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Extraction, Identification And Characterisation Of Flavonoid From Naregamia Alata

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***Abstract:** Naregamia alata, a glorious medicinal plant used by the ethnic groups from time immemorial. A proper phytochemical and pharmacological study is the need for scientific validation, which shall open new pharmacological avenues for this magnificent plant. Such studies are crucial for clinical experimentation and in the development of novel drugs. Bioactive flavonoids have been isolated successfully from Naregamia alata, which were further confirmed the presence of flavonoids as per the results obtained by FT-IR and UV Spectroscopy.These data reveals the experimental plant contain flavonoids as the major active principle.*

***Index Terms:** Flavonoid, Hepatocellular damage, Hepatoprotective activity, Hepatotoxic, NaOH-HCl method, Naregamia alata, Phytochemical study, Soxhlet extraction.*

I. INTRODUCTION

Many plant foods in the ancient Indian ayurvedic medical system and in folk medicine have been described to have curative value. In recent years, there has been a surge of interest in plant foods as source of phytochemicals, which may have a useful role in the prevention of diseases such as cancer, diabetes, cardiovascular diseases, cataract, gallstones, etc. Recently, findings on the role of phytochemicals (peresent in fruits and vegetables) in the prevention of cancer and heart disease and the underlying mechanisms, have been lucidly discussed.

A wide range of 'non-nutrient' chemicals are present in foods with different biological activity and potency. Bioactive phytochemicals in food include antioxidants such as flavonoids and their major biological function include oxygen free radiclal quenching, inhibition of lipid peroxidation etc. Although these chemicals have been reported to be present in a wide range of plant foods, their efficacy in disease prevention has been clearly established only in the case of some chemicals or class of chemicals and studies on natural sources of these bioactive molecules are still scarce.

Flavonoids are polyphenols of plant origin that are among the most important compounds in human diet to their widespread distribution in foods and beverages. The flavonoids consist of a large group of low-molecular-weight polyphenolic substances that are diverse in chemical structure and characteristics. They are found in fruits, vegetables, tea, and wine and are an integral part of the human diet. The flavonoids exhibit a wide variety of biological activities, including antiviral, antibacterial, anti-inflammatory, anti-carcinogenic, and antioxidant actions. Flavonoids are capable of scavenging ROS and chelating metal ions such as iron and copper, which play vital roles in the initiation of free radical reactions. Several studies have shown the flavonoids to act as scanvengers of superoxide anions, singlet oxygen, hydroxyl radicals, and lipid peroxyl radicals. By donating hydrogen atoms to peroxyl radicals the flavonoids also terminate lipid peroxidation chain reaction. Hence, the flavonoids may protect tissues against oxygen free radicals and lipid peroxidation events that may be involved in pathological diseases such as CHD and cancer. They have been reported to have antiviral, anti-allergic, anti-platelet, anti-inflammatory, antitumor, antioxidant, antirombotic, hypolipidemic and hypoglycemic activities. A number of epidemiological studies implicate the role of flavonoids in reducing the risk of coronary heart disease.

Considerabe works has been done on plants to treat cancer, and some plant products have been marketed as anticancer drugs, based on traditional uses and scientific reports. These plants may promote host resistance against infection by re-stabilizing body equilibrium and conditioning the body tissues. Several reports describe that the anticancer activity of medicinal plants is due to the presence of antioxidants in them[9]. In fact, the medicinal plants are easily available, cheaper and possess no toxicity as compared to the modern (allopathic) drugs. Thus, the various combinations of the active components of these plants after isolation and identification can be made and have to be further assessed for their synergistic effects. The rate with which cancer is progressing, it seems to have an urgent and effective effort for making good health of humans as well as animals. On the basis of literature survey *Cyperus rotundus* (commonly known as muttana in Malayalam), *Flacourtia indica* (commonly known as karimulli in Malayalam) and *Naregamia alata* (commonly known as nilanarakam in Malayalam), were selected for the study.

Naregamia alata belong to family Meliaceae. It has been used in traditional medicines in India and elsewhere in the treatment of rheumatism, itch, malarial and chronic fevers, wounds, anaemia, enlarged spleen, ulcers, vitiated conditions of pitta and vata, halitosis, cough, asthma, splenomegaly, scabies, pruritis, dysentery, dyspepsia and catarrh. This plant has been reported to possess antifungal activity, antibacterial activity and antidysentery activities.

Flacourtia indica belong to family Flacourtiaceae. It is an indigenous medicinal plant widely distributed in Bangladesh and India. This plant has been reported as an effective remedy for the treatment of a variety of diseases. Fruits are used as appetizing and digestive, diuretic, in jaundice and enlarged spleen. Barks are used for the treatment of intermittent fever. Roots are used in nephritic colic and gum is used in cholera.

Cyperus rotundus belong to family Cyperaceae. The plant has been reported to possess various pharmacological activities such as diuretic, carminative, emmenagogue, anthelminthic, analgesic, anti-inflammatory, anti-dysenteric, antirheumatic activities. In addition the plant has been reported to possess antiplatelet activity, antibacterial activity, anticonvulsant and antioxidant activity, wound healing activity, lipid lowering activity, antispasmodic activity, antimutagenic and radical scavenging activity, antimalarial activities.

II. OBJECTIVES OF THE PRESENT STUDY

- ✓ Extraction of Flavonoids from *Naregamia alata*.
- ✓ Identification & Characterization of flavonoids.

III. REVIEW OF LITERATURE

A. INDIAN FOLK MEDICINE

Nature always stands as a golden mark to exemplify the outstanding phenomena of symbiosis. In the western world, as the people are becoming aware of the potency and side effect of synthetic drugs, there is an increasing interest in the natural product remedies with a basic approach towards the nature. Throughout the history of mankind, many infectious diseases have been treated with herbals. Today estimate that about 80% of people in developing countries still relays on traditional medicine based largely on species of plants and animals for their primary health care. Herbal medicines are currently in demand and their popularity is increasing day by day. About 500 plants with medicinal use are mentioned in ancient literature and around 800 plants have been used in indigenous systems of medicine.

Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. The world Health Organization (WHO) has listed 21,000 plants, which are used for medicinal purposes around the world. Among these 2500 species are in India, out of which 150 species are used commercially on a fairly large scale. India is the largest producer of medicinal herbs and is called as botanical garden of the world[17]. There are over 1.5 million practitioners of traditional medicinal system using medicinal plants in preventive, promotional and curative applications. There are very few medicinal herbs of commercial importance which are not found in India. The various indigenous systems such as Siddha, ayurveda, Unani and Allopathy use several plant species to treat different ailments. The use of herbal medicine becoming popular due to toxicity and side effects of allopathic medicines. This led to sudden increase in the number of herbal drug manufactures. Medicinal plants play an important role in the development of potent therapeutic agents.

B. METABOLITES: PRIMARY VS SECONDARY

Metabolites are compounds synthesized by plants for both essential functions, such as growth and development. Metabolites are organic compounds synthesized by organisms using enzyme-mediated chemical reactions called metabolic pathways. Primary metabolites have functions that are essential to growth and development and are therefore present in all plants.

Secondary metabolites are chemicals produced by plants for which no role has yet been found in growth, photosynthesis, reproduction, or other “primary’’ functions. These chemicals are extremely diverse; many thousands have been identified in several major classes. Each plant family, genus, and species produces a characteristic mix of these chemicals, and they can sometimes be used as taxonomic characters in classifying plants. Humans use some of these compounds as medicines, flavoring, or recreational drugs.

Secondary metabolites can be classified on the basis of chemical structure (for example, having rings, containing a sugar), composition (containing nitrogen or not), their solubility in various solvents, or the pathway by which there are synthesized (e.g., phenylpropanoid, which produces tannins). A simple classification includes three main groups: the terpenes (made from mevalonic acid, composed almost entirely of carbon and hydrogen), phenolics (made from simple sugars, containing benzene rings, hydrogen and oxygen), and nitrogen-containing compounds (extremely diverse, may also contain sulfur).

Many higher plants are major sources of natural products used as pharmaceuticals, agrochemicals, flavor and fragrance ingredients, food additives, and pesticides[20]. The search for new plant derived chemicals should thus be a priority in current and future efforts toward sustainable conservation and rational utilization of biodiversity[21]. In the search for alternatives to production of desirable medicinal compounds from plants, biotechnological approaches, specifically, plant tissue cultures, are found to have potential as a supplement to traditional agriculture in the industrial production of bioactive plant metabolites.

C. FLAVONOIDS

Phenolic compounds constitue one of the main classes of secondary metabolites. They display a large range of structures and they are responsible for the major organoleptic characteristic of plant-derived foods and beverages, particularly color and taste properties and they also contribute to the nutritional qualities of fruits and vegetables. The most and the most important natural pigments are carotenoids which are tetrapyrrole derivatives of naturally occurring phenolic compounds ubiquitously distributed in plant kingdom. Among these compounds, flavonoids constitute one of the most ubiquitous groups of all plant phenolics. So far, over 8,000 varieties of flavonoids have been identified.

Flavonoids are polyphenols of plant origin that are among the most important compounds in human diet due to their widespread distribution in beverages and foods products of a pant origin, such as fruit, vegetables, wine, tea, and cocoa. They can occur both in the free form (glycones) and as glycosides, and differ in their substituents (type, number and position) and in their insaturation. The most common classes are flavones, flavonols, flavonones, catechins, isoflavones and anthocyanidins, which account for around 80% of flavonoids. Figure 1 shows the basic C6-C3-C6 phenyl-benzopyran backbone. The position of the phenyl ring relative to the benzopyran moiety allows a broad separation of these compounds into flavonoids (2-phenyl-benzopyrans), isoflavonoids (3-phenyl-benzopyrans) and neoflavonoids (4-phenyl-benzopyrans). Division ino further groups is made on the basis of the central ring oxidation and on the presence of specific hydroxyl grous. Most common flavonoids are flavones (with a C2-C3 double bond and C4-oxo function), flavonols (flavones with a 3-OH group) and flavonones (flavones analogues buta C2-C3 single bond), and abundant isoflavonoids include isoflavones (the analogue of flavones). 4-arylcoumarin (a neoflavonoid with a C3-C4 double bond) and its reduced form, 3,4—dihydro-4-arylcoumarin, are the major neoflavonoids. Other natural compounds, such as chalcones and aurones also possess the C6-C3-C6 backbone, and are henceforth included in the general group of flavonoids.

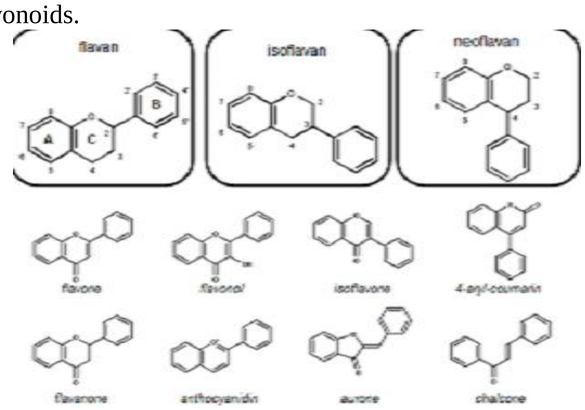

Figure 1: Structure of the structural backbones of the main flavonoid groups (flavan, isoflavan and neoflavan) and of relevant flavonoid classes. Atom numbering and ring nomenclature are also included

Flavonids are mainly water soluble compounds and can be extracted with 70% ethanol and remain in the aqueous layer, following partition of the extract with petroleum ether. Flavonoids are phenolic and hence change in colour when treated with base or with ammonia; thus they are easily detected on chromatograms or in solution.

D. HEPATOPROTECTIVE ACITVITY BY FLAVONOIDS

Plant drugs are relatively non-toxic, safe and even free from serious side effects. Liver damage is very common since liver has to detoxicate many toxic substances. Most of the hepatotoxic chemicals damage liver cells primarily by producing reactive species which form covalent bond with the lipids of the tissue. Liver cell injury caused by various toxic chemicals like certain anti-biotic, chemotherapeutic agents, carbon tetrachloride (CCl_4), thioacetamide (TAA) etc, excessive alcohol consumption and microbes is well studied. Due to excessive exposure to hazardous chemicals, sometimes the free radicals generated are so high that they overpower the natural defensive system leading to hepatic damage and cause jaundice, cirrhosis and fatty liver.

Carbon tetrachloride (CCl_4) is a well known hepatotoxin and exposure to this chemical is known to induce oxidative stress and causes liver injury by the formation of free radicals. The carbon tetrachloride mechanism begins with the trichloromethyl radical ($\cdot CCl_3$) by the action of the mixed function of cytochrome P-450 oxygenase system. This free radical, which is initially formed as unreactive, reacts very rapidly with oxygen to yield a highly reactive trichloromethyl peroxy radical ($\bullet OOCCl_3$). Both radicals are capable of binding to proteins, lipids or abstracting a hydrogen atom an unsaturated lipid, thus initiating lipid peroxydation. This process of lipid peroxidation can significantly damage hepatic plasma membranes. The increased levels of SGOT, SGPT, ALP and TB (Serum biochemical parameters) are conventional indicators of liver injury.

Increase in organ to body weight ratio is an indication of pathological alterations like inflammation and toxicity to the affected organ. This can be revealed in the biochemical parameters and histopathology also. Liver injury is often associated with a rise in serum AST (Aspartate transaminase), ALT (Alanine transaminase) and ALP (Alkaline phospatase) levels. Liver protective plants contain a variety of chemical constituents like phenols, coumarins, lignans, essential oil, monoterpenes, carotinoids, glycosides, flavonoids, organic acids, lipids, alkaloids and xanthenes. The hepatoprotective activity is probably due to the presence of flavonoids in all few herbal plants.

Flavonoids are polyphenolic compounds that occur ubiquitously in plants. The effect of these substances in human nutrition and well being are of considerable importance and extensive study. The beneficial effects of flavonoids on human health are mainly based on anti-inflammatory, anti-carcinogenic, anti-bacterial, hypolypidemic, hypoglycemic and antioxidant properties.

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E. FLAVONOIDS ARE NUTRACEUTICAL

"Nutraceutical" is a term coined in 1979 by Stephen DeFelice. It is defined "as a food or parts of food that provide medical or health benefits, including the prevention and treatment of disease". The major active nutraceutical ingredients in plants are flavonoids. As is typical for phenolic compounds, they can act as potent antioxidants and metal chelators. They also have long been recognized to possess anti-inflammatory, antiallergic, hepatoprotective, antithrombotic, antiviral and anticarcinogenic activities.

F. ANTIOXIDANT ACTIVITY

The best-described property of almost every group of flavonoids is their capacity to act as antioxidants. The flavones and catechins seem to be the most powerful flavonoids for protecting the body against reactive oxygen species (ROS). Body cells tissues are continuously threatened by the damage caused by free radicals and ROS which are produced during normal oxygen metabolism or are induced by exogeneous damage. Free radicals and ROS have been implicated in a large number of human diseases. Quercetin, kaempferol, morin, myricetin and rutin, by acting as antioxidants, exhibited beneficial effects such as anti-inflammatory, antiallergic, antiviral, as well as anticancer activity. The scavenging activity of flavonoids has been reported to be in the order: Myrcetin > quercetin > rhamnetin > morin > diosmetin > naringenin > apigenin > catechin > 5,7-dihydroxy-3',4',4'-trimethoxyflavone > robini > kaempferol > flavone.

G. ANTIMICROBIAL ACTIVITY

Flavonoids and esters of phenolic acids has also been investigated for their antibacterial, antifungal and antiviral activities.

H. ANTIBACTERIAL ACTIVITY

Antibacterial activity has been displayed by a number of flavonoids. Quercetin has been reported to completely inhibit the growth of *Staphylococcus aureus*. Most of the flavonones having no sugar moiety showed antimicrobial activities whereas none of the flavonols and flavonolignans tested showed inhibitory activity on microorganisms.

I. ANTIFUNGAL ACITIVITY

A number of flavonids isolated from the peelings of tangerine orange, when tested for fungistatic activity towards *Deuterophoma tracheiphila* were found to be active; nobiletin and langeritin exihibited strong and weak activities, respectively, while hesperidin could stimulate fungal growth slightly.

J. ANTIVIRAL ACTIVITY

Naturally occurring flavonoids with antiviral activity have been recognized since the 1940s but only recently have attempts been made to make synthetic modifications of natural compounds to improve antiviral activity. Quercetin, morin, rutin, dihydroquercetin (taxifolin), apigenin, catechin, and hesperidine have been reported to possess antiviral acivity against some of the 11 types of viruses.

K. ANTI-INFLAMMATORY ACTIVITY

The anti-inflammatory activity of flavonoids in many animals models have been reported. Flavones/flavonol glycosides as well as flavonoid aglycons have been reported to exert significant anti-inflammatory activity in the animal model of both acute and chronic inflammation when given orally or topically. Hesperidin, a citrus flavonoid, possess significant anti-inflammatory and analgesic effects. Recently apigenin, luteolin and quercetin have been reported to exhibit anti-inflammatory and antiallergic properties of flavonoids are the consequence of their inhibitory actions on arachidonic acid metabolism. Among flavones/flavonols kaempferol, quercetin, myricetin, fisetin were reported to possess LO and COX inhibitory activities.

L. ANTIDIABETIC EFFECTS

Flavonoids, especially qercetin, has been reported to possess antidiabetic activity. Vessal *eta al* reported that quercetin brings about the regeneration of pancreatic islets and proprably increases insulin release in strptozotocin-induced diabetic rats.

M. EXPERIMENTAL PLANT

✓ *NAREGAMIA ALATA*

Naregamia alata (commonly known as nilanarakam in Malayalam) belong to family Meliaceae. It has been used in traditional medicines in India and elsewhere in the treatment of rheumatism, itch, malarial and chronic fevers, wounds, anaemia, enlarged spleen, ulcers, vitiated conditions of pitta and vata, halitosis, cough, asthma, splenomegaly, scabies, pruritis, dysentery, dyspepsia and catarrh. This plant has been reported to possess antifungal activity, antibacterial activity and antidysentery activities.

Naregamia alata is a rare shrub, 15-45 cm tall, found mainly on rocky or grassy slopes in western peninsular India. Leaves are divided into 3 leaflets, each of which is wedge-shaped-obovate, quite entire, and stalkless. The leaf stalk is winged. White flowers, 2.5-5 cm long, arise solitary in leaf axils. Sepal five, 5 mm long. Petals are 5, very ong, strap-shaped, distinct, free from the stamen tube. Filaments are united into a long tube, inflated and spherical at the tip. Capsule is 3-cornered, 3-valved valves circular, 8mm long. Seeds 2 in each cell, curved.

The juice is used for the treatment of rat poison and is for doing 'naseum' in snake treatment. The roots of the plant is used is used for preparing pills and tablets.

Naregamia alata has been used in traditional medicines in India and elsewhere in the treatment of rheumatism, itch, malarial and chronic fevers, wounds, anaemia, enlarged spleen, ulcers, vitiated conditions of pitta and vata, halitosis, cough, asthma, splenomegaly, scabies, pruritis, dysentery,

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dyspepsia and catarrh. The in vitro antioxidant assay of the methanol extract was done by DPPH radical scavenging assay and showed moderate activity. Reducing power assay and super oxide radical scavenging also showed moderate activity. IC50 values of DPPH radical scavenging and super oxide radical scavenging are compared.

Hepatoprotective activity of *Naregamia alata* in animal model reported by Banerjee Soma *et al*. Protective effect of plant extract against carbon tetrachloride attributed the presence of flavanoid.

In another study M. Chinna Eswaraiah et al reports hepatoprotective activity of metanol extract from stem of Naregamia alata against carbon tetrachloride induced toxicity in rats.

Nalini Mabel *et al* showed antibacterial activity of the petroleum ether, chloroform and methanol extracts against gram positive and gram negative bacteria were done using disc diffusion method. The chloroform extract showed remarkable activity against the tested organisms, while the others showed only moderate activity. Antifungal activity also done and petroleum ether extract having highest activity than the others.

Figure 2: Naregamia alata

IV. MATERIALS AND METHODS

A. EXPERIMENTAL PLANT

The aerial parts of *Naregamia alata (Nilanarakam*) were collected from authenticated Ayurvedic medicinal plants shop in Payyanur, Kerala and authenticated.

B. EXTRACTION

The aerial parts of *Naregamia alata* were collected from the local market and identified. It was shade dried and crushed in to powdered from 80% Methanolic extracts were prepared by soxhlet extraction method. The fractions were washed using petroleum ether, discarded the organic fraction for removal of lipids and other fatty substances and the aqueous fraction was retained. The aqueous fraction was treated with diethyl ether, collected the organic fraction [FI]. The aqueous fraction [FII] was treated with ethyl acetate and retained organic [FIII] and aqueous [FIV] fractions. All the fractions collected [FI, FII and FIV] were dried in a fume hood and were stored in a dessicator. Confirmation test for the presence of flavonoids were done by using NaOH-HCl method.

C. TESTS FOR FLAVONOIDS

- ✓ Shinoda Test: To the extract, added 5 ml of 95% ethanol and few drops the concentrated HCl. To this solution 0.5g of magnesium turnings were added. Observance of pink coloration indicated the presence of flavonoids.
- ✓ A small quantity of the extract was heated with 10 ml of ethyl acetate in boiling water for 3 minutes and the mixture was filtered. The filtrate was then shaken with 1 ml of 1% aluminium chloride solution and observed for light yellow color. It indicated the presence of flavonoids.
- ✓ The yellow solution turns colorless on adding diluted NaOH and HCl, which confirmed the presence of flavonoids.

D. IDENTIFICATION OF FLAVONOIDS BY UV-VISIBLE SPECTROSCOPY

Diethyl ether and ethyl acetate of the plant were evaporated to dryness and dissolved in ethanol and subjected to UV-Visible spectroscopy. Absorption spectra was measured using Spectrometer (Shimadzu UV-1700 Spectrophotometer).

E. IDENTIFICATION OF FLAVONOIDS BY IR SPECTROSCOPY

The samples were mixed with 200 mg KBr (FT-IR grade) and pressed into a pellet. The sample pellet was placed into a sample holder and FT-IR spectra were recorded. (FT-IR spectrometer-8400S Shimadzu).

V. RESULTS AND DISCUSSIONS

A. UV-VISIBLE SPECTROSCOPY

UV-Visible spectral data (Fig 3,4) and standard data for spectral analysis of flavonoids (Table 1), it can be observed that the major component in the samples are mainly flavonoids.

a. SPECTRAL CHARACTERISTICS OF MAIN FLAVONOID CLASSES (TABLE 1)

Principal maxima (nm)	Indication
475-560	Anthocyanins
390-430	Aurones
365-390	Chalcones
350-390	Flavonols
250-270	Flavonols
330-350	Flavones and biflavonyls
250-270	Flavones and biflavonyls
275-290	Flavanones and flavanonols
255-265	Isoflavones

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(Ref. Phytochemical methods – a guide to modern techniques of plant analysis, JB Harborne, Third edition, page no 62).

Table 1

b. UV-VISIBLE SPECTRA'S OF DIFFERENT EXTRACTS OF NAREGAMIA ALATA

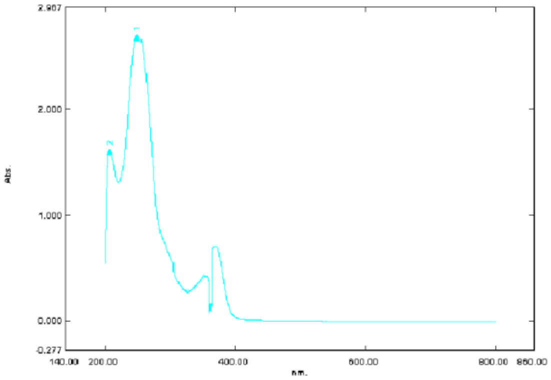

Figure 3: Diethyl ether extract

Wavelength nm.	Absorbance
249.00	2.683
207.50	1.598

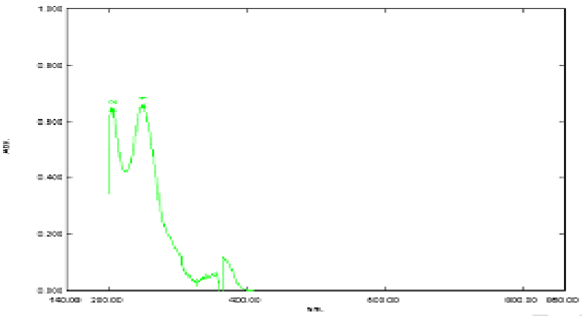

Figure 4: Ethyl acetate extract

Wavelength nm.	Absorbance
250.50	0.656
206.00	0.643

B. FT-IR SPECTROSCOPY

IR spectral data (Fig 5,6) and standard data for spectral analysis of flavonoids.

a. FT-IR SPECTRA'S OF DIFFERENT EXTRACTS OF NAREGAMIA ALATA

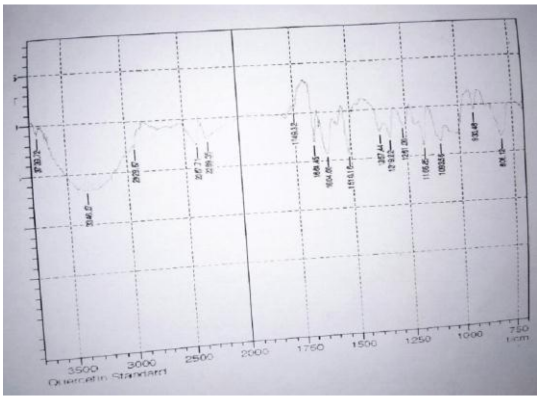

Figure 5: Quercetin standard

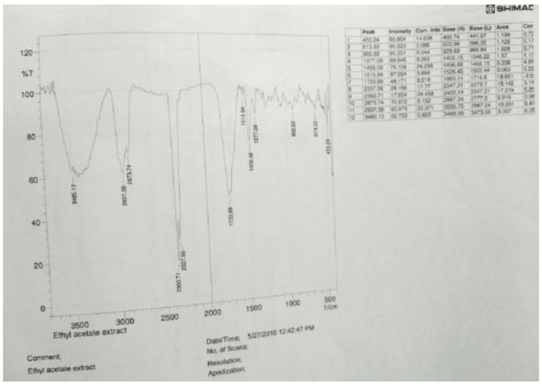

Figure 6: Ethyl acetate extract

b. FT-IR ANALYSIS

FT-IR was used for identifying the functional groups and thereby confirming the isolates from *Naregamia alata* were flavonoids. The functional groups present in the analyte will make vibrations of specific wave numbers. Along with the isolates a standard flavonoid (Quercetin) was also done[Fig 5]. The spectral analysis showed the following wave numbers with functional groups; 3400-3450 cm^{-1} indicating O-H stretching vibration of phenols, 2890-2940 cm^{-1} indicating C-H stretched alkanes, 1655-1665 cm^{-1} indicating C=O aryl ketone stretching vibrations and 1600-1615 cm^{-1} indicating C---C aromatic ring stretching vibrations[51]. The FT-IR fingerprinting provided the presence of OH stretched phenol, C=O aryl ketone and C---C aromatic ring in isolates from the plant, *Naregamia alata*, which confirms they were flavonoids.

VI. CONCLUSION AND SCOPE

Naregamia alata, a glorious medicinal plant used by the ethnic groups from time immemorial. A proper phytochemical and pharmacological study is the need for scientific validation, which shall open new pharmacological avenues for this magnificent plant. Such studies are crucial for clinical experimentation and in the development of novel drugs. Bioactive flavonoids have been isolated successfully from *Naregamia alata*, which were further confirmed the presence of flavonoids as per the results obtained by FT-IR and UV Spectroscopy. These data reveals the experimental plant contain flavonoids as the major active principle. Studies to show the Hepatoprotective activity of the flavonoids isolated from the experimental plant is under progress.

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