



STUDIES ON PRELIMINARY PHYTOCHEMICAL, FLUORESCENCES AND MINERAL ANALYSIS OF *ALBIZIA AMARA* LEAVES

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ABSTRACT

Medicinal plants have been used for centuries as remedies for human and animal diseases because of their therapeutic values. *Albizia amara* is a potent medicinal plant which has important medicinal properties and traditional uses. It is locally known as 'Arappu' This plant is also known as "oil cake tree". The screening of preliminary phytochemical analysis of four different extracts (petroleum ether, acetone, ethanol and water). Among the four tested extracts, most of the phytonutrients were present in the water extract. Further study was carried out by using the leaf powder, ethanol and water extracts using fluorescence, minerals and FT-IR analysis. Fluorescence is an important phenomenon displayed by various phyto-constituents present in plant materials. Mineral elements also are headed in minute quantities for the proper functioning of the human system, health growth and development. FT-IR analysis confirmed the presence of different functional groups in this plant. The observed results of the leaf showed that the mineral content supported the traditional use of the plant. Elemental analysis affords them interesting the fluorescent analysis of powered drug plays an important role in the determination of quality and purity of the drug. Arappu is a common traditional plant, it is concluded in our findings, used as body coolant and antidandruff agent.

KEYWORDS: *Albizia amara*, therapeutic, mineral and coolant.

INTRODUCTION

Medicinal plants have been used for centuries as remedies for human and animal diseases because of their therapeutic values. Hence, plant derived drugs remain an important resources especially in many countries of the world to combat diseases. Medicinal herbs have been used as several forms in various indigenous medicinal system like Siddha, Ayurveda, Unani, Western and Chinese traditional medicine system. The Phytochemical constituents of plants were considered important as it increased the acceptability of traditional medicine.

The genus *Albizia* mostly consist of approximately 150 species, most of them are trees and shrubs confined to tropical and subtropical region of Asia, Africa and Australia. *Albizia amara* commonly moderate sized, branched drought tolerant deciduous tree grows up to 10 meters tall belongs to the family Fabaceae and usually found in dry forest of South India in Tamil Nadu, Andhra Pradesh and Karnataka. It is broadly distributed in Africa, from Sudan and Ethiopia southwards to Zimbabwe, Botswana and the Transvaal. *Albizia amara* is locally known as 'Arappu' This plant is also known as "oil cake tree" usually grown in dry areas of Tamil Nadu, Karnataka and Andhra in India.

Tap leaves of *A.amara* are used as source for animal fodder and may also be used as firewood. It is folk remedy for curing various diseases viz., dandruff, diarrhoea, common cold, wounds and gonorrhoea. The seeds of *Albizia amara* (Fabaceae) used as an astringent, treating piles, diarrhoea, leprosy, leucoderma, erysipelas and abscesses. The leaves and flowers have been applied to boils, eruptions, and swellings, also regarded as an emetic and as a remedy for coughs, ulcer, dandruff and malaria. ^[1] *Albizia* species are socially significant as high quality timber yielding and as a valuable resource for gum. There is a growing interest in correlating phytochemical constituents of plant with its pharmacological activity. Herbal medicines have stolen the show and are being credited due to their safety, efficacy and availability from indigenous sources.

MATERIALS AND METHODS

Collection of the Plant material

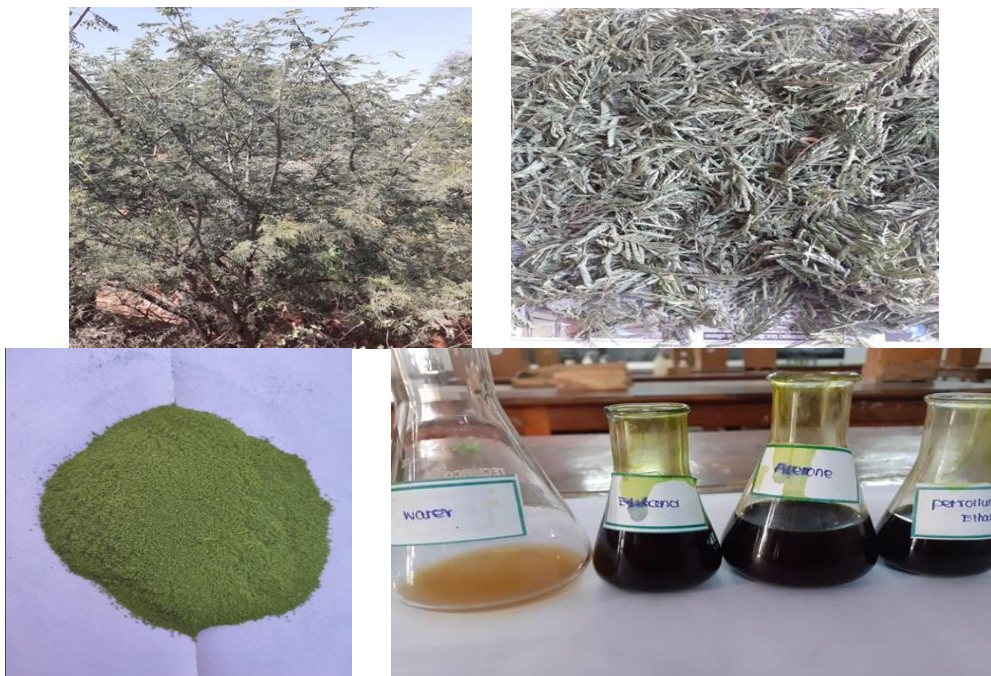
Leaves of *Albizia amara* were collected in January, 2020 in Sathiyathapuram, Theni District, Tamilnadu, India.

Preparation of the plant extract

Shade dried leaves were pulverized to power using a mechanical grinder at PG and Research Department of

Botany, Sri Parasakthi college for Women, Courtallam. Required quantity of powder *Albizia amara* (10gm) was weighted and transferred to stopper flask and treated with petroleum ether, acetone, ethanol, water until the powder is fully immersed. The flask was shaken every hour for the 6 hrs and then it was kept a side and again

shaken after 12 hrs. The extract was filtrate was concentrated at 30°C under reduced pressure in a rotary evaporator. The extract was filtered using muslin cloth. The crude extract was then dissolved in 1ml specific solvents, and evaporated and used for further experiments.



Phytochemical screenings

The leaf extracts were analyzed for the presence of flavonoids, alkaloids, glycosides, steroids, phenols, saponins, terpenoids, cardiac glycosides and tannins according to standard methods.^[2,3]

Fourier Transform Infrared Spectrophotometer (FTIR)

Fourier transform infrared spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. The wavelength of the light absorbed is characteristic of the chemical bonds as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined.

Leaf powder, ethanol and water extracts of the leaf material were used for the FTIR analysis. 10mg of the ethyl acetate extracts was encapsulated in 100mg of KBr pellet, in order to prepare translucent sample discs. The ethyl acetate extracts samples of plant specimen loaded in FTLR Spectroscope (Shimadzu, IR tracer-100), with a scan range from 400-4000cm⁻¹ resolution of 4cm⁻¹. This study was carried out in Instrumentation centre, ANJA Collage, Sivakasi.

Mineral Analysis

The *Albizia amara*, ethanol and water extracts were analyzed for the presence of potassium, Phosphorous,

calcium, sulphur, magnesium and nitrogen according to standard methods,^[4] *Albizia amara* leaf powder using fluorescence analysis under 365 nm.

RESULTS AND DISCUSSION

The phytochemical screening of the Petroleum ether, Acetone, Ethanol and water extracts was summarized in Table 1. The obtained results were showed the presence of all the tested phyto-constitutes in water extracts. In Ethanol extract, Alkaloids, Steroids, Tannins, Phenols, Terpenoids and Cardiac glycosides were present and flavonoids and glycosides were absent. Acetone extracts showed the presence of Alkaloids, Phenols and Cardiac glycosides and Absence of in Flavonoids, Steroids, Saponin, Glycosides and Terpenoids.

All the tested phyto compounds were absent in Petroleum ether extract. This suggests that the *A.amara* offer a wide array of phyto chemicals. In accordance with our findings reported to similar Abdel karim *et al.*, 2016.^[5] The preliminary phyto chemical studies the methanolic extract of *Albizia chinensis* showed negative result for same test. The occurrence of negative results was may be due to the poor quantity of the phytochemicals.

The various phytochemical compounds detected are known to have beneficial important in medicinal response modifiers, because of their inherent ability to modify the body's reaction to allergies and virus and

they should their anti-allergic, anti-inflammatory, anti-microbial, and anti-cancer activities.^[6]

Plant steroids are known to be important for their cardiostimulant activities, possess insecticidal and antimicrobial properties. They are also used in nutrition, herbal medicine and cosmetics.^[7] It should be noted that steroidal compounds are of importance and interest in pharmacy due to their relationship with such compounds as sex compounds.^[8]

Saponin is used as mild detergent and intracellular histochemical stain. It is also used to allow antibody access in intracellular proteins. In medicine, it is used in hypercholesterolemia, hyperglycaemia, anticancer, anti-inflammatory properties.^[9]

Generally, glycosides are non-volatile and fragrance cleaving the glycoside bond yields the aglycone, which itself may be volatile and fragment, glycosides serves as defence mechanism against predation by many microorganisms, insects and herbivores.^[10] Phenols are a class of low molecular weight secondary, metabolites found in most land plants. Phenolic compounds are the largest group of phytochemicals and accounts for most of the antioxidant activity in plants or plant products.^[11] Cardio glycosides on the other hand are known by inhibiting the Na⁺ /K⁺ pump. This causes an increasing in the level of sodium ions in the calcium ion. This inhibition the amount of Ca²⁺ ions available for concentration of the heart failure and cardio arhythmia.^[12]

Terpenoids are found to be responsible for anti-activity. Plant terpenoids are widely used as industrially relevant chemicals, including flavours, fragrances, pesticides, disinfectants, and pharmaceuticals. Recently, there has been a renaissance of awareness of plant terpenoids used as available in biological resource for societies that will have to become less reliant on petrochemicals.^[13] Polyphenols are a set of natural compounds that act as free-radical terminators and showed the anti-oxidant activity. Flavonoids are secondary metabolites that show anti-oxidant and radical-scavenging activities.^[14]

The preliminary phytochemical studies of the methanolic extract of *Albizia chinensis* showed the negative results for the same test. The occurrence of negative results was may be due to the poor quantity of the phytochemicals.^[15]

The *A.amara* and *A.saman* species are globally distributed throughout the tropical region, and are widely used as folk remedy for curing various diseases.^[16]

Phytochemical screening of ethyl acetate successive extract *Albizia lebeck* leaves showed the presence of glycosides, tannins, saponins, flavonoids, carbohydrates, proteins and amino acids. Methanolic successive

extract showed the presence of alkaloids, tannins, saponins, flavonoids and carbohydrates.^[17]

The phytochemical screening of chemical constituents in *Albizia lebeck* study showed that leaves were rich in flavonoids, tannins, and saponins. They are well known to show much of medicinal activity as well as physiological activity. The presence of flavonoids in the present study is support the opinion of Mousallamy (1998),^[18] who noted the presence of flavonoids in *Albizia lebeck* leaves and the presence of tannins and saponin, absence of steroids was confirmed by Ueda 2003.^[19] These findings were similar to our reports.

The medicinal value of plant depends upon the bioactive phyto-constituents of the plant and which shows various physiological effects on human body . So the knowledge of phyto-constituents present in the plant can be important to detect with the help of phytochemical screening. The presence of secondary metabolites like alkaloids, glycosides, phenols, flavonoids, tannins, saponins, tri-terpenes, etc. in the plant has great pharmaceutical interest. This phytochemical screening provide control, efficiency and safety of the herbal product. Phyto-medicines return traces its origin to the fact that medical elite has been convinced that synthetic drugs could not conquest of infectious disease, and if they do these are not without side effects.

Raskinet *al.*, 2002^[20] reported that rediscovery of the connection between plants and health is responsible for launching new generation of botanical therapeutically, multicomponent botanical drugs, dietary supplement and functional food. The World Health Organization (WHO) is encouraging, promoting ,and facilitating the effective use of herbal medicine in development countries for the presence of bioactive compounds. About 1% of the total known medicinal plants species is acknowledged to therapeutic value for human health benefits.

The fluorescence colour is specific for each compound. A non-fluorescence compound may fluorescence is mixed with in purifies that are fluorescent. The fluorescence character of leaf *Albizia amara* was studied under normal light intensity and UV 365 nm. Out of the 12 test, one of the test was varied in colours in day light and UV 365 nm, and one of the other test was varied in colour, when compared to normal powered control to commercial. The results of fluorescent analysis of leaf powder were depicted in table-2. The leaf powder showed the characteristic coloration upon treatment with multifarious chemical reagents.

Fluorescence is an important phenomenon displayed by various phyto-constituents present in plant materials. Some of the phyto-constituents were showed the fluorescence in the visible range in daylight. The

ultraviolet light produces fluorescence in many natural products, which do not visible in fluorescence in daylight. Some of the substance may be often converted into fluorescent derivatives by using different chemical reagents and chemicals though they are not qualitatively some crude drugs using fluorescence.^[21]

The mineral analysis of the leaf showed that the presence of all the mineral content which supported the medicinal use of the plant (Table 3). The higher concentration of sodium in the matured leaves is an indicator that the plant is not good for hypertension patient which could result in high blood pressure, however the presence of these minerals (Na, P, Ca, K) makes them a good traditional medicine for the treatment of various diseases.

The qualitative determination of mineral elements present in plants is important because the concentration and type of minerals must often be stipulated of a food. Mineral elements also are headed in minute quantities for the proper functioning of the human system, health growth and development. The content of mineral elements in plants depends to a high degree on the soils abundance including the intensity of fertility.

Ca is the main constituent of the skeleton and is important for regulating many vital cellular activities such as nerve and muscle function, hormonal actions, blood clotting and cellular mortality. Calcium is essential for healthy bones, teeth and blood. The health of the muscles and nerves depends on calcium. It is required for the absorption of dietary vitamin B, for the synthesis of the neurotransmitter. Iron is the most well known in biological system. It performs a wide range of biological functions. Iron occupies a unique role in the metabolic process. The role of iron in the body is clearly associated with haemoglobin and the transfer of oxygen from lungs to the tissue cells. Iron deficiency is the most prevalent nutritional deficiency in humans. Iron is an essential element for human beings and animals and is an essential

component of haemoglobin. It facilitates the oxidation of carbohydrates, protein and fat to control body weight, which is very important factor in diabetes. Phosphorous maintain blood sugar level, normal heart contraction dependent on phosphorous also important for normal cell growth and repair. It helps in the process of ossification of bones by getting deposited in the form of calcium phosphate.

Mineral elements possess a very important role in human nutrition. Though they are required in minute quantities they are essential for proper functioning of the entire human system. This can further be investigated in a wide scale for the purpose of drug development against various deficiencies. The mineral analysis of this plant may therefore yield to the conclusion that it may act as a good source of diet to fight against deficiency disorders of Iron, Calcium and Phosphorous.

The FT-IR spectrum was used to identify the functional groups of the active components present in the region of IR radiation, when dried leaf powder and ethanol and water extracts were passed into the FT-IR spectrum, the functional groups of the components was supported based on its peaks ratio. The results of FT-IR analysis confirmed the presence of N-H, O-H, C=C, C-H, C-O functional groups. (Figures 1). FT-IR spectroscopy is proved to be a reliable and sensitive method for detection of bio molecular composition.

Sheyinet *et al.*, 2015^[22] reported the FT-IR analysis of *A. lebeck* leaves was confirmed the presence of amide, alkynes, alkanes, carboxylic acids, alkenes, aromatics, aliphatic amines and alkyl halides compound which shows major peaks at 3654.12, 3307.55, 2918.44, 2849.92, 1643.73, 1454.46, 1054.13, and 510.34, respectively. The dry ethanolic extracts of *A. lebeck* leaves in FT-IR analysis proved that the presence of alcohols, phenols, alkanes, carboxylic acids, aromatics, ketones and alkyl halides compounds.

Table-1 Preliminary phytochemical screening of *Albizia amara* leaf extracts.

| Name of the Phytoconstituents | Test | Petroleum ether | Acetone | Ethanol | Water |
|-------------------------------|----------------------|-----------------|---------|---------|-------|
| Alkaloids | Mayer's test | — | + | ++ | ++ |
| | Dragendroff reagent | — | + | +++ | ++ |
| Flavonoids | HCL | — | — | — | + |
| | Sodium Hydroxide | — | — | — | +++ |
| Steroids | | — | — | ++ | ++ |
| Tannins | Lead acetate | — | — | — | + |
| | Ferric chloride | — | + | ++ | +++ |
| Saponin | Sodium bicarbonate | — | — | + | + |
| Phenols | Ferric chloride | — | + | ++ | +++ |
| | Ellagic acid | — | + | ++ | +++ |
| | Folinicaltec reagent | — | — | — | +++ |
| Glycosides | | — | — | — | ++ |
| Terpenoid | | — | — | ++ | +++ |

(—) Absent, (+) Present, (++) Moderately present, (+++) Highly present

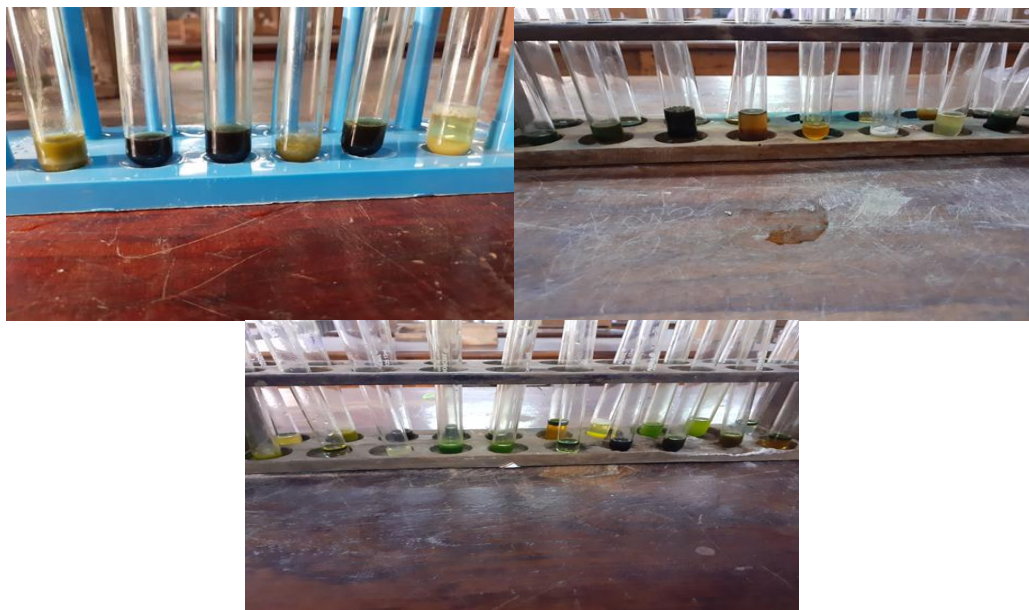


Plate-2: Screening of preliminary phytochemical analysis of *Albizia amara* leaf extracts.

Table-2: Fluorescence analysis of *Albizia amara* leaf powder.

| S.No | Sample | Visible / Day light | 365 nm uv light |
|------|--|---------------------|-------------------|
| 1 | Powder as such | Green | Green |
| 2 | Powder + 1N Aqueous NaOH | Pale Green | Green |
| 3 | Powder + 1 NHCL | Light green | Pale green |
| 4 | Powder + Con. H ₂ SO ₄ | Light green | Pale green |
| 5 | Powder + 50% H ₂ SO ₄ | Light green | Pale green |
| 6 | Powder + Acetic acid | Green | Florescence green |
| 7 | Powder + Ferric chloride | Light green | Green |
| 8 | Powder + HNO ₃ +NH ₃ | Brown | Green |
| 9 | Powder + Petroleum ether | Florescence Green | Dark green |
| 10 | Powder + Chloroform | Green | Brown colour |
| 11 | Powder + Methanol | Brown colour | Green |
| 12 | Powder + Ethanol | Green | Dark green colour |

Table-3: Mineral analysis of ethanol and water extracts of *A. amara* leaves.

| S.No | Name of the minerals | Water | Ethanol | Observation |
|------|----------------------|-------|---------|-------------|
| 1 | Potassium | + | + | Yellow |
| 2 | Phosphorous | + | + | Green |
| 3 | Calcium | — | + | Crystal |
| 4 | Sulphur | + | + | White |
| 5 | Magnesium | + | — | Crystal |
| 6 | Nitrogen | — | + | Dark blue |

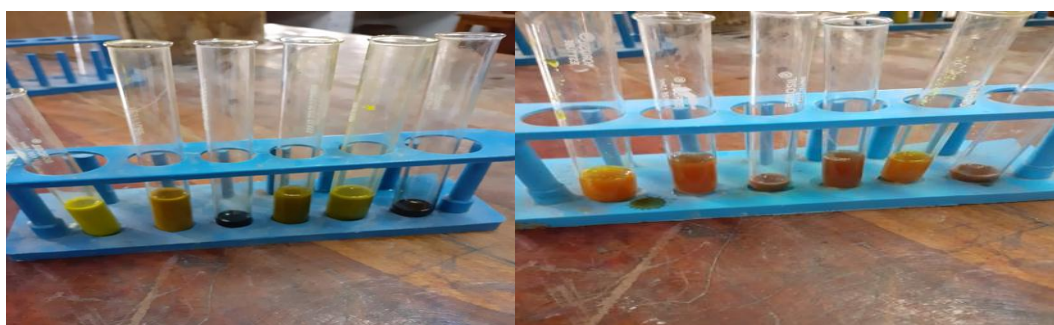
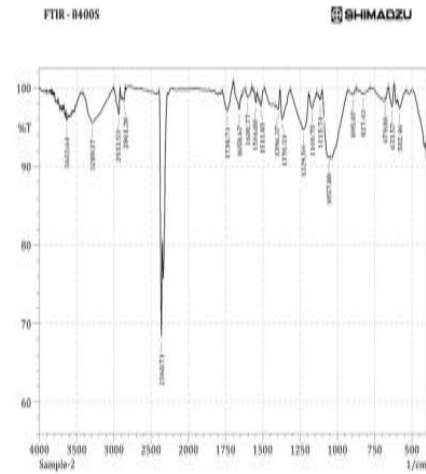


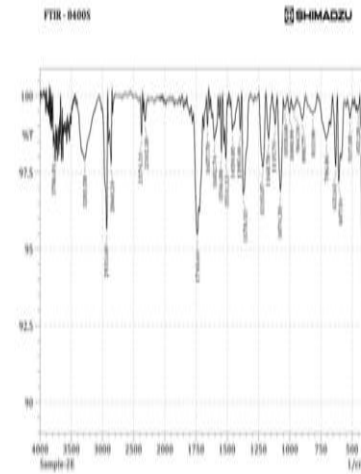
Figure-1: FT-IR analysis of powdered material, ethanol and water extracts of *Albizia amara*.



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| No. | Peak | Intensity | Corr. Intensity | Base (H) | Base (L) | Area | Corr. Area |
|-----|---------|-----------|-----------------|----------|----------|-------|------------|
| 1 | 362.46 | 97.394 | 1.37 | 997.89 | 539.07 | 0.390 | 0.102 |
| 2 | 628.97 | 97.614 | 2.148 | 688.68 | 618.11 | 0.222 | 0.260 |
| 3 | 679.88 | 96.437 | 0.216 | 682.79 | 688.68 | 0.090 | 0.02 |
| 4 | 827.41 | 96.262 | 0.681 | 871.76 | 782.19 | 0.178 | 0.107 |
| 5 | 899.67 | 95.142 | 0.639 | 930.69 | 874.66 | 0.136 | 0.078 |
| 6 | 1057.60 | 97.146 | 1.276 | 1102.24 | 1057.17 | 1.450 | 0.500 |
| 7 | 1116.74 | 98.432 | 0.621 | 1175.39 | 1152.24 | 0.100 | 0.044 |
| 8 | 1169.75 | 97.739 | 1.927 | 1190.99 | 1138.89 | 0.282 | 0.241 |
| 9 | 1229.54 | 94.712 | 4.797 | 1215.30 | 1190.99 | 1.767 | 1.528 |
| 10 | 1370.33 | 98.04 | 3.77 | 1384.79 | 1315.36 | 0.841 | 0.888 |
| 11 | 1386.37 | 97.271 | 1.842 | 1418.85 | 1384.79 | 0.214 | 0.128 |
| 12 | 1613.28 | 97.688 | 1.467 | 1621.73 | 1607.88 | 0.188 | 0.087 |
| 13 | 1648.6 | 98.101 | 1.383 | 1687.41 | 1638.12 | 0.104 | 0.042 |
| 14 | 1651.77 | 96.78 | 1.223 | 1618.17 | 1673.81 | 0.142 | 0.148 |
| 15 | 1656.67 | 97.291 | 1.141 | 1667.24 | 1648.17 | 0.313 | 0.260 |
| 16 | 1736.71 | 97.037 | 1.139 | 1771.5 | 1667.24 | 0.480 | 0.500 |
| 17 | 2060.71 | 98.216 | 17.724 | 2091.57 | 2048.17 | 1.849 | 1.793 |
| 18 | 2061.2 | 98.381 | 1.267 | 2088.2 | 2061.5 | 0.213 | 0.12 |
| 19 | 2923.53 | 98.079 | 3.223 | 3004.89 | 2915.2 | 0.625 | 0.624 |
| 20 | 3289.37 | 98.887 | 3.888 | 3418.7 | 3054.89 | 4.838 | 3.898 |
| 21 | 3688.76 | 98.838 | 0.616 | 3648.18 | 3623.01 | 0.281 | 0.042 |

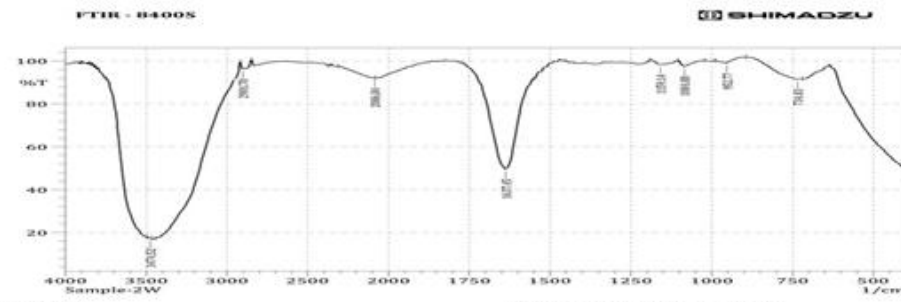
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| No. | Peak | Intensity | Corr. Intensity | Base (H) | Base (L) | Area | Corr. Area |
|-----|---------|-----------|-----------------|----------|----------|-------|------------|
| 1 | 362.46 | 98.921 | 0.11 | 445.17 | 437.61 | 0.023 | 0.005 |
| 2 | 628.97 | 98.265 | 0.602 | 684 | 686.68 | 0.077 | 0.049 |
| 3 | 679.88 | 97.184 | 1.628 | 619.11 | 586.68 | 0.21 | 0.104 |
| 4 | 827.41 | 97.681 | 2.212 | 887.88 | 819.11 | 0.176 | 0.178 |
| 5 | 798.68 | 98.984 | 0.788 | 763.76 | 693.76 | 0.335 | 0.187 |
| 6 | 819.9 | 98.42 | 0.688 | 873.89 | 779.19 | 0.148 | 0.149 |
| 7 | 889.77 | 98.227 | 0.887 | 883 | 873.89 | 1.11 | 0.887 |
| 8 | 1064.34 | 98.68 | 0.133 | 971.02 | 968.68 | 0.04 | 0.048 |
| 9 | 1090.68 | 98.441 | 0.481 | 1017.36 | 968.68 | 0.041 | 0.032 |
| 10 | 1038.6 | 98.988 | 0.338 | 1063.1 | 1017.36 | 0.037 | 0.037 |
| 11 | 1074.26 | 98.805 | 0.028 | 1089.55 | 1063.1 | 0.073 | 0.06 |
| 12 | 1116.74 | 98.088 | 0.848 | 1117.38 | 1104.17 | 0.88 | 0.811 |
| 13 | 1169.75 | 98.987 | 1.478 | 1196 | 1138.89 | 0.142 | 0.138 |
| 14 | 1215.97 | 97.688 | 2.428 | 1245.99 | 1196 | 0.333 | 0.358 |
| 15 | 1370.33 | 98.781 | 1.288 | 1384.79 | 1315.36 | 0.477 | 0.484 |
| 16 | 1386.37 | 98.884 | 1.185 | 1418.85 | 1384.79 | 0.205 | 0.087 |
| 17 | 1609.68 | 98.888 | 0.418 | 1618.82 | 1604.21 | 0.088 | 0.074 |
| 18 | 1611.12 | 97.838 | 1.411 | 1620.17 | 1608.89 | 0.217 | 0.204 |
| 19 | 1668.6 | 98.177 | 1.880 | 1687.41 | 1667.19 | 0.381 | 0.381 |
| 20 | 1669.74 | 98.987 | 0.888 | 1618.19 | 1669.37 | 0.197 | 0.184 |
| 21 | 1677 | 98.205 | 1.105 | 1688.21 | 1669.36 | 0.041 | 0.048 |
| 22 | 1746.88 | 98.487 | 1.714 | 1771.5 | 1726.88 | 0.81 | 0.84 |
| 23 | 2016.18 | 98.983 | 0.713 | 2038.4 | 2017.09 | 0.128 | 0.088 |
| 24 | 2974.21 | 98.883 | 1.584 | 3004.89 | 2982.89 | 0.1 | 0.081 |

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| No. | Peak | Intensity | Corr. Intensity | Base (H) | Base (L) | Area | Corr. Area |
|-----|---------|-----------|-----------------|----------|----------|--------|------------|
| 1 | 734.83 | 94.703 | 0.976 | 834.81 | 719.4 | 2.903 | 0.347 |
| 2 | 952.72 | 99.099 | 1.935 | 959.41 | 894.91 | 0.178 | 0.274 |
| 3 | 1104.46 | 97.684 | 0.746 | 1104.17 | 1021.24 | 0.2 | 0.447 |
| 4 | 1159.14 | 98.44 | 0.043 | 1189.03 | 1104.17 | 0.2 | 0.486 |
| 5 | 1637.46 | 99.688 | 0.05034 | 1770.83 | 1637.46 | 27.749 | 24.328 |
| 6 | 2066.84 | 92.164 | 0.07 | 2310.96 | 2063.89 | 0.369 | 0.144 |
| 7 | 2901.7 | 99.362 | 2.449 | 2917.12 | 2848.67 | 0.784 | 0.63 |
| 8 | 3474.82 | 17.382 | 0.353 | 3497.67 | 3465.84 | 23.998 | 0.177 |

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CONCLUSION

Based on the findings in this work, it can be concluded that the *Albizia* used as a body coolant and anti-dandruff agent. The dried leaf powder and also the four

leaf extracts of this plant were investigated for the evaluation of phytochemical profile. Among the tests, water extract showed high content of phyto-constituents. This study revealed that the presence of phytochemical constituents which enhance the medicinal value of the

plant. The fluorescent analysis and FT-IR spectrum studies of the powdered sample and the extracts were affords them as an interesting drug that plays an important role in the determination of quality and purity of the drug.

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