Phytochemical Analysis and Antibacterial Screening of Asparagus Flagellaris (Kunth) Bak, used in the Traditional Treatment of Sexually Transmitted Diseases and Urinary Infections

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Abstract

The phytochemistry and antimicrobial effect of the stem, bark and leave of Asparagus flagellaris were studied. The phytochemical screening of the stem, bark and leaves showed appreciable amount of flavonoid and moderate amount of carbohydrate, cardiac glycoside and saponin while reducing sugar, ketones and pentose were detected in traces. The ethanol extract inhibited the growth of six organisms viz Escherichia coli, Corynebacteria, Klebsiella, Neiserra gonorrhoeae, Shegiella dysentariae and Candida albicans, at various concentrations, while the aqueous extract were susceptible on five organism namely Corynebacteria, Streptococcus pyogene, Proteus specie, Neiserra gonorrhoeae, and Treponema palladium.

Key words: *Asparagus flagellaris, phytochemical, ethanol extract, aqueous extract and antibacterial screening.*

Introduction

Medicinal and aromatic plants have been used for many centuries and are still popular in today's alternative therapies. Herbal remedies and alternative medicines are used throughout the world and in the past herbs often represented the original sources of most drugs (Sa'ad et al 2005). Asparagus flagellaris (Kunth) Bak. is scantly distributed in the Northern part of Nigeria. Asparagus flagellaris belongs to the family Asparagaceae. The plant is widespread in tropical Africa (Vander Burg 2004). Asparagus flagellaris grows to a height of about 1 meter and is scandent or more or less erect plant with arching spiny branch let (Burkil, 1985). The plant is widely used as a medicine in Africa. The branch lets (cladodes) are the main ingredient of a medicine to combat guinea-worm and of an ointment for hair growth (Vander Burg 2004). The stem, bark and leaves have a variety of medicinal uses; they are added to food or baths for treating syphilis, gonorrhoea and other transmitted diseases. The hot infusion is used to cure diarrhea and urinary infections (Dalziel 1937; Burkil, 1985; Vander Burg 2004).

Material and Method

Plant material

The stem, bark and leaves of Asparagus flagellaris Kunth were collected at Angwan-Baduku, Kokona local Government Area of Nasarawa State, Nigeria. The plant was identified by Prof. J. A. Akinniyi Department of Chemistry Faculty of Science University of Maiduguri, Nigeria. The plant materials were air dried and grounded into fine powder using pulverizer.

Micro Organisms

The test organisms include both gram positive and gram negative organism. The organisms used include Streptococcus pyogene, Staphylococcus aureus, Shigella dysentariae, Escherichia coil, Proteus specie, Entrobacter specie, Salmonella typhi, Corynebacteria, Pseudomonas aeruginosa, Klebsiella, Neisseria gonorrhoeae, Treponema pallidum and Candida albicans. The microorganisms were obtained from University of Maiduguri Teaching Hospital and University of Maiduguri Veterinary microbiology research laboratory. The bacterial strain were grown in the nutrient broth at 37^0C and maintained on nutrient agar slants at 4^0c.

Aqueous Extraction

The aqueous extraction of the water-soluble ingredients was carried out using the method described by Azuzu (1988). 15g of each of the grounded bark and leaves were extracted by soaking for 2 days using 35ml of distilled water in a 250ml sterile conical flask. The extracts were filtered using whatman filter paper No. 1. The filtrates were concentrated in vacuum at 60^0c and stored in universal bottles and refrigerated at 4^0C prior to use.

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Ethanol Extraction

The ethanol extraction of the active ingredient of the stem bark and leaves was carried out using the method described by Harbone (1994). 25g of the grinded sample were soxhlet extracted using 250ml of absolute ethanol. The extraction lasted for 8 hours. The volatile oil obtained was concentrated by evaporation using water bath at 100^0c.

Antimicrobial Screening

Clinical and pure isolates of Streptococcus pyogene, Staphylococcus aureus, Shigella dysentariae, Escherichia coil, Proteus specie, Entrobacter specie, Salmonella typhi, Corynebacteria, Pseudomonas aeruginosa, Klebsiella, Neisseria gonorrhoeae, Treponema pallidum and Candida albicans were obtained from University of Maiduguri Teaching Hospital and University of Maiduguri Veterinary microbiology research laboratory. All the cultures were tested for purity. Each inoculum was prepared by inoculating the stock culture into freshly prepared nutrient agar and incubated aerobically at 37^0C for 6hours. Five concentrations of the plant extracts were prepared, 100, 200, 400,700 and 1000mg/ml and one milliliter aliquot of each of the solutions of the extracts thus prepared was tested for bioactivity on the clinical isolates using the diffusion method of Bauer et al (1966). All bacteria were incubated for 24hours at 37^0C and the fungi for 48hours at 37^0C, after which they were examined for zones of inhibition of growth. Observed zones of inhibition of growth were measured and recorded in millimeter (mm).

Preliminary Phytochemical Analysis

The qualitative phytochemical analysis of Asparagus flagellaris stem bark and leaves powder was tested as follows: Carbohydrate Molisch's test: (4gm of powdered plant was boiled in 50ml of distilled water and filtered while still hot). 2ml filtrate + 3drops of conc. H_2SO_4, purple colour at the interface indicate the presence of carbohydrate. Monosaccharide: Barfoed's test (2ml filtrate + Barford's reagent and heat on water bath for 2 minutes.) Red precipitate indicated the presence of monosaccharide. Fehling test was used to determine the presence of free and combined sugar. Ketone; Resorcinol test (2ml of filtrate + few crystals of resorcinol + equal volume of conc. HCl) then heat over a spirit lamp flame. A rose colouration indicated the presence of ketonic sugar. Pentose: (2ml of filterate + 2ml HCl containing little phloroglucinol and heated over a spirit lamp flame). Red colouration indicated the presence of pentose. Tannins; (200mg plant material in 10ml distilled water, filtered). A 2ml filtrate + 2ml $FeCl_3$, blue black precipitate indicated the presence of tannins. Alkaloids: (200mg plant material in 10ml methanol, filtered). A2ml filtrate + 1%HCl +steam 1ml filtrate + 6 drops of Wagner's reagent. Brownish-red precipitate indicated the presence of alkaloids. Saponins (frothing test, 0.5 ml filtrate + 5ml distilled water. Frothing persistence meant saponin present). Cardiac glycosides (Killer-kiliani test: 2ml filtrated + conc.H_2SO_4). Green- blue colors indicated the presence of cardiac glycosides. Steroids (Liebermann-Burchard reaction): (200mg plant material in 10ml chloroform, filtered).A 2ml filtrate + 2ml acetic anhydride + conc. H_2SO_4. Blue-green ring and red ring indicated the presence of steroid and terpenoids respectively. Flavonoids: (200mg plant material in 10ml ethanol, filtered). A 2ml filtrate + conc.HCl + magnesium ribbon. Pink-tomato red color indicated the presences of flavoniods (Harbone, 1973).

Table 1 shows the result for the phytochemical screening of Asparagus flagellaris stem bark and leaves. The result shows that there are appreciable amount of flavonoid, and moderate amount of carbohydrates, cardiac glycoside and soponin were detected. Free reducing sugar, ketone and pentose were seen in traces, where as non-reducing sugar, monosaccharide, tannins, phlobatannins, alkaloid, terpenoid, steroid, free and combined anthraquinone are completely absent.

Table 2 showed the antimicrobial activity of the aqueous and ethanol extracts of Asparagus flagellaris stem bark and leaves. The result indicated that the aqueous extract inhibited the growth of five microorganisms at different concentration, where as the ethanol extract is susceptible to six organisms at various concentrations.

Discussion

Plant substances continue to serve as viable source of drugs for the world population and several plant-based drugs are in extensive clinical use (Roja and Rao, 2000). For the past few decades, a number of plants have been widely used for the treatment of various diseases due to their antioxidant properties. For instance Akinniyi et al (2007) showed that the leaves and stem back of Vitex milnei used in the treatment of diarraheae inhibited the growth of Shigella dysentariae, corynebacteria and salmonella typhi in vitro. The methanol extract of Lippia multiflora commonly known as bush tea and traditionally used in the treatment of fever, gastro-intestinal disturbances, cough and colds inhibited the growth of Staphylococcus aureus, bacillus subtilis and Shigella dysentariae (Mshelia et al, 2007). Zaria et al (1995) also demonstrated that the water extracts of Monodora tenuiflora and Xylopia quintassii used by the folklore in treatment of diarrhea inhibited the growth of bacteria in vitro. The fungus Candida albicans was susceptible to the ethanol and methanol extract of Gymnema montanum at various concentrations (Ramkumar, 2007). This study also point in the same direction.

Table 2 reveals that the ethanol extracts of the stem bark and leaves of Asparagus flagellaris was more active on the test organisms than the aqueous extract. The activities of the extracts on the organisms are concentration dependent with the exception of the activity of the aqueous extract on proteus species. This reduction in the activity at a higher concentration may be because only small amount of the active ingredient is required to inhibit the growth of the organisms, this is because only small amount is needed to attack a specific site in the organism and high concentration will cause accumulation and blockage of sensitive site thereby causes the reduction (Youman et al, 1967).

The highest activity was observed with the aqueous extract of the stem bark and leaves on Corynebacteria at 1000mg/ml with the inhibition zone of 34mm which shows that the active components are more soluble in water. Treponema palladium was only inhibited by the aqueous extract, which means that the active component was extractable only with water.

The ethanol extract was active on Escherichia coli, Corynecbacteria, Klesiella, Neiserra gonorrhoeae, Shegeilla dysentariae and Candida albicans, while the aqueous extract was active on Corynebacteria, Strephylococcus pyogene, Neiserra gonorrhoeae, and Treponema palladium. Both the ethanol and aqueous extracts were susceptible to Corynebacteria and Neiserra gonorrhoeae although the aqueous extracts are more susceptible than the ethanol extract on the two organisms. This means that the active ingredient in extracts that are active on the two organisms may be the same and soluble in both water and ethanol. The susceptibility increases with increase in concentrations in both extracts which shows that more number of the active components are available to attack more organisms thereby increasing the zones of inhibition. The higher inhibition zone in the case of aqueous extract means that the active ingredients are more soluble in water than the ethanol.

The ethanol extract was active on Klebsiella and Shegiella dysenteries at all concentrations, which means that the active components in the stem, bark and leaves was soluble in ethanol and active on this organisms even at a very small concentration. The range of zone of inhibition in klebsiella is very large compared to that of Shegiella dysentariae, which means that the active ingredient that attack specific site in the organism may not be the same or the active ingredient has more penetrating effect in the case of Klebsiella than Shegiella dysentariae (Youman et al, 1967).

The antimicrobial activity of the plant extracts may be due to the presence of non-toxic glycosides that under go hydrolysis to release phenolics that are toxic to microbial pathogens (Aboaba et al, 2001) since the phytochemical screening Asparagus flagellaris root shows the presence of glycoside. The presence of appreciable amount of flavonoid in the plant may be responsible for its use in African medicine for protection against allergies, inflammation, aggregation of microbes and tumors (Okwu and Okwu, 2004; Farquar, 1996). Flavonoids represent the most common and widely distributed groups of plant phenolics. Flavonoids are free radical scavengers' super antioxidants and potent water soluble which prevent oxidative cell damage and have strong anticancer activity.

The activity of the ethanol extract on Escherichia coli shows that it is susceptible only at high concentration of 400mg/ml and above, that is because the active ingredient was sensitive only when in large quantity. The growth of the fungus Candida albicans was inhibited by only the ethanol extract of the root at a concentration of 400mg/ml. The activity of the ethanol extract on the fungus may be due to the presence of saponins in the root (Aboaba et al, 2001).

Conclusion

The importance of herbal studies and its significance in African medicine has continuing to be of interest to scholars in many fields. This study revealed the potency of *Asparagus Flagellaris* in the treatment of Sexually Transmitted Diseases and Urinary Infections.

The ethanol extracts of stem, bark and leaves of the plant was found to be active on Escherichia coli, Corynecbacteria, Klebsiella, Neiserra gonorrhoeae, Shegeilla dysentariae and Candida albicans. The aqueous extract was found to be active on Corynebacteria, Strephylococcus pyogene, Neiserra gonorrhoeae, and Treponema palladium. The ethanol and aqueous extracts were active on Corynebacteria and Neiserra gonorrhoeae; this shows that the active ingridient on this orgarnisms was soluble in both solvents.

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Table 1: Results for Phytochemical Screening of Asparagus Flagellaris Stem Bark and Leaves

S.NO	Phytochemical Constituents	Stem Bark and Leaves
1	Carbohydrates	++
2	Free reducing sugar	+
3	Non reducing sugar	-
4	Monosaccharide	-
5	Ketone	+
6	Pentose	+
7	Tannins	-
8	Phlobatannins	-
9	Flavonoid	+++
10	Alkaloid	-
11	Terpenoid	-
12	Steroid	-
13	Cardiac glycoside	++
14	Free anthraquinone	-
15	Combined anthraquinone	-
16	saponins	++

+++ Appreciable amount ++ moderate amount + trace amount - complete absence

Table 2: Antimicrobial Activity of the Ethanol and Aqueous Extracts of Asparagus Flagellaris Stem Bark and Leaves

S.No.	Microorganism	Zones of inhibition (mm)									
		Ethanol extracts (mg/ml)					Aqueous extracts (mg/ml)				
		100	200	400	700	1000	100	200	400	700	1000
1	Escherichia coli	0	0	6	10	14	0	0	0	0	0
2	Salmonella typhi	0	0	0	0	0	0	0	0	0	0
3	Corynebacteria	0	0	13	15	18	0	10	20	27	34
4	Klebsiella	15	17	19	25	29	0	0	0	0	0
5	Staphylococcus aureus	0	0	0	0	0	0	0	0	0	0
6	Streptococcus pyogene	0	0	0	0	0	0	6	10	11	17
7	Pseudomonas areginosa	0	0	0	0	0	0	0	0	0	0
8	Proteus specie	0	0	0	0	0	8	12	14	12	12
9	Neiserra gonorrhoeae	0	4	7	13	15	5	14	17	18	22
10	Treponema pallidium	0	0	0	0	0	0	2	5	11	15
11	Shegiella dysentariae	14	14	17	18	18	0	0	0	0	0
12	Candida albicans	0	0	3	5	9	0	0	0	0	0
13	Entrobacter	0	0	0	0	0	0	0	0	0	0

The reported zones of inhibition exclude the diameter of the well.