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Thin Layer Chromatographic Analysis of Flavonoid in Mulberry Leaf Extract

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Abstract: ***Morus alba L., also known as white mulberry, has long been used in traditional medicine to treat a variety of health conditions. The leaves of the plant are rich in polyphenols such as quercetin 3-(- malonylglucoside), rutin, isoquercitin, and cyaniding-3-rutinoside apigenin, luteolin, caffeic acid, gallic acid and umbelliferone, chlorogenic acid, kaempferol, and flavonoids. In the present study, qualitative tests of leaf extracts showed significant indication of the presence of Flavonoids were found to be present in the leaf extracts, with a maximum concentration by using methanol and ethanol solvents. Using thin layer chromatography (TLC) profiling of plant extracts gives an impressive result that directing towards the existence of phytoconstituents. In addition, the alcoholic extracts for mulberry leaves contain a higher content of bioactive compounds, which can be used for further researches on this plant.***

Keywords- Thin layer chromatography, Mulberry leaf extract, separation, flavonoids, quercetin

I. INTRODUCTION

Natural plants are used as very good source of nutrition persistent food as well as source of various chemical constituents operative in curing various diseases which may demand as the biologically active constituents. At the present natural plants are very much in petition in the form of drugs because of their fewer side effects, they are considered the potential resources of various bioactive compounds and are also easily available from the natural sources. In the same context Morus alba, the Mulberry plant which is basically famous for sericulture, the fabrication of silk done through the silkworm and the leaves are also used to diminish the symptoms of diabetes in vernacular medicine as well as for improving cardio-metabolic risks, including antihyperglycemic, antihyperlipidemic, anti-obesity, antihypertensive, antioxidative, anti-inflammatory, anti-atherosclerotic and cardioprotective effects [1] in Chinese medicine used to treat constipation, to tonify the blood, prematurely grey hair, cough, edema, to promote urination, fever, headache, dry & sore eyes [2] and so many more, So, the leaves is used further in this study to explore some more about the biological activity of leaves.[3]

The mulberry plant is part of the 68-species genus Morusha, a family of unisex flowering plants in the Utricles subclass of the Moraceae. The plant is a 20 to 30 feet high shrub or tree, often the size of a small apple tree, with thin, glossy, light green leaves that have five lobes, one lobe, two lobes, three lobes, or no lobes at all. Morus Alba L, also referred to as the White Mulberry, may easily be grown from seeds as well as huge cuttings of roots. Typically, the plantation is raised on a block foundation with intervals between the plants and the rows of 6 feet by 6 feet or 8 feet by 8 feet. The plants are typically pruned once a year, in July - August, during the monsoon season, to a height of 5 to 6 feet, and then left to grow with no more than 8 to 10 shoots at the top [4]. The plant is widely dispersed over Asia, Africa, Latin America, South Europe, North Africa, and Japan.

Mulberry leaf extract is a natural product derived from the leaves of the mulberry plant, which is commonly found in Asia, Europe, and North America. The extract has been used for centuries in traditional medicine to treat a variety of health conditions. The active components of mulberry leaf extract include flavonoids, such as quercetin, kaempferol, and Rutin, as well as various other compounds, such as alkaloids and polysaccharides. These components are believed to have antioxidant, anti-inflammatory, and anti-diabetic properties, among others. Mulberry leaf extract has been studied for its potential benefits in managing blood sugar levels and improving insulin sensitivity. It may also have a positive effect on cardiovascular health, reducing the risk of heart disease and stroke. Morus alba L. Leaves have been used as a substantial source of medicine, drink, and functional foods in many countries. It is used in drinks as green tea with several other herbal drugs like Tulsi and ashwagandha because of its immune boosting antioxidants like Chlorogenic acid, Rutin, soquercitrin, and astragalin. Anticancerous alkaloids like 1- deoxynojirimycin, morroles B–F [5], (2R,3R,4R)-2- hydroxymethyl-3,4-dihydroxypyrrolidine-N-propionamide from the root bark and 4-O-R-D-galactopyranosylcalystegine B2 and 3β,6β-dihydroxynortropane from the fruits [6], mulbaines A, B & C6 [7].

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Eighteen important amino acids include calcium, potassium, sodium, magnesium, zinc, iron, copper, manganese, chromium, selenium, arsenic, vitamins and there's no caffeine property. Other chemical constituents present in leaves are coumarins, flavonoids, anthocyanins and polyphenolsincluding quercetin 3-(- malonylglucoside), rutin, isoquercitin, cyaniding-3- rutinoside apigenin, luteolin, quercetin, morin, caffeic acid, gallic acid, umbelliferone, chlorogenic acid, and kaempferol [8].The plant extract rich in polyphenols used as a non-toxic natural healing agent, which also have high prospective applications as skin-whitening agents due to its potent tyrosinase inhibitor property [9]. Phytoconstituents in mulberry leaf extract can be identified using a variety of analytical techniques. Some of the commonly used methods are Thin-layer chromatography (TLC): This technique involves separating the constituents of the extract on a thin layer of stationary phase material and then visualizing them by spraying them with a chemical reagent or exposing them to a UV light. This method is useful for identifying flavonoids, alkaloids, and other organic compounds.

II. EXPERIMENTAL WORK

A. *Material & Method*

In this study, quercetin, was used as standards; it was obtained from OZONE INTERNATIONAL(INDIA). All solvents were of analytical grade and were obtained from VISHAL CHEMICALS (INDIA). Thin layer chromatography silica gel 60 F254 aluminium plates, measuring 20 x 20 cm in size and having a 0.1 mm thickness were obtained from Merck (Darmstadt / Germany), glass beakers, glass pipettes, qualitative filter papers (FILTROS UK).

B. *Collection of Plant Material*

Mulberry leaf extract was collected from herbal creation (natured by nature) with certificate of analysis.

C. *Preparation of Standard Solution*

Quercetin (1mg/10ml) was prepared by dissolving 1 mg of quercetin in 10 ml of methanol in a standard flask.[10]

D. *Preparation of Sample Solution*

Accurately weighed extract was dissolved in a required amount of methanol. And the stalk solution was prepared.

III. PRELIMINARY PHYTOCHEMICAL SCREENING

A. *Test For Flavonoid*

1) *Shinoda Test:* Pieces of magnesium ribbon and HCL concentrated were mixed with aqueous crud plant extract after few minute and yellow colour show presence of flavonoid.
2) *Alkaline Reagent Test:* 2ml pf 0.2% NaOH mixture was mixed with aqueous plant crud extract; concentrated yellow colour was produced, which become colourless when we added 2 drops of diluted acid to mixture. This result showed presence of flavonoid.
3)

IV. THIN LAYER CHROMATOGRAPHY (TLC)

According to the TLC mentioned in the appendix VIB of Chinese pharmacopoeia version 2010, pipette 10 microliter of sample solution and standard solution respectively put them on the silicone gel slate, which is with CMC Na adhesive. take acetone: chloroform: water (8:2:1) as a development solvent. Put the slate in the developing chamber which is in the saturated state. After developing, take it out and dry in the air. In the chromatograph of the sample solution, it appears the same colour speckle at the same place of standard solution [12].

$$Rf = \frac{Distance\ traveled\ by\ the\ solute}{Distance\ traveled\ by\ the\ solute\ front\ TLC\ plate}$$

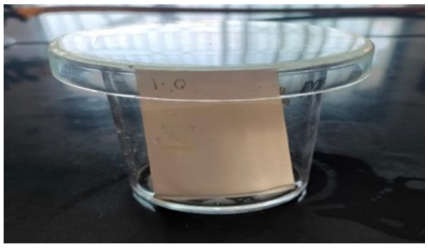


Figure 1 Developing chamber

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V. RESULT

A. *Presence of Flavonoid in Leaf Extract*



Figure 2 presence of flavonoid

B. *Thin Layer Chromatographic Analysis*

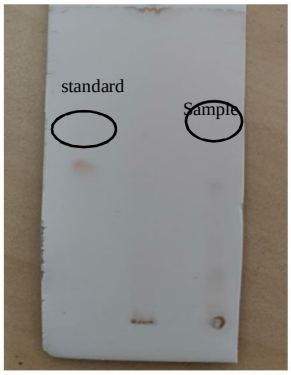


Figure 3 Visible light

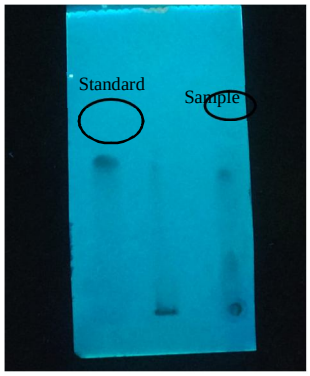


Figure 4 long UV (365nm)

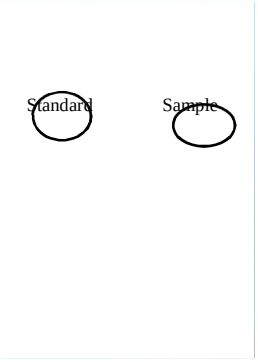


Figure 5 short UV (254nm)

By using the qualitative phytochemical screening of plant extracts the chemical constituents can be provided for pharmacological and pathological discovery of the pharmaceutical agents [13]. In the present study, qualitative tests leaf extracts showed significant indication about the presence of Flavonoids was found to be present in the leaf extracts of Morus alba leaves, with a maximum concentration by using methanol and ethanol solvents. TLC profiling of leaf extracts gives an impressive result that directing towards the presence of phytochemicals. Various phytochemicals give different Rf values in different solvent system. This variation in Rf values of the phytochemicals provides a very important clue in understanding of their polarity and also helps in selection of appropriate solvent system for separation of pure compounds by column chromatography. Mixture of solvents with variable polarity in different ratio can be used for separation of pure compound from plant extract. The selection of appropriate solvent system for a particular plant extract can only be achieved by analysing the Rf values of compounds in different solvent systems [14,15]. Different Rf values of the compound also reflect an idea about their polarity. This result will help in selection of appropriate solvent system for further separation of compounds from these plant extracts.

VII. CONCLUSION

Using thin layer chromatography (TLC) procedure with quercetin as the standard, it is possible to identify flavonoids in a sample. Quercetin is a common flavonoid that is often used as a standard in TLC because of its well-known characteristics, such as its Rf (retention factor) value which is 0.32 and UV absorbance properties. By comparing the Rf values and UV absorbance patterns of the unknown sample to those of quercetin, it is possible to determine the presence of flavonoids in the sample. The Rf value of a compound in the sample match those of quercetin, it is likely that the compound is a flavonoid. However, it is important to note that TLC is a qualitative technique, meaning it can determine the presence or absence of compounds but not their precise identity or concentration. In conclusion, using TLC with quercetin as the standard can provide a quick and easy way to identify flavonoids in a sample. In addition, the methanol and ethyl acetate extracts for Moringa oleifera leaves contain a higher content of bioactive compounds, which can be used for further researches on this plant.

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A. Conflict of interest

The author declares that they have no conflicts of interest.

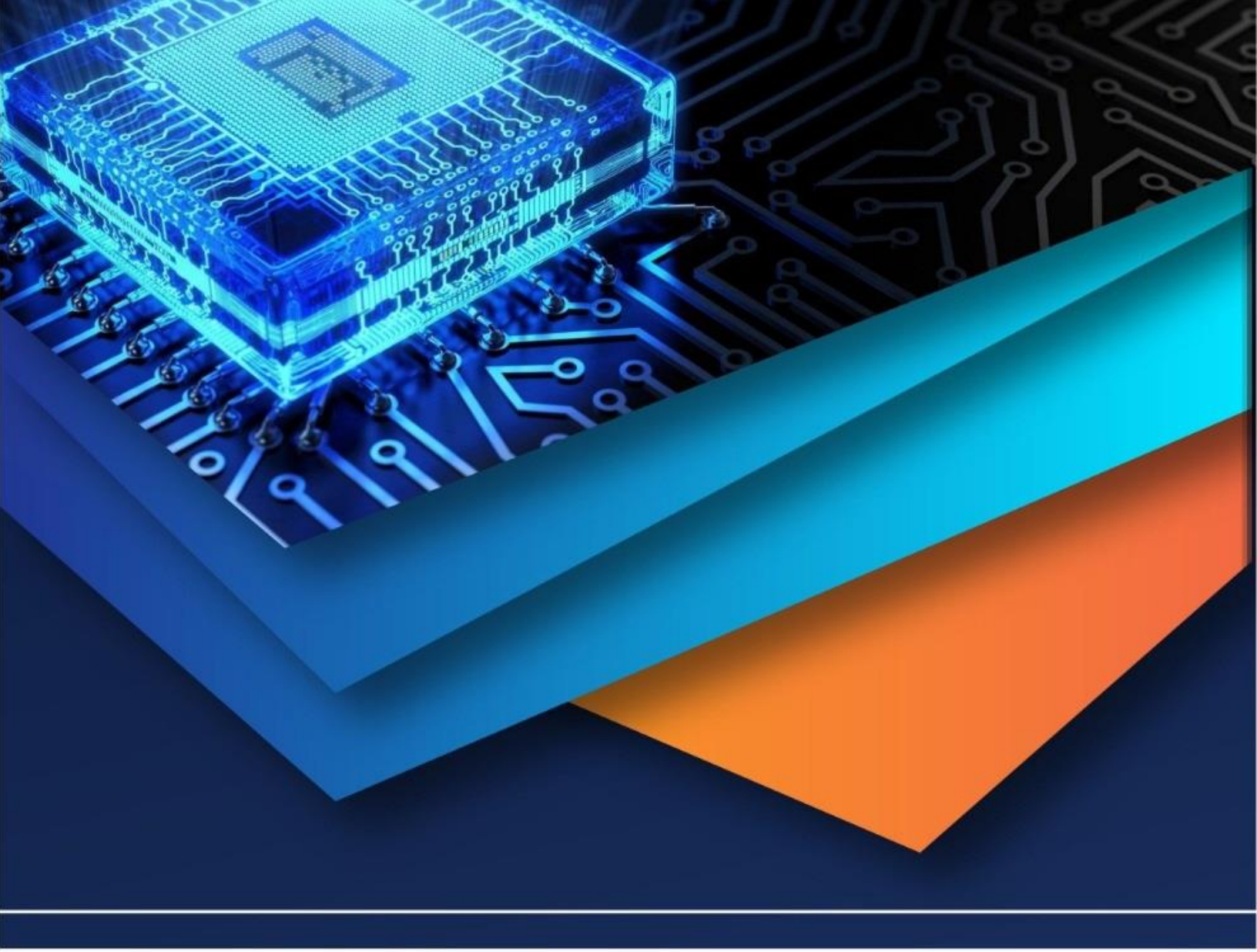
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