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PHYTOCHEMICAL SCREENING, ANTIMICROBIAL ACTIVITIES AND QUANTITY ESTIMATES OF BIOACTIVE COMPOUNDS IN EXTRACTS OF *HOLARRHEA FLORIBUNDA*

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ABSTRACT

This study was carried out to determine the antimicrobial activities of extracts of *Holarrhea floribunda* and to determine the quantity of bioactive compounds in the extract with the most active antimicrobial activities. Extracts of *Holarrhea floribunda* were prepared using different solvents such as ethyl acetate, methanol, chloroform and acetone and were screened for their antimicrobial activities. Phytochemical screening and quantity estimation of detected phytochemicals were carried out on the most active extract which is the methanol extract. It revealed the presence of alkaloid, flavonoid and saponin in the methanol extract. Quantity estimation also revealed that the alkaloid is 38.2 %, flavonoid is 8.2% and saponin is 11.5 % in the methanol extract of *Holarrhea floribunda*. The result obtained revealed that the higher quantity of bioactive compounds in the methanol extract may be responsible for the high antimicrobial activities of this extract. The antimicrobial activities of the methanol extract could be responsible for the traditional uses of *Holarrhena floribunda* in the treatment of boils, eczema, diabetes mellitus and as stimulant, carminative, diuretic and antipyretic.

Keywords: Antimicrobial activities , *Holarrhea floribunda*, quantity estimation, bioactive compounds

INTRODUCTION

The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. Medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. Many of the indigenous medicinal plants are used as spices and food plants. They also sometimes added to foods meant for pregnant women and nursing mothers for medicinal purposes (1). Different parts of the *Holarrhena floribunda* have been used by traditional healers in the treatment of various ailments and disease conditions (11). In the northern part of Nigeria, a decoction of the leaves is used for

treating boils, eczema and diabetes mellitus (2). Crushed leaves are applied on the forehead to treat headaches (10). Infusion made from the leaves and the inflorescence is used as stimulant, carminative, diuretic and antipyretic (9). This present study investigates the antimicrobial activities of extracts, presence and quantity of active principles and chemical compounds present in the extracts of these medicinal plants.

MATERIAL & METHODS

The leaves of *Holarrhea floribunda* were plucked from Ijebu-Ode, Ogun State, Nigeria and air dried. The leaves were then pulverized. Five solvents were used in the extraction process – hexane, ethyl acetate, methanol, chloroform and acetone. Maceration method of extraction was used. The pulverized leaves were soaked initially in hexane for 3 days. The mixture was decanted. Then the remaining solvents were added and decanted. The resulting solvents were distilled, and crude extracts recovered.

ANTIMICROBIAL SCREENING TEST

AGAR DIFFUSION METHOD

An overnight culture of each organism was prepared. 0.1 ml of each organism was taken into 9.9 ml of sterile distilled water to give 10 ml at 10^{-2} dilution. 0.2 ml was taken into sterile molten nutrient agar at 45 ^{0}C. This was aseptically poured into the sterile plants and allowed to set in the bench for about 45 minutes. Concentrations of 200 to 6.25 mg/ml of sample extracts were prepared. A sterile cork borer was used to create wells/holes inside the set plate. Different prepared concentrations of the sample were introduced into the wells. All the concentrations were introduced into the wells with negative & positive control; concentration of 10 mg/ml of gentamicin was used as positive control for bacteria. These were allowed to stay on the bench for two hours before incubation at 37 ^{0}C for 18-24 hrs (3).

SURFACE – PLATING FUNGI

Surface plate method was used for anti-fungi assay. Molten sabour dextrose agar (SDA) was poured aseptically in the sterile plates, allowed to cool and set for about 45 mins. Then 0.2 ml of 1:100 dilution of the organism was spread on the surface using a sterile pread. Then a sterile cork borer was made to create wells inside the set plate. Tioconazole was used for positive control for fungi. All these plates were then incubated at 20-26 ^{0}C for 48 hours (5)

PHYTOCHEMICAL SCREENING TESTS USING STANDARD METHODS (7)

-Test for Saponins

About 0.5 g of extract was shaken with water in a test tube. Observation of frothing is indicative of the presence of saponins.

-Test for Alkaloids

About 0.5 g of the extract was stirred with 5 ml of 10 % aqueous hydrochloric acid on a steam bath. 1 ml of the filtrate was treated with a few drops of Dragendoff reagent. Observation of a

precipitate is indicative of presence of alkaloids.

-Test for steroids

0.5 g of the extract was dissolved in 2 ml of chloroform. H_2SO_4 was carefully added. The formation of a reddish brown colourinterphase is a positive test for steroids.

-Test for Tannins

About 1 g of the extract was stirred with 10 ml of distilled water, warmed and filtered. Ferric chloride reagent was added to the filtrate. The formation of blue-black precipitate is indicative of the presence of tannins.

-Test for reducing sugar

A small portion of extract was dissolved in distilled water and warmed with fehling solution A and B. Observation of bride red precipitate is a positive test for reducing sugar.

-Test for Anthraquiniones

2 g of methanol extract was shaken with 5ml benzene, filtered and 10 ml aqueous H_2SO_4 was added to the filtrate. The mixture was shaken. Observation of pink, red or violet colour is indicative of anthraquinone (5).

QUANTITY ESTIMATION OF PHYTOCHEMICALS BY STANDARD METHODS (4)

Flavonoid

10 g of the plant sample was extracted repeatedly with 100 ml of 80 % aqueous methanol at room temperature, filtered the whole solution with filter paper, transfer the filtrate into crucible or petri dish beaker and evaporate to dryness on a water bath and weighed to a constant weight (8).

ALKALOID

5 g of the plant sample was weighed using analytical balance into a 250 ml beaker and 200 ml of 10 % acetic acid in methanol was added and coppered for 4 hours. It was then filtered and concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitate was completed. The solution was allowed to settle, and precipitate was collected and washed with dilute ammonium hydroxide before it was then filtered. The residue is the alkaloid which was dried in this oven and weighed (12).

SAPONIN

20 g of the sample was weighed into conical flask and 100 ml of 20 % aqueous ethanol was added. The sample was heated over a water bath for 4 hours with continuous stirring at about 55 ^{0}C. The mixture was filtered, and the residue re-extracted with another 200 ml of 20 % ethanol. The combined extracts were reduced to 40 ml on a water bath. After evaporation, this sample was dried in the oven to a constant weight. This concentrated solution was transferred into a 250 ml separating funnel and 20 ml of diethyl either was added and shaken vigorously. The aqueous layer was recovered while the other layer was discarded. The purification process was repeated 60 ml of n-butanol was added. The combined n-butanol extract was washed

twice with 10 ml of 5 % aqueous Nacl. The solution was evaporated on a water bath and the sample dried in the oven to a constant weight (6).

Results and Discussion

TABLE 1: Antimicrobial Screening Result of Methanol Extract of *Holarrhena Floribunda*

concentration		Inhibition zone (mm)								
Mg/ml	*Staphylococcus aureus*	*Escherichia coli*	*Bacillus subtilis*	*Pseudomonasaerugi nosa*	*Salmonella typhi*	*Klebsiella pneumonae*	*Candida albicans*	*Asperigillus niger*	*Rihozpus stolonifer*	*Pencillum notatum*
200	28	26	28	26	24	24	20	18	16	16
100	24	24	24	20	18	18	16	16	14	16
50	20	20	20	18	16	16	14	14	12	14
25	18	16	18	14	12	12	12	10	10	10
12.5	14	12	14	12	10	10	10	10	-	-
6.25	12	10	12	10	10	10	10	-	-	-
+ve	40	38	38	38	40	38	28	28	28	26

TABLE 2: Antimicrobial Screening Result of Chloroform Extract of *Holarrhena Floribunda*

concentration		Inhibition zone (mm)								
Mg/ml	*Staphylococcus aureus*	*Escherichia coli*	*Bacillus subtilis*	*Pseudomonasaerugi nosa*	*Salmonella typhi*	*Klebsiella pneumonae*	*Candida albicans*	*Asperigillus niger*	*Rihozpus stolonifer*	*Pencillum notatum*
200	18	14	16	14	14	16	14	12	14	14
100	14	12	12	12	12	14	12	10	12	12
50	12	10	10	10	10	10	12	10	10	10
25	10	-	-	-	-	10	-	-	-	-
12.5	-	-	-	-	-	-	-	-	-	-
6.25	-	-	-	-	-	-	-	-	-	-

+ve	40	38	38	38	36	36	28	28	28	28

TABLE 3: Antimicrobial Screening Result of Ethyl Acetate Extract of *Holarrhena Floribunda* Extract

concentration		Inhibition zone (mm)								
Mg/ml	*Staphylococcus aureus*	*Escherichia coli*	*Bacillus subtilis*	*Pseudomonasaeruginosa*	*Salmonella typhi*	*Klebsiella pneumonae*	*Candida albicans*	*Asperigillus niger*	*Rihozpus stolonifer*	*Pencillum notatum*
200	24	22	20	22	20	22	18	16	14	16
100	20	18	18	18	18	18	14	14	12	14
50	18	14	14	14	16	14	12	10	10	12
25	14	12	12	12	12	12	10	10	-	10
12.5	10	10	-	10	10	10	-	-	-	-
6.25	10	10	-	-	-	-	-	-	-	-
+ve	38	38	36	36	36	34	28	28	28	26

TABLE 4: Antimicrobial Screening Result of Acetone Extract of *Holarrhena Floribunda* Extract

concentration		Inhibition zone (mm)								
Mg/ml	*Staphylococcus aureus*	*Escherichia coli*	*Bacillus subtilis*	*Pseudomonasaeruginosa*	*Salmonella typhi*	*Klebsiella pneumonae*	*Candida albicans*	*Asperigillus niger*	*Rihozpus stolonifer*	*Pencillum notatum*

200	20	18	18	18	18	18	14	14	14	14
100	18	14	14	14	16	14	12	12	12	12
50	14	12	12	12	14	12	12	10	10	10
25	12	10	10	-	10	10	-	-	-	10
12.5	10	-	-	-	-	-	-	-	-	-
6.25	-	-	-	-	-	-	-	-	-	-
+ve	40	38	38	38	40	38	28	28	28	28

TABLE 5: Antimicrobial Screening Result of Hexane Extract of *Holarrhena Floribunda* Extract

concentration	Inhibition zone mm)								
Mg/ml	*Staphylococcus aureus*	*Escherichia coli*	*Bacillus subtilis*	*Pseudomonasaerugi nosa*	*Salmonella typhi*	*Klebsiella pneumonae*	*Candida albicans*	*Asperigillus niger*	*Rihozpus stolonifer*	*Pencillum notatum*
200	14	14	12	12	12	12	14	12	14	12
100	12	12	10	10	10	10	12	10	12	10
50	10	10	-	-	-	10	10	-	10	-
25	-	-	-	-	-	-	-	-	-	-
12.5	-	-	-	-	-	-	-	-	-	-
6.25	-	-	-	-	-	-	-	-	-	-
+ve	40	38	38	38	40	38	28	28	26	26

Table 6 : Phytochemical Screening of *Holarrhea Floribunda*

Phytochemicals	Present
Alkaloid	+
Flavonoid	+
Saponin	+

Table 7: Quantity Estimation of Phytochemicals in *Holarrhea Floribunda* Extract

Phytochemicals	Percentage %
Alkaloid	38.2
Flavonoid	8.2
Saponin	11.5

In Table 1, The methanol extract showed activity against all micro organisms used from concentration of 200 mg/ml to 25 mg/ml. At the lower concentration of 12.5 and 6.25 mg/ml, the extract was inactive against fungi. Careful study of the other extracts antimicrobial activity revealed that the methanol extract was the most active based on activity against all micro-organisms at the 200 to 25 mg/ml which other extracts do not exhibit this broad activity. The methanol extract was further investigated for the presence of bioactive natural products by carrying out phytochemical screening tests. In table 6, it revealed the presence of alkaloids, flavonoids and saponins. Furthermore, the quantity estimates of these bioactives phytochemicals were investigated. In Table 7, alkaloid was discovered to be 38.2 %, flavonoid was 8.2 % and saponins was 11.5 %. Alkaloids have a pronounced action on nervous system thereby producing physiological and psychological results. They also contribute to the treatment of malaria, diarrhea and stomach ache as reported traditionally in the use of the plant. While flavonoid possesses wound healing activities and, also reduces blood pressure and stops bleeding (anti- inflammatory) and saponins is an excellent remedy for tissue repair healing and its toxic to insects at particular concentration. An infusion of the bark of *Holarrhena floribunda* is used commonly as a substitute for quinine, an alkaloid known for its antimalarial activities. The 38.2 % alkaloid in the leaf of this plant should be isolated and screened for antimalarial activities so that there can be a substitute for the alkaloid quinine. The presence of alkaloid 38.2 %, flavonoid 8.2 % and saponins 11.5 % with their various pharmacological activities could be responsible for the broad antimicrobial activities of the methanol extract of this plant. Consequentially, the antimicrobial activities of the methanol extract could be responsible for the traditional uses of *Holarrhena floribunda* in the treatment of boils, eczema ,diabetes mellitus and as stimulant, carminative, diuretic and antipyretic. It can be suggested that extensive research showed focus more on the

isolation of the phytochemicals present in the plant for further antimalarial and antibiotics drugs so as to discover new drugs. This study further corroborated or justified the use of *Holarrhena floribunda* by the traditional people who don't have access to adequate healthcare in the rural areas.

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