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PHYTOCHEMICAL STUDIES ON FICUS ELASTICA LEAVES

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ABSTRACT

Ficus (*Moraceae*) comprises one of the largest genera of angiosperms about 800 species and 2000 varieties of shrubs, herbs and woody trees. Literature survey has revealed little information on the plant in every aspect. So, an attempt has been made to study the pharmacognostical properties of this plant. Its acid value, saponification value, peroxide value, refractive index and free fatty acid values were 11.16 mg KOH/g, 16.83 mg KOH/g, 15 meq H_2O_2/g, 1.3970 and 5.61% respectively.

KEYWORDS: Ficus elastic, saponification value, peroxide value, refractive index, free fatty acid value.

1. INTRODUCTION

Ficus (*Moraceae*) comprises one of the largest genera of angiosperms about 800 species and 2000 varieties of shrubs, herbs and woody trees. These are found in tropical and subtropical worldwide forests[1,2] it is collectively known as fig trees. *Ficus* species are hemi-epiphytic plants.[3] During the early stage of development when competition for sunlight is harsh they use their host to overcome problems of low light conditions.[4] Half the number of ficus species in the world is monoecious and the others are gynodioecious. All of gynodioecious ficus species are old world species.[5] In Egypt, many *Ficus* species are found in gardens, streets, parks and outside the canal banks. Higher plants have been used extensively as a source in medicinal for numerous active constituents for treating human diseases because they contain of high therapeutic value.[6]

Ficus elastica Roxb. Ex Hornem belongs to family *moraceae* commonly known as rubber tree, rubber fig, rubber bush, Indian rubber bush, Assam rubber tree native to northeast India

and southern Indonesia.[7,8] The plant is locally known as India rubber tree.[9] *F. elastica* is also a common house plant. These trees can grow up profusely without any argonomic management and survive well under extreme conditions such as limited water supply and high temperature.[10] It is adaptable for annual pollarding with also a potential fiber value and is distributed in Tamil Nadu.

F. elastica is widely spread evergreen tree upto 30m tall. Its leaves are 7 to 20cm long, with smooth edges and blunt pointed tips. The leaves are thick with deep green colour and about a foot long.[11] Herbivores graze *F. elastica* only little but shy away.[12] *Ficus elastica* was introduced as an ornamental more than 100 years ago.[13] Flora of Pakistan and flora of west Punjab is listed as an "environmental weed, garden thug, naturalized weed" in global compendium of weeds.[14] It is large spreading tree that creates dense shade restricting growth beneath it, and tolerant of shade, drought, and s wide range of soil types.[15] Plants steroids and alkaloids are mostly poisonous even in minute quantities. They are often toxic to man and animals and have dramatic physiological activities and hence they have wide uses in medicines.[16] *F. elastica* possesses antimicrobial activity and the leaves extract is used for treatment of skin allergies, skin infection, anemia, neurodegenerative disorders and hepatic problems, it is also used as diuretic agent. In addition several chemical constituents from *F.elastica* leaves have been investigated.[17] The current study was designed to estimate the oil contents of leaves of the *ficus elastic* and to determine their physicochemical properties and fatty acid profile for their future fruitful application in the industry. The aim of the present work is to evaluate phytochemical constituent of leave Indian rubber (Ficus elastic).

2. EXPERIMENTAL WORK

2.1. Plant Profile

Ficus elastica: Rubber Tree is a beautiful tree native to NE India, the eastern Himalayas, and SE Asia. Rubber Tree is popular because it is very easy to grow and care for. They can get big pretty quickly, as they are vigorous growers and don't ask for much attention. It grows up to 30-40 m tall, but is usually seen as a smaller garden tree.

It has broad shiny oval leaves 10-35 cm long and 5-15 cm broad. Leaf size is largest on young plants (occasionally to 45 cm long), much smaller on old trees (typically 10 cm long). The leaves develop inside a reddish sheath at the tip of branches, which looks very attractive. It grows larger as the new leaf develops. When it is mature, it unfurls and the sheath drops off the plant. Inside the new leaf, another immature leaf is waiting to develop. The small stalkless, yellow-green oval figs, 1 cm long, grow in pairs. This is a popular house-plant, and grows in all Indian conditions. The tree can yield a milky white latex, which has been used to make rubber.

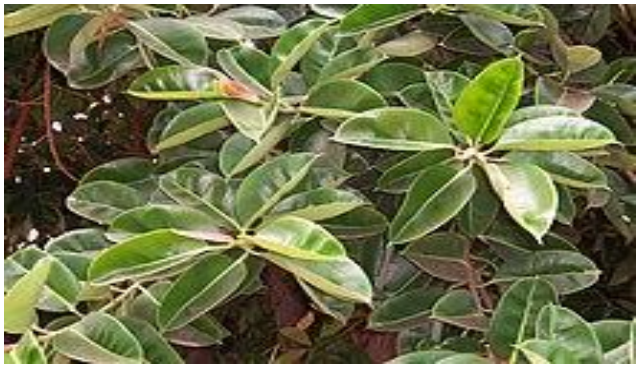

Figure. 1.1 Rubber.

Ficus elastica, the **rubber fig**, **rubber bush**, **rubber tree**, **rubber plant**, or **Indian rubber bush**, **Indian rubber tree**, is a species of plant in the fig genus, native to eastern parts of South Asia and southeast Asia. It has become naturalized in Sri Lanka, the West Indies, and the US State of Florida. 2 3.

2.2. MATERIAL METHOD

2.2.1. Sample Preparation: *Ficus elastica* leaves were collected from the garden. Leaves were washed and cut into small pieces to increase surface area of leaves for maximum extraction of oil. *Ficus elastica* leaves were powder and extract are prepared as per following.

S. No	Solvent	ml	Amount of leaf powder
1.	Petroleum ether	200ml	10gm
2.	Methanol	200ml	10gm
3.	Water	200ml	10gm
4.	Cellulose		1gm

2.2.2. Extraction of oil: 100 g leaves were filled in the thimble and oil was extracted through soxhlet extractor for 6 hours by using *n*-hexane.[18] After extraction, solvent was removed by rotary evaporator under reduced pressure and yield was calculated. It was then stored in the refrigerator for subsequent evaluation of physicochemical properties and GC-FID analysis for fatty acid profile.

2.3. Phytochemical analysis

2.3.1) Test for Alkaloids

2.3.1.1) Dragendorff's test: To the 1 ml of extract, add 1 ml of Dragendorff's reagent (potassium bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.

2.3.1.2) Mayer's test: To the 1 ml of extract, add 1 ml of Mayer's reagent (Potassium mercuric iodide solution). Whitish yellow or cream coloured precipitate indicates the presence of alkaloids.

2.3.1.3) Hager's test: To 1 ml of extract add 3ml of Hager's reagent (saturated aqueous solution of picric acid) yellow colored precipitate indicates the presence of alkaloids.

2.3.1.4) Wagner's test: To the 1 ml of extract add 2 ml of Wagner's reagent (iodine in potassium iodide) formation of reddish brown precipitate indicates the presence of alkaloids.

2.3.2) Test for Saponins: Take small quantity of alcoholic and aqueous extract separately and add 20 ml of distilled water and shake in a graduated cylinder for 15 minutes lengthwise. Layer of foam indicates the presence of Saponins.

2.3.3) Test for Glycosides

2.3.3.1) Legal's test: Dissolve the extract in pyridine and add sodium nitroprusside solution to make it alkaline. No formation of pink to red colour shows absence of glycosides.

2.3.3.2) Baljet's test: To 1ml of the test extract, add 1ml of sodium picrate solution and the yellow to orange color reveals the presence of glycosides.

2.3.3.3) Keller-Killani test: 1gm of powdered drug is extracted with 10ml of 70% alcohol for 2 minutes, filtered, add to the filtrate, 10ml of water and 0.5ml of strong solution of lead acetate and filtered and the filtrate is shaken with 5ml of chloroform. The chloroform layer was separated in a porcelain dish and remove the solvent by gentle evaporation. Dissolve the cooled residue in 3ml of glacial acetic acid containing 2 drops of 5% ferric chloride solution. Carefully transfer this solution to the surface of 2ml of concentrated sulphuric acid. A reddish brown layer forms at the junction of the two liquids and the upper layer slowly becomes bluish green, darkening with standing.

2.3.3.4) Borntrager's test: Add a few ml of dilute Sulphuric acid to 1ml of the extract solution. Boil, filter and extract the filtrate with chloroform. The chloroform layer is treated with 1ml of ammonia. The formation of red color of the ammonical layer shows the presence of anthraquinone glycosides.

2.3.4) Test for Carbohydrates

2.3.4.1) Molisch's test: To 2ml of the extract, add 1ml of a-naphthal solution, add concentrated sulphuric acid through the side of the test tube. Purple or reddish violet color at the junction of the two liquids reveals the presence.

2.3.4.2) Fehling's test: To 1ml of the extract, add equal quantities of Fehling solution A and B, upon heating formation of a brick red precipitate indicates the presence of sugars.

2.3.4.3) Benedict's test: To 5ml of Benedict's reagent, add 1ml of extract solution and boil for 2 minutes and cool. Formation of red precipitate shows the presence of sugars.

2.3.5) Test for Tannins

i) Take the little quantity of test solution and mixed with basic lead acetate solution. Formation of white precipitates indicates the presence of tannins.

ii) To 1ml of the extract, add ferric chloride solution, formation of a dark blue or greenish black color product shows the presence of tannins. The little quantity of the extract is treated with potassium ferric cyanide and ammonia solution. A deep red color indicates the presence of tannins.

iii) To the test extract, add strong potassium dichromate solution, a yellow color precipitate indicates the presence of tannins and Phenolic compounds.

2.3.6) Test for Flavonoids

Shinoda's Test

I) The alcoholic extract is treated with magnesium foil and concentrated HCl give intense cherry red color indicates the presence of flavonones or orange red color indicates the presence of flavonols.

II) The extract is treated with sodium hydroxide; formation of yellow color indicates the presence of flavones.

III) The extract is treated with concentrated H2SO4, formation of yellow or orange color indicates flavones.

2.3.7) Test for Steroids

Salkowski test: Dissolve the extract in chloroform and add equal volume of conc. H_2SO_4. Formation of bluish red to cherry color in chloroform layer and green fluorescence in the acid layer represents the steroidal components in the tested extract.

2.3.8) Test for Proteins

2.3.8.1) Biuret test: Add 1ml of 40% sodium hydroxide solution and 2 drops of 1% $CuSO_4$ solution till a blue color is produced, and then add to the 1ml of the extract. Formation of pinkish or purple violet color indicates the presence of proteins.

2.3.8.2) Ninhydrin test: Add two drops of freshly prepared 0.2% Ninhydrin reagent (0.1% solution in n-butanol) to the small quantity of extract solution and heat. Development of blue color reveals the presence of proteins, peptides or amino acids.

2.3.8.3) Xanthoproteic test: To 1ml of the extract, add 1ml of concentrated nitric acid. A white precipitate is formed, it is boiled and cooled. Then 20% of sodium hydroxide or ammonia is added. Orange color indicates the presence of aromatic amino acids.

2.3.8.4) Millon's test: 1ml of test solution is made acidify with sulphuric acid and add Millon's reagent and boil this solution. A yellow precipitate is formed indicates the presence of protein.

2.4. Physico-chemical analysis

The extracted oil was immediately analyzed for peroxide value and saponification value following the method described by the Association of Official Analytical Chemists.[19]

Estimation of the percentage free fatty acids as oleic acid was done following the method of Cock and Van Rede.[20] The refractive index of the oil (at room temperature) were determined with Abbe refractometer.[21] The state and colour of the oil were noted using visual inspection at room temperature.

2.5. GC-FID analysis of the Ficus elastica leaves oil

The fatty acids were then esterified with methanol in the presence of boron trifluoride. Esterifies fatty acids were extracted with n-hexane and then evaporated on low heat. Then the analysis was performed on gas chromatograph (GC-14A) coupled with flame ionizer detector and data processing. A PEG capillary column (25m × 0.2 mm id) was used for fatty acid. The column was operated with temperature programming from 150 to 200 ^{0}C. The injection and detector temperature were maintained at 250 to 300 ^{0}C respectively. Flow gas of carrier gas (Nitrogen) was 20 mL/min at split ratio of 1:50. Identification of the component was based on their retention time as compared with those obtained from methyl esters of known fatty acids, analyzed under similar conditions.

3. RESULT AND DISCUSSION

The physic chemical properties of the oil are reported in the table 1. The color of the oil was light green which may be due to the presence of some contents of chlorophyll. The presence of peroxide and free fatty acid indicates the oxidation process in the leaf. Natural system for the protection and sustain the life of plants indicates that presence of oil in the leave may be due to protection of excessive loss of water. Furthermore, due to oil contents leaves may be more flexible and have capacity to bear harsh weather.

Qualitative chemical examination

The extracts obtained were subjected to qualitative chemical tests for the identification of various plant constituents. All tests are summarized in the given table.

1. Carbohydrates

A. Molish's test: 0. 1g of extract dissolved in water, added 2-3drops of 1 % alcoholic-napthol & 2ml conc. H^2 SO_4

S. No	Solvent	Inference
1.	Petroleum ether	– ive
2.	Methanol	– ive
3.	Water	– ive
4.	Cellulose	– ive

B. Fehling's test(for reducing sugar): To 5ml of extract solution, 5ml of freshly mixed Fehling's A & B solution added and boiled for 5 minutes on water bath.

S. No	Solvent	Inference
1.	Petroleum ether	– ive
2.	Methanol	+ive
3.	Water	+ive
4.	Cellulose	– ive

C .Benedict's test: To 5ml of extract solution, 5ml of benedict's reagent added and boiled for 5 minutes.

S. No	Solvent	Inference
1.	Petroleum ether	– ive
2.	Methanol	+ive
3.	Water	+ive
4.	Cellulose	– ive

Saponins glucoside

A Foam test: Powdered drug shaken vigorously with water.

S. No	Solvent	Inference
1.	Petroleum ether	– ive
2.	Methanol	– ive
3.	Water	– ive
4.	Cellulose	– ive

B Cardiac glucoside

Legal's test: Dissolve the extract in pyridine and add sodium nitroprusside solution to make it alkaline. No formation of pink to red colour shows absence of glycosides.

S. No	Solvent	Inference
1.	Petroleum ether	– ive
2.	Methanol	– ive
3.	Water	– ive
4.	Cellulose	– ive

C For deoxysugar

Keller-Killani test: 1gm of powdered drug is extracted with 10ml of 70% alcohol for 2 minutes, filtered, add to the filtrate, 10ml of water and 0.5ml of strong solution of lead acetate and filtered and the filtrate is shaken with 5ml of chloroform. The chloroform layer was separated in a porcelain dish and remove the solvent by gentle evaporation. Dissolve the cooled residue in 3ml of glacial acetic acid containing 2 drops of 5% ferric chloride solution. Carefully transfer this solution to the surface of 2ml of concentrated sulphuric acid. A reddish

brown layer forms at the junction of the two liquids and the upper layer slowly becomes bluish green, darkening with standing.

S. No	Solvent	Inference
1.	Petroleum ether	+ive
2.	Methanol	– ive
3.	Water	– ive
4.	Cellulose	– ive

C. For anthraquinone glycoside

Borntrager's test is employed for presences of anthraquinones

S. No	Solvent	Inference
1.	Petroleum ether	-ive
2.	Methanol	– ive
3.	Water	– ive
4.	Cellulose	– ive

For Shinoda's Test: I) The alcoholic extract is treated with magnesium foil and concentrated HCl give intense cherry red color indicates the presence of flavonones or orange red color indicates the presence of flavonols.

II) The extract is treated with sodium hydroxide; formation of yellow color indicates the presence of flavones.

III) The extract is treated with concentrated H2SO4, formation of yellow or orange color indicates flavones.

S. No	Solvent	Step 1	Step 2	Step 3	Step 4
1.	Petroleum ether	+ive	-ive	-ive	+ive
2.	Methanol	– ive	– ive	– ive	– ive
3.	Water	– ive	– ive	– ive	+ive
4.	Cellulose	– ive	– ive	– ive	– ive

Test for Tannins

S. No		Petroleum ether	Methanol	Water	Cellulose
1.	Solution of $FeCl_3$	+ive	-ive	-ive	+ive
2.	Lead Acetate	+ive	– ive	– ive	+ive
3.	Gelatin test	– ive	+ive	– ive	-ive
4.	Bromine water	– ive	– ive	– ive	– ive
5.	Potassium dichromate	– ive	– ive	– ive	– ive
6.	Acetic acid solution	– ive	– ive	– ive	– ive
7.	Dilute Iodine solution	– ive	– ive	– ive	– ive
8.	Potassium permagnate	– ive	– ive	– ive	– ive

Test for Steroids

Salkowski test

Dissolve the extract in chloroform and add equal volume of conc. H_2SO_4. Formation of bluish red to cherry color in chloroform layer and green fluorescence in the acid layer represents the steroidal components in the tested extract.

S. No		Petroleum ether	Methanol	Water	Cellulose
1.	**Salkowski test**	+ive	-ive	-ive	-ive

Libermann Britch test

S. No	Petroleum ether	Methanol	Water	Cellulose
1.	+ive	-ive	+ive	-ive

Libermann Reduction test

Petroleum ether	Methanol	Water	Cellulose
+ive	-ive	-ive	-ive

Alkaloids test

1.1) Dragendorff's test

To the 1 ml of extract, add 1 ml of Dragendorff's reagent (potassium bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.

Petroleum ether	Methanol	Water	Cellulose
-ive	+ive	+ive	+ive

1.2) Mayer's test

To the 1 ml of extract, add 1 ml of Mayer's reagent (Potassium mercuric iodide solution). Whitish yellow or cream coloured precipitate indicates the presence of alkaloids.

Petroleum ether	Methanol	Water	Cellulose
+ive	+ive	+ive	-ive

1.3) Hager's test

To 1 ml of extract add 3ml of Hager's reagent (saturated aqueous solution of picric acid) yellow colored precipitate indicates the presence of alkaloids.

Petroleum ether	Methanol	Water	Cellulose
+ive	+ive	+ive	-ive

Table. 1: Physico-Chemical Characteristics of *Ficus elastica* oil.

Physicochemical Parameters	Results
Colour Light	Green
Acid value (mgKOH\g)	11.16
Free fatty acid (%)	5.61
Saponification value (mgKOH\g)	16.83
Peroxide value (meq H2O2/g)	15
Refractive index at room temperature	1.3970
State at room temperature	liquid

Fatty acids were esterified with methanol (C2H5OH) the presence of boron triflouride (BF3) then its profile was determined by GC-FID. The results showed that it contains lauric acid 3.36%, myristic acid, 3.12%, butyric acid 3.38%, isobutyric acid 6.50%, valeric acid 6.76%, isovaleric acid 23.59%, caprylic acid 5.41%, caproic acid 27.34% and capric acid 20.28%.

Lauric acid is 12 carbon chain fatty acid (C12:0) and its main application in cosmetic product is for the treatment of acne.[21] Myristic acid (C14:0) in the form of ester isopropyl myristate is used in cosmetic and topical medicinal preparations where good absorption through the skin is desired.[7] Butyric acid a four carbon fatty acid is used in the preparation of various butyrate esters. Due to pleasant tastes or aromas it is used used as perfume and food additives. It is also used as an animal feed supplement due to ability to reduce pathogenic bacterial colonization.[8] Due to powerful odor it is used as a fishing bait additive[9] and in anti abortion protesters to disrupt abortion clinics.[10] It is added to imitate the flavor of chocolate produced by Hershey process. Isobutyric acid also known as 2-methylpropanoic acid are used to eliminate calcium in leather industry. Valeric acid (Pentanoic acid) its primary use is in the synthesis of its esters. Volatile esters of valeric acid tend to have pleasant odors and are used in perfumes and cosmetics. Ethyl valerate and pentyl valerate are used as food additives because of their fruity flavors.[11] Isovaleric acid is common name of 3-Methyl butanoic acid it w seen that it was primary cause of flavors which are added to wine caused by Brettsnomyces yeasts.[12] It is anticonvulsant agent in valerian.[13] It uses to synthesize beta-methyl butyric acid by microbial oxidation via fungus. Caprylic acid is 8 carbon chain fatty acid (C8:0) and it is used commercially in the production of esters used in perfumery and also in manufacture of dyes. Caprylic acid is an antimicrobial pesticide used as a food contact surface sanitizer in commercial food handling establishment on dairy equipments breweries, wineries and beverage processing plants and as disinfectants in health care facilities. In addition Caprylic acid is used as an algaecide, bactericide and fungicide in nurseries, greenhouses, gardens centers. The acid chloride of caprylic acid is used in the

synthesis of the perfluoroctanoic acid.[14] Capric acid (C10:0) is other name of Decanoic acid it is saturated fatty acid used in the manufacture of esters for artificial fruit flavors and perfumes, as an intermediate in chemical synthesis and industrially in the manufacture of perfumes, lubricants, greases, rubber dyes, plastics, food additives and pharmaceuticals.[15,16] Caproic acid (C6:0) also known hexanoic acid and is a medium chain triglycerides (MCT) which are widely used for parenteral nutrition in individuals requiring supplemental nutrition and are being more widely used in food drugs and cosmetics. it is fatty acid found naturally in various animals fats and oils, it is also one of the component of vanilla. The primary use of caproic acid is in the manufacture of its esters for artificial flavors and in the manufacture of hexyl derivatives such as hexylphenols,[17] Caprylic acid, Caproic acid along with Capric acid these total 15% in goat milk fat.

4. SUMMARY AND CONCLUSION

Extracted *Ficus elastica* leaves oil was analyzed for physicochemical properties and for fatty acids. It gave 19.28% yield. Its acid value 11.16 mg KOH/g, Saponification value 16.83 mg KOH/g, free fatty acid value 5.61%, peroxide value, 15 meq H2O2/g and refractive index 1.3970 respectively. Fatty acid profile was determined by GC-FID after esterification of the oil with methanol in the presence of boron trifluoruide. It contains lauric acid 3.36%, myristic acid, 3.12%, butyric acid 3.38%, isobutyric acid 6.50%, valeric acid 6.76%, isovaleric acid 23.59%, caprylic acid 5.41% and capric acid 20.28%, caproic acid 27.34%.keeping in view fatty acids profile and with the already published literature support we can say, that *Ficus elastica* oil is well suitable for cosmetic products. *Ficus elastica* leaves were cut into small pieces and extraction of oil was carried out through soxhlet apparatus by using n-hexane for 6 hours. Solvent was separated by rotary evaporator at reduced pressure and green colored oil with 1.92% yield was obtained. Its physicochemical properties were estimated by following standard procedures. Its acid value, saponification value, peroxide value, refractive index and free fatty acid values were 11.16 mg KOH/g, 16.83 mg KOH/g, 15 meq H2O2/g, 1.3970 and 5.61% respectively. Fatty acid profile was determined by gas chromatograph coupled with flam ionization detector (GC-FID) after esterification of the oil with methanol in the presence of boron trifluoruide. It contains lauric acid 3.36%, myristic acid 3.12%, butyric acid 3.38%, isobutyric acid 6.50%, caprylic acid 5.41%, caproic acid 27.34%, valeric acid 6.76 %, isovaleric acid 23.59% and capric acid 20.28%. The oil of *F. elastica* contains lower molecular weight saturated fatty acids which are suitable for application in soap and cosmetic industry**.**

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