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# PRELIMINARY SCREENING OF PHYTOCHEMICALS AND ANTIOXIDANTS IN AYURVEDIC MEDICINAL PLANT MIRABILIS JALAPA LINN.

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**ABSTRACT :**

The records of pre-historic civilization in different parts of the world revealed considerable range of medicinal plants to cure human ailments. *Mirabilis jalapa* belongs to family Nyctaginaceae. *Mirabilis jalapa* has been extensively used in almost all folklore remedies around the world for treating a variety of conditions. It has been reported that indigenous Mexican population uses various decoctions and preparations of *Mirabilis jalapa* for muscular pain, diarrhoea, dysentery, and abdominal colic. The plant has been extensively studied for a variety of bioactive principles and screened for different pharmacological activities. The ethanolic extract of the leaves and the stem was found to have potent antinociceptive activity in experimental mice. The plant has also proved to posses antibacterial, antiviral, and antioxidant activity. This article briefly reviews the botany, pharmacology, biochemistry and therapeutic application of the plant. This is an attempt to compile and document information on different aspects of *Mirabilis jalapa* and highlight the need for research and development.

**Key words**: *Mirabilis jalapa*, Gul-abbas, phytochemical, Antioxidant.

**INTRODUCTION:** India is a country known for ancient scripts, the number system, and invention of zero and Vedas. As a model system for studying ethylene independent floral senescence, we have selected *Mirabilis jalapa*, Four O'clock, also known as Marvel of Peru, an Belladi Notti. Four O'clock plant belonging to family Nyctaginaceae It is known as Beauty of night, Marvel of Peru in English [1]. The whole plant used as anti-inflammatory, antiviral, anti-bacterial, anti candidal, anti fungal, anti spasmodic and anti nociceptive seeds are Carthartic and vermifuge [2].Phytochemical investigation of plant *Mirabilis jalapa* Linn. have revealed the presence of flavanoids, tannins, phytosterol, glycosides. It is rich source of essential fatty acids like palmitic acid, oleic, linoleic, linolenic and stearic acids. Three C-glycosylflavones in the leaves of *Mirabilis jalapa*, the host plant of the lycaenid butterfly pale grass blue (Pseudozizeeria maha), were identified as 6-C-glucosylluteolin (isoorientin), 6-C-glucosylapigenin (isovitexin) and isovitexin 7-methyl ether (swertisin). Flavonoids (specifically flavanoids such as the catechins) are "the most common group of polyphenolic compounds and secondary metabolites in the human diet and are found ubiquitously in plants. In preliminary studies, UCLA cancer researchers proposed that smokers who ate foods containing certain flavonoids, such as the flavan-3-ols (catechins) found in strawberries and grain and black teas, kaempferol from brussel sprouts and apples, and quercetin from beans, onions and apples, may have reduced risk of developing lung cancer.Flavonoids were found to be strong topoisomerase inhibitors and induce DNA mutations in the MLL gene, which are common

findings in neonatal acute leukemia. Some flavonoids have inhibitory activity against organisms that cause plant disease e.g. *Fusarium oxysporum* [3]. "Antioxidants are a type of complex compounds found in our diet that act as a protective shield for our body against certain disastrous enemies.

The study was obtaining the Anti-inflammatory activity of the alcoholic, aqueous pet ether extract from leaves of *Mirabilis jalapa* Linn, by carrageenan-induced paw edema, formaline induced,paw edema, cotton pellet induced granuloma models in wister albino rats [4] [5]. Petroleum ether, chloroform and methanol crude extract of leaves and bark of *Mirabilis jalapa* Linn were screened for cytotoxicity by brine shrimp lethality bioassay.In same study methanol extract of bark was screened for antioxidant using the DPPH free radical scavenging assay [6]. The methanolic acetone,chloroform, ethanolic extract leaves of *Mirabilis jalapa* were tested for their antibacterial activity against different pathogenic resistant Gram positive Gram negative clinical isolates and minimum inhibitory concentration was determined by disc diffusion method The methanolic extract exhibited lowest MIC against *staphyloccus aureus* (39 micro gram/ml ) and *Asperigillus flavus* (45micro gram/ml) [7] [8]. Methanolic extract of *Mirabilis jalapa* Linn. was evaluated anti-bacterial activity against the Gram positive bacteria viz. *Staphylococcus aureus*, *Bacillus substilis* and the Gram negative bacteria viz. *Escherichia coli*, *pseudomonas aeruginosa* [9]. A bioassay guided fraction of an organic extract of the cells mass form manipulated plant cell culture of *Mirabilis jalapa* Linn. resulted in the isolation of the three new phenolics compounds. Two of the phenolics compounds showed inhibitory activity against *Candida albicans* [10].

Fig. 1- New Seedling & Fig. 2- Budding, Flowering and Fruiting Stages of *Mirabilis Jalapa*

**MATERIALS & METHODS:** Reagent- Methanol, Ethanol, Whatman filter paper, $HgCl_2$, KI, Distilled water, Picric acid, Iodine, Ammonia, Sulphuric acid, Chloroform, Con.$H_2So_4$, α-Naphthalol, Led acetate, Silica gel, Ethyl acetate, acetone, Acetic acid, Anisaldehyde $H_2So_4$, Toluene, n-butanol, diethyl ether, Sodium Chloride.

Apparatus: Test tube, Graduated cylinder, Whatman filter paper, funnel, Beaker, Pestal motor, Water bath, soxhlet apparatus, Oven, Crucible, Separator

funnel, Spectrophotometer, Conical flask, Magnetic Stirrer, Refrigerator.

Plant Material:

The present study was carried out on *Mirabilis jalapa* A traditionally important medicinal herb, growing in Eastern India. Phytochemical and antioxidant activity were identified in *Mirabilis jalapa* leaf.

Method

Collection of plant:

To collect specimens of *Mirabilis jalapa* (MJ) Four O'clock is an annual to short-lived perennial plant that has Magenta or pink flowers. Mature plants can grow up to 75cm tall and plant collecting session is between April to June. Firstly plant is collected from the rode side field. Now the sample shade dries than make powder with the help of the blender.

Preparation of the plant extracts

5g. of shade dried *Mirabilis jalapa* samples were ground at a high speed with blender and extracted upto clear sample in methanol with the soxhlet apparatus.

Soxhlet Extraction: A schematic representation of a Soxhlet extraction 1: Stirrer bar 2: Still pot (the still pot should not be overfilled and the volume of solvent in the still pot should be 3 to 4 times the volume of the soxhlet chamber) 3: Distillation path 4:

Thimble 5: Solid 6: Siphon top 7: Siphon exit 8: Expansion adapter 9: Condenser 10: Cooling water in 11: Cooling water out

Material used: Leaves

Solvent used: Methanol

Preparation of methanolic extract with the help of soxhlet apparatus.

Requirement– Methanol, Measuring cylinder, soxhlet apparatus, Beaker, whatman paper, distilled water, funnel, eppendrof tube etc.

A Soxhlet extractor is a piece of laboratory apparatus [11] invented in 1879 [12].It was originally designed for the extraction of a liquid from a solid material. However, a Soxhlet extractor is not limited to the extraction of liquids. Typically, a Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a significant solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance.

Procedure- Weight 12 gm of powder (leaves) of *Mirabilis jalapa*. 120 ml methanol in round bottom distillation flask. Put the sample on whatman filter paper; make the thimble of filter paper placed in the Soxhlet assembly. Placed the assembly on heating mental at $60^0$C. After 12 hrs, the extract was filtered through whatman no. 1 filter paper in a Buchner funnel. The solvent was evaporated in a rotary vaccum evaporator model then crude extracts were stored in amber glass vials in refrigerator at $4^0$C. Crude extracts were diluted with methanol for further investigation.

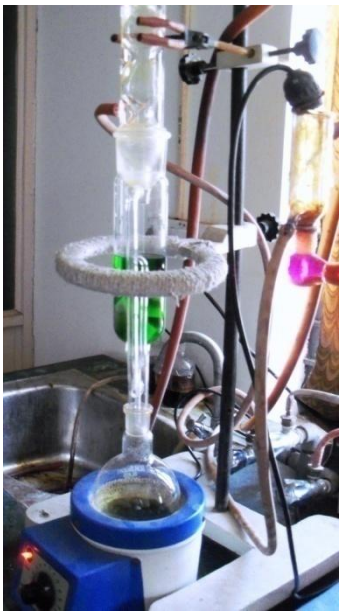

Fig-3. Methanolic extract with the help of Soxhlet appratus.

Preparation of methanolic extract with the help of Microwave apparatus.

Requirement- Flask, Measuring cylinder, microwave apparatus, Crucible, Beaker Whatman paper, distilled water etc.

Procedure- First of all take fresh healthy leaves of *Mirabilis jalapa* and dried shad then until they dried properly.After dried used blend then into the blender and from a thin powder. Now this powder is used for the experiments. Now plant extract is available in the form of powder. We take 25gm of powder and mix 25ml distilled water & kept it in beaker for boiling in the microwave for 15 min also at 80-100$^0$C. After boiling we kept it for cooling. Now we weight the empty crucible after heating in oven to dry at 60$^0$C. Now we take filter paper for filterated the boiled sample in crucible with the help of whatman paper. After filtration the sample into the crucible we weight it & than kept it into the oven for totally evaporation at 60$^0$C. After evaporation the extract is remain which is used for the experiment.

**Preliminary phytochemical screening**

Phytochemical analysis of all the procedure [13] By this analysis the presence of several phytochemical listed in table: 2. was tested for phytochemical analysis as follows:

**Detection of Alkaloids** [14]

Mayer's Reagent: Dissolve 1.358 g of $Hgcl_2$ in 60 ml of water and pour into a solution of 5g of KI in 10 ml of $H_2O$, add distilled water to make the volume 100 ml. (White precipitate with most alkaloids in slightly acid solution).

Hagar's Reagent: Dissolve 1g of picric acid in 100 ml of water.

Wagner's Reagent: To one ml of the methanolic extract sample in a test tube was mixed with one ml of Hager's reagent/Wagner's reagent.

Observation- The appearance of coloured precipitates indicated the presence of alkaloids.

**Detection of Flavonoids** [15]

Reagent: Dilute ammonia solution concentrated sulphuric acid

Procedure- To 5ml of the dilute ammonia solution apportion of the aqueous extract

was added, followed by addition of concentrated sulphuric acid.

Observation- Appearance of yellow coloration indicated the presence of flavonoids.

**Detection of Saponins** [14]

Reagents: Test tube, graduated cylinder

Procedure- The extract was diluted with distilled $H_2O$ and made up to 20 ml. The suspension was shaken in a graduated cylinder for 15 min.

Observation- 2 cm layer of stable foam indicated the presence of saponins.

**Detection of Terpenoids**

Reagent: Chloroform and concentrated $H_2So_4$

Procedure- 5 ml of aqueous extract was mixed with 2 ml of Chloroform and concentrated $H_2So_4$ to form a layer.

Observation- A reddish brown coloration on the interface showed the presence of terpenoids.

**Detection of Carbohydrates** [14]

Reagent: Molish reagent: Prepare reagent by dissolving o.5 g reagent grade napthol in 10 ml of 95% ethanol. Store the reagent at room temperature.

Procedure - To one ml of the sample few drops of molish reagent were added. There after con. $H_2So_4$ was sided along the walls of the test tube.

Observation - Appearance of purple ring at the interface indicated presence of carbohydrates.

**Detection of Tannins** [15]

Apparatus: Test tube.

Reagent - 10% of lead acetate solution: Add 1 gm of lead acetate in 10 ml of distilled H2O and mix properly.

Procedure - To 1 ml of sample in a test tube, 10% of lead acetate solution was added mixed well.

Observation - The presence of yellow precipitates indicated tannins.

All above experiments were performed in triplicates.

**Thin Layer Chromatography (TLC)**

**Instrumentation and Experimental Procedures:** The phytochemical analysis of this plant revealed the presence of flavonoids, saponins, alkaloids, carbohydrates & tannins etc. this extract was further subjected to TLC to confirm the presence of major group like alkaloids, flavonoids, saponins, etc. in the extract .Individual substances separated out based on RF value.

The solvent evaporated dried extracts were redissolved in methanol. TLC performed on Merck Silica Gel 60 glass plate using different eluents analyzed the fraction obtained. The chromatograms were observed in UV/VIS before and after processing with spraying agent. The flavonoids and phytochemicals were identified by comparison to co-chromatographed standards and available literature data[16]

Preparation of TLC Plate: 42 g of silica gel was dissolved in 25 ml chloroform, 25 ml methanol.Prepared the TLC plates by spreading the gel on it.Marking the TLC Plate: The silica gel TLC plates were marked by using pencil.

Activation of TLC Plate: Placed the TLC plate in an oven at 50-60 for 15-20 min to "activate it". Activation involves driving of water molecules that bond to the polar sites on the plate.

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Activation of TLC Plate: Placed the TLC plate in an oven at 50-60 for 15-20 min to "activate it". Activation involves driving of water molecules that bond to the polar sites on the plate.Spotting the TLC Plate: The narrow end of capillary was placed into the extract. When extract rises into the capillary then touch the capillary on the silica plate very carefully.Allowed the solvent to completely evaporate from the spot.

Developing the TLC Plate:

The TLC plate was placed very carefully in the developing bottle containing mobile phase solvent system.Left it for some time so that solvent front can move.

Drying the Plate: Placed the slide in an oven at Temperature 50-60$^0$C to evaporate the solvent.

| | | |
|---|---|---|
| Sample detail | : | *Mirabilis jalapa* leaves. |
| Adsorbent | : | Merck Silica Gel 60 on glass plate |

Solvent system and detecting agents for Thin Layer Chromatography- Three solvents systems were applied to achieve the banding profile of Mirabilis extract, two solvent systems for phytochemical identification and other one solvent system used for antioxidant compounds. These are as follows

Solvent system- 1

| | | |
|---|---|---|
| Solvent system | : | Benzene : Ethanol : Ammonia (18: 2: 0.2) |

Solvent run : 10 cm
Detection : Iodine vapors gave pinkish

Solvent system- 2
Solvent system : Chloroform: Ethanol (8:2)
Solvent run : 10cm
Detection : Iodine vapor gave pinkish red spot

Solvent System- 3
Solvent system : Chloroform: Ethanol (8:2)
Solvent run : 10cm
Detection : Spraying with DPPH

TLC Analysis of the Fractions:

For each extract, three different solvent systems were used as developing systems. These were Benzene: Ethanol: Ammonia (18:2:0.2), Chloroform: Ethanol (8:2). In first two cases, the spots were visualized by exposure of the plates to iodine vapor and last Chloroform: Ethanol (8:2), plate spots were detected with spraying of methanolic solution of DPPH.

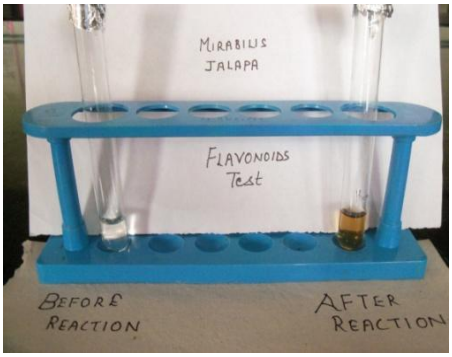
**RESULT:** Results observed after performing various experiments were extreme good and indicated that *Mirabilis jalapa* has extreme scope as medicinal as well as antiaging components. The spectrophotometric analysis of *Mirabilis jalapa* crude extract represent various λ max, which indicates that *Mirabilis jalapa* extract have a pool of phytochemicals, which may have different type of medicinal activities. Phytochemical characteristics verified with various test results given below.The preliminary phytochemical analysis in present Alkaloids, flavonoids, saponins, tannins, Terpenoids. But these are not present Carbohydrates, Glycosides. Show these secondary metabolites localized in leaf, collected in the plant.

Table- 1: Nature and Percentage yield of extracts of *Mirabilis jalapa*

| Sr. no. | Name of the extract | Nature | Color | % Yield (w/w) |
|---|---|---|---|---|
| 1. | Methanolic | Shade | Green | 0.62 |
| 2. | Microwave | Shade | Green | 0.53 |

Firstly two types of extracts were made, which was observed and found that 0.62% methanolic extract was sticky in nature and dark black in color, methanolic extract characteristics was same as methanolic in nature while the yield was 0.53% in microwave phase of *Mirabilis jalapa.*

Qualitative Phytochemicals Analysis of Mirabilis *jalapa*



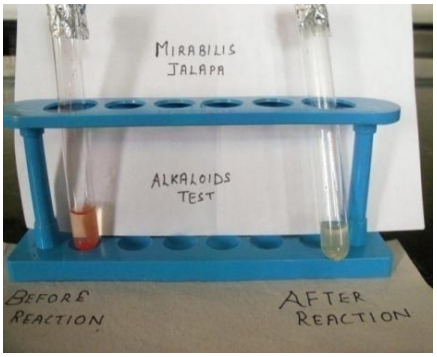



Fig.4 Flavonoids Test of *Mirabilis jalapa*; color changed from colorless to light yellow after reaction, while in Alkaloids test white color precipitates were present after reaction.


Fig.5 Saponins Test of *Mirabilis jalapa* After vigorous shaking a thick layer of foams was present show high amount of saponins, while in Terpenoids test *Mirabilis jalapa* color has been changed after reaction, Appearance of reddish brown coloration indicated presence of Terpenoids.

Details of the Qualitative Phytochemicals Tests

**Table -2: Phytochemical consitutentes of *Mirabilis jalapa* Alkaloids and Flavonoids are as follows:**

| S.No. | Phytochemical Name | Reagents test | Observation | Test Result |
|---|---|---|---|---|
| 1. | Alkaloids | Mayer's reagent Hagar's reagent | White Precipitates | ++ |
| 2. | Flavonoids | Dilute ammonia solution, concentrated sulphuric acid | Yellow colour | +++ |

**Table -3: Phytochemical consitutentes of *Mirabilis jalapa* Saponins and Terpenoids are as follows:**

| S.No. | Phytochemical Name | Reach or reagentes test | Observation | Test Result |
|---|---|---|---|---|
| 1. | Saponins | Vigorous shaking of plant extract | 2 cm layer of stable foam indicated the presence of Saponins | +++ |
| 2. | Terpenoids | Chloroform and concentrated $H_2So_4$ | Reddish brown coloration | ++ |

**Table -4: Phytochemical consitutentes of *Mirabilis jalapa* Saponins and Terpenoids are as follows:**

| S.No. | Phytochemical Name | Reach or reagentes test | Observation | Test Result |
|---|---|---|---|---|
| 1. | Saponins | Vigrous shaking of plant extract | 2 cm layer of stable foam indicated the presence of Saponins | +++ |
| 2. | Terpenoids | Chloroform and concentrated $H_2So_4$ | Reddish brown coloration | ++ |

Table -5: Phytochemical consitutentes of *Mirabilis jalapa* Carbohydrate are as follows:

| S.No | Phytochemical Name | Reach or reagentes test | Observation | Test Result |
|---|---|---|---|---|
| 1. | Carbohydrate | Molish reagent | No reaction | _ |

**Table -6: Phytochemical consitutentes of *Mirabilis jalapa* Tannin are as fallows:**

| S.No. | Phytochemical Name | Reach or reagentes test | Observation | Test Result |
|---|---|---|---|---|
| 1. | Tannin | 10% of lead acetate solution: Add 1 gm of lead acetate in 10 ml of distilled H2O and mix properly. | The presence of yellow precipitates indicated tannins. | +++ |

(Absent = - , Present = +), (- Low Concentration), (++ Medium Concentration), (+++ High Concentration)

**Table -7: Thin layer chromatography of *Mirabilis jalapa* extracts showing experimental conditions and RF values of sample constituents**

| S.No. | *Mirabilis jalapa* | Solvent system | Identification reagents/Detection | Rf values |
|---|---|---|---|---|
| 1. | Methanolic water | Benzene : Ethanol: Ammonia (18:2:0.2) | Iodine vapours | 0.25<br>0.375<br>0.60 |
| 2. | Methanolic water | Chloroform: Ethanol (8:2) | Iodine vapours | 0.33<br>0.40<br>0.43 |

**Table -8: TLC analysis for antioxidant compounds in *Mirabilis jalapa.***

| | | | | |
|---|---|---|---|---|
| 1. | Methanolic Water | Chloroform: Ethanol(8:2) | DPPH | 0.75<br>0.35 |

Anti-oxidant compound were identified by Direct Bioautographic analysis. The methanolic extracts of *Mirabilis jalapa* were dissolved in respective solvent and chromatographed on percolated silica gel plates. The samples were loaded on plates as bands. The plates were developed in selected solvent systems. The plates were dried in air flow for 3hrs then sprayed with 0.008% solution of DPPH in methanol using TLC sprayer. Plates were placed in dark for 20min. for any reaction to be happened. Anti-oxidant compounds were identified as white spots on dark background. The RF value of these spots was calculated. The separation of antioxidant in the extract of *Mirabilis jalapa* in solvent system of Chloroform: Ethanol (8:2) maximum 2 spots having RF value as 0.89 and 0.85, While Solvent System; Toluene: EA (5:7) have minimum spots RF value is 0.25 these chromatograms indicates the presence of antioxidant components in *Mirabilis jalapa*.

## DISCUSSION:

In the present study, total phytochemical content was determined. The phenolic and flavonoid content in the test extracts was found to be higher in *Mirabilis jalapa*. In

general, phenolic compounds were commonly found in plants and have reported several biological activities including potential antioxidants and free radical scavengers apart from primary defense role. Earlier reports revealed that *Mirabilis jalapa* especially their flavonoids have the antioxidant activity. The nature of the active antioxidant TLC bands of the methanolic extracts of *Mirabilis jalapa* with two different extraction systems Microwave and Soxhlet. The selection of appropriate solvent system for a particular plant extracts can only be achieved by analyzing the RF values of compounds in different solvent system. In the present state of affairs, TLC profiling of all the plant extract and leaves in different solvent system indicated the presence of diverse type of phytochemicals in these plant. Different RF values of the compound also reflect an idea about their polarity. This information will help in selection of appropriate solvent system for further separation of compound from these plant extracts.

The antioxidant activity of medicinal plants is mainly related to their bioactive compounds, such as phenolics, flavonols, and flavonoids. On the basis of antioxidant activity, a sample can be classified into one of four major groups, viz., very high, moderate, low and absent antioxidant content. Preliminary phytochemical analysis of aqueous and methanolic extracts of *Mirabilis jalapa* revealed the presence of carbohydrates, alkaloids, flavonoids, glycosides, saponin, tannins, fixed oil and fats. *Mirabilis jalapa* extracts have been shown to have various antioxidant activities. Thus, the experimental data of these previous reports showed that *Mirabilis Jalapa* must have contained strong antioxidant constituents. The extracts of *Mirabilis jalapa* showed several resolved TLC with moderate antioxidant activity of resolved bands showed strong antioxidant activity and few spots with weak antioxidant activity of resolved bands

**CONCLUSION:** The present study concluded the presence of several phytochemicals and antioxidants in the *Mirabilis jalapa* provides useful information of *Mirabilis jalapa* on pharmacological activities and potential applications of such compounds as natural antioxidants phytomedicinal products. *Mirabilis jalapa* must have contained strong antioxidant constituents. In general, phenolic compounds were commonly found in plants and have reported several biological activities including potential antioxidants and free radical scavengers apart from primary defence role. Further studies are being carried out on the other species of Mirabilis of different geographical habitats in different seasons in order to provide accurate amount of chemo types with complete data of antioxidant activity and characterization of the active principle components having specific pharmacological activities, which can be used to treat various chronic and oxidation related diseases in animals and human beings.

The present study concluded the presence of antioxidants in the *Mirabilis jalapa* provides useful information of *Mirabilis jalapa* on pharmacological activities and potential applications of such compounds as natural antioxidants in different food/pharmaceutical products. Further studies are being carried out on the other species of *Mirabilis jalapa* of different

habitats in order to provide complete data of the antioxidant activity and characterization of the principle antioxidant agents, which can be used to treat various oxidative stress-related diseases in humans. Author also have various review and research articles on medicinal and Ayurvedic systems of plants, published on *Tribulus* terrestris,[17] *Acorus* calamus[18], *cathranthus roseus*[19], *Oxalis corniculata*[20], *Cuscuta reflexa*[21], *Solanum nigrum*[22], *Murraya* koeingii[23], *and Simarouba* Glauca[24]. These became popular articles for further investigations on particular medicinal herbs. These articles also have been provided very keen interest to students and researchers to make great achievements in medicinal plants research.

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