Studies on the leaves of *Ficus rumphii* Blume (Family: Moraceae)

Submitted by

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Exam Roll No. - M4PHA19026

Registration No. - 140850 of 2017-2018

DEPARTMENT OF PHARMACEUTICAL TECHNOLOGY

JADAVPUR UNIVERSITY

Under the guidance of

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India

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Thesis Submitted In Partial Fulfillment of The Requirements For The Degree of Master of Pharmacy

Department of Pharmaceutical Technology

Faculty of Engineering and Technology

JADAVPUR UNIVERSITY

2019

FORWARDING CERTIFICATE

This is to certify that the thesis entitled "Studies on the leaves of *Ficus rumphii* Blume (Family: Moraceae)" submitted by Milantirtha Mete, with (Exam. Roll No - M4PHA19026, Registration No - 140850 of 2017-2018) for the partial fulfillment of degree of Master of Pharmacy, Jadavpur University, is absolutely based upon his own research project work under my supervision, The Pharmacognosy & Phytotherapy Research Laboratory, Division of Pharmacognosy, Department of Pharmaceutical technology, Jadavpur University, Kolkata-700032.

He has included his finding into this thesis. His thesis has not been submitted before for any degree/diploma or any other academic award elsewhere. I am satisfied that he has carried out his thesis with proper care and confidence to my entire satisfaction.

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CERTIFICATE OF APPROVAL

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Declaration of Originality and Compliance of Academic Ethics

I hereby declare that this thesis contains literature survey and original research work by me (Milantirtha Mete), as a part of my Master of Pharmacy studies. All information in this document have been obtained and presented in accordance with academics rules and ethical conduct.

I also declare that, as required by these rules and conduct, I have fully cited and referenced all materials and results that are original to this work.

Name : Milantirtha Mete

Examination Roll Number: M4PHA19026

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Thesis : Studies on the leaves of *Ficus rumphii* Blume (Family: Moraceae)

Signature with Date:

CONTENT

Dedicated to

my parents

List of abbreviations

TLC-Thin layer chromatography

TPC-Total phenolic contain

TFC-Total flavonoid content

Mcg-microgram

Conc-concentration

IH-Index Herbariorum

NHE- National Health Educators

LPCP -Lauryl Pentachloro-phenate

R_f- Retardation factor

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INTRODUCTION

Ficus rumphii is a deciduous tree that can grow to a height of 20 meters. The plant often begins life as an epiphyte, growing in the branch of another tree; as it grows older it sends down aerial roots which, when they reach the ground quickly form roots and become much thicker and more vigorous. They supply nutrients to the Fig, allowing it to grow faster than the host tree. The aerial roots gradually encircle the host tree, preventing its main trunk from expanding, whilst at the same time the foliage smothers the foliage of the host.
[Tropical Plants Database, 2019] Eventually the host dies, leaving the Fig to carry on growing without competition.

Ficus, the Fig genus, consists of over 800 species and is one of about 40 genera of the mulberry family(Chopra *et al.*, 1999). The Fig species of greatest commercial importance is *Ficus bengalensis* (The banyan tree) Ficus carica Linn. (Common Fig) Other notable species of Ficus are *Ficus religiosa* . (the Bo tree which sheltered the Buddha as he divined the "Truths"), *Ficus* elastic Roxb. Ex. Hornem. (The rubber tree), and *Ficus racemosa* (syn. glomerata, the giant cluster tree). All *Ficus* latex-like material within their vasculatures, affording protection and self-healing from physical assaults . Plants have been the major source of drugs in Indian system of medicine and other ancient systems in the world. Earliest description of curative properties of medicinal plants is found in Rig-Veda. Charaka Samhita and Sushrusha Samhita give extensive description on various medicinal herbs (Ghani *et al.*, 1998) Present in most tropical and subtropical forests. Throughout the world and distributed all over India from sub Himalayan region and in the deciduous forest of Deccan and south India. They are very large, fast growing, evergreen tree up to 3.0 meters, with spreading branches and many aerial roots. Leaves stalked, ovate-cordate, 3-nerved, entire, when young downy on both sides; petiole with a broad smooth greasy gland at the apex, compressed, downy; Bark smooth, light grey-white,1.27cm thick wood moderately hard, grey or grayish white. Fruit in axillary pairs, the size of a cherry, round and downy (Islamul *et al.*, 2018). Some species recorded in India which are Ficus bengalensis (Indian banyan), *Ficus auriculata*, syn. *Ficus roxburghii*, *Ficus carica* (common edible Fig), *Ficus religiosa* etc.

The plant is harvested from the wild for local use as food and medicine. The tree is often cultivated as an ornamental and shade tree along avenues.

Plants are the oldest and the most important source of medicines at the end of nineteenth century, after synthesis of aspirin, research on herbal products was halted and researchers started focusing on synthetic and semi-synthetic drugs (Mosmann *et al.*, 2011). But from last few decades, there is an upsurge in the use and research of natural origin, especially botanical drugs. About 25% of the prescribed drugs are derived from higher plants and this value is increased to 50%, if animal and microbial products are also included (Islamul *et al.*, 2018).

Ficus bengalensis, belonging to family -Moraceae, is commonly known as Banyan tree (English), Darakht-e-Reesh and Bargad (Unani medicines) and Bohar (Urdu)

. It is native to a wide area of Asia i.e. India, Burma, Southeast Asia, Southern China, Thailand, and Malaysia. The tree is cultivated in botanical gardens and parks throughout the tropical regions of the world .Many pharmacological activities and useful phytoconstituents of this plant have been reported. Besides other useful chemical

Constituents, the tree also contains a high amount of good quality natural rubber (Remington *et al.*, 2005).

Species: *Ficus Rumphii Blume*

Family: Moraceae

Tribus: Ficeae

Genus: *Ficus*

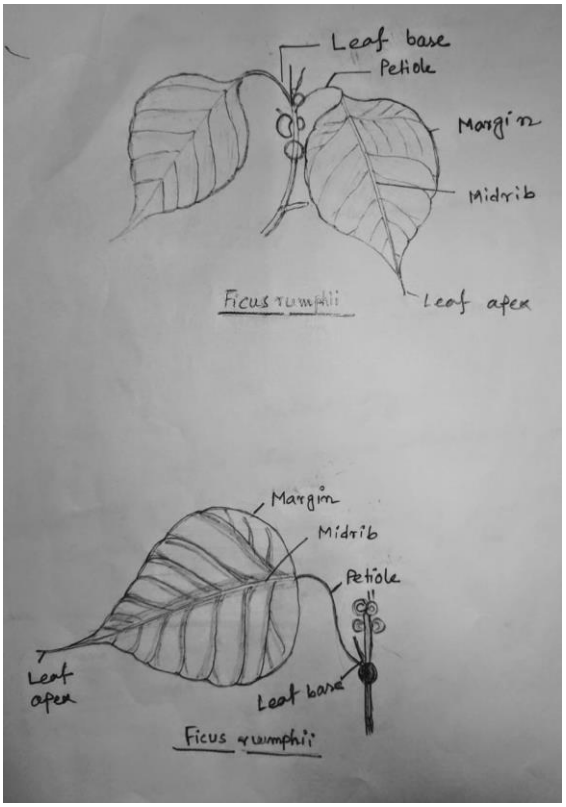

Fig 1: drawing of a portion of leaf of *Ficus rumphii*.

Cultivation Procedure:

Fig trees have a unique form of fertilization, each species relying on a single, highly specialized species of wasp that is itself totally dependent upon that Fig species in order to breed. The trees produce three types of flower; male, a long-styled female and a short-styled female flower, often called the gall flower. All three types of flower are contained within the structure we usually think of as the fruit (Brickell et al.,2008).

The female fig wasp enters a Fig and lays its eggs on the short styled female flowers while pollinating the long styled female flowers. Wingless male Fig wasps emerge first, inseminate the emerging females and then bore exit tunnels out of the Fig for the winged females. Females emerge, collect pollen from the male flowers and fly off in search of figs whose female flowers are receptive. In order to support a population of its pollinator, individuals of a Ficus spp. must flower asynchronously. A population must exceed a critical minimum size to ensure that at any time of the year at least some plants have overlap of emission and reception of fig wasps. Without this temporal overlap the short-lived pollinator wasps will go locally extinct (Armstrong *et al*., 1998).

Medicinal Uses:

Medicinal plants, since times immemorial, have been used in virtually all cultures as a source of medicine. The widespread use of herbal remedies and healthcare preparations, as those described in ancient texts such as the Vedas and the Bible, and obtained from commonly used traditional herbs and medicinal plants, has been traced to the occurrence of natural products with medicinal properties.

The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has also been widely observed Furthermore, an increasing reliance on the use of medicinal plants in industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural herbal remedies. The World Health Organization has estimated that 80% of the world's population use botanical medicine for their primary health care needs (Hoareau *et al*, 1999)

Uses:

a. The latex and fruits are emetic and anthelmintic, and used to treat itch.
b. The latex is given internally as a vermifuge and for the relief of asthma.
c. The bark yields a rough cordage.
d. The soft wood is used as a fuel and for the production of charcoal. (Tropical Plants Database, 2019)

Medicinal and Biological applications of *Ficus*

Every part of *Ficus* plant, including leaves, roots, twigs, bark and fruits have their own medicinal uses. The Fig fruits are important as both food and traditional medicine, contain laxative substances, flavonoids, sugars, vitamins A and C, acids and enzymes. Many *Ficus* species are commonly used in traditional medicine to cure various diseases (Hoareau *et al*., 1999) They have long been used in folk medicine as astrigents, carminatives, stomachics, vermonicides, hypotensives, antihelmintics and antidysentery drugs .The genus Ficus constitutes an important group of trees with immense medicinal value. Many species of the genus Ficus were reported to exhibit significant pharmacological properties including antimicrobial, antidiabetic, anticancer and antiulcer. *Ficus rumphii* extract shows anti inflammatory activity. Bark of this plant is used as astringent, aphrodisiac, demulcent, depurative and emollient. It is also useful against inflammation, diarrhoea, diabetes, burning sensation, leprosy, scabies, wounds and skin diseases.The leaf extract of Ficus exasperata has antiarthritic and antioxidant properties (Balsas *et al*., 1979).

Literature Review

This chapter provides a brief review of existing literature on various aspects of *Ficu*s such as taxonomy and distribution, ecology, reproductive biology, physiology, phytochemical, pathological, nutrient composition, Fig and pollinator mutualism, seed dispersal, phyto sociology, ecology, ethnobotany and conservation. A section on NBR deals with the floristic studies on *Ficus* of this region. (Berg *et al.*, 2005) The chapter concludes with the gaps in the earlier studies.

In English the word "Fig" means giving care about something. The word ficolin, which appears similar to Ficus and refers to a lectin like compound combining the first parts of the words for fibrinogen and collagen. *Ficus* constituted one of the largest genera of medicinal plants with about 750 species of woody plants, trees and shrubs primarily occurring in subtropical and tropical regions throughout the world. The genus is remarkable for the large variation in the habits of its species. The most important species of Ficus are *F. bengalensis, F. carica, F. racemosa* and *F. elastica*. *Ficus Caricais* commonly referred as "Fig". Various parts of the plant like bark, leaves, tender shoots, fruits, seeds, and latex are medicinally important. The Fig is a very nourishing food and used in industrial products. It is rich in vitamins, mineral elements, water, and fats. Figs are one of the highest plant sources of calcium and fiber. According to USDA data for the Mission variety, dried figs are richest in fiber, copper, manganese, magnesium, potassium, calcium, and vitamin K, relative to human needs.The genus, Ficus, consists of over 800 species and is one of about 40 genera of the mulberry family, Moraceae. There is significant genetic diversity among different varieties of Fig, which contain remarkable pharmacological activities and are of commercial importance. Literature survey indicated that Fig have been cultivated over 1100 years and these are among the earliest cultivated plants for human use (Ahmad *et al*, 2013).

Taxonomy and Distribution:

The genus *Ficus* included seven species, four being Indian *Ficus bengalensis, F. indicia, F. racemosa and F. religiosa*. An illustrated account of 31 wild *Ficus* species of Kenya, with a key to identification; description o f the natural history of the genus, biotic community associated with the Fig trees, ethno botany and distribution maps of the area. A taxonomic revision of the family Moraceae, genus Ficus which descriptions and a key was given of 19 species, 12 varieties and 3 forms found in Taiwan. A study on *Ficus semicordata* which exists as two main variants and its taxonomy in Nepal. The distribution patterns of Ficus in

tropical North East Africa (Berg *et al.,* 1990) discussed the phyto geography of about 150 species of *Ficus* in Africa. In study on the Moraceae of the Vista Chinese Reserve, Rio de Janeiro, presented descriptions of identification key, geographical distribution and illustrations for nine native species and two introduced species (Berg et al.,2005). Collected 19 species of *Ficus* on an expedition to the Moluccas and presented with taxonomic and ecological notes on each specimen. The related names of *F. carica* on the basis of historical documents and field investigations in China. India has one of the largest diversity of *Ficus*, which is about 65 species, in the world. A detailed investigation on taxonomy, anatomy and palynology of South Indian species of Ficus. The differentiation of normal and back pocketed leaves of *F. irisnae*. In taxonomic account of Moraceae in Madhya Pradesh recorded five genera and 29 species of the family (Corner *et al*., 1958).

Physiological Studies

Diurnal changes in CO_2 exchange and transpiration were studied in leaves of one year old seedlings of five species of Ficus viz, *F. rumphii, F. glomerata, F. auriculata, F. religiosa* and *F. pallmata* at an altitude of 500m at Srinagar (Etter *et al*., 1990).

Phytochemical Studies

The isolation and identification of acetates of n - triacotanol, beta – amyrin and gluanol is reported from petroleum-ether extracts of the dried bark powder of *F. hispida*. Extracts from 87 species belonging to 8 genera of the family Moraceae where screened for testing phototoxic constituents (Ahmad *et al*, 2013). Reported three new triterpene constituents viz. calotropenyl acetate, lupeol acetate and oleanolic acid from the leaves of *F. carica*. An insulin sparing derivative, dimethyl ether of leucopelargonidin 3.0.alpha - rhamnoside was isolated from the bark of *F. benghalensis* by Cherianefa (Gibbs *et al*., 1927).

Ethnobotanical Studies

Many Ficus species are medicinally used as stringent, carminatives, stomachic, vermicides, hypotensive, anthelmintic and antidysenterics. The distribution, morphology and medicinal uses of *F. racemosa* were studied with particular reference to the use of bark ash to treat menorrhagia (Gupta *et al*., 2006).A detailed review on the ethno botany of various *Ficus* species in India. Similarly a review on the traditional uses of *Ficus* species was done Latex from *F. insipida* is taken orally, either unprocessed or mixed with alcohol, as an anthelmintic conducted a study on the use of various species of Ficus in traditional African medicine. Recommended six species of Ficus, viz. *F. glomerata, F. hispida, F. ausiculata, F. semicardata, F. subinaisa and F. palmate*, as multipurpose tree crops for social Forestry and agro forestry the central Himalayan Hills, Ethnobotanical study on *Ficus* carried out in North Eastern India. In study on the ethno medicinal plant diversity in Southern Uttar Pradesh identified *Ficus racemosa* as one among the plants used by the indigenous people for treating paralysis (King et al., 1888). Conducted experiment on the fruit extracts of *F. sycomorus, F. benjamina, F. bengalensis and F. religiosa* for their bioactivity (Leong *et al*., 2008).

Objective: The current study evaluates the antioxidant and pharmacognostic property of the leaves from *Ficus rumphii* (Family- Moraceae).

Materials and Methods

1. Collection of plants Leaves: Mature Leaves are collected from Arambagh, vill – Telua, P.O-Bhalia, Hooghly, West Bengal during months of September. After collection of plants leaves, leaves are shaded dry of pulverized into a grinder mixer to obtain powder form. The plant was authenticated from Botanical survey of India, Central National Herbarium, Howrah-711103, West Bengal, India. The Specimen Voucher No (JAD/MM-01). It was deposited in Pharmacognosy & Phytotherapy Research Laboratory, Division of Pharmacognosy, Department of Pharmaceutical Technology, Jadavpur University, Kolkata-700032, India. The leaves were washed properly, shade dried for 20 days, then cut into small pieces and powdered by a mechanical grinder. The powdered material was stored in an airtight container for experimental use.

Herbarium Introduction:

The "Herbarium" has two meanings; one is a repository or storehouse of collected plant Specimens and second is a plant specimen according to accepted international standards. If the term "Herbarium" followed by a code assigned by of "Index Herbariorum (IH)" authorities in the parenthesis, it shall be consider as repository and the plural is "herbaria". If the term "Herbarium" is not followed by the code in parenthesis we shall be consider it as a "plant specimen" and plural is "herbarium specimens". It is now estimated that there are nearly 350,000,000 specimens that are documented from the world's vegetation. These herbarium specimens are available at approximately 3,000 herbaria in the world, with approximately 12,000 associated curators and plant specialists (Ahmed et al., 2016)

2. Identification and preparation of Herbarium Sample:

A Herbarium is defined as a collection of plants that usually have been dried, pressed, preserved on sheets and arranged according to any accepted system of classification for future reference and study.

In fact, it is a great filleting system for information about plants, both primary in the form of actual specimens of the plants, and secondary in the form of published information, pictures and recorded notes.

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GOVERNMENT OF INDIA
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सत्यमेव जयते

भारतीय वनस्पति सर्वेक्षण
BOTANICAL SURVEY OF INDIA
केंद्रीय राष्ट्रीय पादपालय
CENTRAL NATIONAL HERBARIUM
हावड़ा / HOWRAH – 711 103

संख्या/No.: CNH/Tech.II/2019/30

दिनांक/ Date: 14 -05-2019

To,
Mr. Milantirtha Mete
Master in Pharmacy
Jadavpur University
West Bengal

Sub.: Identification of one plant specimen – reg.

Dear Mr. Mete,

Please refer to your letter dated 08th May 2019 along with a plant specimen for identification. The specimen has been identified by the concerned expert as:

Sl. No.	Specimen No.	Scientific Name	Family
1.	JD/ MM-01	**Ficus rumphii** Blume	Moraceae

The receipt of ₹ 50/- (Rupees fifty only) Receipt No. TR-5, C-160595 dated 14-05-2019 is enclosed herewith.

Your specimen is returned herewith.

Yours sincerely

14/05/19

(K. KARTHIGEYAN)
Scientist –'D'
वैज्ञानिक "डी" Scientist 'D'
केन्द्रीय राष्ट्रीय पदपालय
Central National Herbarium
भारतीय वनस्पति सर्वेक्षण
Botanical Survey of India
हावरा / Howrah-711103

Certificate for plant Identification

3. The preparation of a herbarium involves:

(i) Field visits,

(ii) Collection of specimens

(iii) Drying

(iv) Mounting on a herbarium sheet,

(v) Preservation,

(vi) Labelling and

(vii) Proper storage.

4. Poisoning and drying the specimen:

Poisoning kills the plants and prevents the formation of abscission layer and thereby the leaves, flowers and fruits will be intact with the specimen (twig) will not be getting detached from the plant. The poisoning is generally done by dipping the whole plant in a saturated solution of mercuric chloride in ethyl alcohol (usually 20 gm in a litre of alcohol). The plant is again put between the blotters in the presser till it gets completely dried. Mercuric chloride is corrosive for metals, and hence enamel trays and disposable gloves are used. Lauryl Pentachloro-phenate (LPCP) is also used (3.75% in white spirit) for poisoning the specimens. It is safer than mercuric chloride and leaves the plant features more intact. The solution can also be applied to mounted specimens by spraying. Then, the specimens are spread out for pressing and drying. It is important that the plants are put under sufficient pressure; otherwise more time will be required to achieve a good desiccation, besides they could be damaged by dampness and moulds. Every specimen in the press must be linked with the data in the field note book . Detailed notes should be entered in the field note book at the time of collection in the field itself. The best one can do is to use a tag for each specimen. Bulky plant parts can directly be placed in contact with corrugated material to speed up drying. Instead of blotting paper we can use newspapers, which are cheap and readily available. Once a specimen has become dry and stiff, it is ready for mounting. (Ahmed *et al.*, 2016)

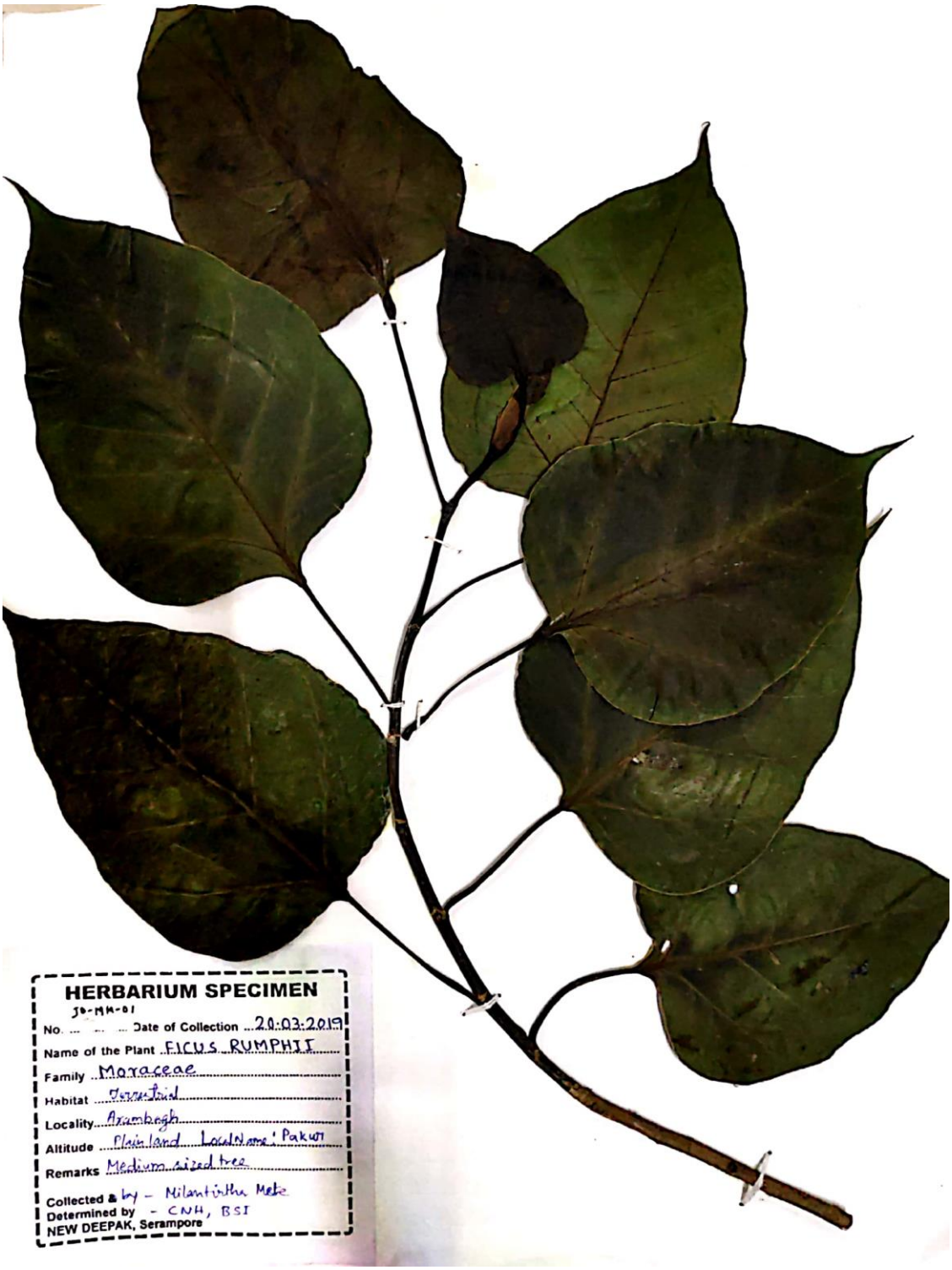

Fig 2: Herbarium of *Ficus rumphii*

3. Transversection of *Ficus Rumphii*

Ficus rumphii leaf is divide into three main parts namely, the epidermis, mesophyll and the veins. A typical leaf is made up of several layers of cells which are sandwiched between two layers called epidermis. The epidermal layer of leaf protects the tissues that lie between them and also helps in the process of gaseous exchange (kokate,1994 practical Pharmacognosy) Epidermis is divided into two types - upper epidermis and lower epidermis. Mesophyll is a primary a photosynthetic tissue of leaf that includes the palisade mesophyll and the spongy mesophyll.

Veins provides support to the leaf that consists of xylem and phloem vessels that involved in the transport of water to leaf and synthesized food to the rest of the plant structures respectively.(Kunwar *et al.*,2006)

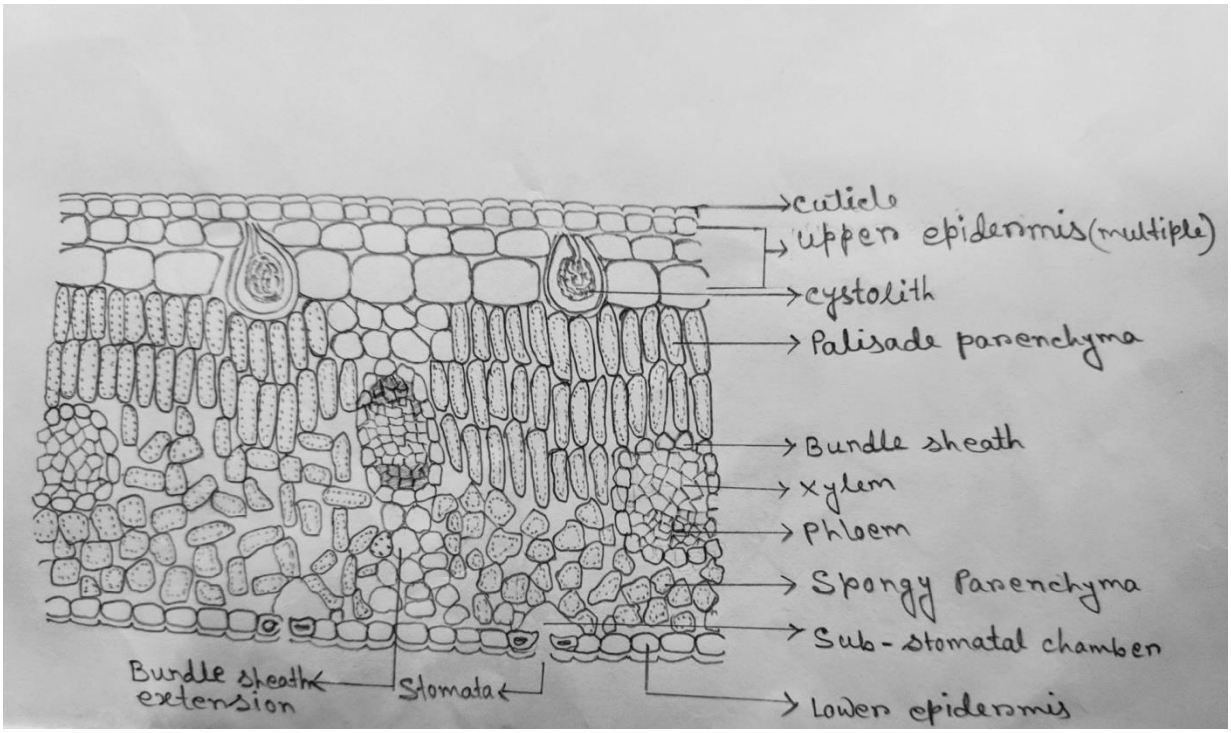

Fig 3: Celluler drawing of a portion of leaf of *Ficus rumphii* in transverse section.

4. Extraction of Plants Leaves:

Extraction of *Ficus rumphii*, By the help of Sohxlet Apparatus:

i)Weight of Leave Extract : 64.9 gm

Solvent Use: Pet ethar by hot percolation method.

ii) Weight of Leave Extract: 45.9 gm

Solvent Use: Methanol

1. Chemical group test (Phytochemical Screening) of *Ficus Rumphii*.

The need of the hour to understand and emphasize the consequence of the pharmacognostical study and standardization of the natural products obtained while searching for a mother molecule that could finally lead to a breakthrough achievement in the field of natural product chemistry, thereby, saving thousands of lives. Yet, one cannot achieve so, without authenticating the first crude form whosoever is supplied with, then the demand for a process arises which calls for parameters to be testified, approved and presented as both references and standards.

The study also provides a check on any adulteration that might have posed an obstacle difficult to overcome during the entire duration of study. For a check on adulteration, a comprehensive study at almost every step was carried out with caution (Mandal *et al.*, 2015).

The group of phytochemicals plenty in *Ficus rumphii* extracts were: alkaloids, glycosides, tannins, saponins, etc. The group tests were performed accordingly.

The extract is diluted with water in a ratio of 1:20

Samples of the made-up solution are taken (1-2ml) in test tubes, and subjected to various group tests; such as:

• Test for Alkaloids.

The extract was mixed with a few drops of 2% H_2SO_4 and warmed for a couple of minutes, cooled and filtered. The filtrate was then divided into 4 parts equally, and few drops of the following reagents added to observe the results (Mandal et al., 2015).

1. Dragendroff's reagent- A reddish color precipitate, indicates presence of alkaloids.

2. Hager's reagent- a dirty yellow precipitate, indicates the presence of alkaloids.

3. Wagner's reagent- A red color precipitate, indicates alkaloids.

• Test for Tannins.

1. Ferric Chloride Test- 1ml of stock solution was taken and a few drops of ferric chloride were added, a greenish black coloration indicated presence of tannins (Mandal *et al.*, 2015).

2. Lead Acetate Test- 1ml stock solution was taken in a test tube, to it 1-3 drops of $FeCl_3$ solution freshly prepared; was added. A green coloration indicated presence of tannins (Mandal *et al.*, 2015).

• Test for Glycosides.

The stock solution was taken (2ml) in attest tube, to it 1ml glacial acetic acid and 1-2 drops of $Fecl_3$ was added. Along the side of the test tube 0.5ml of 98% conc H_2SO_4 was added. A brown color ring formation at the junction indicates presence of cardiac glycosides, especially de oxy sugar characteristics (Mandal *et al.*, 2015).

• Test for Flavonoids.

2ml of stock solution was taken in a test tube; to it 0.5ml of 50% nitric acid was added, shaken vigorously for 10seconds. Few drops of conc H_2SO_4 was added along the sides of the test tube. Yellow coloration confirms the presence of Flavonoids, which disappears on standing.

2. Benedict's Test: Another test tube benedicts reagent was added, heated, formation of red precipitate confirms reducing sugars are present (Mandal *et al*., 2015).

• Test for Saponins:

Foam Test: 40mg powdered drug was taken and 5ml of distilled water was added, and vigorously shaken. A stable froth if obtained was shaken with a few drops of olive oil to give an emulsion (Mandal *et al*., 2015).

• Test for Terpenoids.

Salkowaski's Test- Extract (0.2ml) to that 1ml of chloroform was added and then 1ml conc H_2SO_4 was added, a reddish brown discoloration at the interference indicated presence of terpenoids (Mandal *et al*., 2015).

• Test for Fats and Fixed Oils.

Spot on filter paper with a drop of stock solution, on application indicates presence of fixed oils and fats (Mandal *et al*., 2015).

• Test for Proteins.

2ml of Biuret reagent was added to 2ml stock solution, shaken well while being heated on a water bath. Red or violet coloration indicates presence of proteins.

The preliminary phytochemical analysis of the extracts carried out using pet ethar and methanolic extracts and on the powdered specimens using standard procedures to identify the various constituents (Mandal *et al*, 2015).

2. Test for Anthraquinone Glycosides:

Borntrager's test - Boil the test material with 1ml of dilute sulphuric acid in a test tube for 5min (anthracene glycosides are hydrolyzed to aglycone and sugars by boiling with acids)

centrifuge or filter while hot (if centrifuged hot, the plant material can be removed while anthracene aglycones are still sufficiently soluble in hot water, they are however insoluble in cold water), pipette out the supernatant or filtrate, cool and shake with an equal volume of dichloromethane (the aglycones will dissolve preferably in dichloromethane) separate the lower dichloromethane layer and shake with half its volume with dilute ammonia. A rose pink to red color is produced in the ammonical layer (aglycones based on anthroquinones give red color in the presence of alkali) (Mandal *et al*., 2015).

Modified Borntrager's test - Boil 200mg of the test material with 2ml of dilute sulphuric acid, 2ml of 5% aqueous ferric chloride solution for 5min and continue the test as above. As some plant contains anthracene aglycone in a reduced form, if ferric chloride is used during the extraction, oxidation to anthroquinones takes place, which shows response to the Borntrager's test (Mandal *et al*., 2015).

3. Test for Steroids

i. Salkowski's test :- a red color produced in the lower chloroform layer when 2 ml of organic extract was dissolved in 2 ml of chloroform and 2 ml concentrated sulphuric acid was added in it, indicates the presence of steroids (Mandal *et al*., 2015).

ii. Liebermann Burchard test: - development of a greenish color when 2 ml of the organic extract was dissolved in 2 ml of chloroform and treated with concentrated sulphuric acid and acetic acid indicates the presence of steroids (Mandal *et al*.,2015).

4.Test for Reducing Sugar:

Fehling's test- Two milliliters (2 ml) of the aqueous solution of the extract in a test tube was added into 5 ml mixture of equal volumes of Fehling's solutions I and II and boiled in a water bath for about 2 min. The brick-red precipitate was indicative of the presence of reducing sugars (Mandal *et al.*, 2015).

5. Test for Flavonoids:

Shibita's reaction test -One gram (1g) of the water extract was dissolved in methanol (50%, 1-2 ml) by heating, then metal magnesium and 5 - 6 drops of concentrated HCL were added. The solution when red was indicative of flavonols and orange for flavones (Mandal *et al.*, 2015).

6. Test for Carbohydrates

- **Molisch's Test:**

Alcoholic alpha naphthol forms furfural and furfural derivatives, such as hydroxymethylfurfural, by the concentrated sulphuric acid acting on the sugar. This compound forms a reddish-violet coloured ring at the junction of the two liquids. Molisch's reagent is 5% solution of alpha naphthol in alcohol (Mandal et al., 2015).

Procedure:

Add 2 drops of Molisch's reagent to 2 ml of sugar solution in a test tube. Mix thoroughly, add 2 ml of conc. H_2SO_4 by the side of the test tube slanting the tube. Then erect the test tube slowly. The formation of reddish violet ring at the junction of two liquids indicates the presence of carbohydrates.

7. Total Phenolic Content of *Ficus Rumphii* :

Phenolic acids are plant metabolites widely spread throughout the plant kingdom. Recent interest in Phenolic acids stems from their potential protective role, through ingestion of fruits and vegetables, against oxidative related diseases such as coronary Heart disease, stroke and cancers. Phenolic compounds were essential for the growth and reproduction of plants and are produced as a response for defending injured plants against pathogens. The importance of antioxidant activities of Phenolic compounds and their possible usage in processed foods as a natural antioxidant requires more research in this area (Rashid *et al.*, 2018). These compounds have demonstrated promising health benefits for humans. Therefore, the aim of this study was to examine the distribution of Phenolic compounds in West Bengal traditional herbs so that it could become as platform for further investigation to evaluate a potential source of Phenolic compounds to be used for agriculture and pharmaceutical purposes.

Methods:

Pre-treatment of plant samples: All the plants leaves were washed using tap water three times and one time with distilled water to clean it completely from contaminants. Then, it was dried in the drying oven (60°C) for several days. The dried samples were grounded into powder using mixer blender.

Determination of total phenolic content: The amount of total phenolics in extracts was determined according to the Folin-Ciocalteu procedure. Samples (2ml, triplicates) were introduced into test tubes; 1.0 ml of Folin-Ciocalteu reagent and 0.8 ml of sodium carbonate (7.5%) were added. The tubes were mixed and allowed to stand for 30 min. Absorption at 765 nm was measured (UV-vis spectrophotometer). The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram dry material (Rashid *et al.*, 2018)
Instrument Used – Model name-**SpectraMax M5** Manufactured by Molecular devices.

8. Antioxidant assay through DPPH

Previously described the method by Sharma and Bhat (2009) was used with slight modification. Total antioxidant activities of different fractions and NHE were measured on the basis of electron donating ability by bleaching a purple solution of 2, 2-diphenyl 1 picrylhydrazyl (DPPH) at different concentrations (100-500µg/ml).Samples were added to 0.5 ml of 0.2 mol/l DPPH solution. The samples were kept at dark for 45 min, and

absorbance was taken against a blank at 517 nm. Ascorbic acid (AA) was used as a positive control and results were expressed as half maximum inhibitory concentration (µg/ml) in the triplicate assay. The percentage of scavenging ability of different fractions and NHE were calculated using the following equation (Rashid *et al*, 2018)

DPPH scavenging activity (%)

$$= \text{Absorbance of Control} - \text{Absorbance of sample} / \text{Absorbance of Control} * 100$$

Where Absorbance of control is the Absorbance of (DPPH+ Methanol) and Absorbance Of sample is the Abs of (DPPH+ samples or standard).

Chromatographic Analysis:

Herbal preparations are taken as a reliable medication in many cases of disorders and diseases, hence, a comprehensive study and analysis of the materials both in their extractable form and post-formulation stage is to be performed, to understand the probable desirable action that is to be identified and achieved; once it enters further scrutinization.

Principle

Chromatography acts a preliminary check, a multifaceted segregation technique, where plant material (herbal preparations), are checked based on the different phytochemicals obtained, from an array of phytochemicals. Therefore, fingerprints of the certain 'botanical drug' is obtained, which solidifies the evidence of certain group of phytochemicals which have some hypothecated applications, once a knowledge library is used as a reference. Column Chromatography (CC), Thin Layer chromatography (TLC), High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), Gas Chromatography coupled with

Mass Spectrometry (GC-MS), Liquid Chromatography (LC), Liquid Chromatography coupled with Mass Spectrometry (LC-MS) etc. The technique which was employed here, TLC and Column Chromatography.

9. Column Chromatography of *Ficus Rumphii*:

The extract (82 gm) of *F. rumphii* was subjected to column chromatography for fractionation. The column was run with solvent of increasing polarity. Starting with 100% petroleum ether, polarity of the solvent (mobile phase) was increasing gradually by adding increasing by adding increasing proportions of ethyl acetate and methanol. The subsequent elutes were collected in 100 ml flasks. All the fractions from ethyl acetate extract were tested on TLC under UV light, in iodine chamber and by vanillin-sulfuric acid spray (Elansary *et al.*, 2015)

10. TLC of Ficus *Rumphii* Blume. :

Introduction to Thin Layer Chromatography

Thin layer chromatography (TLC) and high performance thin layer chromatography (HPTLC) – now also called planar chromatography – are, like all chromatographic techniques, based on a multistage distribution process. This process involves: a suitable adsorbent (the stationary phase), solvents or solvent mixtures (the mobile phase or eluent), and the sample molecules. For thin layer chromatography the adsorbent is coated as a thin layer onto a suitable support (e.g. glass plate, polyester or aluminium sheet). On this layer the substance mixture is separated by elution with a suitable solvent. The principle of TLC is known for more than 100 years now. The real break-through of TLC as an analytical method, however, came about 35 years ago as a consequence of the pioneering work of Egon Stahl . After some time of stagnation thin layer chromatography has gained increasing importance as an analytical separation technique, which is probably due the effects of instrumentalisation and automatisation. At the same time the applicability of thin layer chromatography was enhanced by the development of new adsorbents and supports.

Today MACHEREY-NAGEL offers a versatile range of ready to-use layers, which are the result of 30 years of continuous research and development. (Mandal *et al.*, 2015)

Evaluation of a Thin Layer Chromatogram

The evaluation depends on the purpose of a chromatographic analysis. For qualitative determination often localization of substances is sufficient. This can be easily achieved by parallel runs with reference substances. A parameter often used for qualitative evaluation is the Rf value (retardation factor) or the 100fold value hR_f. The R_f value is defined as follows:

R_f = distance travel by solute/distance travel by solvent

i.e. the Rf values are between 0 and 1, best between 0.1 and 0.8 (i.e. 10 – 80 for hR_f). If reproducible R_f values are to be obtained it is, however, essential that several parameters such as chamber saturation, constant composition of solvent mixtures, constant temperature etc. are strictly controlled. A quantitative evaluation is possible by suitable calibration measurements. For this purpose either the area of a substance spot is measured or a photometric evaluation is performed directly on the layer. The latter procedure, however, requires a higher instrumental expense. The following paragraphs describe the most frequently used methods for evaluation in TLC.

TLC (Thin Layer Chromatography) is a planner Chromatography in Ficus *Rumphii* Extarct for determination of components present in *Ficus Rumphii* leaves. Silica Gel G is mixed with water. This mixture is spread as a thick slurry on a clean glass plate. The resultant plate is dried and activated by heating in an oven for thirty minutes at 110°C (Mandal *et al.*, 2015)

Mobile phase used in TLC of Ficus *Rumphii* Blume.
(Pet ether: Choloform) 1:1
(Hexane: Ethyl Acetate) 1:1

TLC system components consists of

TLC chamber: This is used for the development of TLC plate. The chamber maintains a uniform environment inside for proper development of spots. It also prevents the evaporation of solvents, and keeps the process dust free.

Mobile phase: This comprises of a solvent or solvent mixture the mobile phase used should be particulate-free and of the highest purity for proper development of TLC spots. The

solvents recommended are chemically inert with the sample, a stationary phase.

Filter paper: This is moistened in the mobile phase, to be placed inside the chamber. This helps develop a uniform rise in a mobile phase over the length of the stationary phase.

11. **Total Flavonoid content:** The total flavonoid content of crude extract was determined by the aluminium chloride colorimetric method. In brief, 50 µl of crude extract (1 mg/ml ethanol) were made up to 1 ml with methanol, mixed with 4 ml of distilled water and then 0.3 ml of 5% $NaNO_2$ solution; 0.3 ml of 10% $AlCl_3$ solution was added after 5 min of incubation, and the mixture was allowed to stand for 6 min. Then, 2 ml of 1 mol/L N_aOH solution were added, and the final volume of the mixture was brought to 10 ml with double-distilled water. The mixture was allowed to stand for 15 min, and absorbance was measured at 510 nm. The total flavonoid content was calculated from a calibration curve, and the result was expressed as mg Rutin equivalent per g dry weight (Rashid *et al.*, 2018)

12. Introduction to column chromatography

Column chromatography is a chromatography technique used to separate mixture of chemical substances into its individual compounds. Column chromatography is a widely used method for the purification or separation of chemical compound mixture

Principles of column chromatography

Column Chromatography consists of two phases: one mobile phase and one contiguous stationery phase. The stationery phase is solid and the mobile phase is liquid. The compound mixture moves along with the mobile phase through stationery phase and separates depending on the different degree of adhesion (to the silica) of each component in the sample or the compound mixture. (Mandal *et al.*, 2015)

Explanation

The stationery phase: A glass tube with a circle large inlet and a small outlet with a plug or tap, named as column is used for this column chromatography. The column is placed vertically with a stand where the outlet is downward.

A piece of cotton wool is entered into the outlet and placed over the plug if there are no glass wool present to stop escaping the stationery phase from the column. There are two procedures to prepare the column by packing with silica or alumina:

Dry method: In dry method at first the column is filled with dry powdered silica. Then the mobile phase, a suitable solvent is flushed through it until all the silica are wet and settled. From this point till the end always the column need to keep wet with solvent.

Wet method: In wet method firstly slurry of silica and solvent is prepared and then poured onto the column using a funnel. More solvent must be used until the silica is settled into it.

Result and Discussion:

1. Chemical Group Test: (Phytochemical screening)

Reagent Uses	Pet Ethar	Methanol
Alkaloids		
Dragendorff's (Red ppt.)	+	+
Mayer's (White ppt.)	+	+
Wagner's (reddish brown ppt.)	+	-
flavonoids		
Shinoda test(Extract +0.5ml HCL +Magnesium Metal(Reddish color)	+	+
Carbohydrates		
Molisch Reagent (conc. H2SO4 added, Appearance of violet ring at the junction)	-	-
Starch (Treated with iodine)	-	-
Protein		
Millon's(Red color ppt)	-	-
Test for tannins and phenolic compounds		
Lead Acetate test	+	+
Test for triterpenoids		
Salkowski test	+	+

"+" denotes present "–" denotes not present

Table 1: Phytochemical analysis of methanol and Petroleum ethar extracts from leaves of *Ficus Rumphii.*

Both the petroleum ether and methanol extract were found to contain, alkaloids ,flavonoids, triterpenoids and tannins.

Carbohydrate, protein were absent in the both extracts.

2. Anti Oxidant Activity: Different concentration were taken, that is 20,40,60,80 and 100 µg/ml.

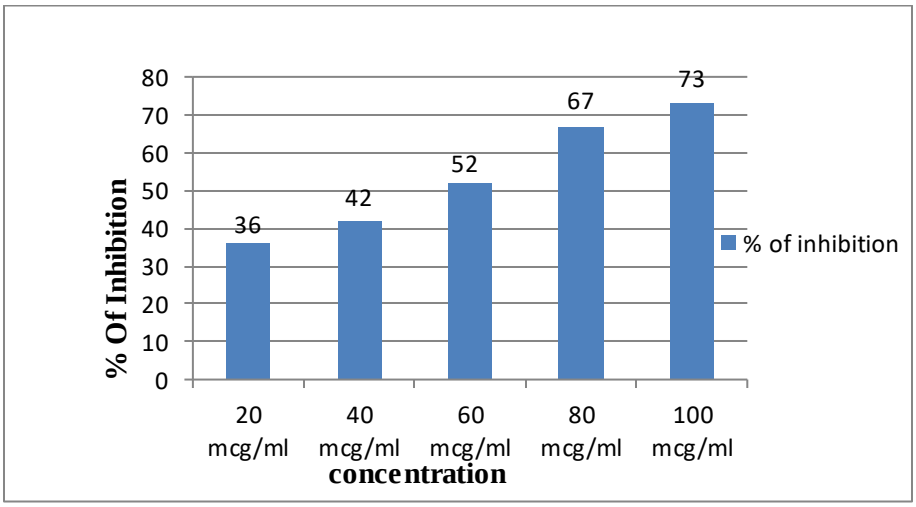

Fig 4: Graphical representation of % of inhibition against the concentration, in petroleum ether extract to determine the anti oxidant property.

For petroleum ether extract, maximum percentage of inhibition (73%) was seen in 100 µg/ml.

And minimum percentage of inhibition (36%) was seen in 20 µg/ml. Percentage of inhibition (42%) was seen in 40 µg/ml, 60 µg/ml, and 80µg/ml. had a percentage of inhibition 52 % and 67%.

3. Total Phenolic Content (TPC):

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Solvent Used in Extract	Total Phenolic content (mg GAE/gm extract)
Petroleum ethar, (60-80)ºC	0.90
Methanol	1.10

Table 2: TPC value of extracts from leaves of *Ficus rumphii*

Total phenolic content is used determination of the presence of phenols in extract sample. Methanol extract of leave of *Ficus rumphii* has higher TPC value than Petroleum ethar extract of the same plant extract

4. Total Flavonoids Content (TFC):

Sample Extract	Total Flavonoid Content (mg quercetin/gm extract)
Petroleum Ether, (60-80)ºC	6.5
Methanol	16.8

Table 3: TFC Value of extracts from leaves of *Ficus rumphii*

The total flavonoid content of crude extract was determined by the aluminium chloride colorimetric method. Methanol Extract of leaves of *Ficus Rumphii* has higher Total Flavonoid Content Value than the Petroleum ether extract of the same plant.

5. Thin Layer Chromatography (TLC)

Calculate the Retardation factor (R_f) Value.

Extract	Number of Spot	Rf Value
Petroleum ethar, (60-80)ºC	3	0.052,0.142,0.089
Methanol	3	0.035,0.026,0.041

Table 4: R_f Value of methanol extract and petroleum extract of *Ficus rumphii* leaves.

TLC is very common procedure for determining the number of compounds present in a mixture. Using silica Gel g as a absorbent, and Chloroform: Benzene, as a mobile phase and also Ratio of mobile phase mixture is 7:3.

Three spot are visible in petroleum ethar extract and also three spot are visible in methanol extract.

6. Column Chromatography:

Fig 5: Column Chromatography of *Ficus rumphii* extract.

Petroleum ethar is used as mobile phase in this column Chromatography of *Ficus rumphii* extract. Two visible bands are seen, orange and greenish yellow. Orange layer are run by the petroleum ethar solvent and collected in beaker for further study like TLC. When cotton was colourless means the remaining extract has no affinity to run by this solvent .Then change the mobile phase, use benzene as a mobile phase and collected the greenish yellow eluent in a beaker for TLC.

CONCLUSION

Medicinal plants are an effective source of both traditional and modern medicines; herbal medicine has been shown to have genuine utility. The results of this present studies showed that petroleum ether and methanol extract of *Ficus rumphii* leaves possess antioxidant activity. The plant possesses higher phenolic and flavonoid content in methanol extract of the leaves than the petroleum ether of the same extract. Antioxidant activity of *Ficus rumphii* extract was determined by scavenging assay. The petroleum ether and methanol extract of *Ficus rumphii* contained various phytochemicals like alkaloids, flavonoids, Triterpenoids, phenols and tannins. Based on the findings of the current assignment, it was confirmed that the leaves of *Ficus rumphii* possessed antioxidant activity which may be effective against various ailments. The results of these studies exhibit that *Ficus rumphii* has the promising characteristics of a potential antioxidant and may be effective in prevention of various diseases.

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