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SHORT COMMUNICATION

FREE RADICAL SCAVENGING, PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITIES OF *Hymenoacadia acida* LEAVES

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Abstract

Hymenocadia acida belongs to the family Phyllanthaceae. Leaves, bark and roots are used to treat hypotension, diabetes, epilepsy and schizophrenia. In this study, antioxidant and antimicrobial activities of leaves extract of *Hymenocadia acida* were investigated. The extract was also screened for the presence of phytochemicals. The antioxidant activity was investigated using DPPH radical scavenging activity while antimicrobial activity was investigated using surface plate and agar diffusion methods. At concentration of 1.0 mg/mL, the leaves extract displayed significant antioxidant activities. The IC_{50}value calculated was 0.5 mg/mL. The extract was also active against all microorganisms used at concentration of 50, 100 and 200 mg/mL. Phytochemical tests revealed the presence of flavonoids, terpenoids, alkaloids, steroids, saponins and cardiac glycosides. This study revealed that *Hymenocadia acida* is a good source of antioxidants, and also justifies or provides scientific evidence to support the use of this plant in the treatment of fever, diarrhea and mouth infections due to its antibacterial, antifungal activities and presence of bioactive secondary metabolites.

Keywords: Antioxidant, Antimicrobial, DPPH, Phytochemicals, *Hymenocadia acida*

Introduction

Hymenocadia acida belongs to the family Phyllanthaceae. It is a small tree that grows to 10 m. The leaves are used in northern Nigeria to manage sickle cells diseases. Leaves, bark and roots are used to treat hypotension, diabetes, epilepsy and schizophrenia (Ibrahim *et al.,* 2007). Compounds isolated include cyclopeptide alkaloids from the roots (Emmy *et al.,* 2016). The intake of antioxidant compounds present in food is an important health protecting factor. Natural antioxidants present in foods have attractedconsiderable interest because of their presumed safety and potential nutritional and their therapeutic effect (Sulekha *et al.,* 2009). Because extensive and expensive testing of food additives is required to meet safety standards, synthetic antioxidants have generally been eliminated from many food applications. The increasing interest in the search for natural replacements for the synthetic antioxidants has led to the antioxidant evaluation of a number of plant sources. Mediterranean diet which is rich in natural antioxidants leads to limited incidence of cardio-and cerebrovascular diseases (Frei *et al.,*1988). Free oxygen radicals plays cardinal role in the etiology of several diseases like anthritis, cancer e.t.c. These free radicals are highly

toxic and thus generate oxidative stress in plants. In plants, these free radicals are deactivated by antioxidants. Antioxidants constituents of plants act as radical scavengers and helps in converting the radicals to less reactive species (Sulekha *et al.,*2009)

Materials and Methods

Materials

2,2-diphenyl-1-picrylhydrazyl, Whatman No. 1 filter paper, rotary evaporator, methanol Ascorbic acid, UV spectrometer, Dragendoff reagent, Hydrochloric acid, with Fehling solution A and B. Dextrose Agar, Ferric chloride reagent, Sulphuric acid.

The leaves of the plant were collected in Ibadan, Oyo State and authenticated at the Forestry Research Institute of Nigeria, Ibadan, Oyo state with voucher specimen FHINO. 109676.The leaves of *Hymenocadia acida* were plucked and air dried.

Methods

Preparation of Crude Extracts

The plants material was air dried at room temperature for 72 hours. The leaves were then pulverized. Methanol was used as extraction solvent. Maceration method of extraction was used. The pulverized leaves were soaked with methanol for 3 days. The mixture was decanted. The resulting solvents were distilled and crude extracts recovered.

Determination of DPPH Radical Scavenging Capacity

The effect of the extract on DPPH radical was estimated adopting the method of (Liyana *et al.,* 2002). A solution of 0.135 mL DPPH in methanol was prepared and 1.0 mL of this solution was mixed with 1.0 mL of extract in methanol containing 0.02-0.1 mg of extract. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. the absorbance of the mixture was measured spectrophotometrically at 517 run. Ascorbic acid was used as standards. Half maximal inhibitory concentrationIC_{50} value was calculated using Graph pad software.

Antibacterial Testing

Agar diffusion method used for antibacterial assay. 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 1:100 (10^{-2}) dilution from 10^{-2} dilution. 0.2 ml was taken into sterile molten nutrient ager at 45^{0}C. This was aseptically poured into the sterile plates 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. In the wells, different prepared concentrations of the sample were introduced. All the concentration was introduced into the wells with negative and 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 h (Afolayan, 1997).

Surface plate method was used for antifungal 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 spreader. Then a sterile cork borer was made to create wells inside the set plate. Ticonazole was used for Positive control for fungi. All these plates were then incubated at 20 - 26^{0}C for 48 hours (Afolayan,1997).

Phytochemical Screening Tests

The extract was screened for thepresence of phytochemicals using standard methods (Sofowora ,1993).

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 Flavonoids

To 2 ml of extract, 1 ml of 2N sodium hydroxide was added. Presence of yellow color indicates the presence of flavonoids

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 Tannins

To 1 ml of extract, 2 ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

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 colour interphase is a positive test for steroids

Test for Terpenoids

0.5 ml of the extract was treated with 2 ml of chloroform and conc. sulphuric acid. Formation of red brown colour at the interface indicates the presence of terpenoids placed in unknown environment with obstacles, it moves and avoids all obstacles with considerable accuracy.

Results and Discussion

Table 1: Free radical scavenging capacity of the methanol extract of *Hymenocadia acida* leaves

CONCENTRATION mg/mL	**ABSORBANCE (nm)**			
		Ascorbic	Scavenging Activity %	
	Sample	Acid	Sample	Ascorbic acid
0.625	1.045	0.580	21	52
0.250	0.455	0.520	29	58
0.125	1.244	0.560	23	56
0.5	0.507	0.420	42	60
1.0	0.788	0.406	44	68

Table 2: Antimicrobial activities of methanol extract of *Hymenocadia acida* leaves Concentration

Concentration	**Inhibition Zone**							
(mg/ml)	**(mm)**							
	S. aureus	*E. Coli*	*B. subtilis*	*P. aeruginosa*	*S. typhi*	*K. pneumonae*	*C. albicans*	*P. notatum*
6.25	-	-	-	-	-	-	-	-
12.5	10	-	10	-	-	-	-	-
25	12	10	12	-	-	-	10	-
50	14	12	14	10	10	10	12	10
100	16	14	16	14	14	12	14	12
200	18	16	18	16	16	14	18	14
+ve	38	40	38	40	38	36	28	28

Table 3: Phytochemical screening of *Hymenocadia acida* leaves

Phytochemicals	Present
Alkaloid	+
Flavonoid	+
Saponin	+
Tannin	-
Steroids	-
Terpenoids	-

The methanol extract was active against all the micro-organisms used at concentration of 200 to 50 mg/ml. At concentration of 12.5 mg/ml, it was active against *staphylococcus aureus* and *Bacillius substilis* only. This extract is more active on bacteria than fungi. At concentration of 1.0 mg/ml, the leaves extract displayed significant antioxidant activities. The extract gave a significant IC_{50} value of 0.50 mg/mL as compared with the standard ascorbic acid with IC_{50} value of 0.25 mg/mL. This indicates than *Hymenocadia acida* is a good source of antioxidant. This gives added value to the pharmacological uses of this plant. It is well known that Mediterranean diet which is rich in natural antioxidants preventscardio-and cerebrovascular diseases (Frei *et al.,*1988). Phytochemical screening also revealed the presence of secondary metabolites such as alkaloid, flavonoid and saponin. Alkaloids have a pronounced action on nervous system thereby producing physiological and psychological results while flavonoid possess wound healing activities and also reduces blood pressure. The presence of alkaloids and flavonoids give an added value to the traditional use of the plant.

Conclusion

The presence of this phytochemicals and its antimicrobial activity against microorganisms causing diseases could be responsible for the traditional medicinal uses of the plant. The antioxidant activity should be investigated further to isolate and determine the type of antioxidants present in the leaves.It can be deduced from the study that the plant can be utilized as a good source of antimicrobial and antioxidants constituents

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