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In vitro preliminary screening of bioactive compounds of different parts of *Adansonia digitata* L. a globally endangered tree

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Abstract

The present investigation was undertaken to screen the different parts of *Adansonia digitata* L. for the presence of bioactive compounds. The preliminary phytochemical screening was performed by using various solvent extracts of different parts such as leaf, seed, fruit wall and floral parts of *A. digitata*. The phytochemical analysis of the dry seeds, leaf, floral extracts, fruit wall and bark of stem was carried out to determine the presence of bioactive compounds using the standard qualitative procedures. The phytochemical analysis revealed that all the solvent extracts of different parts were rich in possession of flavonoids, alkaloids, glycosides, tannins, sterols, phenols, saponins, coumarins and quinones. From the present study it can be concluded that extracts of different parts of this valuable tree can be utilized in the preparation of various active principles of medicines to alleviate many diseases.

Keywords: Adansonia digitata, Phytochemical screening, Solvent extracts.

Introduction

Medicinal plants are of great importance to the health of individuals and communities and play a major role in indigenous medicine [1]. Plants produce a diverse array of 1,00,000 low molecular mass secondary metabolites; estimates of the total number in plants exceed 5,00, 000 [2]. Many of these natural products have vital role as mediators of ecological interactions; that is, they have functions in ensuring a continued survival of particular organisms in often hostile environments where there is competition with other organisms [3].

The production of synthetic drugs is highly expensive and the time taken for their preparation is also very long and their usage is associated with many side effects but plant based drugs have long history of use, have less or no side effects and are cheaper when compared to modern drugs. Hence, it has become an important to focus on natural products research which requires the utilization of virtual screening methods to find novel lead compounds.

The tree species of *Adansonia digitata* L. is an important medicinally and it is globally endangered. It is commonly called as "*baobab tree"* possessing multipurpose properties widely used for food, medicine, fuel, timber, fodder [4]. All the parts of the plant are medicinally important possessing antioxidant, anti-inflammatory, analgesic, antipyretic, anti-dysenteric, anti-diarrhoea and excipient properties [5-7].

It is called "*Kalpvriksha"* in India as all the parts of the tree are reported to be used as food stuffs and medicines. The literature survey reveals that the several plant parts of this globally endangered medicinally important multipurpose tree *A. digitata* have interesting anti-oxidant, anti-viral and anti-inflammatory properties, and baobab has been used extensively since ancient times in traditional medicine. However, for baobab, the nutritional and medicinal data are widely scattered and research is fragmentary. Hence, we have under taken the present investigation on *in vitro* preliminary phytochemical analysis in different aerial parts of *A. digitata*.

Materials and Methods

Plant Material

The plant material (leaves, flowers, fruit wall, seeds, and stem bark) was washed thoroughly with distilled water and was shade dried for two months. Each sample of the material was ground separately into fine powder and stored in air tight containers at ambient temperature.

Preparation of Crude Extracts

10 gms of each sample was soaked for 24hr in a conical flask containing 100 ml petroleum ether, methanol, acetone and water (aqueous) solvents separately. The extracts were filtered through Whatmann No.1 filter paper. The supernatants were collected, covered, labeled and used for the screening of various phytochemicals.

Phytochemical Analysis

The chemical tests for the screening of phytochemicals in the plant under study (*A. digitata*) were carried out in the different solvent

extracts of various parts using the standard methods and procedures [8-10].

Tests Performed For The Presence Of Phytoconstituents

Tests for Alkaloids

Dragendorff's test: To 1 ml of each of the sample solution taken in a test tube few drops of Dragendorff's reagent (potassium bismuth iodide solution) was added. A reddish brown precipitate was observed indicating the presence of alkaloids.
Meyer's test: To 1ml of each of the sample solution few drops of Meyer's reagent (potassium mercuric chloride solution) was added. A creamish white precipitate was formed indicating the presence of alkaloids.
Wagner's test: To few ml of each of the sample solution, Wagner's reagent (Iodine in potassium iodide) was added, which resulted in the formation of reddish brown precipitate indicating the presence of alkaloids.
Hager's test: To 1 ml of each of the sample few drops of Hager's reagent (Picric acid) was added. Yellow precipitate was formed reacting positively for alkaloids.
Tannic acid test: When few ml of 10% Tannic acid was added to 1ml of each sample, a buff colour precipitate was formed giving positive result for alkaloids.
$FeCl_3$ test: One drop of $FeCl_3$ solution was added to each of the test sample, formation of yellow precipitate was resulted reacting positively for alkaloids.

Tests for Glycosides

Raymond's test: Test solution when treated with dinitrobenzene in hot methanolic alkali giving a violet colour
Legal's test: When the test samples were treated with pyridine and sodium nitroprusside solution blood red colour appears
Bromine water test: When treated with bromine water test solution gives yellow precipitate.
Kellar Kiliani test: 1ml of concentrated sulphuric acid was taken in a test tube then 5ml of extract and 2ml of glacial acetic acid with one drop of ferric chloride were added, formation of a blue colour.
Concentrated Sulphuric acid test: Conc.H_2SO_4 was added to test sample which resulted in appearance of reddish colour.
Molisch test: When alpha naphthol and concentrated H_2SO_4 were added to test samples reddish violet ring at junction of two layers was resulted.

Tests for Tannins and Phenolic Compounds

Ferric chloride test: When few drops of ferric chloride were added to sample solution a blackish precipitate appears.
Gelatin test: When gelatin and water were added to test samples formation of white precipitate was resulted.
Lead acetate: Few ml of test samples were taken in different test tubes followed by the addition of aqueous basic lead acetate. It results in the formation of reddish brown bulky precipitate.
Alkaline reagent: When sodium hydroxide solution was added to the sample solution results in the formation of yellow to red precipitate.
Mitchell's test: Tannins give a water soluble iron–tannin complex with iron and ammonium citrate or iron and sodium tartarate.
Ellagic acid test: When 5% glacial acetic acid and 5% sodium nitrite were added to test samples a muddy niger brown colour appears, which is a positive result for phenols.

Tests for Flavonoids

Zinc Hydrochloride reduction test: To test the sample solution for the flavonoids added a mixture of zinc dust and concentrated hydrochloric acid results in red colour.
Lead acetate test: When aqueous basic lead acetate was added to test sample produces reddish brown precipitate.
Ferric chloride test: To few ml of test samples taken separately, few drops of ferric chloride were added which resulted in the formation of blackish red precipitate.
Shinoda test (Magnesium hydrochloride reduction test): To the test solution few fragments of magnesium ribbon and concentrated hydrochloric acid were added drop wise and reddish to pink colour was resulted.
Alkaline reagent test: When sodium hydroxide solution was added to the test samples formation of intense yellow colour, which turns to colour less on addition of few drops of dilute acid indicates the presence of flavonoids.

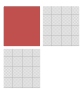
Tests for Sterols

Libermann-Buchard test: When samples were treated with few drops of acetic anhydride, boiled and few drops of concentrated sulphuric acid from the sides of the test tube were added, shows a brown ring at the junction of two layers and the upper layer turns green which shows the presence of steroids.
Salkowski test: Few drops of concentrated sulphuric acid were added to the test samples in chloroform, a red colour appears at the lower layer indicates the presence of sterols

Tests for Fats and Oils

Stain test: Press the small quantity of each extract between two filter papers, the stain on filter papers indicates the presence of the oils.
Saponification test: Added a few drops of 0.5N alcoholic potassium hydroxide to various extracts with a drop of phenolphthalein separately and heat on water bath for 1-2hours, formation of soap or partial neutralization of alkali indicates the presence of oils and fat.

Tests for Lignins



Labat test: When gallic acid is added to the test sample,it results in the formation of olive green colour.
Furfuraldehyde test: When furfuraldehyde is added to the test sample a red colour appears indicating the presence of lignin.

Tests for Quinones

Alcoholic KOH test: When alcoholic KOH was added to the test samples red to blue colour appears reacting positively for quinines.

Tests for Saponins

Foam test: 5ml of extract was shaken vigorously to obtain a stable persistent froth. The froth was then mixed with three drops of olive oil and observed for the formation of an emulsion, which indicated the presence of saponins.

Results and Discussion

Phytochemical Screening of different parts of *A. digitata*

Leaf extracts

Analysis of phytochemicals in leaf extracts of *A. digitata* showed the presence of flavonoids, alkaloids, glycosides, tannins, sterols, phenols, saponins, coumarins (Table-1). Whereas quinones were found to be negative. Leaf extracts of all the solvents were found to be strongly positive for bioactive compounds.

Floral extracts

Phytochemical screening of floral extract of *A. digitata* resulted the presence of alkaloids, glycosides, flavonoids, tannins, sterols, phenols, lignins and saponins while quinones were absent in all the extracts (Table-2).
Alkaloids were screened positive in all the extracts except in Wagner's test. Glycosides were present in all the solvent extracts and detected strongly in acetone, methanol, petroleum ether and water. Tannins were screened positive only in aqueous extract, but were absent in all other extracts analysed. Flavonoids were weakly present in all the extracts except in water in which they were reported to be strongly present. Sterols, phenols, saponins and lignins were positive in all the extracts while quinones were negative in all the extracts.

Fruit wall extracts

All of the four organic solvents (methanol, acetone, petroleum ether,water) of fruit wall extracts showed positive results for the presence of alkaloids, flavonoids, tannins, glycosides, sterols, phenols, saponins and coumarins. Whereas fruit wall extracts of all the solvent extracts showed negative results for quinones and phenols (Table-3).
Tannins were screened positive in all the extracts and flavonoids were weakly present except in methanol and petroleum ether extracts in which they were found to be strongly present. Saponins were positive in all the extracts. Lignins were found feebly in all the extracts.

Seed extracts

All the solvent extracts of seed of *A. digitata* were positively resulted for the presence of alkaloids, glycosides, tannins, phenols, lignins, saponins while quinones, flavonoids and coumarins were completely absent in all the solvent extracts. Alkaloids, glycosides, tannins, phenols and saponins were found to be strongly positive (Table-4).

Stem bark extracts

The screening for the phytochemicals present in the stem bark extracts of *A. digitata* revealed the presence of low concentrations of alkaloids, flavonoids, sterols and lignins and absence of quinones. All the organic solvent extracts of bark were strongly positive for tannins, saponins, phenols. Alkaloids were weekly detected in methanolic extract and chloroform extracts of bark. Glycosides were absent in all the solvent extracts (Table-5).
The importance of medicinal plants as a source of active drugs emerged from the chemical profile that produces a clear physiological action on the biological system. Flavonoids, alkaloids, tannins and phenolic compounds have been established as the most important bioactive compounds of plants [11].
Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids [12, 13]. The importance of the alkaloids, saponins, steroids and tannins in various antibiotics used in treating common pathogenic strains has recently been reported by Kubmarawa, 2007[12].
Knowledge of the chemical constituents of plants is desirable because such information will be value for synthesis of complex chemical substances [14-16]. Therefore, it becomes an important need to look forward in search of medicinal plants for their medicinal value and also isolation and characterization of specific chemical compound that plays a vital role in therapeutic potential in curing various ailments.

Table-1: Analysis of Phytochemicals in Leaf extracts of *Adansonia digitata*

Phytochemical test		Methanol Extract	Petroleum ether Extract	Acetone Extract	Chloroform Extract	Aqueous Extract
ALKALOIDS	Dragendorff's test	+	+	+	+	+
	Mayer's test	+	-	+	+	-
	Wagner's test	+	+	+	+	+
	Hager's test	+	+	+	+	+
	Tanicacid test	+	+	+	+	+
GLYCOSIDES	Raymond's test	-	-	-	-	+
	Legal's test	-	-	-	+	+
	Bromine water test	+	+	+	+	+
	Kellar Kiliani test	-	-	-	-	+
	Conc. H_2SO_4 test	-	-	-	-	+
	Molisch test	+	+	+	+	+
TANNINS	$FeCl_3$ test	-	-	-	-	-
	Gelatin test	+	+	+	+	+
	Lead acetate test	-	+	-	+	-
	Alkaline reagent test	+	+	+	+	+
	Mitchell's test	-	-	-	-	-
FLAVONOIDS	Zn-HCl reduction test	+	-	-	+	-
	Lead acetate test	-	+	+	-	+
	$FeCl_3$ test	+	-	-	+	+
	Shinoda's test	+	-	-	-	-
	Alkaline reagent test	+	+	+	+	+
COUMARINS		+	+	+	+	+
STEROLS	Libermann Burchard test	+	+	+	-	+
	Salkowski test	+	-	-	+	+
FATS &OILS	Stain test	+	+	+	+	+
	Saponification test	-	-	+	-	+
PHENOLS	$FeCl_3$ test	+	+	+	+	+
	Elagic acid test	+	-	-	+	+
LIGNINS	Labat test	-	-	-	-	-
	Lignin(furfuraldehyde) test	+	+	+	+	+
QUINONES	Alcoholic KOH test	-	-	-	-	-
SAPONINS	Foam test	+	+	+	+	+

+ = Present; - = Absent

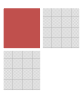


Table-2: Analysis of Phytochemicals in Floral extracts of *Adansonia digitata*

Phytochemical test		Methanol Extract	Petroleum ether Extract	Benzene Extract	Chloroform Extract	Aqueous Extract
ALKALOIDS	Dragendorff's test	+	+	+	-	+
	Mayer's test	+	-	-	+	+
	Wagner's test	+	+	+	-	+
	Hager's test	+	+	+	+	+
	Tanic acid test	+	+	+	-	+
GLYCOSIDES	Raymond's test	+	+	+	-	+
	Legal's test	+	+	+	-	+
	Bromine water test	+	+	+	+	+
	Kellar Kiliani test	+	+	+	-	+
	Conc. H_2SO_4 test	+	+	+	+	+
	Molisch test	+	+	+	-	+
TANNINS	$FeCl_3$ test	+	+	+	+	+
	Gelatin test	+	+	+	+	+
	Lead acetate test	+	+	-	+	+
	Alkaline reagent test	+	+	+	+	+
	Mitchell's test	-	-	+	+	+
FLAVONOIDS	Zn-HCl reduction test	+	-	-	-	+
	Lead acetate test	+	+	-	-	+
	$FeCl_3$ test	+	+	+	-	+
	Shinoda's test	-	+	-	-	-
	Alkaline reagent test	+	+	+	+	+
COUMARINS		+	+	+	+	+
STEROLS	Libermann Burchard test	+	+	+	+	-
	Salkowski test	+	_	+	+	-
FATS & OILS	Stain test	+	+	+	+	+
	Saponification test	+	+	+	+	+
PHENOLS	$FeCl_3$ test	-	-	-	-	+
	Elagic acid test	+	-	-	-	+
LIGNINS	Labat test	-	-	-	-	-
	Lignin(furfuraldehyde) test	-	-	-	+	+
QUINONES	Alcoholic KOH test	-	-	-	-	-
SAPONINS	Foam test	+	+	+	+	+

+ = Present; - = Absent

Table-3: Analysis of Phytochemicals in Fruit wall extracts of *Adansonia digitata*

Phytochemical test		Methanol Extract	Petroleum ether Extract	Benzene Extract	Chloroform Extract	Aqueous Extract
ALKALOIDS	Dragendorff's test	-	+	+	+	+
	Mayer's test	+	-	-	-	-
	Wagner's test	-	+	+	+	+
	Hager's test	-	+	+	-	+
	Tanicacid test	-	+	+	-	+
GLYCOSIDES	Raymond's test	-	-	-	-	-
	Legal's test	-	-	-	-	-
	Bromine water test	-	-	-	-	-
	Kellar Kiliani test	-	-	-	-	-
	Conc. H_2SO_4 test	-	-	-	-	-
	Molisch test	-	-	-	-	-
TANNINS	$FeCl_3$ test	-	-	-	-	-
	Gelatin test	-	-	-	-	-
	Lead acetate test	-	-	-	-	-
	Alkaline reagent test	-	-	-	-	-
	Mitchell's test	-	-	-	-	-
FLAVONOIDS	Zn-HCl reduction test	+	-	+	-	+
	Lead acetate test	+	+	-	+	+
	$FeCl_3$ test	+	+	+	-	+
	Shinoda's test	-	+	-	-	-
	Alkaline reagent test	+	+	+	+	+
COUMARINS		-	-	-	-	-
STEROLS	Libermann Burchard test	-	-	-	-	-
	Salkowski test	+	_	+	+	-
FATS & OILS	Stain test	+	+	+	+	+
	Saponification test	+	+	+	+	+
PHENOLS	$FeCl_3$ test	-	-	-	-	-
	Elagic acid test	-	-	-	-	-
LIGNINS	Labat test	-	-	-	-	-
	Lignin(furfuraldehyde) test	-	+	-	+	-
QUINONES	Alcoholic KOH test	-	-	-	-	-
SAPONINS	Foam test	+	+	+	+	+

+ = Present; - = Absent

Table-4: Analysis of Phytochemicals in Seed extracts of *Adansonia digitata*

Phytochemical test		Methanol Extract	Petroleum ether Extract	Benzene Extract	Chloroform Extract	Aqueous Extract
ALKALOIDS	Dragendorff's test	+	+	+	+	+
	Mayer's test	+	+	+	+	+
	Wagner's test	+	+	+	+	+
	Hager's test	+	+	+	+	+
	Tanicacid test	+	+	+	+	+
GLYCOSIDES	Raymond's test	-	-	-	-	-
	Legal's test	+	+	+	+	+
	Bromine water test	+	+	+	+	+
	Kellar Kiliani test	-	-	-	-	-
	Conc. H_2SO_4 test	+	-	+	+	-
	Molisch test	-	-	-	-	+
TANNINS	$FeCl_3$ test	-	+	-	-	-
	Gelatin test	-	+	-	-	-
	Lead acetate test	-	-	-	-	-
	Alkaline reagent test	-	+	-	-	=
	Mitchell's test	-	+	-	-	-
FLAVONOIDS	Zn-HCl reduction test	-	-	-	-	-
	Lead acetate test	-	-	-	-	-
	$FeCl_3$ test	-	-	-	-	-
	Shinoda's test	-	-	-	-	-
	Alkaline reagent test	-	-	-	-	-
COUMARINS		-	-	-	-	-
STEROLS	Libermann Burchard test	+	+	+	+	+
	Salkowski test	+	_	+	+	-
FATS & OILS	Stain test	+	+	+	+	+
	Saponification test	+	+	+	+	+
PHENOLS	$FeCl_3$ test	+	+	+	+	+
	Elagic acid test	+	+	+	+	+
LIGNINS	Labat test	-	-	-	-	-
	Lignin(furfuraldehyde) test	+	+	+	+	+
QUINONES	Alcoholic KOH test	-	-	-	-	-
SAPONINS	Foam test	+	+	+	+	+

+ = Present; - = Absent

Table-5: Analysis of Phytochemicals in Stem bark extracts of *Adansonia digitata*

Phytochemical test		Methanol Extract	Petroleum ether Extract	Benzene Extract	Chloroform Extract	Aqueous Extract
ALKALOIDS	Dragendorff's test	-	+	+	+	+
	Mayer's test	+	-	-	-	-
	Wagner's test	-	+	+	+	+
	Hager's test	-	+	+	-	+
	Tanicacid test	-	+	+	-	+
GLYCOSIDES	Raymond's test	-	-	-	-	-
	Legal's test	-	-	-	-	-
	Bromine water test	-	-	-	-	-
	Kellar Kiliani test	-	-	-	-	-
	Conc. H_2SO_4 test	-	-	-	-	-
	Molisch test	-	-	-	-	-
TANNINS	$FeCl_3$ test	-	-	-	-	-
	Gelatin test	-	-	-	-	-
	Lead acetate test	-	-	-	-	-
	Alkaline reagent test	-	-	-	-	-
	Mitchell's test	-	-	-	-	-
FLAVONOIDS	Zn-HCl reduction test	+	-	+	-	+
	Lead acetate test	+	+	-	+	+
	$FeCl_3$ test	+	+	+	-	+
	Shinoda's test	-	+	-	-	-
	Alkaline reagent test	+	+	+	+	+
COUMARINS		-	-	-	-	-
STEROLS	Libermann Burchard test	+	+	+	+	+
	Salkowski test	+	_	+	+	-
FATS & OILS	Stain test	+	+	+	+	+
	Saponification test	+	+	+	+	+
PHENOLS	$FeCl_3$ test	-	-	-	-	-
	Elagic acid test	-	-	-	-	-
LIGNINS	Labat test	-	-	-	-	-
	Lignin(furfuraldehyde) test	-	+	-	+	-
QUINONES	Alcoholic KOH test	-	-	-	-	-
SAPONINS	Foam test	-	-	-	-	-

+ = Present; - = Absent

Conclusion

The phytochemical analysis of *A. digitata* performed for the presence of various bioactive compounds revealed the high concentrations of alkaloids, glycosides, flavonoids, tannins, saponins, phenols, lignins and lower concentrations of quinones and sterols. Based on our results, we conclude that the various solvent extracts of different parts of *A. digitata* have immense potential to act against various ailments due to the presence of medicinally important phytochemicals, hence can be employed in the preparation of pharmaceuticals. Further studies are required for identification of active constituents responsible and to understand the mechanism of action of these bioactive compounds.

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