

PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF STEM BARK EXTRACTS OF *PERINARIA MACROPHYLLA* SABINE, AGAINST *STAPHYLOCOCCUS AUREUS* AND *ESCHERICHIA COLI* (CLINICAL BACTERIAL ISOLATES)

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ABSTRACT

Antimicrobial and phytochemical screening of ethanolic, aqueous and chloroform extracts were carried out against Escherichia coli and Staphylococcus aureus. Plant extracts were obtained by cold percolation method. A disc diffusion method was employed for antimicrobial screening against the test organisms. Results show that ethanolic and aqueous extracts had exhibited antimicrobial activity against the test organisms. Both the test organisms have shown resistance against chloroform extract. Activity of ethanolic and aqueous extracts was greater at the higher concentration of 3000µg/disc and 2000µg/disc respectively. Meanwhile the activity was found to be high against S. aureus (12mm, 11mm) and (13mm, 11mm) than the E. coli (10mm, 8mm) and (9mm, 7mm). From the result obtained all the test organisms have shown resistance against the lower concentration of 1000µg/disc and 100µg/disc. Augmentine and Gentamicine were the antibiotics used as the control disc and they showed greater activity (30mm) and (27mm) than the crude extracts. The secondary metabolites screened were Alkaloids, Saponins, Glycosides, Tannins, Flavonoids and Steroids. Effort has been intensified in the development of antibiotics for the treatment of various diseases, but bacteria is profoundly developing resistance to the antibiotics and proliferate in the environment to cause harm. Consequently this rekindles interest in the use of natural plant materials in the manufacture of drugs. Based on the research findings the plant, Perinaria macrophylla, may be recommended as a promising candidate in the development of conventional antibiotics against S.aureus and E. coli.

KEYWORDS: Antimicrobial, Phytochemicals, Extracts, Inhibition, Perinaria, Staphylococcus aureus, Escherichia coli

INTRODUCTION

Most of the developing countries have adopted traditional medicinal practice as integral part of their culture. This practice of traditional medicine has remained the most affordable and easily accessible means of treatment in the primary health care of poor resource communities (Sofowara, 1993). However one of the problems of such practice is lack of scientific proves to confirm the efficacy. It has now become the responsibility of the scientific community to carry out researches to confirm the efficacy of the plant material so as to contribute towards fostering realistic approach to traditional medicine and further contribute to health care delivery system. Plants are used medicinally and are source of many potent and powerful drugs (Kubmarawa, *et, al*; 2009). Recently there is an increasing growing interest in obtaining sample of plant materials and or/ethnobotanical uses of plants with a view to explore the possibilities for commercial medical products. This developed interest in healing power of plant has been attributed to the current global

upsurge of bacterial resistance towards antimicrobial drugs (Sale and Doughari, 2008). This has brought considerable progress in understanding the nature of valuable component in plant for the treatment of various diseases.

Perinaria macrophylla belongs to the family chrysobalanaceaea. The plant family has 17 genera and 35 species (Castilho, 2000). Chrysobalanaceaea is a family of trees and shrubs distributed throughout the tropical region especially in Africa. *Perinaria* is 30ft tall with stout densely tormentors branhlets and knarled bole, flower crowded and produces fruit with rough skin and edible. *Perinaria* has been reported to contain sugar protein and steroids. Pharmacologically, the decoction of the barks and leaves are used as mouth wash, internal trouble and for imflamed eye (Audu, 2005). An antioxidants has been found in the plants which may be soluble flavonoids and glycosides. Deterpenoids has been isolated from other species of *Perinaria*. However, to date no detail of the chemical constituent of this plant has been reported (Audu, 2005).

This paper seeks to investigate the antimicrobial activity of the plant extracts and determine some of the secondary metabolites present in the plant extracts.

MATERIALS AND METHODS

SCREENING FOR ANTIMICROBIAL ACTIVITY

Processing of plant materials

The plant material was collected from Diggol village of Dambatta local government area of Kano state Nigeria. The plant was identified and confirmed in the herbarium of Forestry Department Audu Bako College of Agriculture, Thomas, Dambatta. The plant material used was stem bark. This was dried under shade and grounded into powder using clean mortar and pestle. The powdered material was also sieved and fine powder was obtained (Bukar *et al*; 2009).

Extraction method

Plant extracts were obtained by cold percolation method (Veeramuthu, 2006). Ethanol, water and chloroform were the solvents used in the extraction method. For all the solvents 50g of the dried plant materials were percolated in 500ml of the solvents in a 750ml capacity flask. The mixtures were agitated intermittently and kept for two weeks for ethanol and chloroform, while aqueous was kept for only one week. The mixtures were filtered through Whatman No.1 filter paper. The filtrate were evaporated to dryness using water bath at 40°C for water and ethanolic extract, while chloroform extract was allowed to dry overnight at an ambient temperature. The residues were stored at 4°C for subsequent use (Veeramuthu, 2006).

Preparation of sensitivity disc

Whatman No.1 filter paper was punched using paper puncher of which discs of 6.0mm in diameter were obtained. These were placed in sterile capped bijoux bottles and sterilized in a hot air oven at 160°C for one hour (Bukar *et al*; 2009).

Preparation of concentrate for sensitivity test

Two grammes (2g) of each plant extract was dissolved in 2ml of appropriate diluents to arrive at 1.0g/ml (1000000µg/ml) which serves as stock solution (Shamsuddeen *et al*.; 2008).

From the stock four concentrations were prepared. 0.3ml was added to 0.7ml of diluent, 0.2ml to 0.8ml, 0.1ml to 0.9ml, 0.01ml to 0.99 making 1ml each. One hundred (100) sterilized discs were placed into each bottles which gave disc potencies of 3000µg/disc, 2000µg/discs, 1000µg/discs, and 100µg/disc respectively.

Test organisms

The test organisms used in the research were bacterial isolates obtained from Aminu Kano Teaching Hospital (AKTH). The isolates were *Escherichia coli* and *Staphylococcus aureus*. The organisms were confirmed in the microbiology laboratory of Biological Sciences, Bayero University Kano. The stock culture were maintained on nutrient agar at 40°C in a refrigerator in accordance with procedure by Scott (1989).

Standardization of inoculum

Each culture of the isolates were standardized by culturing on nutrient agar for 24hrs at 37°C. The overnight culture were diluted in normal saline (0.5 w/v) until turbidity matched with 0.5 macfarland standard to give a mean of 3.3×10^6 Cfu/ml (Deeni and Hussain 1991).

Antimicrobial susceptibility

Disc diffusion method was employed to screen for antimicrobial activity of the plant extracts. Mueller Hilton agar plates were used for the inoculation of organism. The test organisms were streaked evenly on the surface of the agar plates with the use of sterilized wire loop. With the aid of sterile pair of forceps, impregnated paper discs containing the extracts of materials at different concentrations 3000µ/discs, 2000µ/discs, 1000µ/disc and 100µ/disc were arranged radially and pressed slightly and firmly to the inoculated agar surface to ensure even contact. The plates were incubated at 35°C for 24hrs. The degree of sensitivity was determined by measuring the diameter in millimetre of the visible zone of inhibition of the microbial growth produced by the diffusion of the extracts (Stokes and Ridgeway 1980).

Phytochemical screening

Phytochemical screening of the extracts was carried out according to the methods of (Abalaka *et al*; 2010) and Sofowora (1993). Active compounds tested were alkaloids, tannins, saponins, flavonoids and glycosides Steroids.

Alkaloids : One ml of hydrochloric acid was added to 3ml extracts in a test tube, about two drops of Meyers reagent to one ml of the extracts was added, creamy and turbid precipitate was an indication of the presence of alkaloids.

Tannin : Ferric chloride was added to 1ml of the extract, presence of blue black and blue green precipitate indicated the presence of tannin

Saponins : this was carried out by frothing test, 2ml of the extracts was vigorously shaken in the test tube for two minutes, presence of frothing in the test tube indicated positive test.

Flavonoids : a piece of magnesium ribbon was added to 1ml of the extract followed by the addition of hydrochloric acid drop wise, a magenta colouration indicated the presence of flavonoids.

Glycosides : Ten ml of H₂SO₄ was added to 1ml of the extracts and the mixture was heated in boiling water for 15 minutes. 10ml Fehlings solution was added and the mixture boiled. A brick red precipitate was confirmatory for the presence of glycosides.

Steroids : Salkowski test: 5 drop of concentrated H₂SO₄ was added to 1ml of the extracts in a test tube. Red colouration indicates the presence of steroids.

RESULTS

Table 1 shows the diameter of the zone of inhibition of the test organisms against the plant extracts. Result shows that ethanolic and aqueous extracts showed activity against the test organisms and the organisms were resistant against the chloroform extracts. The

activity of aqueous and chloroform extracts was greater against *S.aureus* (13mm,11mm) and (12mm, 11mm) than the *E. coli* (10mm, 8mm) and (9mm, 7mm). Meanwhile the activity was observed at the higher concentrations 3000µg/disc and 2000µg/disc respectively, while the test organisms showed resistance against the lower concentration of 1000µg/disc and 100µg/disc. From the result it was observed that the antibacterial activity was enhanced by the increase in the concentration of the extracts i.e the higher the concentration of the extracts the greater the zone of inhibition. Augmentine and Gentamicine were the antibiotics used as the control discs and they showed greater activity than the crude extract of the plant materials. The secondary metabolites screened from the plant extracts were Alkaloids, Saponins, Flavonoids, Glycosides, Steroids and Tannins (Table 2).

TABLE 1. The diameter (mm) of the zone of inhibition produced by ethanolic, aqueous, and chloroform extracts of *Perinaria macrophylla* at various concentrations.

Extracts	organisms	Concentrations µg/disc/zone of inhibition/mm				
		3000	2000	1000	100	control
Ethanolic extracts	<i>Escherichia coli</i>	10	8	NI	NI	30
	<i>Staphylococcus aureus</i>	12	11	NI	NI	27
Aqueous extracts	<i>Escherichia coli</i>	9	7	NI	NI	30
	<i>Staphylococcus aureus</i>	13	11	NI	NI	27
Chloroform extract	<i>Escherichia coli</i>	NI	NI	NI	NI	30
	<i>Staphylococcus aureus</i>	NI	NI	NI	NI	27

KEY: NI - No inhibition, Control discs, Gentamycine against (*E. coli*)
 Augmentin Against (*Staphylococcus aureus*)

Table 2: Phytochemical component from the plants extracted using various solvents

Active compounds	Extracts of the plant material		
	Ethanol	Aqueous	chloroform
alkaloids	+	+	+
Tannin	+	+	-
Saponins	+	+	-
Flavonoids	+	+	-
Steroids	+	+	+
Glycosides	+	+	+

KEY = + Present - Absent

DISCUSSION

The research shows that, plant extracts had exhibited antibacterial activity against the test organisms with the exception of chloroform extracts, this may be due to better solubility of the active components in water and ethanol. The activity of ethanolic and water extracts was observed at the higher concentrations 3000µg/disc and 2000µg/disc respectively. However there was greater resistance at the lower concentration of 1000µg/disc and 100µg/disc. This may be due to the fact that the concentrations were not strong enough to cause inhibition against the test organisms. The activity showed by

the plant extracts may be due to the presence of active components. The zone of inhibition produced by the test organisms indicated the susceptibility to the plant extracts. Sani, and Aliyu (2011) reported that the plant was found to be used in ethnomedicine by the traditional healers of Kano, Northern Nigeria. Therefore, the activity of stem bark extracts of *Perinaria macrophylla* justifies the traditional usage of the plants in ethnomedicine. The zone of inhibition produced by the test organisms indicated the susceptibility to the plant extracts.

According to the research findings zone of inhibition by the bacterial isolates varied. (Mann *et al.*, 2008) also reported that the position of the zone edge (diameter of the zone of inhibition) is determined by the initial population density of the organisms, their growth rate and diffusion of the antimicrobial agent which clearly indicated the differences in the zone of inhibition observed. Augmentine and Gentamicine were used as control discs against the organisms. These antibiotic showed greater activity than the crude extract. This is possible because the material are well refined industrial products, therefore their activity may be more pronounced than the crude extracts.

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CONCLUSION

The extracts of *Perinaria macrophylla* sabine, had exhibited antibacterial activity against the test organisms. The activity may be due to the presence of secondary metabolites in the plant extracts. Effort has been intensified in the development of antibiotics for the treatment of various diseases, but bacteria are profoundly developing resistance to the antibiotics and proliferate in the environment to cause harm. Consequently this has rekindles interest in the use of natural plant materials in the manufacture of drugs. Based on the research findings the plant *Perinaria macrophylla* may be considered as promising candidate for the development of conventional drugs (antibiotic) against the bacterial organisms.