

Phytochemical Study of Some Ethnomedicinal Plants Used as Anti-Ageing Source in Ranchi District of Jharkhand

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Abstract

Anti-ageing means substances that have capacity to prevent or slow down the process of becoming old. The medicinal properties shown by different medicinal plants are due to the chemical constituent present in the plant. These phytochemical play a significant role for the treatment of several ailments related to ageing problems. To examine and investigate the phytochemicals present in the selected medicinal plants used as anti-ageing source in Ranchi District of Jharkhand was the main purpose of this study. The phytochemicals flavonoids, tannin, phenolic compounds alkaloids are the important bioactive components of plants. The Phytochemical study have been studied in leaves, flowers of *Calotropis procera* (Asclepiadaceae), *Cassia tora* (Caesalpinaceae), *Clitoria ternatea* (Fabaceae), *Mimosa pudica* (Mimosaceae), *Hibiscus rosa-sinensis* (Malvaceae), *Azadirachta indica* (Meliaceae), *Centella asiatica* (Apiaceae). Ethnobotanical inventories depend on conducting interviews with tribal people, formal surveys in the field, collection and identification of plants. The dried leaf, flower, whole, plants were subjected to primary phytochemical screening through cold maceration method. The phytochemical analysis of seven plants was studied in which *Mimosa pudica* was found suitable for anti-ageing purpose.

Keywords: Anti-ageing; Ethnomedicinal Plants; Phytochemical Properties; Bioactive Compound

Introduction

Ageing is a complex biological process characterized by a gradual loss of physiological integrity, leading to the decline of almost all physiological function and increased vulnerability to death (Lopez-Otin., *et al.* 2013; Lenart and Krejci, 2016). It has been proposed that human age in "spare parts" (in French: en pieces detaches), a process characterized by increasing losses of vital function, some occurring faster, as the elastic function, and other relatively slowly as the nervous conductivity [1,2]. Since time immemorial, people of Ranchi district of Jharkhand have been using medicinal plants to tackle different ailments which also related to ageing problem. Traditional folk treatment from wild plants has always guided researchers to search for novel medications to develop healthy life. This is the era, which is aggressively destroy the life style, mental ability discretion of human beings. Every human being wants to postpone their ageing. Related to this there are many products available in market synthetic as well as natural or herbal product.

Now a days herbal product demand is on pick as it has no any side effect. Phytochemicals of natural plants have less/no side effects comparison with synthetic one. Herbal products are easily trustable to human. The present study has brought light on some interesting plants having such chemical compounds which have the potential of ability to control ageing problems [3,4].

Materials and Methods

The plants were collected from local environment of Ranchi District of Jharkhand. These plants were authenticated by a taxonomist in Ranchi University P.G Dept. of Botany Prof. Kunul Kandir. Ranchi Jharkhand. It was ensured that the plants were healthy and uninfected. Then parts of the plants washed with tap water about 2 - 3 times. After that they kept in for drying. After drying, sample was grinded to get fine powder with the help of mechanical blender. Then for the future use with proper labeling, the powder stored in air tight plastic container. The Powdered material was weighed using Meltzer weighting balance until constant weight was obtained. The aqueous extract of each sample was prepared by soaking 100 gm of dried sample in 200 ml of distilled water for 12hr. The extract was filtered using Whatman filter paper no. 42. The extract was then stored in the suitable condition [6-16].

Extraction procedure used

Plants tissue homogenization in solvent has been widely used by researchers dried or wet, fresh plants parts are grinded in a blender to fine particle, put in certain quantity of solvent and shaken vigorously for 5 - 10 minutes or left for 24hrs. after which the extract is filtered. The filtrate then may be dried under reduced pressure and dissolved in the solvent to determine the concentration.

Phytochemical screening

Various chemical tests were carried out on the aqueous extract and on the powdered specimens using standard procedure to identify and detect the chemical constituent present in them.

Extraction through cold maceration

Chemical group tests

Various chemical tests have been done to trace the chemicals present in them. The extracts obtained were subjected to qualitative line for the identification and detection of constituents present in them.

Test of alkaloids

Small quantity of solvent free alcoholic extracts were stirred separately with a few drops of dilute hydrochloric acid and filtered. The filtrate was carefully tested with various alkaloidal reagents for example Mayer's reagent, Dragendroff's reagent and Hager's reagent.

Following reactions show the presence of alkaloids:

Extract + Dil. HCl + Mayer's reagent → Yellowish buff. precipitate.

Extract+ dil. HCl + Hager's reagent → Yellow buff. precipitate.

Test of amino acid

The small quantity of alcoholic extract dissolved in few milliliter of water with Ninhydrin gives pink color which indicates the presence of Amino Acid:

Extract + Ninhydrin → Pink ppt. formed.

Test of flavonoids

Extract and sulphuric Acid (10%) heated in a water bath and then cooled, diethyl ether and chloroform is added to it and then divided into three parts in three test tubes. 1 ml of dilute sodium carbonate or 1 ml of dilute ammonia is added, yellow color confirms the presence of flavonoids.

Test of reducing sugar

Extract and Benedict's reagent heated together brick red precipitate confirms the presence of reducing sugar:

Extract + Benedict's reagent → Brick red precipitate.

Fehling solution A and Fehling solution B mixed in equal quantity then boiled in water bath then equal volume of extracts added to it, purple color shows the presence of reducing sugar:

Fehling solution A + Fehling solution B (boiled) + Equal volume of extract → Purple color appears.

Test of tannin

Alcoholic Extract is tested with 10% aqueous potassium dichromate. Yellow precipitate confirms the presence of tannin:

Extract + 10% aq. $K_2Cr_2O_7$ → Yellow brown ppt.

Test of resins

- A few ml of extract is added to 5 ml of distilled water. Appearance of turbidity shows the presence of resin.
- 3 ml extract is added to a few ml, of acetone and 3 ml of dilute HCl then heated on a water bath for 30 minutes Pink color indicates the appearance of resins.

3 ml Extract + Acetone + 3 ml dil HCl (heated) → Pink color

- Small quantity of alcoholic extract is added to a few drop of Ferric Chloride solution, appearance of green color shows the presence of resin.

Extract of few drops of Ferric Chloride → Green color appears.

Test of saponin

3 ml of extract mixed with 10 ml of distilled water and shaken in a graduated cylinder for 15 min. 1 cm thick layer of foam indicates the presence of Saponin, Wallis (1955).

Extract + Distilled Water (Shaken) → Stable foam.

Detection of steroids

Few ml. of extract is added to 1 drop of chloroform, acetic anhydride and conc. sulphuric acid. Reddish violet color indicates the presence of steroids.

Extract + Chloroform + Acetic anhydride + conc. H_2SO_4 → Reddish violet color

Few ml. of extract added to one drop of each of conc. Sulphuric acid and Chloroform. Appearance of reddish blue color shows presence of steroid.

Extract + Conc. H_2SO_4 + Chloroform → Reddish blue color

Chemicals and reagents

Reagents and chemicals during phytochemical study were as follows.

Benedict's reagent

86.5 gms sodium citrate and 50 gms of sodium carbonate is dissolved in 300 ml distilled water. 8.65 gms of copper dissolved in 50 ml of distilled water. Both the solutions are and diluted in 500 ml of distilled water.

Fehling's reagent

Equal volume of Fehling's solution A and B. Solution A - 35 gms. copper sulphate ($CuSO_4 \cdot 5H_2O$) dissolved in 500 ml distilled water.

Fehling's solution B - 50 gms. sodium Hydroxide (NaOH) and 173 gms sodium potassium tartrate dissolved in 500ml distilled water.

Dragendorff's reagent

It is a solution of potassium Iodide and Bismuth nitrate. Bismuth Nitrate $[\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}]$ - 8 gms of Bismuth nitrate is dissolved in 2 ml liquid ammonia (NH_3).

Potassium iodide

27.2 gms is dissolved in 50 ml distilled water.

Wagner's reagent

A solution of Iodine and potassium iodide in distilled water.

Iodide - 1.27 gms,

Potassium iodide - 2 gms,

Distilled water - 100 ml.

1.27 gms of iodine and 25 gms potassium iodide is dissolved in 100 ml of distilled water.

Mayer's reagent

It is a solution of potassium mercuric iodide. It gives a pale yellow precipitate except with the alkaloids of purine groups and few other.

Mercuric Chloride (HgCl_2) - 1.36 gms. of HgCl_2 dissolved in 60 ml of distilled water.

Potassium Iodide (KI) - 5 gms of KI is dissolved in 20 ml of distilled water.

Water (H_2O) - 30 ml H_2O is dissolved in the above mixture.

Hager's reagent

A saturated solution of picric acid with cold water.

Ferric chloride solution

A 5% w/c solution of ferric chloride in water.

Dilute sulphuric acid

Conc. H_2SO_4 1.8 ml and 100 ml water. This dilute solution of H_2SO_4 gives strength of 200.

Chloral hydrate

80 gm of chloral hydrate is dissolved in 20 ml of water with continuous heating and stirring.

Phloroglucinol solution

0.1 gm of phloroglucin is dissolved in 10ml. of 96% alcohol.

Result and Discussion

The present investigation is based on the search of phytochemicals present in seven plants viz. *Lawsonia inermis* Linn., *Clitorea ternatea* Linn., *Azadirachta indica* A. Juss., *Hibiscus rosa-sinensis* Linn., *Cassia zora* Linn, *Mimosa pudica* Linn., *Centella asiatica* Linn. For the search of their chemical constituents a thorough study has been done during the research period. The work was done in following the steps as Collection of the plants, detail morphological studies, physical evaluation of dried and powdered plant parts, extraction and chemical tests of the extracts.

The present investigation is also based on the phytochemical screening the of some plants used as anti-ageing source. Modern researches on the indigenous medicinal plants have revealed the presence of active principle. The first plant taken for the phytochemical research is *Lawsonia inermis* Linn. commonly known as Henna. Ethanol extract of plant confirm presence of chemical alkaloid, amino acid, saponins, reducing sugar, steroid, flavonoid and tannin. Various chemical constituents are found in the leaves, stem and flower. The essential oil of leaves contain alkaloid, vasicine, vaccine and maintain are highly effective. The leaves also contain flavonoid which enhancing the healing property.

The second plant taken for the phytochemical research is *Clitorea ternatea* Linn. commonly known as Aparajita. It is a sacred tree, referred to as treasure of the goods, phyto-chemical studies have been reported from the last few years. The results of the qualitative phytochemical study revealed that the methanol extracts of leaf of *Clitorea ternatea* Linn. showed the presence of alkaloid, flavonoid, steroid, tannin, resin terpenoid. The methanolic extract of *Clitorea ternatea* Linn. showed antimicrobial activity against gram positive bacteria. In the chemical test of methanol extract shows that it contains amino acid, saponin, tannin, reducing sugar. The various chemical constituents reported in the leaves, stem, flower etc.

The third plant taken for the research is *Azadirachta indica* A. Juss. In the chemical test of methanol extract showed that it contains alkaloids, steroid, tannins, amino acid and saponins. The plant contains steroid, ascorbic acid, carotene, calcium and anabolic steroid etc.

The fourth plant taken for research is *Hibiscus rosa-sinensis* Linn. It has a long history of medicinal use. The plant contains essential oil, alkaloid, starch grain, saponins, flavonoids, terpenes and steroids etc. In the chemical test of plant ethanol extract showed alkaloid, steroid, saponin, flavonoid and amino acid.

The fifth plant which is taken for the research *Cassia tora* Linn. In India the local people have the rich traditional medicinal knowledge about chakwad. The plant contains alkaloids, flavonoids, phenols, steroid, terpenoid, saponins, tannins and galactosides. In the chemical test of the plant ethanol extract showed that it contains alkaloid, steroid, flavonoid, tannin, reducing sugar and amino acid. The leaves contain tannic acid, mannitol, resin, carotene and steroids are highly effective against tumor on skin. Leaves also contain chrysophanic acid glucoside, cantharidin alkaloids quinalinolids effective against tumor.

The sixth plant taken for the research is *Mimosa pudica* Linn. The plant contains fatty acid, protein and steroid etc. It shows presence of alkaloid, reducing sugar, flavonoid, tannin, resin and saponin.

The seventh plant taken for the research is *Centella asiatica* Linn. The chemical test of *Centella asiatica* in methanol extract shows presence of alkaloid, amino acid, steroid, flavonoid, saponin, tannin and reducing sugar. It also contain chemicals as diterpene, tinosporic acid, polysaccharides, glycosides, protein, calcium and phenol which help in improving memory.

All parts of the plant from root to fruit possess secondary metabolites which are very effective in treatment of ageing related problem. Leaves contain flavonoids which fight against arthritis. After the field work in laboratory taxonomical study and phytochemical study were carried out.



Figure

Plant's Name	Alkaloid	Reducing Sugar	Steroid	Tannin	Amino Acid	Resin	Flavonoid	Saponin
<i>Lawsonia inermis</i> Linn.	+	+	+	+	-	+	-	+
<i>Clitorea ternatea</i> Linn.	-	+	-	+	+	+	+	+
<i>Azadirachta indica</i> A. Juss.	+	+	+	+	-	+	+	+
<i>Hibiscus rosa-sinensis</i> Linn.	+	+	+	+	+	+	+	-
<i>Cassia tora</i> Linn.	+	+	+	+	+	+	+	
<i>Mimosa pudica</i> Linn.	+	+	+	+	+	+	+	+
<i>Centella asiatica</i> Linn.	+	+	+	+	-	+	-	+

Table: Chemical group test of some plants.

Present: +; Absent: -.

Conclusion

For extraction of chemical constituents method of cold maceration was adopted. The preliminary phytochemical screening shows the presence of different types of chemical constituents. These plants contain a number of secondary metabolites or the phytochemicals viz. alkaloid, reducing sugar, steroid, tannin, amino acid, resin, flavonoid, saponin etc.

Alkaloid, Resin, Tannin steroid are present in all seven plants - *Azadirachta indica* A. Juss., *Hibiscus rosa-sinensis* Linn., *Cassia tora* Linn., *Lawsonia inermis* Linn., *Mimosa pudica* Linn., *Centella asiatica* Linn., *Clitorea ternatea* Linn. Amino Acid is absent in *Lawsonia inermis* Linn., *Centella asiatica* Linn., *Azadirachta indica* A. Juss. Flavonoid is absent in *Lawsonia inermis* Linn., and *Centella asiatica* Linn.

Steroid is absent in *Clitorea ternatea* Linn., Saponin is absent in *Cassia tora* Linn., and *Hibiscus rosa-sinensis* Linn. Alkaloid is absent in *Clitorea ternatea* Linn. In *Mimosa pudica* Linn. alkaloid, reducing' sugar, steroid, tannin, amino acid, resin, flavonoid and saponin are present. On the basis of present investigation, it is concluded that these plants are of great medicinal value and they can cure various diseases due to phytochemicals present among them. The present investigation attempts to attract the attention of researchers and common people towards the medicinal use of plants. It is an attempt for collection, identification, taxonomical studies and chemical analysis of specific chemical constituents present in these plants. The main aim of this investigation is to motivate the people for the effective herbal cure of various diseases without any risk and side effects. These plants contain a number of secondary metabolites. The investigation was based only on qualitative analysis of the chemicals present. Further investigation is required on quantitative analysis of chemicals.

This would be a guideline to the researchers for further investigation.

The present work is an attempt to attract the attention of researcher and on people towards the medicinal use of plants which are used as anti- ageing source. Traditional medicines remained as the most affordable and easily accessible source of treatment in the primary health care system among the people. All over the world the ethnic people have protected the flora and fauna with which they have emotional and symbolic relationship.

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