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Research Paper

Analysis of Ethanolic Extract Caesalpinia Coriaria Pods Using Thin Layer Chromatography (TLC) and Radial Diffusion Assay Techniques

Medical Science

KEYWORDS : *Caesalpinia Coriaria*, TLC, Inkimaram, RDM, Tannin

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ABSTRACT

Medicinal plants have been resurgence in the consumption and demand. These plants are finding use as pharmaceuticals, neutraceticals, cosmetics and food supplements. Even as traditional source of medicines and they continue to play pivotal rule. In this study mainly focused on the analysis of ethanol extract Caesalpinia Coriaria pods in two different way analyses. The antioxidant activity of ethanol extract showed the significant result. The presence of antioxidant bioactive compound in the pod extract could possibly contribute to the antioxidant activity.

1.INTRODUCTION

Millions of rural households use medicinal plants in a self-help mode. Over 20,000 practitioners of the Indian System of Medicine in the oral and codified streams use medicinal plants in preventive, promotive and curative applications in Tamil Nadu. There are estimated to be about 1000 manufacturing units in Tamil Nadu. In recent years, the growing demand for herbal product has led to a quantum jump in volume of plant materials traded within and across the countries. An estimate of the EXIM Bank puts the international market of medicinal plants related trade at US$ 60 billion per year growing at the rate of 7% only. Though India has a rich biodiversity, the growing demand is putting a heavy strain on the existing resources. While the demand for medicinal plants is growing, some of them are increasingly being threatened in their natural habitat.

In this work detailed to study of *Caesalpinia Coriaria* pods components us two different techniques. That the techniques are Thin layer chromatography (TLC) and Radial diffusion assay.

2. MATERIALS AND METHODS

2.1 PLANT DESCRIPITION

KINGDOM : Planate
DIVISION : Magnoliophyta
CLASS : Magnolipsida
ORDER : Fabales
FAMILY : Fabaceae
SUB FAMILY : Caesapinioideae
GENUS : Caesalpinia
SPECIES : Coriaria
TAMIL NAME :Inkimaram, Kodivelam, Kodichittal.

2.2 COLLECTION OF PLANT EXTRACT

Dry pods of Casalpinia coriaria free from diseases were collected from captain Srinivasa drug research institute, Arumbakkam, Chennai, Preparation of aqueous, Ethanol, Ethyl acetate extract using Soxhlet apparatus (Franz von soxhlet, 1879). The extract kept in refrigerator 4°C for future investigation.

2.3 CHEMICAL NATURE

Analysis of the dark pods, shed from the tree gave (moisture – free basis)

Tannin - 66.6 - 85.4

Non tannin - 6.7 - 27.5

PH of infusion - 3.2 – 3.4

(Infusion on standing ellagic acid)

The tannins in the pod comprise pyrogallal type of hydrolysable tannin and consist of gallo tannin & ellagi tannin.

2.4 THIN LAYER CHROMATOGRAPHY

Separation and identification of condensed tannins (proanthocyanidins) by thin layer chromatography

2.4.1 MATERIALS

1. Silica gel G

2. Standard: 1% tannic acid solution

10mg of tannic acid dissolved in 1ml of distilled water

3. Plant extracts:

0.1mg of ethanol extract of *Caesalpinia coriaria* dissolved in 1ml of ethanol.

4. Developing Solvent:

Dichloromethane, Ethyl acetate was mixed in ratio of 5: 1. This was used as developing solvent.

5. Locating Reagent: 0.1% Ferric chloride reagent

1g of ferric chloride was dissolved in 100ml of distilled water from the above mixture 1ml was taken and diluted to 100ml using distilled water.

6. UV illuminator

7. TLC tank

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2.4.2 PROCEDURE

2.4.3 PREPARATION OF SLURRY

Slurry of silica gel was prepared using distilled water in the ratio 1:2 (w/v). Place dry clean TLC plate base over a plain surface. Stir the slurry thoroughly for 1-2 minutes and poured on the glass plate evenly.Leave the glass plate to dry in room temperature for 15-20 minutes and then the plate is kept in oven at 200°C for 2 hours to remove the moisture and to activate the adsorbents on the plate.

2.4.4 APPLICATION OF SAMPLE

Leave 2-5 cm from end of the glass plate, equal distance from the edges. Apply the sample and standard by means of capillary tube into small spots. All the spots should be placed at equal distance from both the ends of the plate. Allow the sample to dry and spotting repeated for 2-3 times for more concentrated spot of the sample.

2.4.5 DEVELOPING OF CHROMATOGRAM

Pour the developing solvent into tank of depth 2.5cm. Allow it to stand for at least one hour with the cover plate over the top of the tank. This ensures that the atmosphere within the tank gets saturated with the solvent vapour. This is called equilibration. After this remove the cover plate and place the TLC plate vertically inside the tank. Replace the cover plate and the separation of compounds occurs as the solvent moves upwards. Develop the chromatogram at constant temperature in order to avoid enormous solvent running effect. Once the solvent reaches the top of the plate, remove the plate from the tank and allowed to dry it and then proceeded for identification of separated compounds.

2.4.6 IDENTIFICATION OF SAMPLE

After drying, the plate was sprayed with 0.1% ferric chloride locating reagent .The plate was viewed for sample under UV illuminator. R_F values were calculated and compared with standards.

R_F can be calculated using the formula

$$R_F = \frac{\text{Distance moved by the solute}}{\text{Distance moved by the solvent front}}$$

RADIAL DIFFUSION ASSAY (HAGERMAN , 1987)

2.5.1 MATERIALS

1.1% Agarose
2. Sodium chloride
3. Well puncher
4. Distilled water
5. Glass slides
6. Bovine serum albumin (BSA)

2.5.2 METHOD:

- 1% Agarose in distilled water, heated till it changes to colourless solution .To this 50mg of BSA was dissolved and cooled to bearable warm.
- The agarose solution was poured into the slide and the well is punched.
- The extracts of aqueous, ethanol, ethyl acetate of 20 micro litres was poured into each well.
- The slides are kept overnight to observe the result.

3. RESULT

3.1 THIN LAYER CHROMATOGRAPHY

Separation and identification of condensed tannins (proanthocyanidins) by thin layer chromatography

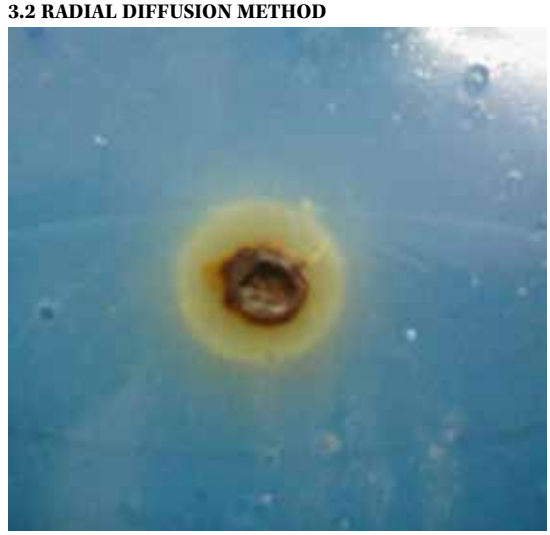
STD Test

$$R_F = \frac{\text{Distance moved by the solute}}{\text{Distance moved by the solvent front}}$$

SAMPLE	SOLVENT FRONT	SOLUTE FRONT	Rf
Standard tannic acid	15.0cm	13.1	0.87cm
Ethanol extract of pods	15.0cm	13.0	0.86cm

The fluorescent band was observed under UV illuminator and presence of condensed tannins was observed.

3.2 RADIAL DIFFUSION METHOD



Interaction of tannin in ethanol extract with BSA embedded in agar

This method depends on the formation of complexes between tannins present in the ethanol extract of ***C.coriaria*** pod and the

protein (bovine serum albumin) embedded in agar.

The ethanol extract placed in a well in the agar diffused and precipitated the albumin. In this case an opaque circle was formed around the well. The diameter of the circle formed indicates the amount of tannins present in the aqueous extract

DISCUSSIONS

Xanthine oxidase is an endogenous source of free radicals. Free radicals are molecules containing unpaired electrons. The unpaired electron is a highly reactive "hot potato" that either "burns" a molecule (causes oxidative damage) or is passed from molecule to molecule causing turning the recipient into free radical and neutralizing the donor.

Antioxidant destroys the free radicals in the body. Free radicals are by product of oxygen metabolism that can damage cells and are among the causes of many degenerative diseases. They also associated with aging process.

In this study help to further analysis of *Caesalpinia coriaria* pods phytochemical analysis and activity of Xanthine inhibitor assay from different sources. It also helps to analysis secondary metabolites. The isolation of the bioactive compound from extract needs future work.

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