Antibacterial, Antioxidant and Phytochemical Analysis of Edible Parts of Potent Nutraceutical Plant – *Adansonia digitata*

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Abstract

***Adansonia digitata* Linn. (*Bombacaceae*), commonly known as baobab has a great potential to be exploited by food and beverages, nutraceutical and natural cosmetic section. Leaves, stem, bark, fruit pulp and seeds of this tree are employed as food stuff as well as medicine hence aptly called as small pharmacy. The present study aims at assessment of nutraceutical potential of methanolic extracts of edible parts of Baobab viz., leaf (MEL), stem (MESt), fruit pulp (MEFP) and seed (MESe) in terms of antioxidant and antibacterial activity. Antibacterial activity was evaluated by Agar Disc Diffusion method against *Klebsiella pneumoniae*, *Proteus mirabilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Bacillus subtilis*, *Nocardia* sp. and *Staphylococcus aureus*. All extracts exhibited very little or no zone of inhibition indicating less sensitivity towards bacteria. The oxidative stress due to free radicals is one of the causes for majority of diseases in humans. Antioxidant activity of edible parts of *A. digitata* was evaluated on the basis of DPPH free radical assay. IC_{50} was found to be 22 µg/ml (MESt), 23 µg/ml (MEL), 50 µg/ml (MEFP) and 94 µg/ml (MESe) indicating strong antioxidant activities in all edible parts of the plant. Phytochemical analysis of these extracts indicated presence of anthracene, bitter principles, coumarin, flavonoid, lignan and tannin in different plant parts.**

INTRODUCTION

Adansonia digitata Linn. (*Bombacaceae*), commonly called as baobab is famous for its peculiar habit with irregularly shaped huge broad trunk rapidly narrowing upwards (Fig. 3A). The tree provides food, water and shelter (Swaminathan and Kochhar, 2003); young leaves and fruit pulp are edible and used as a vegetable and are a staple food source for rural populations in many parts of Africa (Gebauer et al., 2002). Seeds and fruit find immense industrial applications as thickening and flavouring agent and beverages (Sidibe and Williams, 2002). Baobab is also called as "tree of life" as its parts share both "high nutritional-high medicinal properties".

As a part of the research program to evaluate the nutraceutical potential of *Adansonia digitata*, preliminary investigations were done on antioxidant and antibacterial activity of different edible parts of the baobab tree.

All these edible parts show significant medicinal properties. Leaves and seeds are internally given as an astringent, sudorific, tonic and febrifuge (Kirtikar and Basu, 1933; Anonymous, 1985). Seeds are used in dysentery and infusion of leaf is used in diarrhea, fever, inflammation, kidney and bladder diseases, blood clearing and asthma (Gruenwald and Galizia, 2005). Also cooked ground and given in toothache and gingivitis. The fruit pulp can be utilized as an excipient in tablet formulations; it has properties of a lubricant, glidant and diluent (Anonymous, 1985, 2001). Pulp is aperient and demulcent and is used in the treatment of fevers, dysentery and haemoptysis (Kirtikar and Basu, 1933). The dried pulp is the richest known source of vitamin C, vitamin A and calcium having six times more ascorbic acid content than orange (Vertuani et al., 2002). It is administered with water during chronic bronchial asthma, itching in case of allergic dermatitis urticaria. Stem bark is bitter and has been used as a substitute for cinchona bark (Anonymous,

1985). It contains a crystalline bitter principle adansonin and β-sitosterol (Kirtikar and Basu, 1933; Anonymous, 1985) and finds use in traditional medicine for treating sickle cell anaemia.

Water soluble fraction of fruit pulp of *Adansonia digitata* is reported to have stimulating effects on the proliferation of important gastrointestinal bacteria (Gruenwald and Galizia, 2005). Hence preliminary study was taken up to see the effect of aqueous methanolic extracts on the growth of normal microbial flora of GIT and other pathogenic bacteria.

The food rich in vitamin C content show remarkable capacity to reduce reactive oxygen species levels and protect the body against early ageing, cancer and other diseases. High ascorbic acid content of Baobab fruit pulp projects it to be strong nutritional antioxidant. Different edible parts of baobab were evaluated for free radical scavenging activity using DPPH assay.

Phytochemical analysis was also done to detect different secondary metabolites as they impart medicinal and other properties useful for humans and can subsequently provide phytochemical leads for pharmaceutical development.

MATERIALS AND METHODS

Plant Materials

Different edible parts of *Adansonia digitata* L. (Fig. 3B-E) were collected from nearby areas, cleaned, shade dried, powdered and used for extractions.

Extractions

The aqueous methanolic (1:4) extracts of edible parts of baobab viz., leaf (MEL), stem (MESt), fruit pulp (MEFP) and seed (MESe) were prepared. The extracts were filtered and evaporated in water bath to obtain residue, 200 mg/ml solution of this residue was used for investigations.

Antibacterial Determinations

Antibacterial activity was evaluated by Agar Disc Diffusion method as per NCCLS (1993) against 8 different bacteria namely *Klebsiella pneumoniae*, *Proteus mirabilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Bacillus subtilis*, *Nocardia* sp. and *Staphylococcus aureus*. Discs (6 mm in diameter) were impregnated with different extracts (200 µg/ml) and incubated at 37°C (except *Pseudomonas aeruginosa* – 32°C) with different bacterial strains on LB media. Inhibition zone was recorded inclusive of discs.

Antioxidant Determinations

The free radical scavenging activity of MEL, MESt, MEFP and MESe was measured in terms of hydrogen donating or radical-scavenging activity using the stable radical DPPH (Blois, 1958). 0.1 mM solution of DPPH (2,2-Diphenyl-1-picryl hydrazyl) in aqueous methanol (1:4) was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution at different concentrations (10-100 µg/ml). The reaction mixture was left in the dark at room temperature 37°C for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid was used as standard reference. The ability to scavenge DPPH radical was calculated by the following equation: DPPH radical scavenging activity (%)=$[(Abs_{control}-Abs_{sample})]/(Abs_{control})]\times 100$. The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value is defined as the concentration (in µg/ml) of extracts that inhibits the formation of DPPH radicals by 50%.

Phytochemical Evaluations

Phytochemical screening of extracts was done by TLC method using different solvent systems and spray reagents by standard procedures (Wagner and Bladt, 1996;

Harborne, 2005) for the detection of anthracene, bitter principles, coumarin, hydroxy cinnamic acid, flavonoid, lignan, tannin and saponin. The class of compounds was identified from its colour test, its solubility, R_f properties, its UV spectral characteristics and direct comparison with authentic materials.

RESULTS AND DISCUSSION

Antibacterial Activity

Table 1 summarizes antibiotic data. The results indicated extremely variable sensitivity of different bacterial strains. Growth inhibition was observed only in *Escherichia coli*, *Bacillus subtilis*, *Nocardia* sp. and *Staphylococcus aureus* (Fig. 3G). *Proteus mirabilis* and *Salmonella typhi* were insensitive. Interestingly there was growth proliferation in case of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* (Fig. 3H) which when present in excess in GIT can become pathogenic. Bactericidal activity differed in different plant parts with fruit pulp showing antibacterial activity against all sensitive bacteria.

Antioxidant Activity

Figure 2 shows the dose-response curve of DPPH radical scavenging activity of the MEL, MESt, MEFP and MESe, compared with ascorbic acid (Fig. 1). It was observed that MESt and MEL had higher activity than that of MEFP and MESe.

Low IC_{50} value indicates higher antioxidant activity. Stem and leaf showed higher free radical scavenging activity (22 and 23 µg/ml) than fruit pulp (50 µg/ml) and seed (94 µg/ml) which is contradictory to earlier reports by Vertuani et al. (2002) and Lamien-Meda (2008) claiming high antioxidant activity of fruit pulp owing to high ascorbic acid content. The discrepancy might be due to methods and the type of extract used in the present investigation.

Phytochemical Analysis

Table 3 summarizes the results of phytochemical analysis of extracts. Phenolic compounds like anthracene, coumarin, flavonoid, lignan and tannin are reported to impart the antibacterial and antioxidant properties to plants (Lamien-Meda, 2008). TLC analysis of extract indicated presence of hydroxy cinnamic acid and flavonoids in greater quantity than anthracene, lignan and tannin in leaf and stem. Glycosidic bitter principles and coumarin are present in all parts.

CONCLUSIONS

The literature survey indicated that great stress is laid on the nutritional composition of edible baobab plant. For value addition, supporting data in terms of antioxidant and antibiotic potential is necessary. However, present studies revealed very low antibiotic activity. Further, growth stimulating activities in case of pathogenic bacteria namely *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* is a matter of concern. The antioxidant activity is found to be strong and can be used for nutraceutical exploitation of the plant. Further investigations are necessary to evaluate prebiotic activity of edible parts of baobab tree and to identify the exact correlation of phytochemicals with antibiotic and antioxidant activities to claim it as a potent nutraceutical plant.

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Tables

Table 1. Antibiotic activity of extracts against different bacterial strains.

Organism	MEL (200 mg/ml)	MESt (200 mg/ml)	MEFP (200 mg/ml)	MESe (200 mg/ml)
Gram negative bacteria				
Proteus mirabilis	-[1]	-	-	-
Salmonella typhi	-	-	-	-
Escherichia coli	6.49±0.04[3]	-	7.49±0.05	7.02±0.05
Klebsiella pneumoniae	+[2]	+	+	+
Pseudomonas aeruginosa	+	+	+	+
Gram positive bacteria				
Bacillus subtilis	6.51±0.05	-	7.02±0.05	-
Nocardia sp.	-	-	8.4±0.59	-
Staphylococcus aureus	6.52±0.02	-	7.06±0.16	-

[1] no measurable zone.
[2] growth stimulation.
[3] mean value of inhibition zone with ± S.D.

Table 2. Antioxidant activity of extracts.

Concentration (µg/ml)	DPPH radical scavenging (%)			
	MEL	MESt	MEFP	MESe
10	26.26	24.57	12.57	8.47
20	44.65	45.41	19.94	13.82
30	64.62	65.01	42.53	18.42
40	73.90	74.96	43.67	23.48
50	74.21	76.36	50.19	27.49
60	75.47	77.14	57.84	33.28
70	75.79	78.07	61.62	38.63
80	76.41	78.38	70.32	43.98
90	78.14	78.54	71.74	48.14
100	78.62	79.00	75.80	53.64
IC_{50} (µg/ml)	23 µg/ml	22 µg/ml	50 µg/ml	94 µg/ml
IC_{50} (µg/ml) of ascorbic acid	2.5 µg/ml			

Table 3. Phytochemical analysis of extracts.

Compounds▼	MEL	MESt	MEFP	MESe
Anthracene	++	+	-	+
Bitter principles	+++	++	++	++++
Coumarin	++	+	+	+
Hydroxy cinnamic acid	++	++	-	-
Flavonoid	++	++	-	-
Lignan	++	+	-	-
Tannin	+	-	+	-
Saponin	-	-	-	-

Figures

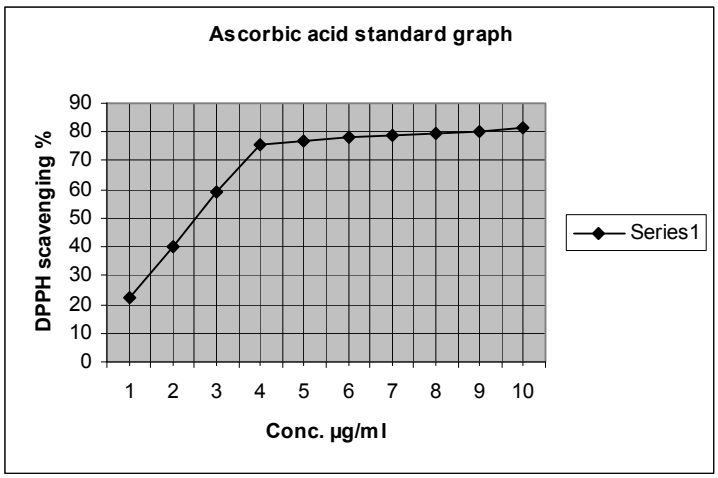

Fig. 1. Ascorbic acid standard graph.

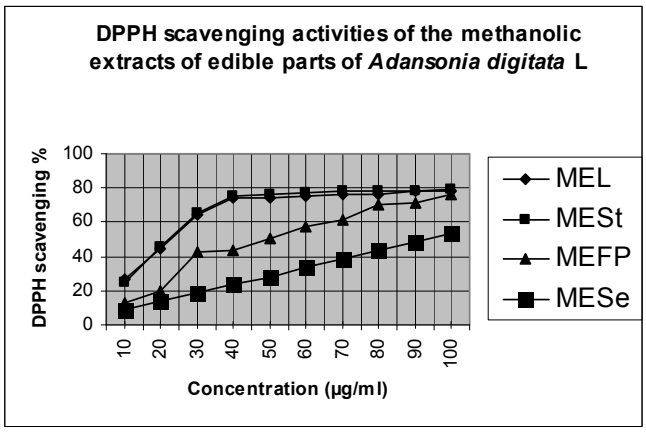

Fig. 2. DPPH scavenging activities of the methanol extracts of edible parts of *Adansonia digitata* L.

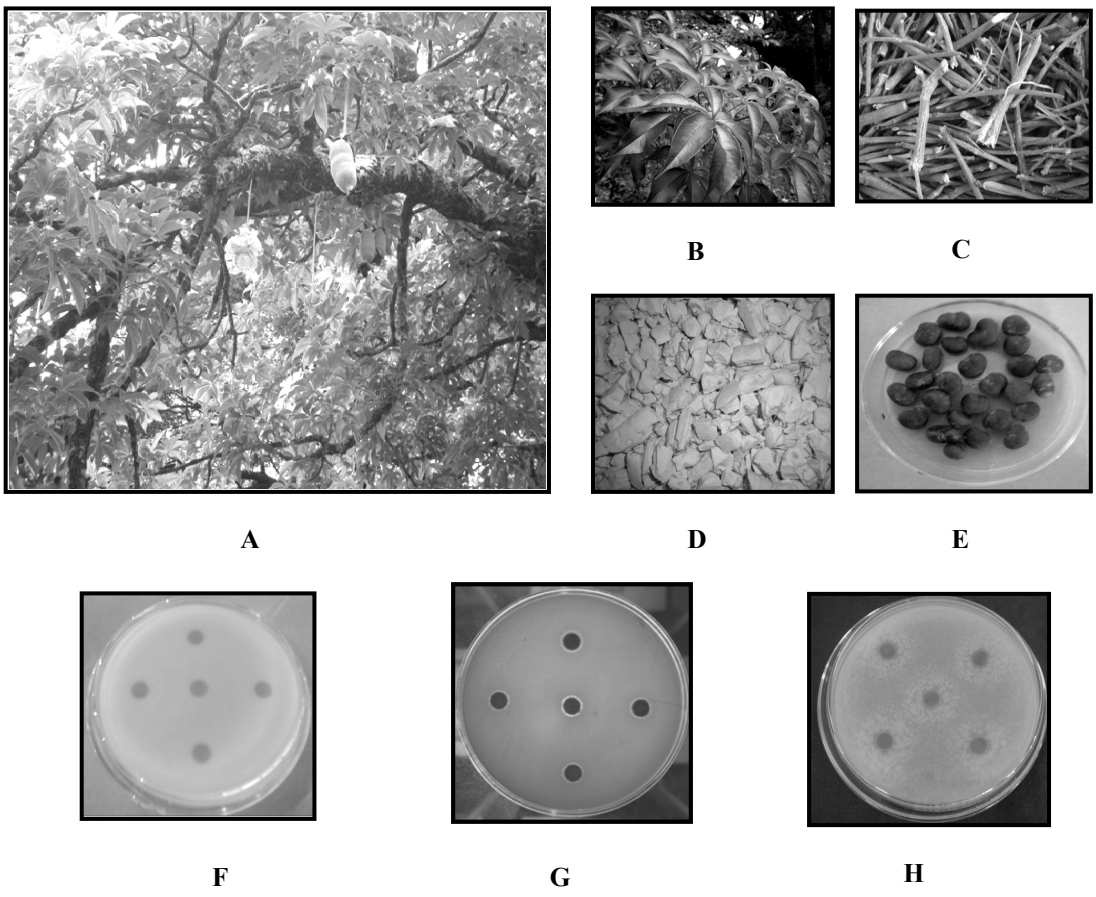

Fig. 3. A- *Adansonia digitata* habit, B- leaves, C- stem, D- pulp, E- seeds, F- *Bacillus subtilis* (no zone), G- *Staphylococcus aureus* (zone of inhibition), H- *Klebsiella pneumoniae* (zone of growth stimulation).