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THIN LAYER CHROMATOGRAPHY OF FLAVANOIDS IN *PITHECELLOBIUM DULCE* METHANOLIC LEAF EXTRACT

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Abstract:

Background: This study focuses on performing the TLC of the flavonoids in the *Pithecellobium dulce* methanolic leaf extract using suitable solvent system. The use of common available plant constituents, flavonoids like kampferol, quercitin can produce anticancer effect. Qualitative estimation of flavanoid is important to screen the antioxidant or anticancer effect of plants.

Objectives: The objectives of the present study are Qualitative estimation of phytochemicals using TLC.

Methods: Powdered leaves are subjected to extraction using soxhlets apparatus using methanol as solvent for 24 hrs. Thin glass plates (20×10 cm) were coated with Silica Gel G. The developed plates were dried and visualised under UV light and are also sprayed with 5% $FeCl_3$ and iodine. The spots thus developed were noted and the Rf values are calculated.

Result: The TLC profiles of PDME is similar to that of standard flavonoid (Sharma Priyanka and Sarin Renu., 2010), it may have the similar biological activities (*Sukantha T. A., 2015)*. Hence, it may show antiangiogenic effect.

Conclusion: The TLC can be used as qualitative analytical tool to determine flavanoids present in different plant.

Keywords:

Profiles, Extracts, Determining, Flavanoids.

INTRODUCTION:

Focusing majorly on the disadvantages of the conventional cancer theraphies, involving the use of synthetic drugs or radiations. The development of ntiangiogenic agents using natural products has remained a significant hope in the mainstream of anticancer research. The use of common available plant constituents, flavonoids like kampferol, quercitin can produce anticancer effect. The TLC can be used as a qualitative analytical tool to determine flavanoids present in different plant.

***Pithecellobium dulce* plant profile**

Pithecellobium dulce (Roxb.) Benth. (Family Leguminosae, sub family Mimosoideae) is one of 100-200 species in this genus. Synonyms are Huamachil, Manil tamarind, Camachile, Wild tamarind.

A large, nearly evergreen tree that grows up to 20 m or more in height, Manila tamarind has a broad crown (to 30 m across) and a short bole (to 1 m thick). At the base of each leaf is normally found a pair of short, sharp spines, though some specimens are spineless. Flower fragrant the color white the flowers have dozens of stamina. Fruit grow in pods, the pod color is pink looks like scorpion's tail, fruit pulp is white and hairy until 4-6 fruits in pod, in some cultivars peel easy, the seeds also edible

Pithecellobium dulce Benth. is a small to medium sized, evergreen, spiny tree up to 18 m height, native of tropical America and cultivated throughout the plains of India and in the Andamans. *Pithecellobium dulce* is the only species that has become widespread outside its origin. It is now common and naturalized in India and tropical Africa, especially along coasts. *Pithecellobium dulce* generic name refers to the curly pod, that mimics an ape's earring (pithekos ellobium), and the species name "dulce" refers to the sweet pod.

Chemical constituents

It contains the Flavanoids, Tannin, 25.36%; fixed oil, 18.22%, olein. A glycoside quercitin has been isolated. Seeds have been reported to contain steroids, saponins,

lipids, phospholipids, glycosides, glycolipids and polysaccharides. Bark yields 37% tannins of the catechol type. Leaves yield quercitin, kaemferol, dulcitol and afezilin.

Therapeutic uses

Abortifacient activity, anti-inflammatory activity, antivenom activity, protease inhibitor activity, antitubercular activity, antimicrobial activity, spermicidal activity, activity against ccl_4-mediated hepatic oxidative impairments and necrotic cell death, anti-ulcerogenic activity, hypolipidemic activity, locomotor activity, adulticidal activity, analgesic and anti-inflammatory activities.

Reported activities of *Pithecellobium dulce* plant

The aqueous extract of the fruits of *Pithecellobium dulce* (AEPD) against carbon tetrachloride (CCl_4)-induced hepatic injury using a murine model has been found to possess free radical (DPPH, hydroxyl and superoxide) scavenging activity (Prasenjit Manna *et al*., 2010). The bark and leaves possess astringent property, and leaves have emollient, abortifacient and antidiabetic properties.

The ethyl acetate, methanolic and aqueous extracts of fruit peel of *Pithecellobium dulce* will have antioxidant and antibacterial potential (Sukantha T.A *et al.*, 2011). The aqueous and alcoholic extracts of leaves of *Pithecellobium dulce* causes significant CNS depression action in albino mice that may be due to increase in the concentration of GABA in brain (M. Sugumaran., 2008).

Oral administration of PDM (125, 250 and 500 mg/kg) for 21 days caused a significant decrease in fasting blood glucose, HbA1C and significant increase in body weight, serum insulin, total protein, and liver glycogen levels in treated diabetic rats. PDM effectively normalized dyslipidemia associated with streptozotocin-induced diabetes. In liver, kidney and pancreas, the activity of antioxidant enzymes and content of reduced glutathione were found to be significantly enhanced, while levels of lipid peroxides were suppressed in treated diabetic rats.

P. dulce exerts gastroprotective effect by down regulating gastric H^+/ K^+-ATPase synthesis and up regulation of mucin secretion in stomach and duodenum. Petroleum ether, Ethyl alcohol and water extracts of the seeds will show anti-ulcer activity in pyloric ligation induced gastric ulcer. Thus *P. dulce* may be included in antiulcer drug formulations either singly or with other known herbal medicines for the prevention and treatment of peptic ulcer (Jayraman Megala, 2015). Though *Pithecellobium dulce* extracts was proved to have many biological activities but none of the previous studies focused on the antiangiogenic activity.

MATERIALS AND METHODS

Reagents and chemicals

Absolute methanol (Research lab fine chem industries, Mumbai), Sodium chloride (Research lab fine chem industries, Mumbai), Hydrocortisone (Himedia laboratories Pvt. Ltd., Mumbai), Silica Gel (Finar Chemicals Ltd., Ahmedabad), Ninhydrin Reagent (Finar Chemicals Ltd., Ahmedabad).

Glass ware

Soxhlets extraction apparatus, measuring cylinders, forceps, syringes, test tubes, glass rods, volumetric flasks, beakers, centrifuge tubes, funnel, pipettes

Equipments

Incubator (Biotechnis India), Microscope (Olympus), Centrifuge, Thin-layer Chromatogram.

EXPERIMENTAL METHODOLOGY

Extraction (Anil Bobade., 2010)

Leaves of the *Pithecellobium dulce* were collected from the local areas of Vijayawada and the plant was authenticated by the Department of Pharmacognosy, Vijaya Institute of Pharmaceutical Sciences for Women. The leaves were shade dried, finely powdered using mixer and blender and seived. The total powder was weighed. Powdered leaves are subjected to extraction using soxhlets apparatus using methanol as solvent for 24 hrs.

Thin layer Chromatography (Sharma Priyanka and Sarin Renu., 2012)

Thin glass plates (20×10 cm) were coated with Silica Gel G. The freshly prepared plates were dried at room temperature, thereafter these were kept at 100 ˚C for 30 minutes to inactivate the enzymes and then cooled at room temperature. Each of the extract was co-chromatographed with flavonoid samples. This plate were developed in air tight chambers saturated with the solvent mixture of benzene, acetic acid and water (125:72:3). The developed plates were dried and visualised under UV light and are also sprayed with 5% $FeCl_3$ and iodine. The spots thus developed were noted and the Rf values are calculated. Several other solvent systems such as n-butanol, acetic acid, water (3:1:1)

were also tested, but the solvent system containing benzene, acetic acid and water (125:72:3) gave better results.

RESULTS AND DISCUSSION

After the TLC of the PDME using the solvent system containing benzene, acetic acid and water. It was observed that the samples had shown good mobility. The corresponding Rf values of the developed spots are measured and compared. As the TLC profiles of PDME is similar to that of standard flavonoid (Sharma Priyanka and Sarin Renu., 2010), it may have the similar biological activities (*Sukantha T. A., 2015)*. Hence, it may show antiangiogenic effect.

SUMMARY

Leaves of the *Pithecellobium dulce* of Leguminosae family was collected from the local areas of Vijayawada. The leaves were shade dried, finely powdered and are subjected to soxhlets extraction using methanol as solvent.Thin Layer Chromatography was performed using benzene, acetic acid and water mixture as solvent system and the corresponding Rf values are noted. As the TLC Profiles of PDME similar with that of standard flavonoids, the therapeutic actions will be similar.

CONCLUSION

The present TLC study of PDME, conclude that the PDME contains phytochemical flavonoids, that may possess a strong antiangiogenic effect.

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