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Extraction of Flavanoids from *Albizia amara* for commercial application

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Abstract: *Albizia amara* commonly called as *Arapu* is a plant of Ayurvedic and traditional importance as indigenous tribes used different parts of the plant because of its medicinal values. The plant is widely used owing to its remarkable properties like reducing body temperature, etc. In addition the bark, pods and roots have been found to contain anti-cancer and anti-ulcer activities. In this study, the ethanolic extract of *Albizi aamara* plant was obtained and filtered followed by treatment with lead acetate and concentrated hydrochloric acid to obtain crude filtrate. Then the filtrate was subjected to lyophilisation. The final lyophilized product was treated with ethyl alcohol and subjected to flavanoid extraction by TLC and HPLC. Flavonol glycosides (quercitin and isoquercitin) present in albizia found to have properties such as body temperature reduction and control of hairfall. Then the extract was mixed with coconut oil and formulated as albizia hair oil possessing the properties of controlling hairfall and reducing dandruff.

Keywords: *Albizia amara*, Flavanoids, HPLC, Oil Formulation, Hair fall.

Dandruff is a major cosmetic problem that poses very great public health concern both in developed and developing countries (Gulfraz *et al.*, 2006). No population in any geographical region would have passed through freely without being affected by dandruff at some stage in their life (Chun*et al.*, 2005). The word dandruff (dandruff, dandriffe) is of Anglo-Saxon origin, a combination of "tan" meaning "tetter" and "drof" meaning "dirty" (Harborne *et al.*,, 1986). Dandruff is a chronic scalp condition characterized by scaling, itching and redness of the scalp. It occurs when scalp sheds epidermal cells in large clumps. The skin of scalp renews itself about once a month. Usually, scalp sheds dead cells in nearly invisible way, but sometimes cell turnover becomes unusually rapid and dead cells are shed as visible flakes called dandruff (Goswami *et al*., 2004).Plant-derived substances have recently become of great interest owing to their versatile applications in treating hair fall and dandruff . *Albizia Amara* which is known to be a traditional plant found in south east region. This is used for most of the medical treatments like hair fall, antidandruff. Albizia plants are widely used therapeutically in treating insomnia, irritability, wounds as antiseptic, antitubucular, antidysentric etc., Albizia leaf extract also contains flavonoids, alkaloids, trepenes, saponinsin which *Albizia Amara* contains two flavonol glycosides (quercitrin and iso quercitrin). Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, i.e. any part of the plant may contain active components (Ghafour et al., 2010). Plants contain different types of compounds such as resins, rubbers, gums, waxes, dyes, flavours, fragrances proteins, amino acids, bioactive peptides, phytohormones, sugars, flavonoids, and bio pesticides. Flavonoids are a group of about 4000 naturally occurring poly phenolic compounds, found universally in foods of plant origin. These are primarily recognized as the pigments responsible for the colours of leaves, especially in autumn. According to the IUPAC nomenclature, they can be classified into: flavonoids, (examples: quercetin, rutin), iso flavonoids, neo flavonoids. The three

flavonoids classes above are all ketone-containing compounds. Flavonoids are also important for human health. Like vitamins, these compounds are not produced endogenously by the body and must be supplied either through the diet or nutritional supplements (Winkel 2002).

Albizia Amara plant leaf is collected from forest region of south east and shade dried in room temperature, and then it is crushed to small pieces using pestle and mortar and finely powdered in an electric grinder. Flavonoids extraction will be done by immersing 100 gram of *Albizia amara* leaf powder in ethyl alcohol in room temperature for 24 hours using magnetic stirrer and the obtained extract will be further concentrated using vacuum rotary evaporator. The crude concentrated extract obtained from the vacuum rotary evaporator will then be filtered using what man No 1 papers and the process will be repeated again using the remaining residue with 300 ml ethyl alcohol in order to ensure the complete extraction during each time. The two filtrates that obtained on filtration with the whatman No1 filter and treated with 100 ml of 1% lead acetate for 4 hrs and allowing the mixture to undergo precipitation. The mixture will then be filtered once again after the precipitation process. To the obtained filtrate a mixture of 250 ml acetone and 30ml of concentrated hydrochloric acid (HCl) will be added and filtered. The resulting pellet will be finally subjected to lyophilization (Freeze- drying) at -50ºC under vacuum for 12 hrs of time period. The extract will be dissolved in ethyl alcohol. The extraction process will be done once again for 1hr and filtered to produce a red coloured filtrate .Finally, the filtrate will be placed in a clean and dry Petri dish away from light at room temperature until deep red brown coloured powder is obtained. The crude extract obtained from the flavonoid extraction process will be dissolved in ethanol and spotted on TLC plates (5x 20 cm) coated with silica gel. These plates are developed in chromatography chamber containing solvent mixture of (butanol, acetic acid and water (70:25:5, v/v/v) and let to stand for 1 hr. The developed plates will be air dried and visualized under ultraviolet light (UV light). The plates will be then placed in a chamber that has been saturated with ammonia vapours that has the ability to observe the colour of spot and plates will also be placed in a chamber saturated with I2 vapours to observe the colour of spot. Rf values will be calculated for the obtained isolated sample. HPLC analysis is done to find the presence of flavonoids in the *Albizia amara* solvent extract which contains partially of methanol and ethanol. Before injecting the sample system wash has been done using distilled water. Column lines are purged and A line is connected with methanol and B line is connected with Acetonitrile in the ratio of 7:3. Then the process is set for 30min at the flow rate of 0.5ml/min. solvent sample is filtered using membrane suction from the 20ul of the sample is injected to the column by using photo diode detector. Then the variable wavelength is detected at339nm.

The *Albizia amara* leaf extract obtained from the ethanolic extract (10g of *Albizia amara* leaf powder and 150ml of ethanol) followed by which the crude extract (6.8g) was obtained on lyophilizing (freeze drying) the earlier obtained extract has been confirmed to contain flavonoids, by the final results of qualitative and various quantitative tests such as Phyto chemical Screening ,TLC and HPLC. The appearance of yellow colour on addition of 1% KOH to the alcoholic extract obtained from *Albizia amara* leaf powder confirms the presence of the flavonoids in the sample.

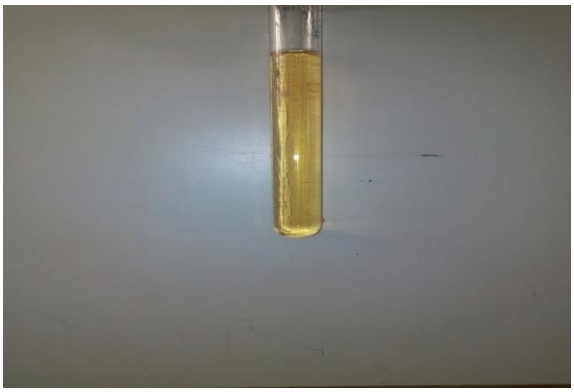

Figure 2: Qualilative analysis of Flavanoids

The separation of the flavonoid compounds present in the *Albzia amara* leaf extract has been sotted in the TLC plates that has been coated with the silica gel. Now these spots over the TLC plates of size 5x20 confirm the presence of the flavonoids.

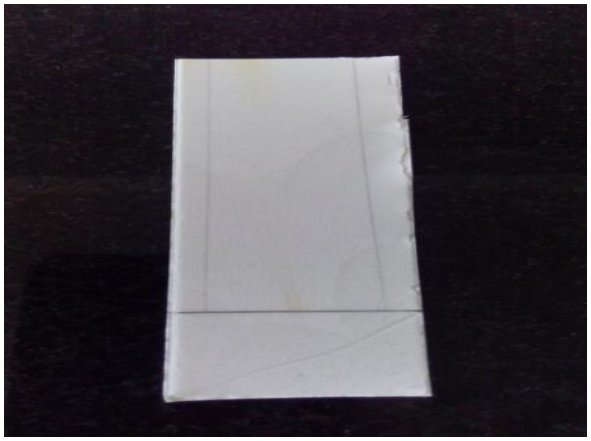

Figure 2: TLC Results

The High Performance Liquid Chromatography (HPLC) technique has been done to detect the presence and quantify the types of flavonoids present in the *Albizia amara* leaf extract. The HPLC graph shows the presence of three different types of flavonoids present in the crude sample such as rutin, isoquercitin and quercetin. The solvent system that has been used for the HPLC analysis of the crude sample is methanol and acetonitrile.

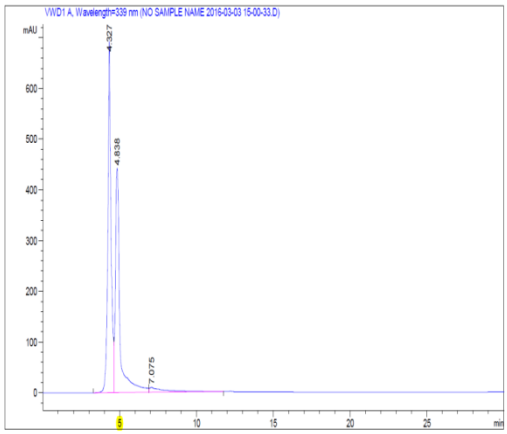

Figure 3: HPLC graph showing presence of flavanoids

From 10 g of *Albizia amara* leaf, 6.8g of crude extract was obtained.The amount of flavanoids present in the ethanolic crude extract of albizia amara leaves were found to be 3.42, 3.13 & 0.25g of rutin, isoquercitin and quercitin respectively. This indicates that flavanoids are active compounds and present in large amount. Finally the purified flavanoids are mixed with pure coconut oil and formulated as medicated oil. This formulated product can be used for commercial applications. It reduces the hiarfall and controls dandruff.

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