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PHYTOCHEMICAL SCREENING USING DIFFERENT PLANT EXTRACTS OF *PERGULARIA DEAMIA* AND *CASSIA AURICULATA*

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ABSTRACT:

Pergularia daemia and *Cassia auriculata* has a long history of use in the Ayurvedic and Unani systems of medicine. It contains a number of phytoconstituents viz. alkaloids, phytosterols, glycosides, amino acids, proteins, phenolic acids, enzymes, vitamins, sugars, minerals, flavonoids, resins, terpenoids etc The plant powder was extracted successively with different organic solvents (i.e. benzene, chloroform, acetone, ethyl acetate, methanol and ethanol). Then the extracts were assessed for the presence of phytochemical components. The results revealed the presence of proteins, carbohydrates, steroids, tannins, flavanoids, alkaloids, glycosides, saponins and resins. Ethanol extract were found to have all major phytochemical constituents in both the plants.

KEY WORD: *Phytoconstituents, Ethanol extract and Organic solvents.*

INTRODUCTION:

Plants have great potential uses, especially as traditional medicine and pharmacopoeial drugs. A large proportion of the world population depends on traditional medicine because of the scarcity and high costs of synthetic medicine. Plant kingdom is being screened for newer and effective chemotherapeutic agents. A number of phytochemical studies have demonstrated the presence of several classes of chemical compounds. The efficacy and safety of herbal medicine have turned the researcher towards medicinal plants for treating chronic disease. Historically, plants have

provided a source of inspiration for novel drug components, as plant derived medicine have made large contribution to human health.

Pergularia daemia

The plant *Pergularia daemia* (Asclepiadaceae) known as “Veliparuthi” in Tamil, “Uttaravaruni” in Sanskrit and “Utranajutuka” in Hindi is a slender, fetid- smelling perennial climber. Leaves opposite, membranous, 3-8 cm long and about as wide, orbicular or deeply cordate, acute or short acuminate at apex, pubescent beneath, petioles 2-9 cm long. Flowers greenish-yellow or dull white tinged with purple, borne in axillary, long-peduncled, drooping clusters. Fruits (follicles) lanceolate, long-pointed, about 4 cm long, covered with soft spines and seeds are pubescent.

P. daemia is used as anthelmintic, laxative, antipyretic and expectorant. Aerial parts of this plant reported the various pharmacological activities like hepatoprotective (Sureshkumar *et al.*, 2006), anti-diabetic (Wahi *et al.*, 2002).

Classification

The plant is classified as shown below

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Gentianales

Family: Apocynaceae

Subfamily: Asclepiadoideae

Genus: *Pergularia*

Species: *P. daemia*

Vernacular names

Uttamani, Veliparuthi, Hariknot plant, Uttamarani, Utaran, Sagovani, Chamardudhi, Uturdi, *Pergularia daemia* (Forssk.) Chiov., *Daemia scandens*, *Daemia cordifolia*, *Daemia garipensis*, *Asclepias daemia* Forssk.

Cassia auriculata

Cassia auriculata is a legume tree in the subfamily Caesalpinioideae. It occurs in the dry regions of India and Sri Lanka. The leaves are alternate, stipulate, paripinnate compound, very numerous, closely placed, slender and pubescent. Its flowers are irregular, bisexual, bright yellow and 2.5 cm long. The fruit is a short legume tipped with long style base, flat, thin, papery, undulately crimped, pilose, pale brown. The root is used in decoctions against fevers, diabetes, diseases of urinary system and constipation. The plant has been reported to possess hepatoprotective (Rao and Vedavathy, 1991), antidiabetic, antiperoxidative and antihyperglycemic (Manickam *et al.*, 2002) and microbicidal activity (Pari and Latha, 2003).

Classification

The plant is classified as shown below

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Fabales

Family: Fabaceae

Subfamily: Caesalpinioideae

Tribe: Cassieae

Subtribe: Cassiinae

Genus: *Cassia*

Species: *C. auriculata*

Vernacular names

Aavartaki, Aadari simbi, Tanners cassia, Avaram, Tarwar. *Cassia auriculata* L., *Cassia densistipulata* Taub.

MATERIALS AND METHODS:

Procurement of drug

About 2kg of whole plant of *Pergularia daemia* Linn. were collected from Tirupur District, Tarapuram (Tamilnadhu).

Drying & size reduction

The freshly collected whole plant of *Pergularia daemia* Linn. were shade dried and then powdered to required particle size.

Extraction

The dried material was reduced to coarse powder in a mechanical grinder and pass through sieve No. 40 to obtain about 800gms powder of desired particle size. It is subjected to successive extraction with benzene, chloroform, acetone, ethyl acetate, methanol and ethanol. The crude extracts were evaporated to dryness in Rotary evaporator under low temperature and reduced pressure.

The different extracts of the leaves of *Pergularia daemia* were subjected to phytochemical screening using the standard methods (Harborne, 1984).

Proteins

Millon's test

To a small quantity of the extract, 5 -6 drops of Millon 's reagent were added. A precipitate which turn red on heating was formed and it indicate the presence of proteins.

Biuret's test

To 1.0 ml of the plant extract, 5-8 drops of 10% KOH solution, followed by one or two drops of 3% CuSO₄ were added. Formation of red or violet colour confirmed the presence of proteins.

Test for amino acid**Ninhydrin Test**

To 2ml of plant extract, few drops of a solution of 0.2 g of ninhydrin (1,2,3-indanetrione monohydrate) in 50 mL of water were added. The test mixture is heated to boiling for 15-20 sec. A blue color appearance indicates the presence of amino acids.

Carbohydrates**Fehling's test**

To 2.0 ml of the plant extract, 1.0 ml of a mixture of equal parts of Fehling's solution 'A' and 'B' was added. The contents were boiled for a few minutes. Formation of red or brick red precipitate indicates the presence of carbohydrates.

Benedict's test

To 0.5ml of the plant extract, 5ml of Benedict's reagent was added and boiled for 5 minutes. Formation of a bluish green colour showed the presence of carbohydrates.

Molisch's test

In a test tube containing 2.0 ml of plant extract, 2 drops of freshly prepared 20 % alcoholic solution of naphthol was added and mixed. To this solution 2.0 ml of concentrated H₂SO₄ was added so as to form a layer below the mixture. Formation of the red violet ring at the junction of the solution indicate the presence of carbohydrates.

Steroids**Libermann –Burchardt's t Test**

To 1.0 ml of the extract, 1.0 ml of conc. H₂SO₄ was added followed by the addition of 2.0 ml of acetic anhydride solution. A greenish colour developed and turned blue which indicate the presence of steroids.

Salkowski's Test

To 2.0 ml of the extract, 1.0 ml of concentrated H₂SO₄ was added carefully along the sides of the test tube. A red colour was produced.

Tannins**Ferric chloride test**

To 1-2 ml of the extract, few drops of 5% aqueous FeCl₃ solution was added. Appearance of dark blue or greenish black colour indicates the presence of tannins.

Gelatin Test

To the extract, 1% gelatin and 10% sodium chloride was added. Appearance of milky white colour indicates the presence of Tannin.

Test for Flavanoids

Sodium Hydroxide test

A small amount of extract was treated with aqueous NaOH and HCl, observed for the formation of yellow orange colour.

Sulphuric acid test

A fraction of extract was treated with concentrated H₂SO₄ and observed for the formation of orange colour.

Alkaloids

Dragendorff's test (Kraut reagent – potassium bismuth iodide)

To 0.5 ml of plant extract, 2ml of HCl was added. To this acidic medium, 1 ml of reagent was added. An orange, red precipitate produced immediately, which indicates the presence of alkaloids.

Wagner's reagent (Iodine-potassium iodide solution)

A few drops of Wagner's reagent were added to the plant extract. Formation of yellow or brown precipitate confirmed the presence of alkaloids.

Meyer's reagent (Potassium Mercuric iodide)

To 1ml of the extract, few drops of Meyer's reagent was added. Formation of white or pale yellow precipitate showed the presence of alkaloids.

Glycosides

Keller-killiani test

To 0.5 ml of plant extract, add 0.4 ml of glacial acetic acid containing a trace amount of ferric chloride. Add 0.5 ml of conc. sulphuric acid along the sides of the test tube. Appearance of blue colour indicated the presence of glucosides (cardiac Glucosides).

Test for Saponins

Foam test

To 2 ml of plant extract, 2ml of distilled water was added and shaken in a graduated cylinder for 15 minutes lengthwise. Formation of 1cm layer of foam indicated the presence of saponins.

Haemolysis test

A suspension of RBC in normal saline was treated with a small amount of decoction. In the presence of saponin, haemolysis was observed.

RESULTS AND DISCUSSION:

Table 1 and 2 shows the results of phytochemical investigation of various extracts of the whole plant of *Pergularia daemia* and *Cassia auriculata*. The result shows that the plant has a number of phytochemical constituents like: alkaloids, steroids, flavonoids, tannins, glycosides, carbohydrates, proteins, amino acids,

saponins and resin. Of all the extracts, ethanol extract contains all major phytochemical constituents except saponins.

Pergularia daemia

Different phytochemicals have been found to have a broad range of activities, which may help in protection against various diseases (Liu, 2003). Phytochemicals found in the leaves of *P. daemia* are flavonoids alkaloids, terpenoids, tannins, steroids and carbohydrates (Karthishwaran et al., 2010).

Phytochemical tests were studied or analysed on the different extracts of the powdered form of the whole plant parts of *Pergularia daemia*. On the basis of phytochemical screening we conclude that this plant have a number of phytochemical constituents like: alkaloids, steroids, flavonoids, tannins, glycosides, carbohydrates, proteins, amino acids, saponins and resin (as shown in table-1). Ethanol extract contains all major phytochemical constituents except saponins. Methanol extract contains all constituents except carbohydrates, tannins and resins. Active principle present in acetone and ethyl acetate extracts are carbohydrate, steroids, flavanoids, alkaloids and glycosides. Chloroform extract contains carbohydrates, flavanoids and alkaloids. Presence of flavonoids and tannins is responsible for free radical scavenging effects. Saponins may be used in traditional medicine as anti infecting agents. A similar phytochemical screening study was also done on *Pergularia daemia* (Usman et al., 2012).

Cassia auriculata

On the basis of Phytochemical tests on the different extracts of the powdered form of the whole plant parts of *Cassia auriculata* it is concluded that the plant has a number of phytochemical constituents like alkaloids, steroids, flavonoids, tannins, glycosides, carbohydrates, proteins, amino acids, saponins and resin (Table-2). Ethanol extract contains all major phytochemical constituents except tannins and resins. Methanol extract contains all constituents except carbohydrates, saponins, tannins and resins. Active principle present in acetone and ethyl acetate extracts are carbohydrate, tannins, flavanoids and glycosides. Active principle in chloroform extract are carbohydrates, steroids, flavanoids, saponins and resins. It was found from previous studies that the main constituents of leaves 3-O-Methyl-dglucose, α -Tocopherol- β -D-mannoside, Resorcinol, n-Hexadecanoic acid, 13-Octadecenal, and 1,2,3,4-Tetrahydroisoquinolin-6-ol-1-carboxylic acid (Anandan et al., 2011). The fatty acid and fatty acid ester derivatives are recorded predominantly in seeds of *Cassia auriculata*. Phytochemical analysis revealed presence of fatty acid esters, fatty acid amide, terpenoids, diterpene alcohols and phytol as major compound groups in the methanol fractions of *Cassia auriculata* seeds (Raj et al., 2012).

CONCLUSION:

It is believed that the information presented in this paper would help the researchers to get aware of this plant and extensive research should be done further for establishing new therapeutic drugs.

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Table 1: The qualitative phytochemical investigation of extracts of whole plant *Pergularia daemia*.

| <i>Pergularia daemia</i> | | | | | | |
|----------------------------|---|---|---|---|---|---|
| Test | A | B | C | D | E | F |
| Proteins | - | - | - | - | + | + |
| Million's test | | | | | | |
| Biuret's test | | | | | | |
| Amino acids | - | - | - | - | + | + |
| Ninhydrin test | | | | | | |
| Carbohydrates | - | + | + | + | - | + |
| Fehling's test | | | | | | |
| Benedict's test | | | | | | |
| Molisch's test | | | | | | |
| Steroids | - | - | + | + | + | + |
| Libermann-Burchardt's test | | | | | | |
| Salkowski's test | | | | | | |
| Tannins | - | - | - | - | - | + |
| Ferric chloride test | | | | | | |
| Gelatin test | | | | | | |
| Flavanoids | - | + | + | + | + | + |
| Sodium hydroxide test | | | | | | |
| Sulphuric acid test | | | | | | |
| Alkaloids | - | + | + | + | + | + |
| Dragendroff's test | | | | | | |
| Wagner's test | | | | | | |
| Meyer's test | | | | | | |
| Glycosides | + | - | + | + | + | + |
| Keller-killiani test | | | | | | |
| Saponins | - | - | + | - | + | + |
| Foam test | | | | | | |
| Haemolysis test | | | | | | |
| Resins | - | + | - | - | - | + |

Table 2: Table showing the qualitative phytochemical investigation of extracts of whole plant *Cassia auriculata*.

| <i>Cassia auriculata</i> | | | | | | |
|--------------------------|---|---|---|---|---|---|
| Test | A | B | C | D | E | F |
| Proteins | + | - | - | - | + | + |
| Million's test | | | | | | |
| Biuret's test | | | | | | |
| Amino acids | + | - | - | - | + | + |
| Ninhydrin test | | | | | | |
| Carbohydrates | - | + | + | + | - | + |
| Fehling's test | | | | | | |
| Benedict's test | | | | | | |
| Molisch's test | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Steroids Libermann- Burchardt's test Salkowski's test | - | + | + | - | + | + |
| Tannins Ferric chloride test Gelatin test | - | - | + | + | - | - |
| Flavanoids Sodium hydroxide test Sulphuric acid test | + | + | + | + | + | + |
| Alkaloids Dragendroff's test Wagner's test Meyer's test | + | - | - | + | + | + |
| Glycosides Keller-killiani test | + | - | + | + | + | + |
| Saponins Foam test Haemolysis test | - | + | - | - | - | + |
| Resins | - | + | - | - | - | - |

(+) → Presence and (-) → Absence

A- Benzene extract

B- Chloroform extract

C- Acetone extract

D- Ethyl acetate extract

E- Methanol extract

F- Ethanol extract