Universal Journal of Pharmacy
Take Research to New Heights

Research Article
ISSN 2320-303X

PRELIMINARY PHYTOCHEMICAL EVALUATION OF *IN VIVO* AND *IN VITRO* PLANT PARTS OF *ADANSONIA DIGITATA* L.: AN ENDANGERED MEDICINAL TREE

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Received 25-05-2014; Revised 17-06-2014; Accepted 13-07-2014

ABSTRACT

Adansonia digitata L. is a native deciduous tree of African savannas and belongs to the family Bombacaceae. The tree faces a crisis of survival and is enlisted as an endangered species in the Red Data book. Currently, fear has been expressed about its regeneration. *Adansonia* has immense medicinal value and possesses various pharmacological properties like anti-bacterial, anti-fungal, anti-malarial, anti-pyretic and almost all parts of the tree are used for healing purposes. To explore its pharmacological uses and isolation of bioactive compounds, phytochemical screening of *in vivo* and *in vitro* parts was carried out in Acetone, Methanol and Aqueous solvents. Results indicate the presence of phytochemicals like Alkaloids, Flavonoids, Saponins, Terpenoids and Tannins in almost all extracts. Fatty acid content was highest in methanol fruit pulp. Presence of phytochemicals may further contribute to anti-microbial activity and isolation of bioactive compounds using cell and suspension culture technology in near future. Looking into its pharmacological uses, its conservation is also initiated using Tissue culture approaches.

Keywords: *Adansonia digitata*, medicinal, pharmacological, anti-bacterial, phytochemical screening, bioactive compound, conservation, alkaloids, cell suspension.

INTRODUCTION

Adansonia digitata L. commonly known as **'Baobab'** belongs to the family Bombacaceae. It is a large, deciduous, succulent tree indigenous to the dry lands of African savannas[1,2]. The tree is found in areas of South Africa, Mali, Benin, Senegal, Ivory Coast, Sudan etc. It can grow up to 25 metres in height, 28 metres in girth and may live for several hundred years.

Adansonia is regarded as the **"Queen of all the carbon storage trees"** and possess high water holding capacity[3]. The tree has mythological significance and is known as **'Kalpavriksha'** in India[4]. The tree is rarely found and only 7-8 trees are located in Bhopal. Every part of the tree is reported to have numerous medicinal and non-medicinal uses[5]. The leaves, bark and fruit pulp have been traditionally used as immuno-stimulants, analgesics etc. in the treatment of diseases like fever, diarrhoea, cough, dysentery, haemoptysis, tuberculosis, microbial infection and worms[6]. The tree is thus named as **"The small pharmacy or chemist tree".** The seeds and oil are used as food, fuel, cosmetics and medicines in the tropical treatment of muscle wounds, dandruff and other skin ailments[7, 8].

Medicinal plants are the richest bio-resource of drugs be it traditional or modern systems of medicine, nutraceuticals, pharmaceutical intermediates or chemical entities for synthetic drugs[9]. There lies a great potential for discovering novel bioactive compounds from the plant kingdom. A variety of chemicals have been isolated from *Adansonia digitata* which belong to the class of flavonoids, steroids and vitamins[10]. Till date, large number of secondary metabolites of medicinal importance i.e., alkaloids, flavonoids and terpenoids etc. have been reported from various plant parts. Pure compounds have also been isolated through preliminary phyto-chemical screening and characterized for their anti-microbial activity against human pathogen cultures[11].

The main objective of the present study was to ascertain the presence of phytochemicals in various *in vivo* parts (leaf, bark and fruit pulp) as well as *in vitro* parts (leaf callus) and to relate further the anti-

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microbial activity and anti-oxidant activity of these extracts to the *in vitro* propagated *A. digitata*.

MATERIAL AND METHODS

Collection of samples

Different parts like leaves, bark and fruits of *Adansonia digitata* were collected from the naturally growing trees at Mandavgarh, District Dhar, Madhya Pradesh **(Plate 1).** The plant was identified by the taxonomists at the Botanical survey of India, Regional Office, Pune and accession number was assigned to *Adansonia digitata* as (SUSADDI).

Method for Callogenesis

In vivo leaves of *Adansonia digitata* **(Plate 2 A)** were washed in running water and mild detergent Labolene for 10 minutes. The leaf explants were washed with sterile water 3-4 times to remove the detergent completely. The explants were surface sterilized using 70% ethanol for 2 minutes, 0.1% mercuric chloride for 12 minutes followed by rinsing with sterile water 4-5 times. These were aseptically cut into small segments and inoculated in MS media supplemented with BAP (0.5-5.0 mg/l) and NAA (0.5-10.0 mg/l). The culture vessels were incubated at 25±2ºC with a photoperiod of 16 hour light and 8 hour darkness. The callus induced was sub-cultured after every 15 days on fresh media of the same composition for 6-8 weeks.

Preparation of Extracts

The plant material (leaf, bark and fruit pulp samples) were collected, cleaned and air dried for 20 days. After complete drying, the samples were grinded to obtain smooth, fine powder **(Plate 3).** The powdered samples were kept in small zip lock bags in air tight bottles with proper labeling at 4°C. 10 gram of the powdered samples was extracted with 100 ml of acetone, methanol and distilled water using a rotary shaker. The samples were filtered with Whatman filter paper (125 mm) and the filtrate was concentrated at 50 ºC under reduced pressure with Buchi Rotavapor for 2-6 hours. The extraction was done three times for each plant sample **(plate 4 A).**

Healthy friable leaf callus obtained on MS medium supplemented with BAP (0.5-5.0 mg/l) and NAA (0.5-10.0 mg/l) was used for the preparation of extract. The callus was grinded with liquid nitrogen (-196°C) for mechanical dissociation of the cells into fine powder. 10 gram of powdered callus was weighed and extracted with 100 ml of Methanol and distilled water. The extraction procedure was same as for *in vivo* parts. The extracts were weighed and stored at 4°C prior to the analysis.

Preliminary Qualitative screening tests

Preliminary tests for qualitative phytochemical analysis[12] **(table 1)** were applied to the prepared aqueous, methanol and acetone extracts of leaf, bark, and fruit pulp as well as aqueous and methanol extracts of leaf callus samples.

Table 1: Qualitative tests for phytochemical screening

S.No	Phytochemicals	Test procedure
1.	Phenolic compounds and tannins	Powdered sample + boiling in distilled water + filtered + 0.1% Ferric chloride gives green/blue black color.
2	Saponins[13]	Powdered sample + boiling in distilled water + filtered. Filtrate + distilled water + shaking to form persistent froth. Froth + olive oil to form emulsion.
3	Flavonoids	Plant extract + few drops of 1% ammonia solution.
4	Terpenoids	**Salkowski test:** Extract sample + Chloroform + conc. sulphuric acid along the walls. Reddish brown color appears at the interface.
5	Glycosides	**Keller-Killani test:** Concentrated sulphuric acid in a test tube + extract sample mixed with glacial acetic acid containing 1 drop of Ferric chloride. This mixture added to conc. sulphuric acid. Brown ring appears.
6	Alkaloids	**Mayer's test[14]:** Extract + ½ drops of Mayer's reagent. Appearance of white, creamy precipitate confirms the presence of alkaloids. **Wagner's test[15]:** Extract + 1/2 drops of Wagner's reagent. Appearance of red precipitate confirms the presence of alkaloids. **Dragendorff's test**: Extracts + few drops of Dragendorff's reagent. An orange brown precipitate is formed.
7	Fixed oils and fats	**Spot test:** Extracts were pressed between two filter papers. Appearance of translucent spots showed the presence of fixed oils. **Saponification test:** Extract + few drops of 0.5 Molar alcoholic potassium hydroxide solution + 1 drop of phenolphthalein + heat on water bath for 2 hours. Formation of soap occurs.
8	Leucoanthyocyanins[16]	Extracts + iso-amyl alcohol. Upper layer appears red in color.
9.	Anthocyanins	Extracts + 2N Hydrochloric acid and ammonia. Appearance of pink red color that later turns into blue-violet.
10.	Coumarins	Extracts + 3 ml of 10% sodium hydroxide. Yellow color is obtained.

11.	Proteins and amino acids[17,18]	**Biuret test:** Extracts +1 drop 2% Copper sulphate solution + 1 ml 95% ethanol + excess of potassium hydroxide. Pink color in ethanol layer appears. **Ninhydrin test:** Extract +2 drops Ninhydrin solution. Appearance of purple color shows the presence of proteins and amino acids. **Xanthoproteic test:** Extract + few drops of conc. nitric acid. Appearance of yellow color indicates the presence of proteins and amino acids.
12.	Gums and mucilages[19]	Extract + 25 ml absolute ethanol, constant stirring. White cloudy precipitate appears.
13.	Carbohydrates[20]	**Benedicts test:** Extract + 0.5 ml Benedict's solution + heat for 2 minutes. Appearance of blue-green-orange-brick red precipitate.
14.	Resins	**Turbidity test:** Extract + 5ml distilled water. Occurrence of turbidity shows the presence of resins.

RESULT AND DISCUSSION

Callus induction in leaf explants

The plant growth regulator combination (BAP+NAA) was tested at different concentrations (mg/l) for callus induction. It was observed that the leaf explants started swelling after 8-10 days of inoculation. BAP (2.0 mg/l) and NAA (10.0 mg/l) was the optimum combination which induced callogenesis in 82.2% of explants (**table 2, plate 2 B, C).** The callus obtained was healthy, green and friable. After repeated sub-culturing, the callus turned pale brown in colour and was used for extraction purpose.

The *in vivo* plant extracts as well as *in vitro* calli extracts were subjected to the phytochemical tests. The results of preliminary phytochemical screening revealed the presence of many bioactive compounds in various extracts **(table 3, plate 4 B).**

Table 2: Effect of BAP and NAA combination on callus induction in leaf explants

Plant Growth regualtors used (mg/l)	% callus induction (mean ± SD)	Callus induction response	Nature of callus
Control	0.00±0.00	-	-
BAP (1.0)+ NAA(2.0)	72.2±1.90	+++	Average, white, friable
BAP(1.0) + NAA(5.0)	71.1±1.90	+++	Average, white, friable, compact
BAP(1.0) +NAA(7.0)	75.5±3.86	+++	Profuse, white, loose
BAP (1.0) + NAA(10.0)	57.7±3.86	++	Profuse, white, loose
BAP (2.0)+ NAA(2.0)	59.9±6.65	++	Average, pale yellow, compact
BAP(2.0) + NAA(5.0)	71.1±1.90	+++	Average, pale white, nodular
BAP(2.0) +NAA(7.0)	74.4±3.81	+++	Profuse, greenish, compact
BAP (2.0) + NAA (10.0)	**82.2±1.90**	**+++**	**Profuse, friable green,**

++ denotes moderate callusing, +++ denotes high callusing, - denotes no callusing

Table 3: Phyto-chemical screening of *in vivo* and *in vitro* plant parts of *Adansonia digitata*

Phytochemicals and their tests	AL	AB	AF	ML	MB	MF	AqL	AqB	AqF	MLC	AqLC
Alkaloids											
Mayer's	+	-	+	+	-	+	+	-	+	+	+
Wagner's	+	-	+	+	+	+	+	-	+	+	-
Dragendorff's	++	-	+	++	+	+	+	-	+	+	+
Flavonoids (Alkaline reagent test)	-	+	+	+	+	+++	+	+	+++	++	+
Saponins (Frothing test)	-	-	++	-	+	+++	-	+	+++	++	-
Terpenoids (Salkowski test)	+	+	+	+	+	+	+	+	+	+	-
Phenolic compounds & tannins (Ferric chloride test)	+	+	+	++	+	+	+	+	+	++	+
Glycosides (Keller-Killani test)	+	+	+	+	+	+	+	+	+	+	+
Leucoanthocyanins	+	-	-	+	-	-	-	-	-	-	

Anthocyanins	+	-	-	++	-	-	-	-	-	-	-
Coumarins	-	-	+	-	-	+	-	-	+	+	-
Fatty acids											
Spot test	-	-	+	-	-	+	-	-	+	-	-
Saponification test	-	-	++	++	+	+++	+	+	++	+	+
Proteins & amino acids											
Biuret's test	-	-	-	-	-	-	-	-	-	-	-
Ninhydrin test	-	-	-	-	-	-	-	-	-	-	-
Xantho-Proteic test	+	-	++	++	-	++	+	-	-	++	-
Gums & mucilages	+	-	++	++	-	++	+++	+	-	+	-
Turbidity test	-	+	++	-	+	+	-	+++	++	+	-
Benedicts test	++	+	+++	+	+	+++	++	+	++	++	++

Where + is low amount, ++ moderate amount and +++ is high amount

AL is Acetone leaf, AB is Acetone bark, AF is Acetone fruit, ML is Methanol leaf, MB is Methanol bark, MF is Methanol fruit, AqL is Aqueous leaf, AqB is Aqueous bark, AqF is Aqueous fruit, MLC is Methanol Leaf callus and AqLC is Aqueous leaf callus.

The results of phytochemical screening **(table 3)** revealed the presence of alkaloids in all the extracts except Acetone and Aqueous bark. Flavonoids are present in all extracts except acetone leaf. Terpenoids, tannins and glycosides are present in all extracts. Leuco-anthocyanins are absent in all samples except aqueous and methanol leaf extracts. These metabolites have been reported to possess antimicrobial properties[13]. High amount of fatty acids are present in methanol fruit and moderate amount in acetone fruit, methanol leaf and aqueous fruit extracts. Reducing sugars are present in all extracts from moderate to high amount. Aqueous bark revealed high amount of resin content. Acetone and methanolic bark extracts as well as aqueous bark and fruit extract samples showed the absence of proteins and amino acids.

As compared to *in vivo* leaf extracts in alkaloids are present in lower amounts in leaf, bark and fruit pulp and callus extracts. Moderate amount of alkaloids were present in acetone leaf and methanol leaf with Dragendorff's test. Flavonoids were present in high amount in methanol fruit pulp, aqueous fruit pulp and moderate amount in methanol leaf callus extracts. Saponins were present in high amount in methanol and aqueous fruit pulp extracts followed by moderate amount in acetone fruit pulp and methanol leaf callus extracts. Terpenoids, tannins and glycosides were present in small amounts in all extracts. Presence of anthocyanins was detected only in acetone and methanol leaf extracts. Large amount of fatty acids were found in methanol fruit pulp followed by moderate amounts in Acetone fruit, methanol leaf and aqueous fruit pulp extracts as per saponification test. Little amount was found in aqueous bark and leaf callus as well as methanol leaf callus extracts. Moderate amount of proteins were detected by Xanthoproteic test in Acetone fruit, methanol leaf and fruit pulp extracts and little amount of proteins were detected in aqueous leaf, fruit and methanol leaf callus extracts.

A variety of phytochemicals have been isolated and characterised from *A. digitata*. They belong to the classes of Alkaloids, Terpenoids, Flavonoids, Vitamins, and Carbohydrates etc[21]. Reports show that preliminary phytochemical screening of the ethanolic extract of *A. Digitata* fruit pulp revealed the presence of, Flavonoids, Saponins, Tannins, Alkaloids and Glycosides. Our results are in parallel with the reports of[22] who reported the presence of terpenoids, glycosides and saponins in the aqueous bark extracts of *Adansonia digitata*. Presence of phytochemicals like Flavonoids, Alkaloids, Terpenes, Saponins and Tannins from methanolic extracts of stem bark of *A. digitata* is also reported[23]. It is also reported that crude methanolic stem bark extracts of *A.digitata* exhibits high anti-malarial activity and were able to reduce the number of *Plasmodium* parasites in mice[24].

CONCLUSION

It is well known that biological activity of plants is attributed to the presence of secondary metabolites. This study showed that Methanolic and Aqueous fruit pulp extracts of *A. digitata* are rich in flavonoids, saponins, fatty acids, gums and mucilage. The *in vitro* leaf callus extracts (methanol and aqueous) have been found to contain moderate amount of Flavonoids, Saponins, sugars. This indicates that cell and suspension culture technology especially callus cultures may serve as model systems to investigate the production and regulation of important secondary metabolites. Further studies on anti-microbial activity and isolation of bioactive compounds will unravel the medicinal potential of this multipurpose tree species.

ACKNOWLEDGEMENTS

The author is grateful to Madhya Pradesh Council of Science and Technology (MPCST), Bhopal for supporting this research. Our thanks to Ms. Raksha Sisodiya for her untiring help during the research work.

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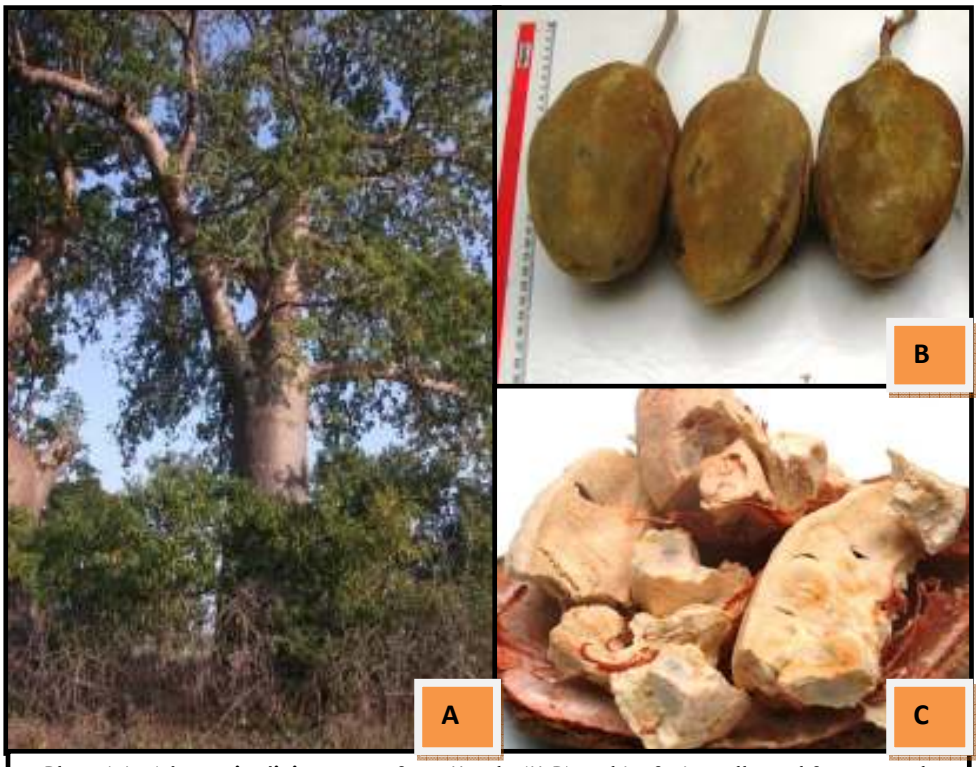

Plate 1 A: *Adansonia digitata* tree from Mandu (M.P) and its fruits collected for research studies
B: Fruits of *Adansonia digitata* 20-30 cm long collected from more than 100 year old tree
C: Breaking of fruit shows pale white coloured fruit pulp

Plate 2: Callus induction in *Adansonia digitata* using leaf explants
2 A: Mature leaf explants used for callus studies 2 B: Light brown, friable callus formed after 2 weeks in MS medium with BAP (2.0 mg/l) and NAA (10.0 mg/l)
2 C: Microscopic image of callus

Plate 3 *In vivo* plant parts processed into smooth fine powder
Plate 3 A: Stem bark powder of *Adansonia digitata*
B: Fruit pulp powder of *Adansonia digitata*
C: Leaf powder of *Adansonia digitata*

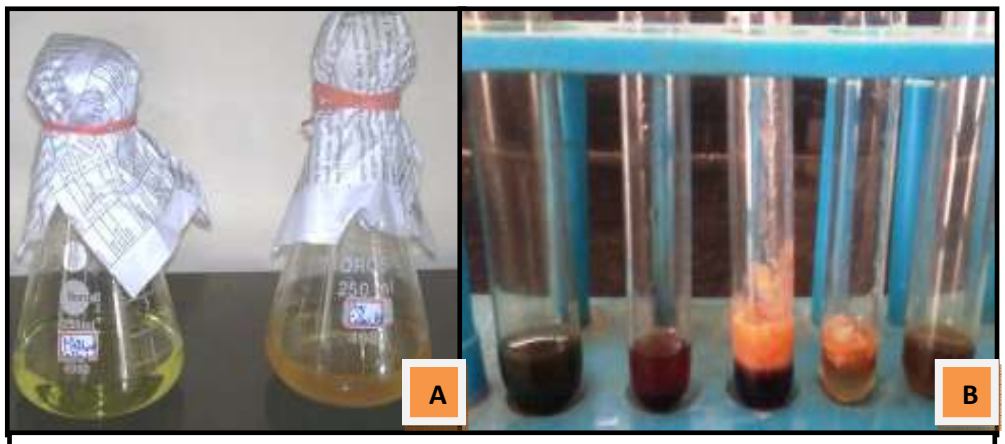

Plate 4 A: Methanolic and aqueous *in vitro* leaf callus extracts
4 B: Phytochemical screening of plant extracts of *Adansonia digitata*

Source of support: Nil, Conflict of interest: None Declared