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Isolation of Active Constituent in Ficus Religiosa Root Bark by Column Chromatography

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ABSTRACT: The primary type of medicine in India has always been herbs. Ficus religiosa (L.), also known as Pepal and a member of the Moraceae family. Phytochemical analysis of Ficus religiosa plant extract discovered the presence of various biochemical compound such as alkaloid ,flavanoids, phenols, tannins,carbohydrate.etc. Traditionally the root bark of the Ficus religiosa used as Anti-inflammatory, Anti-diabetic, analgesic, antioxidant, anticonvulsant.etc.The objective of the study is to isolate the phenolic compounds from Aqueous Ethanolic soxhlet extract by coloumn chromatography. The Phytochemical test, TLC and UV spectra results shows the compound isolated is Phenol derivatives.

KEYWORDS: Ficus religiosa, Extraction, Phytochemical screening, Colum chromatography,

I. INTRODUCTION:

Herbal medicine is the study and use of medicinal properties of plants having ability to synthesis a wide variety of chemical compounds. Phytoconstituents present in herbal medicine have better compatibility with human system. Medicinal plants have healing capabilities. Due to the presence of many complex chemicals with diverse compositions that are discovered as secondary metabolites which present various part of these plants.

Ficus religiosa (L.), which grows up to 170 metres above sea level in the Himalayas, and can be found all over Indian plains.[1].Ficus religiosa is one of the important potential traditional medicines that have been used as a treatment for various illnesses and its indigenous to Indian subcontinent and Southeast Asia.Since Ficus religiosa is so important to Indian culture, mythology, and religion, it is typically planted close to sacred or spiritual locations in Indian cities and villages. Hindus, Buddhists, and Jainists alike regard it as sacred. Synonyms for it include "Ashwattha" in Sanskrit, "Sacred fig" in English, "Pimpala" in Marathi, "Peepal" in Hindi, and "Achuvattam" in Tamil.F. religiosa has been reported to have medicinal properties like antimicrobial, antiulcer, antidiabetic, antiasthmatic, antioxidant, anti-inflammatory, wound healing, hepatoprotective, anti-convulsant, anti-parkinson, anti-amnesic, anti-cancer, acetylcholinesterase inhibitor, memory enhancing, and anti-arthritic.[2,3]

Almost all parts are used in the preparation of herbal medicine.Ficus religiosa has recently been studied for the presence of a wide range of phytoconstituents.This bark contains lanosterol, -sitosteryl-D-glucoside, bergaptol, bergapten, steroids, flavonoids, alkaloids, and phenol. Due to the presents of mthese chemical compounds, it is effective against bacteria such as Azobacter chroococcum, Bacillus cereus, -megaterium, and Streptoccusfacealis.[4]The leaves contain bioactive compounds (campestrol, stigmasterol, isofucosterol, tannins, arginine, serine, aspartic acid, glycine, threonine, alanine, proline, ryptophan, tyrosine, methionine, valine, isoleucine) that help in the prevention of gastric problems.The methanolic,ethanolic and aqueous extracts can be made from bark, leavesand fruits .[5]The fruits have been reported with bioactive compounds such as asparagine,tyrosine, undecane, tridecane, tetradecane, ocimene,limonene, dendrolasine, flavonoids (kaempeferol, quercetin,myricetin) and other phenolic components.[6,7]Leaves contain flavonoids, tannin etc. which effectively cure diseases like vomiting, antivenom, inflammation etc. Traditionally, barks are used as antibacterial, astringent, antidiarrhoeal, in the treatment of gonorrhea etc[2,8].F.religiosa are reported phytoconstituents of phenols,tannins, steroids, lanosterol, stigmasterol, lupen-3-one.The active constituent from the root bark F. religiosa was found to be β-sitosteryl-D-glucoside, The seeds contain phytosterolin, β-sitosterol, and its glycoside, albuminoids.The fruit of F. religiosa contained appreciable amounts of total phenolic contents, total flavonoid.[9]

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Fig:1 Ficus religosa plant

Fig:2 Root bark of F. religiosa

II. MATERIAL

The root bark of the plant Ficus religiosa was collected from Kozhikode, Kerala, India. The plant was authenticated by Dr. A.K. Pradeep, Associate Professor, Dept. of Botany, Calicut University with specimen No. 178206. The root bark was washed thoroughly with tap water, shade dried and then pulverized to fine powder. It is stored in air tight container for further study.

Chemicals: Toluene, ethylacetate, methanol, ethanol, ferric chloride, lead acetate, formic acid distilled water.

Instruments: weighing balance, hot air oven, soxhlet apparatus, Rotary evaporate, heating mantle, TLC plate, U.Vspectrophotometer.

III. EXPERIMENTATION

Collection of plant material

The root barks of Ficus religiosa were collected and washed well to remove adhering foreign particle and soil materials. The washed root barks were cut into small pieces. Then, these were dried under the shade for a month and coarsely powdered. The powder was stored in air tight container.

Macroscopical and physiochemical evaluation

Macroscopical parameters: The Macroscopic evaluation was carried out to know the shape, size, colour, odour, taste and fracture of the material.

Different Physiochemical parameters are carried out to know the values of Foreign Organic matters, Loss of drying, Determination of total ash, Determination of acid insoluble ash, water insoluble ash, and Sulphated ash

Extraction of root bark

50g of collected, washed, dried and coarsely powdered root bark was tightly packed in a thimble. Ethanol and water were used as solvents. Extraction was carried out by hot continuous percolation using Soxhlet apparatus maintained at a temperature 40ºC. 50g powder was extracted using 250 ml water and methanol mixture in the ratio 30:70 and was carried out for 72 hrs or solvent is colourless in siphon tube. After completion of extraction, it was filtered. The filtrate was concentrated to evaporate ethanol and water and the dried extract was collected. The obtained extract was stored in a desiccator.

Phytochemical screening tests:

a) Test for Carbohydrates:

Benedict's test: To 1ml of extract solution, add 2ml of Benedict's reagent and heat on a water bath for 10- 15minutes. Observe the colour change in the test tube.

b) Test for Alkaloids: -

Dragendorff's test: To 2 ml of the extract added 1 ml of Dragendorff's reagent along the side of the test tube. Formation of orange or orange reddish brown precipitate indicated the presence of alkaloids.

c) Test for Tannins:

Ferric chloride test: This detection was based on blue colour formed by the addition of few drops of 5% ferric chloride solution to 2 ml of the extract solution.

d) Test for Flavonoids:

Shinoda test: A few magnesium turnings and 5 drops of concentrated hydrochloric acid was added drop wise to 1 ml of the extract solution. A pink, scarlet, crimson red or occasionally green to blue colour appeared after few minutes confirm the presence of flavonoids.

e) Test for Saponins:

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Foam test: 5 ml of the extract was taken in a test tube was shacked well for five minutes. Formation of stable foam indicates the presence of saponins.

f) Test for Glycoside:

Kellar killani's test: Dissolve the crude extract in water with glacial acetic acid, ferric chloride and add concentrated sulphuric acid, presence of brown ring at the junction indicates the presence of glycosides.

g) Test for phenols: -

Folin ciocalteu test: To the extract solution add Folin ciocalteu reagent. The formation of blue colour indicates the presence of phenols.

Silica gel column chromatography:

Aqueous ethanol extract was subjected to column chromatography in silica gel (60-120 mesh) glass column. About 5 gm of crude Ficus religiosa were mixed with 8 gm of silica gel and loaded on to the column of 30×2cm and eluted with water: ethanol (3:7). All the collected fractions were subjected to TLC using Hexane:chloroform:water (3:3:4) as the developing solvent system and detected as yellow spots by exposing the plate to iodine vapour .Then organic solvent removed from isolated fractions by using rotatory evaporator. The dried isolated fractions were again subjected to the column chromatography in silica gel, the column was slowly eluted with controlled flow rate and temperature with mobile phaseToluene: Ethyl acetate: Methanol: Formic acid (3:3:0.8:0.2) for isolation of phenolic compound. After removing the mobile phase each fraction was analysed by TLC and similar fractions with the Rf values were pooled and the organic solvent was removed by rotary evaporator. The total phenolic content of pooled fractions collected were analysed by UV spectrophotometry at 400 nm. The various photochemical tests are performed to determine the isolated compound. Like phenols, tannins flavonoids. etc

TLC procedure:

Thin layer chromatography can be used to monitor the progress of a reaction, identify compounds present in a given mixture, and determine the purity of a substance. Silica gel G used as adsorbent and toluene: ethyl acetate: methanol: formic acid in the ratio of (3:3:0.8:0.2) as mobile phase. Plate was prepared by pouring silica gel on glass plate and activated by heating at110^0c for 30 minutes, the spots are detected under iodine vapours. Rf value of each fraction was calculated.

UV visible spectroscopy: The isolated compound is tested under U.V spectroscopy of 10µg/ml in 200to 400nmto determine the maximum absorption.

IV. RESULTS AND DISCUSSION:

Ficus religiosa is a member of the Moraceae family and can be found throughout India's tropical and subtropical regions. Several traditional therapeutic properties have been claimed for various portions of the plant. There has been no research on the standardization and separation of the active moiety of the root bark of the Ficus religiosa. As a result, an attempt was made in the current research to isolate the active phenolic compound in root bark of Ficus religiosa. The physiochemical screened value of the crude is shown in table:1, and the percentage yield of each 50gm of crude extraction is 4.8%. (Table:2)

Tab:1; Physiochemical parameter of Ficus religiosa crude powder

PARAMETERS	VALUES	LIMITS
Foreign organic matters	1.6%	NMT 2%
Loss on drying	0.1%	
Total ash	5.5%	NMT 7%
Acid insoluble ash	0.19 mg	NMT 0.3mg

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Water insoluble ash	0.20mg	NMT 50 mg
Alcohol soluble extractives	6.3%	NMT 8%
Water soluble extractives	6.5%	NMT 9%

Tab:2; Percentage yield of extract:

Sl.no	Extract	Percentage yield
1	Water: Methanol (3:7)	4.8%

Tab:3; Preliminary phytochemical analysis of hydro alcoholic extract of Ficus religiosa.

Phytochemical constituents	Extract
Carbohydrate	+ve
Tannins	+ve
Phenolic compounds	+ve
Flavonoids	+ve
Alkaloids	+ve
Saponins	+ve
Terpenoids	-ve
Steroids	+ve

Isolation of active moiety:

In the present study deals with isolation of active molecule in Ficus religiosa root bark. Isocratic elution technique in column chromatography was performed. when the crude extract was from root bark so the active molecules was firstly isolated by high polar mobile phase Water: Methanol (3:7) mixture then eluted compounds is concentrated and packed in new column and eluted with Toluene: Ethylacetate:Methanol:Formic acid(3:3:0.8:0.2) as mobile phase. Then the fraction was concentrated, the residue was tested by TLC in same mobile phase got a single spot on Rf value 0.69 (fig.3).The isolated compound (10µg/ml) is tested in U. V spectroscopy in 400nm get a single peak at 280 nm (λ max). The compound was screened through chemical tests. Exhibited a positive response for Ferric chloride test, lead acetate test.

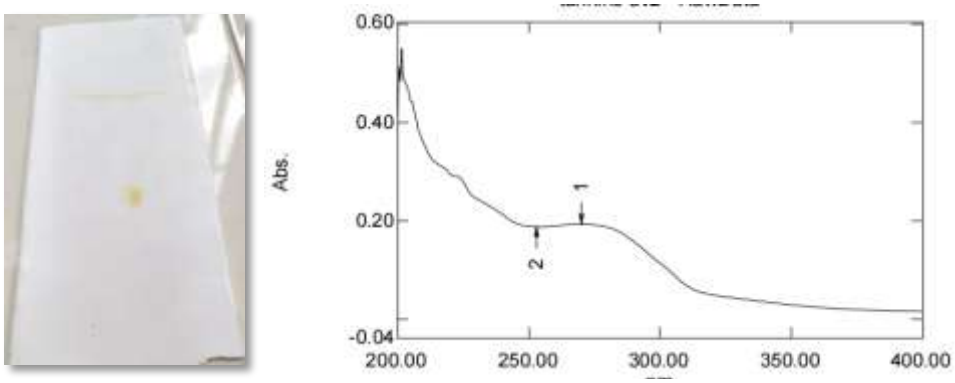

Fig:3, TLC of isolate UV spectra of isolated compound.

V. CONCLUSION:

The study was mainly deals with isolation of active phenolic compounds of root bark of Ficus religiosa by silica gel column chromatography. It was found that the isolated compound was mainly match with phenolic derivative it may be Flavonoid

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or tannin derivatives. Hence detailed structure of the compound can be found throw analytical elucidation technique.

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