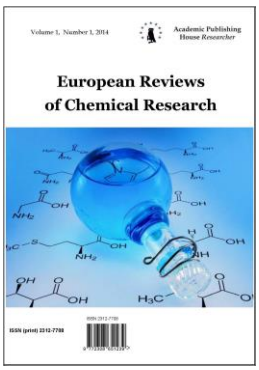

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Extraction and Phytochemical Analysis from the Leave of Vernonia Amygdalina (Shuwaka)

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Abstract

The phytochemical analysis of Vernonia Amygdalina (bitter leaf) was carried out to determine the bioactive compound presenting. The plant had been use locally to treat diseases for decades. The extraction of bioactive compounds was done through aqueous and ethanol extraction. The main bioactive compounds are alkaloids, tannin. saponnin, steroids, glycoside and flavonoids. The plant also exhibited antimicrobial activity on selected microorganisms. These activities may be as a result of the metabolites present tested for and those not tested for.

Keywords: metabolites, dimethyl sulphoxide, antimicrobial, phytomedicine, parasitic.

1. Introduction

Vernonia amygadalina commonly known as bitter leaf (English) oriwo (edo) ewuro (Yoruba) and Shuwaka (Hausa) is a tropical shrub that grows up to 3 meters high in the African tropics and other parts of Africa particularly Nigeria, Cameroon and Zimbabwe (Bekele, 2015). The leaves are dark green in colour with characteristic odour and bitter tasted leafs. It is reputed to have several health benefits ; it is effective against amoebic dysentery, gastrointestinal disorders and has antimicrobial and anti-parasitic activity (Ibisi et al., 2017).

Vernonia amygadalina is a perennial herb belonging to the asterace family, the species is indigenous to tropical Africa and is found wild or cultivated all over sub-Sahara Africa (Longanga et al., 2000). The leafs are eaten after crushing and washing thoroughly , however, almost all parts of the plant are pharmacologically useful; both the root and the leaves are used in phytomedicine to treat fevers, hiccups, kidney diseases and stomach discomfort among others (Ibisi et al., 2017).

Vernonia amygadalina has various culinary and medicinal properties, the medicinal properties of the extract has bactiostastic and bacteriocidal effects on some bacteria as well as antitumorigenic properties (Akinpelu, 1999). Further studies have demonstrated hypoglycemic and hypolidimic effect of the leaves extracts in experimental animals (Nalule et al., 2013).

Many herbalist and native doctors in Africa recommend aqueous extract for their patients for treatment of a variety of ailments form emesis, nausea, diabetes, lack of appetite, dysentery ,gastrointestinal tract problems to sexual transmitted diseases and diabetes mellitus among others

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(Nalule et al., 2013). Therefore this study was primary undertaken to confirm the acclaimed antimicrobial effects of Vernonia amygdalina in Nigeria.

2. Materials and methods

Cold extraction was employed for the extraction process and methanol was the solvent used for the extraction.

Preparation of reagents

Wagners 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.

Mayers reagents

About 1.36 g of mercury chloride was weighed and 5.00 g 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 200 ml 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 3 g of sample was boiled in 50ml 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 5minutes, the mixture was filtered and 5 ml to 10 ml 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 glycoside

25 ml of sulphuric acid was added to extract a test tube and boiled and then 5 ml of fehling solution A and B was added. A brick red precipitate for reducing indicate the presence of glycoside.

Antimicrobial activity

The antimicrobial activity was determined using well diffusion assay(Ekaiko et al., 2016). Dimethyl sulphoxide (DMSO) was used as a negative control and stereptomycine and ciprofloxacine (10/disc) were used as a positive control for bacteria strains and photercine B and nystatin were used as a positive control for fungi, the were done in triplicate bacteria culture were incubated at 37°C for 24 hours while the other fungi cultures were incubated at (30-32°C) for 48 hours, solution of 10mg/ml of sterptomycineciproflaxacine, nystatine and amphoteincin B were used as a standard for comparison. Antimicrobial activity was determined by measurement of zone of inhibition(Gumgumjee, Hajar, 2012).

Extraction preparation for bioassay analysis

About 1ml of the culture (previously) diluted 10^7 (fulme) was inoculated into 20 ml of molten nutrient ager in a petri plate and then spread uniformly using a sterile and a cork borer (5 mm in diameter was used to make wells on media in the plates for introduction of extract).

One hundred microliters 4 mg/ml of each extracts prepared in 20 % (DMSO) were introduced into wells. Each extracts was introduced into each hole in triplicate. The plates were kept at room temperature for extracts to diffuse in to the media before it was placed in the incubator at 37°C for 24 hours. The relative susceptibility of the organism to extract is indicated by clear zones of inhibition produced after incubation. Diameters of inhibition zones were measured by calculating the difference between cork borer (5 mm) and the diameters of the inhibition. Chloramphenicol and dimethyl sucohoxide were used positive control and negative control respectively (Adetunji et al., 2013).

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°C and subsequently allowed to cool about 45 % (a temperature of which the agar remain molten in preparing the plate), some 15-20 ml of the molten agar media were poured into the sterile labeled petri dishes then stored closed upside down in a refrigerator at 4°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 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 5 ml of nutrient broth to obtain different concentration of extracts. The process was repeated several times to obtain other dilution. 25 mg/ml, 12mg/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).

3. Result and discussion

Table 1. Phytochemical analysis of Vernonia amygdalina

S/N	PHYTO CONSTITUENT	ETHANOL	WATER
1	Saponin	++	+
2	Tannin	++	+
3	Flavonoids	++	+
4	Alkaloids	+	+
6	Glycoside	+	+

Key: + = present
- = absent (not detected)

Table 2. Antimicrobial activity of *V amygdalina*

TEST ORGANISM	ETHANOL EXTRACTS zone of inhibition (mm)	AQUEOUS EXTRACTS zone of inhibition (mm)
Escherichia coli	11.3	12.5
Pseudomonas aeruginosa	10.8	12.2
Klebsiella SPP	11.4	11.8
Staphylococcus aureus	11.4	11.4
Candida	11.4	11.8

Qualitative phytochemical screening revealed the presence of natural products in the extracts, both ethanol and water leaf extracts of *Vernonia amygdalina* were found to contain all the phytochemicals tested for. These phytochemicals were all found to be present in both ethanol and water leaf extracts of *Vernonia amygdalina,* this may be attributed to similarity in their polarity indexes.

The extracts exhibited antimicrobial activity by inhibiting the growth of test organisms by their respective zones of inhibition.

The antimicrobial activity may be attributed as a result of the secondary metabolites tested for and those present that were not tested for. These metabolites have been shown to exhibit medicinal and antimicrobial properties(Senguttuvan et al., 2014; Iqbal et al., 2015; Qadir et al., 2015).

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