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Antioxidant Capacity and Antibacterial Activities of Leaf Extract of Adansonia digitata

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ABSTRACT

This research work investigated the antioxidant and antibacterial activities of the leaves of *Adansonia digitata* L.malvaceae (Baobab tree) to justify its use in traditional medicine practice. The leaf extract of *Adansonia digitata* (Baobab tree) was evaluated for antioxidant and antibacterial activities using 2,2- diphenyl -1-picrylhydrazyl (DPPH) method and the disc diffusion method respectively. The extract was also screened for the presence of phytochemicals. At concentration of 0.062 mg/ml, the leaves extract displayed high free radical scavenging activities. The extract was also active against all the tested bacteria but inactive against fungi at concentration of 200 to 100 mg/ml. Preliminary phytochemical tests revealed the presence of flavonoids, alkaloid and saponins. This study revealed that *Adansonia digitata* is a good source of antioxidants and also justifies the use of this plant in the treatment of fever, dysentery, diarrhea, inflammatory ulcers and diuretic activities due to its antibacterial activities.

Keywords: Antimicrobial, Antioxidant, Adansonia digitata, DPPH, Disc diffusion

INTRODUCTION

Mediterranean diet which is rich in natural antioxidants leads to limited incidence of cardiovascular diseases. Antioxidants constituents of plants act as radical scavengers and helps in converting free oxygen radicals that are responsible for cardiovascular diseases to less reactive species (Liyan et al., 2002). Natural antioxidants present in foods have attracted considerable interest because of their presumed safety and potential nutritional and their therapeutic effects (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 (Sulekha et al., 2009). Adansonia digitata L. belongs to the family malvaceae. It is a tree revered in Africa for its medicinal and nutritional value .It is used to treat diarrhoea, malaria and microbial infections. It is used as a forage for ruminants in dry season.(Lockett et al., 2000).The bark has astringent properties and has been used traditionally to alleviate colds, fevers. The leaves may be used as an antiperspirant and they may also have been used to treat fever, kidney and bladder diseases. (Sulaiman et al., 2011). It is also used as a forage for ruminants in dry season (Deshmukh et al., 2013). Adansonia digitata L. is highly valued for a variety of food and artisanal uses and for its cultural symbolism extending over millennia (Kamatou et al., 2011) .The fruit has antiinflammatory, febrifuge and analgesic properties

due to the presence of saponins and sterols and contains astringent compounds (tannins, cellulose) which exert an antidysenteric action due to its osmotic effect and an inhibitory interaction with acetylcholine, the neurotransmitter that is responsible for gut spasms (Vertuani, 2002).

MATERIALS AND METHODS

Materials: Whatman No. 1 filter paper, DPPH, methanol, ascorbic acid, Agar solution, gentamicin, Molten Sabour Dextrose Agar (SDA), Ticonazole, hydrochloric acid, Dragendoff reagent, chloroform, H_2SO_4 distilled water, Ferric chloride reagent, Fehling solution A and B, benzene, aqueous H_2SO_4

Methods: The leaves of the plant *Adansonia digitata* were collected in Ibadan, Oyo State. The leaves of *Adansonia digitata* were plucked and air dried. 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

Preparation of Crude Extract

The plants material was air dried at room temperature for 72 hours, pulverized into powdered form and a portion (100 g) of the material was extracted in methanol at room temperature for 24 hours. Extract was filtered through Whatman No. 1 filter paper and the filtrate evaporated into dryness at 40° C using rotary evaporator.

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Determination of DPPH Radical Scavenging Capacity

The effect of the extract on DPPH radical was estimated adopting the method of Liyana *etal.*, (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. The ability to scavenge DPPH radical was calculated by the following Eq:

DPPH radical scavenging activity (%) = [(Abs_{control}) - Abs_{sample}) /(Abs_{control}]x100

Where,

 $Abs_{control} = Absorbance of DPPH radical + methanol$

Abs_{sample} = Absorbance of DPPH radical + sample extract/standard

Antibacterial Testing

Agar diffusion method (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° 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 were 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 hrs (Grierson and Afolayan, 1999).

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 as Positive control for fungi. All these plates were then incubated at $20 - 26^{\circ}$ C for 48 hours (Afolayan and Meyer, 1997).

Phytochemical Screening

Test for Saponins

About 0.5 g of extract was shaken with water in a test tube. Observation of frothing was 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 was 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 colour interphase was 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 was 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 red precipitate was 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 was indicative of anthraquinone.

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RESULTS AND DISCUSSION

Table 1: Antimicrobial screening of the leaves extract of Adansonia digitata

Concentration		Inhibition zone(mm)						
Mg/ml	Staphylococcus aureus	Escherichia coli	Bacillus subtilis	Pseudomonasaeruginosa	Salmonella typhi	Klebsiella pneumonae		
200	14	12	16	16	12	12		
100	12	10	14	14	10	10		
50	10	-	12	10	-	-		
25	-	-	10	-	-	-		
12.5	-	-	-	-	-	-		
6.25	-	-	-	-	-	-		
+ve	38	40	38	40	38	36		

Table 2: Antioxidant activities of leaves Extract of Adansonia digitata

CONCENTRATION mg/mL	ABSORB	ANCE (nm)			
		Ascorbic			
	Sample	Acid	Scavenging Activity %		
	-		Sample	Ascorbic acid	
1.0	1.238	0.406	51	44	
0.5	0.722	0.420	71	42	
0.25	1.295	0.520	48	29	
0.125	0.931	0.560	63	23	
0.625	0.588	0.580	77	21	

Table 3 : Phytochemical screening of leaves extract of Adansonia digitata

Phytochemicals	Present	
Alkaloid Flavonoid Saponin	+ + + +	

The result in table 1 showed that the methanol extract was active against bacterial only at concentration of 200 to100 mg/ml. At concentration of 25 mg/ml, it was active against Bacillius substilis only. At concentration of 1 mg/ml to 0.625 mg/ml, the free radical scavenging activity of the methanol extract of the leaves of Adansonia digitata showed more activity than the standard ascorbic acid used as shown in the result in table 2. The result in table 3 revealed the presence of alkaloid ,flavonoid and saponin from the phytochemical screening. The free radical scavenging activity of the methanol extract of the leaves indicates that Adansonia digitata is a good source of antioxidant. Previous work done on the fruit pulp of Adansonia digitata indicated that it is a valuable source of vitamin C. The red funicles of the fruit have an impressive antioxidant capacity (Vertuani,2002). This research showed that the leaves of Adansonia digitata also have an impressive antioxidant capacity. This plant therefore will aid in the prevention of degenerative

diseases like cancer and tumour if consumed since antioxidants have been implicated in the prevention of these diseases. These phytochemicals have reported bioactivities, they have a pronounced action on nervous system thereby producing physiological and psychological results, possess wound healing activities and also reduces blood pressure. The antimicrobial activity of the leaves extract of *Adansonia digitata* against diseases causing microorganisms may be responsible for the medicinal uses of this plant. Most antibiotics have lost their effectiveness due to development of resistant strains.

CONCLUSION

The result of this research study has justified the use of the plant for the treatment of infectious diseases caused by bacteria and fungi. The study also suggests that leaves of *Adansonia digitata* may be utilized as a safe natural source for antioxidant and antimicrobial agents.

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