001).

CONCLUSION

The present study revealed that the root of the plant Azanza garckeana has got some antibacterial activity. Antibacterial activities of plants can be explained on the basis of their chemical constituent. Various secondary metabolites have been implicated to exhibit a wide range of biological effect and protection against different diseases. The antibacterial activity of the root bark of this plant may be attributed to flavonoids, alkaloids, cardiac glycosides and

saponin as identified by standard tests. Alkaloids exhibit marked physiological effects such as antibacterial (Owolarafe *et al,* 2014).

From the results it is depicted that *S. aureus* and *E. coli* are the most susceptible. The result of this study showed that the extracts of the root bark of *Azanza garckeana* have varied antibacterial activities against the tested organisms this suggests that the extracts of these plants are broad spectrum in their activities. The present study revealed that all the extracts showed good degree of susceptibility against the test organisms.

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