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Phytochemical and Antimicrobial Activity of Stem Bark Extract of Ficus Plat Platyphylla Extraction and Phytochemical Analysis from the Leave of Vernonia Amygdalina (Shuwaka)

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Abstract

Stem bark sample (100g and 60g) of Ficus platyphylla were thoroughly extracted with ethanol and water respectively. The qualitative phytochemical analysis of the ethanol crude extract showed the presence of saponin, tannis, flavonoids ring, cardiac glycosides and carbohydrates, steroids ring, and Alkaloids. The qualitative was justified by their colour changes with their various reagents. The extract exhibited antimicrobial activity on E coli and S aureus. This appears to because of the secondary metabolites tested for and those present but not tested for.

Keywords: inflammatory, ficus platyphylla, analgrstic, therapeutic, precursor, orthodox.

1. Introduction

Background of the study

Ficus platyphylla is a deciduous plant locally known as “Gamji” in Hausa and widely distributed through the Savannah region of West Africa coast. The plant is used by herbalist for treating several diseases such as insomnia, psychosis, depression, and as analgesic (Menezes et al., 2011).

Ficus Plattyphylla which consists of about 60 species of typical fruiting trees in the flowing plant of family moraceae. It is a large savannah tree which grows up to 60ft high with trusty or pinkish-brown bark and large grey scaly distributed in sub-Sahara Africa. The bark of the plant has been used in the treatment of asthma disease; it’s also help in flushing out contaminated blood from the body of newly delivered women, insomnia epilepsy, pain and inflammation for many years and also malaria (Beyene et al., 2016).

Medicinal plant contains substances used for the therapeutic purpose or used as a precursor for the synthesis of some drug as well as vital chemicals. The origin of the therapeutic used a herbal medicine can be trace to China about 500 years ago(Narendhiran et al., 2014), a large part of the world population still relies on plant as they affordable sources of medicine Herbalists and indigenous healer have used botanical medicines traditionally worldwide for the prevention and treatment of different pathogens, traditionally medicine is of immense value in the healthcare

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system of developing countries, and the world health organization (Narendhiran et al., 2014) estimated that more than 80 % of health care need in this countries are met through traditional healthcare practice (World Health Organisation, 2001).

This is may be possibly because it affordability and accessibility is more than that of orthodox medicine (Beyene et al., 2016).

A lot of herbs have been used traditionally to treat infertility, malara, cholera, tuberculosis, myelin (meca), Discorea villosa (wild yam) Ficus platyphylla has been used for this purpose.

Ficus platyphylla has been evaluate for the scientific basic for the use of this plant in traditional medicine for the treatment of Central Nervous System (C.N.S) disorders(Qadir et al., 2015). The extract of ficus platyphylla has been reported to possess Analgesis(Iqbal et al., 2015), an inflammatory and anti contraceptive activities (Wakeel et al., 2004). Further investigation reported the use of the stem bark in the treatment of tuberculosis, the extract plant are used in Huasa ethnol medicine of Northern Nigeria for the treatment of various ailments (Odey et al., 2012).

2. Main part

Experimental

Preparation of reagents

Warner's reagent

About 3.0 g of potassium iodide was weighed and dissolved in about 40cm^3 of distilled water. To the resulting solution of potassium iodide, 2.0 g of iodide crystal was added and properly stirred to homogenize into solution. This was transferred into 100cm^{3}volumetric flask and filled up to the mark with distilled water.

Mayer's reagent

The Mayer's reagent is freshly prepared by dissolving a mixture of mercuric chloride (1.36 g) and of potassium iodide (5.00 g) in water (100.0 m).

Sample collection and preparation

The sample collection

The stem bark of the plan ficus platyphylla was collected at MARA Ward, Rimin Zayam, Toro Local Government Area, Bauchi State, Nigeria.

Preparation of sample

The plant sample was air dried under shade for about two weeks then tightly parked inside a clean and sterilized paper bag and kept in the laboratory until it required.

Extraction

Ethanol extraction

The 100ml of ethanol was added to 50 g of the Powder and homogenized in a conical flask. The mixture was stirred and allowed to stand for 48 hours at room temperature by occasionally mixing. After 48 hours the mixture was filtered using a watchman NO: 4filter paper, the filtrate was allowed to evaporate to dryness in a water bath at 60^{o}c and the residue was air dried. The percentage yield was calculated accordingly.

Aqueous extraction

A 60g of the powdered plant sample was soaked in 500cm^3 of distilled water; the mixture was heated to boil for 40minutes and then allowed to stand for 48 hours at room temperature. The mixture was filtered thoroughly with a watchman NO4 filter paper and the filtrate heated in a water bath until it completely dried. The residue was weighted and the percentage yield was calculated.

Preparation of reagents

Wagners reagent

About 3.0g of potassium iodide was weighed and dissolved in about 40cm^3 of distilled water; to the resulting solution of potassium iodide, 2.0g of iodide crystal was added and properly stirred to homogenize into solution.

Mayers reagents

About 1.36 g of mercury chloride was weighed and 5.00g of potassium iodide was weighed and dissolved in 100ml of distilled water and diluted to homogenize into solution.

Sample collection and preparation

Healthy looking fresh leaves of Vernonia amygdalina (bitter leaf) free disease were collected in the morning before sun rise in a clean polythene bag at Wuntin Dada Bauchi Local Government and was transported to the Department of Science Laboratory Technology, School of Science and

Technology, AbubakarTatari Ali Polytechnic Bauchi for identification and preparation of the sample. The leaves were washed thoroughly 2-3 times with running tap water and once with sterile distilled water; the leaves were then air dried under shade and then grounded into fine powder using laboratory pistle and mortar.

Extraction

About 100 g of dried powder of plant materials was added to 200ml of sterile distilled water and methanol 70 % w/v respectively in order to obtain water and/or methanol extracts (100 mg/mc). The extraction was done at room temperature for 24 hours for the water extraction and 74 hours for the methanol extraction (Newton et al., 2002) and the fraction purified by filtering through what man No. 1 filter paper. The stock solution of the extraction was then sterilized by filtration through milli pore membrane filters of 0.45 hm pore size. The sterile extracts obtained were then stored sterile in the refrigerator at 4^{o}C until required.

Phytochemical analysis

Phytochemical analysis of the leaves extracts for the presence of some secondary metabolites was done using standard procedures.

Test for tannins

About 3g of sample was boil in 50 ml of distilled water for 30minutes on a hot plates. The mixture was filter and a portion of the filtrate was dilute with sterile water in a 1.4 and 3 drops of 10 % ferric chloride solution was added. A blue colour indicates the presence of tannin.

Test for flavonoids

5 g of sample was weighed and dissolved completely with acetone, the residue on a water bath. The mixture was filter and the filtered was used for the test. 5 ml of 10 % of sodium hydroxide was added to an equal volume of the detained water extracts. A yellow solution indicates the presence of flavonoids.

Test for alkaloids

2 ml of sample extracts was measured in a test-tube to which picric acid solution was added. The formation of orange coloration indicates the presence of alkaloids.

Test for saponins

1 g of sample was weighed into a conical flask in which 10ml of sterile distilled water was added and boiled for 5 minutes, the mixture was filtered and 5 ml to 10ml of sterile distilled water was added in a test tube and was taped and shaken vigorously for about 30 seconds. And it was allowed to stand for half for hour. The honey comb indicates the presence and saponin.

Test for steroid

The crude extract each was mixed with 2ml of chloroform and concentrated hydrochloric acid was added side wise. The appearance of red colour produced in the lower chloroform layer indicated the presence of steroid.

Test for Anthraquanones

0.5 mg of each extract was put into a dry test tu be and 5ml of chloroform was added while shaking for about 5minutes. The mixture was then filtered and the filtrate was shaken with an equal volume of 100 % ammonia solution. The appearance of a pink, violet colour in ammonia layer indicated the presence of free anthraquanones.

Test for carbohydrate

5 mg of each extract were heated with concentrated sulfuric acid. The appearance of blackening effervescence indicated the presence of carbohydrate.

Test for Cardiac glycoside

Crude extract was mixed with 2ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice sulfuric acid was carefully added. A change of colour from violet to blue to greenish indicated the presence of glycosides.

Preparation of the media

The powdered agar media was mixed with water and steam to dissolve the agar and then were sterilized in a autoclave at 121^{o}C and subsequently allowed to cool about 45 % (a temperature of which the agar remain molten in preparing the plate), some 15-20ml of the molten agar media were poured into the sterile labeled petri dishes then stored closed upside down in a refrigerator at 4^{o}C.

Inoculation and application of the extracts

The standard method for preparing inoculums designed by national committees for clinical laboratory standard (1990) was followed. A sterile wire loop was used to pick five colonies of each of the test organism into different labeled test tube containing 5ml nutrient broth.

The broth culture was inoculated over night at 37°C for the bacteria and room temperature for the fungi until a slightly visible turbidity compared to 0.5ml faland stranded (1.5x108cfo(ml).

Minimum inhibitory concentration (MIC)

The initial concentration of the plant extracts (100 g/ml) was diluted using double fold serial dilution by transferring 5 ml of the sterile plant extracts 9 stock solutions into 5ml of nutrient broth to obtain different concentration of extracts. The process was repeated several times to obtain other dilution. 25 mg/ml, 12 mg/ml have obtain different concentrations of extracts, each concentration inoculated with 0.1ml of the standardized bacterial cell suspension and inoculated at 37°C for 24 hours, the growth of the inoculums in broth is indicated by turbidity of broth and the growth of the test organisms were taken as the minimum inhibitory concentration (MIC).

Extraction preparation for antimicrobial activity

16ml nutrient ager plates that has been checked for sterility were seeded with 0.1 of an overnight broth culture of each bacterial isolate in sterile plates The stem bark plates were allowed to set after a uniform distribution of the bacterial isolate following slow rotation of the plates. A standard sterile cork borer of 6ml diameter was used to cut uniform wells on the surface of the ager. The wells filled with 0.1ml of each extracts and allowed to stand for 1hour at room temperature to allow proper diffusion of the extract to occur under sterile conditions. All the plates were incubated at 37°c for 24hours and observed for the zone of inhibition. A zone inhibition and the diameter are measured in millimeters.

Inoculation and application of the extract

The stem bark extract of the plant at different concentration were arranged radically and extract of the plant at different concentration were arranged radically and pressed firmly on to the inoculated ager surface to ensure even contact. Each disc was sufficiently space out and kept at least 15 mm from the edge of the plate to prevent overlapping of zones. The plate inoculated aerobically 37°C for 18 hours. Diameter of zone inhibition was measure using millimeter rule.

Determination of minimum inhibitory concentration (MIC)

Grade concentration of the ethanolic extracts 5ml each were mixed with melted (45°C) 5 ml of double strength Mueller-Hilron ager and poured aseptically into sterile plate. The plates were allowed to set sterile paper discs in duplicates were aseptically placed equidistantly on the set ager. Thereafter 10NL of the standardized bacterial cultures were inoculated on the sterile paper disc aseptically. The plates were allowed to stand for 1 hour and then incubated at 37₀C for 18 hours. Ciprofloxacin was used as standard antibiotics. This procedure was carried out for fungal culture.

3. Results

Table 1. Phytochemical analysis of Ficus playtyphylla

Metabolic	Water extract	Ethanol extract
Saponin	+	+
Tannin	+	+
Flavonoid	-	+
Anthraquanone	-	-
Carbohydrate	+	+
Alkaloid	+	-
Steroid Ring	+	+
Cardiac Glycoside	-	+

Key:

+ = Present

- = Absent

Table 2. Mass of Reactants and Residue

SOLVENT	MASS OF SAMPLE	MASS OF RESIDUE	MASS OF EXTRACT
Water	60g	49.9g	10.1g
Ethanol	100g	83.4g	16.6g

Table 3. Physical Properties of Solvent

SOLVENT	B.P	M.P	COLOUR & TEXTURE	DENSITY
Water	100°C	O°C	Maroon & Solid	100kg/m^3
Ethanol	78.37°C	-114°C	Reddish brown & solid	0.789g/cm^3 at 25°C

Table 4. Antimicrobial Effect of Extract

TEST ORGANISM	CONCENTRATION (mg/ml)	ZONE OF INHIBITION	
	10^{-1}	Water extract	Methanol extract
	10^{-2}	21.00MM	23.00mm
S. Aureus	10^{-3}	17.00MM	18.00mm
	10^{-4}	X	X
	10^{-5}	X	X
	10^{-1}	22.00mm	24.00mm
	10^{-2}	18.00mm	19.00mm
E. Coli	10^{-3}	X	X
	10^{-4}	X	X
	10^{-5}	X	X

Discussion

The qualitative test have been carried out using, ficus platyphylla stem bark extracts and it revealed various composition of phytochemicals, crude extract, and antimicrobial Assay of ficus platyphylla demonstrated the presence of most of the phytochemicals tested. Both the ethanol and water extract revealed the presence of saponins, tannins, flavonoids, alkaloids, cardiac glycoside, steroids, and carbohydrates. This may be so because of the relative similarity in polaritiy (Odey et al., 2012). While antheraquinones is absent in both aqueous and ethanol extract of the plant.

The bioassay of the methanol and water extracts of Ficus platyphylla (Stem bark) gave a minimum inhibitory concentration at 21.00 mm and 22.00 mm for Staphylococcus aureus and Escherichia, coli respectively. The result agrees with other research in similar area in the antimicrobial effects of secondary metabolites(Beyene et al., 2016).

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