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Preliminary Phytochemical analysis of Leaf Extract of Alternanthera Brasiliana

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Abstract: Alternanthera brasiliana L. (Amaranthaceae) is evergreen, perennial herbs, native to tropical and sub-tropical regions of Brazil, Australia & South America. All of its parts are used in traditional system of medicine; leaves are the most important parts which are use medicinally. The present study was carried out to investigate the phytochemical profile of leaf of *Alternanthera brasiliana* L. The leaf powder was successively extracted with petroleum ether, chloroform, methanol and ethanol: water (50: 50). Phytochemical analysis shows the presence of presence of Flavonoids, Carbohydrate, Alkaloids, Amino acid and Tannins & Phenolic compounds. The result of the study could be useful for description and foundation of monograph of the plant.

Keywords: Alternanthera brasiliana, Alkaloids, Flavonoids and Phytochemical.

Introduction: Quality can be defined as the status of a drug that is determined by identity, purity, content and other chemical, physical, or biological properties, or by the manufacturing processes. Quality control is a term that refers to processes involved in maintaining the quality and validity of a manufactured product. For the quality control of a traditional medicine, the

traditional methods are procured and studied, and documents and the traditional information about the identity and quality assessment are interpreted in terms of modern assessment.

World Health Organization (WHO) and European Union (EU) issued several guidelines and acts concerning safe and appropriate use of herbal medicines.[1-3] Safety issues related to herbal medicine are complex, and comprise possible toxicity of natural herbal constituents, presence of contaminants or adulterants, and potential interactions between herbs and prescription drugs.[4]Use of indigenous drugs from plant origin forms a major part of complementary and alternative medicine/traditional medicine (CAM/TM). The world market for herbal medicine, including herbal products and raw materials has been estimated to have an annual growth rate between 5 &15%. The Indian medicinal plants-based industry is growing at the rate of 7–15% annually.[5]

Alternanthera brasiliana (L.) O. kuntze, (Amaranthaceae) is an important herb found as a perennial herb, native to tropical and subtropical regions of Australia and South America; five species have been recorded from India (Figure 1). Through almost all of its parts are used in traditional system of medicines, leaves are the most important parts which are use medicinally.[6] It is a herb indigenous to Brazil, described as perennial, prostrate and branchy, presenting a circular to polygonal stem, long internodes and swollen nodes at which opposite leaves attach. The inflorescence is cymes, composed of hermaphrodite, actinomorphous and monocyclic flowers.[7] It is found especially around tank and ponds.[8]

Alternanthera brasiliana is a brazilin plant occurring in several regions, being known as “penicilina” or terramicina, widely used by rural communities as medicinal agent to cure different disease, such as inflammation, and dolorous or infection processes, wound healing, analgesic, antitumor activity, immunomodulator and lymphocyte proliferation. Alternanthera brasiliana focusing the influence of different kind of lights to produce compound with possible analgesic action.[9] It used against cough & diarrhoea in Brazilian popular medicine.[10] Here the aim was to summarize the more recent common actions and therapeutic application of Alternanthera brasiliana and its active constituent.

Taxonomic classification:

Kingdom - Plantae
Subkingdom - Tracheobionta
Super division - Spermatophyta
Division - Magnoliophyta
Class - Magnoliopsida
Subclass - Caryophyllidae
Order - Caryophyllales
Family - Amaranthaceae
Genus - *Alternanthera*
Species - *brasiliana*

Vernicular name:

Latin - Alternanthera brasiliana

Unani - Machhechhi

Tamil - Ponnonkanni

Siddha - Ponnonkanni

Ayurvedic - Matsyaakshi, matsyaakshika, minaakshi.

Figure 1: Leaves & Flowers of Alternanthera brasiliana

Materials and method:

Collection and Authentication of plant materials: The fresh leaves of *Alternanthera brasiliana* L. Kuntz were collected in the month of November, 2010 from the botanical garden within Ghaziabad (U.P.) India. The plant material was authenticated by Dr. H.B. Sing NISCAIR New Delhi were a voucher specimen no. NISCAIR/RHMD/Consult/-2010-11/1607/205.

Preparation of the extract:

The leaves were cleaned and shade dried in open air for 8-10 days then pulverized to dry power using electric grinder. About 400 gm of the dried leaf powder was extracted with solvent of increasing polarity such as petroleum ether, chloroform, ethyl acetate, methanol, and 50% ethanol & water for 24 hours with each solvent, by extraction using the soxhlet apparatus at a temperature of 30 to 35˚C. Each time before extracting with the next solvent, the powdered material was air dried below 50˚C and then each extract was dried under reduced pressure using a Rotary evaporator. The concentrated extract was reduced to a semisolid mass by drying on water bath at 40±50˚C to obtain 12.92gm petroleum ether, 15.8gm chloroform, 6.64gm ethyl acetate, 43.72gm methanol, 66.88gm 50% ethanol & water extract of leaf of *Alternanthera brasiliana* L. Kuntz. The herb was subjected to phytochemical screening for the verification of the presence of phytoconstituents.

The percentage extractive yield was calculated by formula as mentioned below:

$$\% \text{ Extractive yield (w/w)} = \frac{\text{weight of dried extract}}{\text{weight of dried fruit}} \times 100$$

Determination of Extractive value:- The extractive values of dried leaf and bark (mixture) powder of *Ficus infectoria* were determined with different solvents i.e. petroleum ether, chloroform, methanol, ethanol: water (50:50) and water.

Preliminary physical analysis of dried leaf and bark (mixture) extract:- The property of selective reactivity of phytochemical present in an extract forms the basis of chemical tests for identification of different constituents.

Preliminary analyses of *Ficus infectoria* leaf and bark extract was performed initially to identify various chemical compounds present and to assess physicochemical properties.

The performed preliminary analyses included:

a) Macroscopic evaluation of leaf & bark (mixture) extract:- Macroscopic evaluation of leaf and bark extract was performed with respect to colour, odour, taste, touch etc.

b) Analysis of solubility parameters:- Solubility of prepared leaf and bark (mixture) extract of *Ficus infectoria* was determined in various solvents i.e. distilled water, methanol, ethanol, benzene and chloroform.

Preliminary phytochemical screening:- The various extracts of *Ficus infectoria* i.e. petroleum ether, chloroform, methanol and ethanol: water (50:50) were subjected to qualitative chemical analyses to detect the presence of various phytoconstituents.[8, 9]

Test for Carbohydrate:

A small quantity of the extracts was dissolved separately in 5 ml distilled water and filtered. The filtrates were subjected to the following tests to detect the presence of carbohydrates.

Molish′s test:- Extract filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube separately and 2 ml of concentrated sulphuric acid was added carefully along the sides of the test tubes. Formation of violet ring at the junction may indicate the presence of carbohydrates.

Test for reducing sugar:

Fehiling′s test:- Extract filtrates were treated in equal volumes with 1ml Fehling A and 1ml Fehling B solutions, boiled for one minute separately. The mixtures were boiled for 5-10 minutes on water bath. Reddish brown colour was obtained due to formation of cuprous oxide which indicated the presence of reducing sugar.

Benedict′s test:- Extract filtrates were treated with equal volumes of Benedict′s reagent in test tubes separately. The mixtures were boiled for 5-10 minutes on water bath. Solution appeared green, yellow or red depending on amount of reducing sugar present in each filtrate.

Test for Glycosides:

Test for cardiac glycosides:

Keller kelliani test (test for deoxysugar):- Leaf and bark mixture extract were treated with chloroform and evaporate it to dryness. Separately 0.4 ml of glacial acetic acid containing a trace amount of ferric chloride was added and transferred to a small test tube added with carefully 0.5 ml of concentrated Sulphuric acid by the side of the test tube, blue colour appears in the acetic acid layer indicating the presence of glycosides.

Test for Anthraquinone Glycosides:

Borntrager's test:- Leaf and bark mixture extract were boiled with 1 ml of dilute

Sulphuric acid in a test tube separately for 5 min, filtered while hot, pipette out the supernatant or filtrate, cooled and shaken with an equal volumes of dichloromethane. The lower levels of dichloromethan separated and shaken with half its volume with dilute ammonia. A rose pink to red color appeared in the ammonical layer, indicating the presence of glycosides.

Test for Saponin Glycosides:

Froth test:- Leaf and bark (mixture) extracts were treated with water in a semi-micro tube separately shaken well. The froth appeared thus indicating the presence of glycosides.

Tests for Amino acid and Protein:

Biuret test (General test):- Leaf and bark (mixture) extract were treated with 1 ml 10% sodium hydroxide solution separately and heated. A drop of 0.7% copper sulphate solution to the above mixtures was added. The formation of purplish violet colour may indicate the presence of proteins.

Million′s test (for proteins):- 3 ml test solutions were mixed with 5 ml Million's reagent separately. White precipitate was formed which on heating turned to brick red. It may indicated the presence of amino acids.

Tests for Sterols and Triterpenoids:

Libermann-Burchard test:- Leaf and bark (mixture) extract were treated with few drops of acetic anhydride separately. Boiled and cooled, concentrated sulphuric acid was added from the side of the test tubes. A brown ring at the junction of two layer and the upper layer turning green which indicated the presence of sterols while formation of deep red colour indicated the presence of triterpenoids.

Salkowski's test:- Leaf and bark (mixture) extract were treated in chloroform separately with few drops of concentrated sulphuric acid, shaken well and allowed to stand for some time, red colour appeared in the lower layer indicated the presence of sterols while formation of yellow coloured lower layer indicated the presence of triterpenoids.

Tests for tannins and phenolic compounds:

Ferric chloride test:- Small amount of leaf and bark (mixture) extract were shaken with water separately and warmed. Then about 2

ml of 5% ferric chloride solution was added and observed for the formation of green or blue colour which may indicate the presence of phenols.

Gelatin test:- 1% gelatin solution containing 10% sodium chloride was added to each leaf and bark (mixture) extract. Formation of precipitate indicated the presence of tannins and phenolic compounds.

Iodine test:- Leaf and bark (mixture) extract were treated with diluted iodine solution separately. Appearance of transient red colour indicated the presence of tannins and phenolic compounds.

Nitric acid test:- Leaf and bark (mixture) extract were treated with dilute nitric acid separately. Formation of reddish to yellowish colour indicated the presence of tannins and phenolic compounds.

Test for alkaloids:

About 500 mg of the leaf and bark (mixture) extract were stirred with about 5 ml of dilute hydrochloric acid separately and filtered. Each filtrate was tested with the following reagents:

Dragendroff's test:- Few drops of Dragendroff's reagent (solution of potassium bismuth iodide) were added to each filtrate and observed for the formation of orange yellow precipitate which may indicate the presence of alkaloids.

Mayer′s test:- Few drops of Mayer's reagent (Potassium mercuric iodide solution) were added to each filtrate and observed for the formation of white or cream colour precipitate which may indicate the presence of alkaloids.

Hager′s test:- Few drops of Hager's reagent (saturated aqueous solution of picric acid) were added to each filtrate and observed for the formation of yellow precipitate which may indicate the presence of alkaloids.

Wagner′s test:- Few drops of Wagner's reagent (solution of iodine in potassium iodide) were added to each filtrate and observed for the formation of reddish brown precipitate which may indicate the presence of alkaloids.

Tests for flavonoids:

Shinoda test (Magnesium Hydrochloride reduction test):- To leaf and bark (mixture) extracts, 5ml. 95% ethanol was added

separately. Each mixture was treated with 0.5g magnesium turnings and few drops of conc. HCL. Pink colour, if produced, may confirm the presence of flavonoids.

Alkaline reagent test: - Small quantity of each extract sample was taken and added with lead acetate solution. After few minutes appearance of yellow colour precipitates which indicated the presence of flavonoids.

Observations:

Table 1: Physical characteristics and % yield of *Alternanthera brasiliana* L. Kuntz extract

Solvent	Colour of extract	Odour	Consistency	Sense of touch	Amount of extract (gm)	% yield
Petroleum ether	Yellow	Characteristic	Semisolid	Sticky	12.92	3.32%
Chloroform	Light green	Characteristic	Semisolid	Sticky	15.8	3.95%
Methanol	Dark green	Characteristic	Semisolid	Sticky	43.72	10.93%
Ethanol: water (50:50)	Brownish dark green	Characteristic	Semisolid	Sticky	66.88	16.726%

The % yield was maximum (16.726%) obtained with aqueous: ethanol (50:50) and least (3.32%) with petroleum ether media.

The extractive value of *Alternanthera brasiliana* L. Kuntz leaves was performed by using different solvent. The leaves showed different extractive value with different solvents. The obtained results were shown in table no.2

Table 2: Extractive value of *Alternanthera brasiliana* L. Kuntz extract

Solvent	Extractive value
Petroleum ether	0.8%
Chloroform	1.6%
Methanol	21%
Ethanol: water (50:50)	23.2%

The extractive value was found to be maximum (23.2 %) with aqueous: ethanol (50:50) solvent while minimum (0.8%) with petroleum ether.

Table3: Phytochemical evaluation of *Alternanthera brasiliana* L. Kuntz extracts

Phytochemical Test	Result			
	Petroleum ether	Chloroform	Methanol	Ethanol: Water (50:50)
Test for Carbohydrates				
Molish's test	-	-	+	+
Benedict's test	-	-	+	+
Fehling's test	-	-	+	+
Test for Glycoside				
Legal's Test (test for cardenoloids)	-	-	-	-
Keller killiani's Test (for deoxysugars)	-	-	-	-
Brontrager's Test	-	-	-	-

Froth Test	-	-	-	-
Test For Protein				
Biuret Test	-	-	+	+
Test For Amino Acids				
Millon's Test	-	-	+	+
Ninhydrin Test	-	-	+	+
Test for Phytosterol				
Libermann-Burchard Test	-	-	-	-
Salkowski's Test	-	-	-	-
Test for Phenolics and Tannins				
Ferric Chloride Test	-	+	+	+
Gelatin test	-	+	+	+
Iodine test	-	+	+	+
Nitric acid test	-	+	+	+
Test for Alkaloids :				
Mayer's Reagent	-	-	+	+
Dragendroff's Reagent	-	-	+	+
Hager's test	-	-	+	+
Wagner's test	-	-	+	+
Test for Flavonoids				
Shinoda's Test	-	-	+	+
Lead acetate Test	-	-	+	+

Note- (+) Positive Test, (-) Negative test

Conclusion: Phytochemical screening of petroleum ether, chloroform, methanol and Ethanol: Water (50:50) extracts revealed the presence of carbohydrate, alkaloid, protein, amino acid, tannin & flavonoids by positive reaction with the respective test reagent. Phytochemical screening showed that maximum presence of phytoconstituents in methanolic and Ethanol: Water (50:50) extracts.

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