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Phytochemical Screening, Antimicrobial and Antioxidant Activity of Fruit Extracts of *Hydnora africana* Thumb

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ABSTRACT

This study was aimed to identify phytochemical screening of the medically important species; *Hydnora africana* fruit extracts and to evaluate its potential antimicrobial and antioxidant activities. The samples were collected from Karal village west Nyala, South Darfur State Sudan. The extraction of crude was carried out at Standards and Metrology Laboratories in (El-Obeid) then analyzed at National Research Center in Khartoum in December 2020. Phytochemical screening of secondary metabolites of the plant crude extracts (methanol, acetone, petroleum ether, chloroform and distilled water) were carried out. The results revealed the presence of alkaloids, saponins, tannins, flavonoids, terpenoids, coumarins, quinones, cardiac glycosides, xanthoproteins, glycosides, steroids, phenols, resins and carboxylic acid in different concentrations. The antioxidant activity of the extract of *Hydnora Africana* exhibited A maximum DPPH scavenging activity of acetone extract was 88% whereas for the standard propyl gallate was (82±0.01). The antimicrobial activity of the extracts was evaluted using diffusion assay against two Gram positive bacteria: *Staphylococcus aureus*, *Bacillus subtilis* and two Gram negative bacteria: *Eschericha coli*, *pseudomonas aeruginosa* and fungi *candida albicans*, the methanolic extract was active against fungi *candida albicans*at concentration 100mg/ml. The methanol and acetone extracts were active but the petroleum ether extract was partially active against four types of bacteria, but the chloroform and distilled water extracts were inactive against four types of bacteria and fungi and no distinct inhibition zones at Concentration 100mg/ml. This study Recommend further antimicrobial activity test against others types of bacteria and fungi and use modern analytical methods of bioactive components in the plant.

Key words: *Hydnora africana* Thumb, screening, antioxidant and antimicrobial activities.

INTRODUCTION

Plants are an essential part of human uses since the civilization started. Plant materials remain an important resource to combat serious diseases in the world. The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries. The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body. In the last decades, various plant extracts have been the focus of great interest from researchers because they represent natural resources of new antibacterial agents with possibly novel mechanisms of action. The potential use of these products as an alternative for the treatment of several infectious diseases

has been extensively screened. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. Therefore, it is of great interest to carry out a screening of these plants in order to validate their use in folk medicine and to reveal the active principle by isolation and characterization of their constituents. Systematic screening of them may result in the discovery of novel active compounds [1].

Hydnora Africana is an achlorophyllious plant in the family *Hydnoraceae*, native to South Africa it's parasitic on the roots of member of the family *Euphorbiaceae* it's called jakal food [2]. *Hydnoraceae* are the only angiosperms known to have no leaves or scales and are considered obligate parasites completely dependent on their hosts to survive[3]. The plant grows under ground, except for a fleshly flower that emerges above ground and emits an odor of feces to attract it's natural pollinators, dung beetles and carrion beetles [4]. *Hydnora Africana* parasitic flowering plant *Hydnoraceae* (piperales) and avoid of chlorophyll and majority life cycle under ground or have ability to photosynthesize. The root, fruits, tuber pulp (like patato) are used in folk medicine to treat infection-related diseases such as diarrhoea, dysentry amenorrhoea [5]. Medicinal plants have contributed immensely to health care in Africa *Hydnora africana* is medicinal plant played important roles as a source of antimicrobial and antioxidant [6].

The uses of chemical compounds found in the various plant species have different medicinal effects which have been shown to have scientific basis [7]. The biomolecules (phytochemicals) help the body cell wall and DNA to reduce and neutralize reactive oxygeen species (ROS) such as hydroxyl (OH^-), super oxide (O_2^-), Nitric oxide (NO), peroxyl (RO_2^-), lipid peroxyl (LOO^-). Generated during normal metabolic processes in human body.

Natural antimicrobials can be derived from plants, animals tissues and microorganism The plant have limitless ability to synthesize aromatic substances, mostly phenols or their Oxygen-substituted derivatives [8].Today there is growing interest in chemical composition of plant based medicines, several bioactive constituents have been isolated and studied for pharmacological activity, in view of the potential health benefits of this plant and coupled with the lack of scientific information literature and biochemical information on the safety of human [9].

MATERIALS AND METHODS

Plant material

Fruits of *Hydnora africana* were collected from Karal Village west Nyala City South Darfur State-Sudan in January 2020.The collected samples were identified by plant taxonomist at the Department of Botany, Faculty of Science University of Kordofan. The fruits of *Hydnora africana* were shade dried and coarsely powdered by Hammer mill [10].

Preparation of extracts

To prepare Methanolic, Chloroformic, Acetonic and Petroleum ether extracts 30g of plant separately macerated with above solvents and allowed to stand for 72 hrs and then filtered. The filtrates were evaporated at room temperature and air dried. The dried extracts were stored at 5°C in the refrigerator, until required for use. For the aqueous extract 30g of plant material macerated in 200ml distilled water for 48hrs and filtered with vacuum and the filtrate was kept in refrigerated at 5°C until required for use [11].

Phytochemical analysis

Phytochemical screening of bioactive chemical constituents in the medicinal plant under study was carried out by using standard procedures as described in [12,13].

Preparation of reagent

Preparation of Mayer's reagent

0.355g of mercuric chloride was dissolved in 60ml of distilled water and weighted 5g of potassium iodide was dissolved in 20ml of

distilled water the both solutions were mixed and volume was raised to 100ml with distilled water.

Preparation of Dragendroff 's reagent
Solution A: 0.42g of bismuth nitrate and 5g of tartaric acid were dissolved in 20ml of distilled water. Solution B: 4g of potassium iodide was dissolved in 10ml distilled water. Both solutions (A and B) were mixed in 1:1 ratio.

Phytochemical screening of chemical constituents

Test for flavonoids
The filtrate was used for following tests.

(a) 2ml of filtrate mixed with 3ml of 1% Aluminium chloride in methanol in a test tube. Formation of yellow color indicate the presence of flavonols, flavones and chalcones.

(b) 2ml of filtrate was mixed 3ml of 1% potassium hydroxide in a test tube. A dark yellow color Indicate the presence of flavonoids.

(c) 2ml filtrate and 5ml of the dilute ammonia solution and drops of concentrate H_2SO_4 were mixed. The appearance of yellow coloration indicate the presence of flavonoids.

Test for alkaliods
2ml of various extract was mixed in 4ml 1% HCl, were treated separately with both reagents (Mayer and Dragendroff), after which it was observed whether the alkaloid were present or absent in the turbidity or precipitate formation.

Test for glycosides
2ml of various extract were hydrolyzed separately 2ml conc HCl and boiled for 20 Minute on water bath and hydrolyzed extract were subjected to the following tests: a small amount of alcoholic extracted of samples was dissolved in 1ml water and then aqueous 10% sodium hydroxide was added and formation of yellow color indicate the presence of glycosides.

Test for steroids
1ml of various extract was mixed with 2ml of acetic anhydride followed by 1ml of the color changed from violet to blue or green in some samples indicate the presence of steroids.

Test for phenols
1ml of various solvent extracts for sample, 2ml of distilled water followed by few drops of 10% aqueous ferric chloride solution were added. Formation of blue or green color indicate the presence of phenols.

Test for terpenoids (salkowski test)
2ml of various solvent extract was mixed in 1ml of chloroform followed by the carful addition of 1ml concentrated H_2SO_4. A layer of the reddish brown coloration was formed at the interface thus indicating appositive result for the presence of terpenoids.

Test for saponins
3ml of various solvent extract mixed with 2ml distilled water in a test tube and was shaken vigorously and boiled in water bath the formation of stable persistent forth, was mixed with 3 drops of olive oil and shaken vigorously for the formation of emulsion thus a characteristic of the presence of saponins.

Test for resins
1ml of various solvent extract were treated with few drops of acetic anhydride solution followed by 1ml of conc. H_2SO_4, the resins give colouration ranging from orange to yellow.

Test for tannins
1ml of various solvent extract treated with 1% aqueous ferric chloride ($FeCl_3$) solution the appearance of intense green, purple, blue or black color indicate the presence of tannins.

Test for cardiac glycosides (Keller-Killani test)
2ml of various solvent extract was mixed with 2ml of glacial acetic acid containing one drop of ferric chloride $FeCl_3$ 10% solution, followed by addition of 1ml concentrated sulfuric acid. Brown ring was formed at the interface which

indicated the presence of deoxysugar of cardenoloides. Aviolet ring may appear beneath the brown ring, while in the acetic acid layer, a greenish ring may also form just gradually through out the layer.

Test for carboxylic acid

1ml of various extracts was separately treated with a few drops of sodium Bicarbonate solution. Effervescence (due to liberation of carbon dioxide) indicates the presence of carboxylic acid.

Test for coumarins

1ml of various extracts was treated with 1N NaOH solution, the test tube was placed for 5 minutes in boiling water examined on sun light or under the UV light for yellow color or yellow fluorescence indicate the presence of coumarins.

Test for Quinones

1ml of various extracts was treated separately with alcoholic potassium hydroxide solution. Quinines give coloration ranging from red to blue.

Test for Xanthoproteins

1ml of various extracts were treated separately with few drops of conc. HNO_3 and NH_3 solution. Formation of reddish orange precipitate indicates the presence of xanthoproteins [14].

Preparation of bacterial suspensions

One ml aliquots of a 24 hours broth couture of the test organisms were aseptically distributed on to nutrient agar *slopes* and incubated at 37°C for 24 hours. The bacterial growth was harvested and washed off with 100ml sterile normal saline, to produce a suspension containing About 10^8-10^9 .µ|ml. The suspension was stored in the refrigerator at 4°C till used. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique. Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by micro pipette on to the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37°C for 24 hours, after incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension. Each time afresh stock suspension was prepared, all the above experimental condition were maintained constant so that suspensions with very close viable counts would be obtained [15].

Preparation of fungal suspension

The fungal cultures were maintained on sabouraud dextrose agar, incubated at 25°C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspension in 100ml of sterile normal saline, and the suspension were stored in the refrigerator until used [16].

Test of antibacterial susceptibility

The paper disc diffusion method was used to screen the antibacterial activity of plant extracts and performed by using Mueller Hinton agar (MHA). The experiment was carried out according to the national committee for Clinical Laboratory Standards Guidelines, Bacterial suspension was diluted with sterile physiological solution to 10^8 mg/ml (turbidity=McFarland 0.5). One hundred micro liters of bacteria suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (whatman No.1, 6mm in diameter) were placed on the surface of the MHA and soaked with 20µl of a solution of each plant extracts. The inoculated plates were incubated at 37°C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured.

Antioxidant activity

The test sample were allowed to react with 2.2Di (4-tert-octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at

37°C ,the concentration of DPPH was kept as (300µM). The test samples were dissolved in Dimethyl sulfoxide (DMSO) while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517nm using multiply Reader spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated control group; all tests and analysis were run in triplicate [17].

RESULTS AND DISCUSSION

Phytochemical screening

Phytochemical screening of *Hydnora africana* fruit extracts were carried out to detect the presence or absence of various types of secondary metabolites. The results were shown in table (1). *Hydnora africana* fruit extracts contain very high amount of flavonoids, steroid and phenols in methanolic extract. And high amount of flavonoids, alkaloids, resins, carboxylic acid and coumarins in chloroform extract. And all plant extract contain trace amount of terpenoids and xanthopretins and Quinones. The presence of these metabolites in different plant extracts explains the various uses of this plant in traditional Medicine.

The results of antioxidant activity for the extracts of *Hydnora africana* fruit were shown in table (2). The values of antioxidant activity of *Hydnora africana* fruit considerably (0.01-0.04 mg/ml). When compared with Ciprofloxacin (0.01-0.1 mg/ml). The values of acetone, petroleum ether and methanol are equal with Ciprofloxacin and the standard propyl gallate (0.01).

The presence of flavonoids in crude extract of *Hydnora africana* fruit is importance since they have been reported to exhibit antimicrobial, anti-inflammatry, analgesic, anti-alergic, antioxidant, antitrypanosomal and anti-leishmanial properties [18].

Antioxidant activity

The percentage of DPPH radical scavenging activity presented in table (4.2). Exhibited A maximum DPPH scavenging activity at 88% whereas for the standard (propyl gallate) was found to be 82%. The presence of saponins in this plant may account for management of excess cholesterol and thus reduce the risk of cardiovascular disease [19]. Flavonoids have been shown to exert potent antioxidant activity against the superoxides radical [20].

Assessment of antimicrobial activities of the extracts

The highest sensitivity shown by the tested bacteria against the acetone, petrolum ether and methanol extracts of *Hydnora africana* fruit could suggest the presence of anti-bacterial activity in the plant. Several studies have also been reported by other reserchers for the antibacterial activity of *Hydnora africana* and *Hydnora abyssinica* [21]. The distilled water and chloroform extracts were weak effects of antimicrobial activity.

The antimicrobial activities of the tested extracts were quantitatively assessed by the presence or absence of inhibition zones diameter. Results presented in table (3) clearly demonstrated that the *Hydnora africana* fruit extracts exhibited significant antimicrobial activity against all tested Gram-positive and Gram-negative bacteria. The *Hydnora africana* fruit extracts showed highly activity in methanol extract against *Escherichia coli, Staphylococcus aureus, Bacillus subtilis* and fungus *Candida albicans* and poor activity against *Pseudomonas aeruginosa* in concentration 100mg/ml. The acetone extracts also highly activite against *Escherichia coli, staphylococcus aureus, Bacillus subtilis* and *Pseudomonas aeruginosa* and poor active against *Candida albicans.* But petrolum ether extract has weak activity against *Esecherichia coli* and *Staphylococcus aueus* in concentration 100mg/ml.

Table 1: Phytochemical screening of *Hydnora africana* fruit extracts

S.NO	Constituents	Aqueous	Methanol	Acetone	Pet. Ether	Chloroform
1	Flavonoids	+ +	+ + +	+ ++	+	+ + +
2	Alkaloids	+	+	+	+	+ +
3	Glycosides	+	-	+	-	-
4	Steroids	++ +	++ +	+	+	+
5	Phenols	+ + +	+ + +	+	+	-
6	Terpenoids	+	+	+	+	+
7	Saponins	+	+	+	+ +	+
8	Resins	+	+ +	+ +	-	+ +
9	Tannins	+	+	+ +	+	-
10	Cardiac glycosides	+	+	++ +	+	+ +
11	Carboxylic acid	+	+	+ +	+	+ +
12	Coumarins	+	-	-	+	+ +
13	Quinones	+	+	+	+	-
14	Xanthopretins	+	+	+	+	+

+++: Strong intensity reaction; ++: Medium intensity reaction; +: Weak intensity reaction; - No reaction [2]

Table 2: Antimicrobial activity of *Hydnora africana* fruit extracts (inhibition zone in mm)

S.NO	Extract	*E. coli*	*P. auruginosa*	*B. subtilis,*	*S. aureus*	*C. albicans*
1	Acetone	13	17	16	14	-
2	Pet. Ether	10	-	-	10	-
3	Methanol	16	-	17	15	15
4	Chloroform	-	-	-	-	-
5	Aqueous	-	-	-	-	-

– Gram negative; *E. coli, and P. auruginosa* Gram positive; *B. subtilis, and S. aures Fungi; C. albicans*.
– Activity: <9 inactive, 9-12 partially active, 13-18 active, >18 very activity

Table 3: Antioxidant activity of *Hydnora africana* fruit extracts

S.NO	Extract	%RSA ±SD (DPPH)
1	Petrolum ether	83±0.01
2	Chloroform	63±0.04
3	Distilled water	75±0.04
4	Acetone	88±0.01
5	Methanol	82±0.01

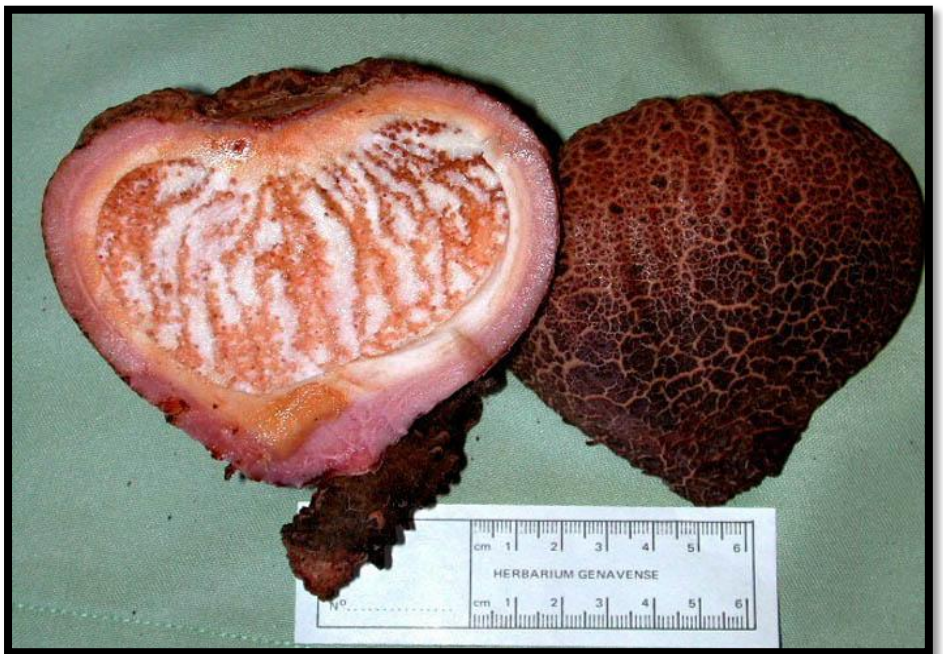

Fig 1. *Hydnora africana* fruit

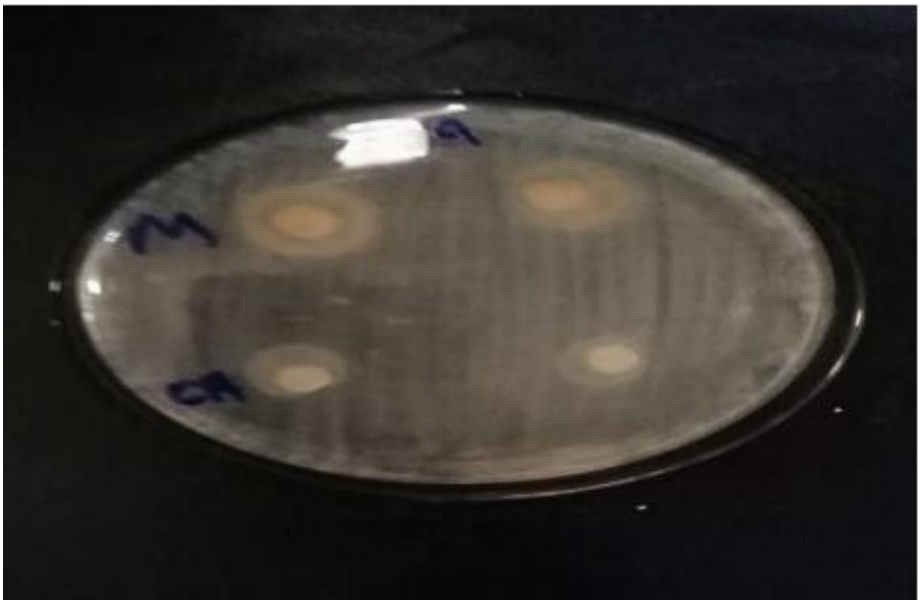

Fig. 2: Inhibition zones of antibacterial activity of *methanol*, chloroform ether extracts against *Staphylococcus aureus.*

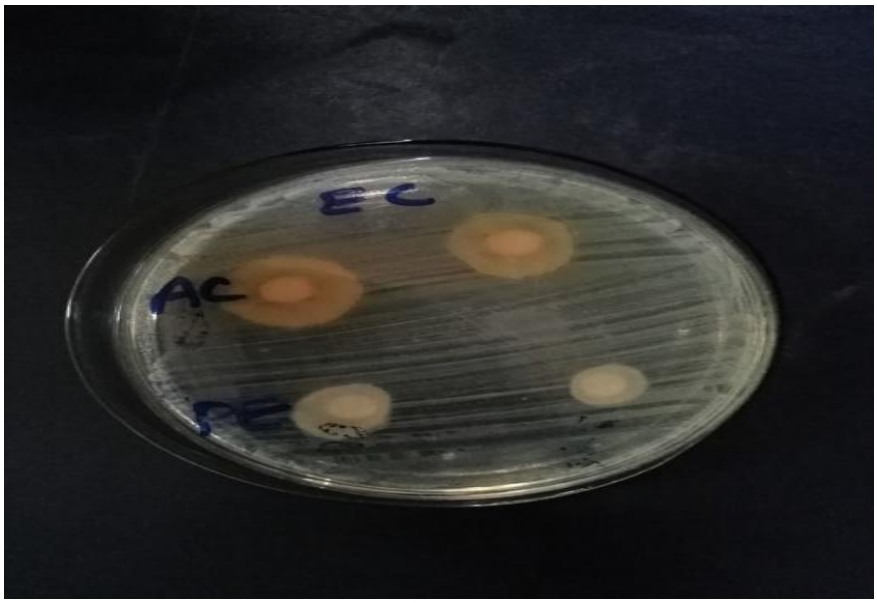

Fig. 3: Inhibition zones of antibacterial activity of *acetone*, petroleum ether extracts against *Escherichia coli.*

CONCLUSION

The results indicated that the water and ethanolic extracts of the *Hydnora africana* have considerable antimicrobial and antioxidant activity.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

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