



Phytochemical Profiling In *Rivina Humilis. L* (Petiveriaceae)

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DOI : <https://doi.org/10.5281/zenodo.22085698>

ARTICLE DETAILS

Research Paper

Received: 29-06-2026

Accepted: 20-07-2026

Published: 20-08-2026

Keywords:

Rivina, *FT-IR*,
Phytochemical,
Pharmacognosy, *Physico-chemical*

ABSTRACT

Medicinal plants are widely used for curing different diseases since ancient times. Among many other curative properties, they have pain relieving, antibacterial, anti-inflammatory, and antidiabetic capabilities. According to the World Health Organization, herbal medicine is the major resource for primary health care for people in developing countries. In addition, a large number of modern medicines are either based on or derived from medicinal plants. The plant selected for the current study is *Rivina humilis* belonging to the family Petiveriaceae. *Rivina humilis L.* is a herbaceous plant commonly found in wastelands of gardens and plains. The leaves of the plant were shade dried and ground to fine powder and subjected to organoleptic, fluorescent and physico-chemical analysis. The leaf powder was subjected to Soxhlet extraction using methanol as the solvent. Phytochemical screening including Qualitative and Quantitative tests of the leaf extract was done according to the standard biochemical procedures. Functional group analysis of the methanol extract was conducted using FT-IR spectroscopy. The organoleptic analysis revealed characteristic colour, taste, odour and nature of the powder of *Rivina humilis*. In fluorescence analysis, on treatment with different solvents, colour changes could be noticed in the leaf powder. The results of the physico-chemical analysis provided important parameters in detection of improper handling of

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drugs. Qualitative and quantitative analysis in the plant confirmed the presence of many important phytochemicals such as tannins, saponins, flavonoids, terpenoids, alkaloids, simple phenolics, coumarins, quinones, acids, lignin and flavanols. The standard FT-IR studies revealed the presence of almost all groups including alcohols, phenols, amines amides, alkanes, alkenes, alkynes, nitrites, carboxyls, aromatics, aliphatic amines, carboxylic acids, esters, ethers, alkyl halides, sulphonates and aryl compounds. The present work would be useful in the synthesis of new drugs of pharmaceutical importance from *Rivina humilis*.

INTRODUCTION

In recent years, many researchers have focused on medicinal plants derived from natural products due to their wide range of pharmacological significance (Endale et al., 2015). Moreover, natural resources of vegetable origin represent an important source of drugs in the process of developing new pharmacologically active compounds (Dar et al., 2017). The World Health Organization established that, in many developing countries, traditional medicine plays an important role in meeting the primary health care needs of the population, and highlights specific types of this medicine (WHO, 2014). They play an important role in the development of the indigenous population of Ecuador (Raskin et al., 2003). These people live in The Andes, an area with different topographies climates, grounds and vegetation, that offers a great variety of medicinal plants (Mamedov and craker, 2012). Herein, 9865 plant species have been identified, being 64% of them indigenous to Ecuador. In Indian subcontinent various studies have presented initial documentations of medicinal plants and their utilization by indigenous tribes on the region (Sofowora, 2008). In some Asian and African countries, up to 80% of the population relies on traditional medicine for their primary health care needs.

The term “pharmacognosy” was used for the first time by the German physician Johann Adam Schmidt (1759-1809) in their published book “Grundriss der Materia Medica” in 1811 and by Anotheus Seydler in 1815 in a work titled Analecta Pharmacognostica. Originally—during the 19th century and the beginning of the 20th century “pharmacognosy” was used to define the branch of medicine or commodity sciences which deals with drugs in their crude, or unprepared form. Crude drugs are the dried, unprepared material of plant, animal or mineral origin, used for medicine.

As late as the beginning of the 20th century, the subject had developed mainly on the botanical side, being particularly concerned with the description and identification of drugs both in their whole state and in powder form. Such branches of pharmacognosy are still of fundamental importance, particularly for botanical products (widely available as dietary

supplements in the U.S., natural health products in Canada), quality control purposes, Pharmacopoeial protocols and related health regulatory frameworks. At the same time, development in other areas of research has enormously expanded the subject.

The advent of the 21st century brought a renaissance of pharmacognosy and its conventional botanical approach has been broadened up to molecular and metabolomic levels. Plant has the ability to synthesis a wide variety of phytochemical compounds as secondary metabolites which shows anti-inflammatory activity (Taylor et al., 2001). It is well known that medicinal materials comprise hundreds of components, and produce their curative effects through mutual effects of many ingredients, so the limited number of specific components cannot availably reflect the real qualities of herbal medicines. Therefore, an inexpensive and effective method to entirely monitor all the constituents of the medicinal materials and their corresponding extract products is required (Leu et al., 2006).

The WHO notes, however, that “inappropriate use of traditional medicines or practices can have negative or dangerous effects” and that “further research is needed to ascertain the efficacy and safety” of such practices and medicinal plants used by traditional medicine systems. As a result, the WHO has implemented a nine-year strategy to “support Member States in developing proactive policies and implementing action plans that will strengthen the role traditional medicine plays in keeping populations healthy. In the written record, the study of herbs dates back 5,000 years to the ancient Sumerians, who described well-established medicinal uses for plants. In Ancient Egyptian medicine, the Ebers papyrus from c. 1552 BC records a list of folk remedies and magical medical practices.

All plants produce chemical compounds as part of their normal metabolic activities. These phytochemicals are divided into primary metabolites such as sugars and fats, which are found in all plants; and secondary metabolites—compounds which are found in a smaller range of plants, serving more specific functions. Some secondary metabolites are toxins used by plants to deter predation and others are pheromones used to attract insects for pollination. It is these secondary metabolites and pigments that can have therapeutic actions in humans and which can be refined to produce drugs—examples are inulin from the roots of dahlias, quinine from the cinchona, morphine and codeine from the poppy, and digoxin from the foxglove. Plants synthesize a variety of phytochemicals, but most are derivatives A typical protocol to Isolate a

pure chemical agent from natural origin is bioassay-guided fractionation, meaning step-by-step separation of extracted components based on differences in their physicochemical properties, and assessing the biological activity, followed by next round of separation and assaying.

The plant selected for the present study is *Rivina humilis*. *Rivina humilis* is a species of flowering plant in the family Petiveriaceae. It was formerly placed in the pokeweed family, Phytolaccaceae. It can be found in the southern United States, the Caribbean, Central America, and tropical South America. Common names include pigeonberry, rougeplant, baby peppers, bloodberry and coralito. The specific epithet means “dwarfish” or “lowly” in Latin, referring to the plant’s short stature.

Leaves are simple, alternate, entire with a wavy margin. Blade is light green, thin textured, ovate to ovate-elliptic, supported by a long slender petiole, 10-50 mm long. The base is round and the apex is acuminate. Upper leaf surface is glabrous and lower surface is covered with few minute hairs along midrib and main veins. Inflorescence is racemes, terminal and axillary. The flowers (2-3 mm in diameter) is shortly pedicellate (2-3 mm). The 4 petals are obovate, white or greenish to rosy; four white stamens. Fruits are glossy, bright red berries, sub globose (ca. 4 mm in diameter with one seed per fruit. Seed ca. 2.5-3.5 mm in diameter, puberulent. Flowering from September to April and multiplies only by seed. Seeds are propagated through dispersal by birds (Umayamma, 2010).

This plant prefers damp, shady sites. Found along river margins and in shady areas in grassland, in villages, particularly in disturbed places and dump sites. It is a perennial plant and the main parts used as herbal medicine are the leaves. However, the juice contains Betaxanthin humilixanthin, may be made from the berries for consumption. The common names are inflammation weed, Pigeonberry, Blood berry, Rouge plant, Baby pepper. It may be used to treat wounds, bruises and cuts. This may be done by making a tea from the leaves and then used to wash the wounds, bruises and cuts and any other skin ailments.

The plant is native to tropical and sub-tropical America but introduced and naturalized in many parts of the world like Madagascar, tropical Southeast Asia, Malaysia, Indonesia.

Philippines, Singapore. Pakistan, Burma. Sri Lanka, Eastern Asia, West Indies and India (Birla, 2017). In India, it is known from Telangana (Present Report), West Bengal (Tiwari et al., 2011), Jharkhand (Shah and Seth, 2010.), Maharashtra, Bihar, Karnataka, Kerala, Meghalaya, Rajasthan, Tamil Nadu, Uttar Pradesh and Andhra Pradesh (Dahanukar et al., 2000). *Rivina* is found as invasive alien weed in New Caledonia, Queensland, Australia, Reunion, Norfolk Island, Fiji, Tonga. French Polynesia. Hawaii, Galapagos Islands and India). For pains including headaches, the tea from the leaf is a good remedy. Other uses include



clearing the fallopian tubes with the decoction and inflammation of the stomach, joints and just about anywhere on the body that is inflamed may be treated by this medicinal herb. This medicinal herb is used primarily in use of cleaning block tubes, infertility or any womb related problem, the herb is also use for menstruation flow problems.

Infrared spectroscopy (IR) has the potential to provide biochemical information without disturbing the biological sample. It is a powerful method for the study of molecular structure and intermolecular interaction in samples. Fourier transform infrared spectrometers, with their high signal-to-noise ratio and high precision in absorbance and wave number measurements have caused a resurgence of interest in the use of infrared spectra for identification of biomolecules.

Fourier transform infrared (FT-IR) spectrometry is a rapid, noninvasive, physico- chemical analytical, time saving technique that does not determine the concentration of individual metabolites but possibly provides a snapshot of the metabolic composition of a tissue at a given time , The analysis can be performed both on pure compounds and complex mixtures, without separation into individual components. It is more sensitive and selective than colorimetric methods and has played a vital role in pharmaceutical analysis in recent years. This technique determines the structure of unknown composition and the intensity of absorption spectra associated with molecular composition or content of the chemical group It measures predominantly the vibrations of bonds within chemical functional groups and generates a spectrum that can be regarded as a biochemical or metabolic “fingerprint” of the sample. By attaining IR spectra from plant samples, it might be possible to detect the minor changes of primary and secondary metabolites At present, particularly in photochemistry. FT-IR has been exercised to identify concrete structures of certain plant secondary metabolites and offers a rapid and non- destructive investigation to fingerprint herbal extracts or powders (Movasaghi et al., 2008).

The aim of the present study is a systematic study on the Pharmacognostic, chemical composition and functional groups elucidation to validate the ethno-medicinal and pharmaceutical potential of *Rivina humilis*.

MATERIALS AND METHODS

PLANT MATERIAL

The plant selected for the study is *Rivina humilis*. It is collected from Pallimon, Kollam district. The leaf part of plant is selected for the study

1. POWDER ANALYSIS

Fresh plant of *Rivina humilis* was collected in polythene bag. Dirt was removed from the collected material. It was shade dried and then powdered in an electric grinder and sieved with fine mesh sieve. The powder was then used for the organoleptic study and solvent extraction.

A. ORGANOLEPTIC STUDY

Organoleptic (literally “impression on the organs”) refers to the evaluation by means of the organs of sense and includes the macroscopic appearance of the plant material, its colour, odour,

and taste, occasionally the sound of ‘snap’ of its fracture and the ‘feel’ of the powder to the touch (Wozniak et al., 1997). The plant powder characteristics like the colour, odour, taste and nature were evaluated.

B. FLUORESCENCE ANALYSIS

The crude drug powder was treated as such with eight different reagents. The solvents used were water, hydrochloric acid, sulphuric acid, nitric acid, sodium hydroxide, acetic anhydride, methanol and acetone. Each solution was loaded on an activated thin gel layer slide and the fluorescence under normal light, short UV (256 nm) and long UV (365 nm) was observed (Chase and Pratt, 1949).

C.

P

PHYSICOCHEMICAL CHARACTERIZATION

Different physicochemical parameters were determined according to the official methods and guidelines on quality control for medicinal plant materials.

1. Loss on drying (Indian Pharmacopoeia, 1992)
2. Foaming index (WHO, 1992)
3. Swelling index (WHO, 1992)
4. Foreign matter (Indian Pharmacopoeia, 1996)
5. pH (Iqbal et al., 2010)

2. PHYTOCHEMICAL SCREENING

A. PREPARATION AND YIELD OF EXTRACT (INDIAN

PHARMACOPOEIA, 1996)

About 15 g of the powdered plant material was subjected to extraction by solvent apparatus using 100 ml methanol. The extract was concentrated under reduced pressure and preserved in refrigerator until further use. The percentage of the crude extract was determined using the following equation.

$$\text{Percentage yield (\%)} = (\text{Weight of the crude extract})/(\text{Weight of the sample}) \times 100$$

B. QUALITATIVE ANALYSIS

Different phytochemical constituents were tested using standard biochemical procedures (Harborne, 1973).

C. QUANTITATIVE ANALYSIS

1. **Determination of Alkaloids (Harborne, 1973)**
2. **Determination of flavonoids (Harborne and Williams, 2000)**

D. FT-IR SPECTROSCOPY

The methanol extract of *Axonopus compressus* was subjected to FT-IR analysis. FT-IR was performed using the spectrophotometer system. The characteristic peaks values were recorded and their functional groups were detected in the region 4000-400 cm^{-1} by employing standard potassium bromide (KBr) pellet technique (Kalsi, 2007). One milligram of methanol extract was mixed with 10mg of KBr (FT-IR grade) and pressed into a pellet. The pellet was immediately put into the sample folder and the FT-IR spectrum was recorded. The analysis was repeated twice for the spectrum confirmation. Using standard FT-IR charts, functional group of peaks were determined (Coates, 2000).

RESULTS**1. POWDER ANALYSIS****A) ORGANOLEPTIC STUDY**

1. colour :pale green
2. Smell : pungent smell3.taste : bitter 4.texture :soft

B) FLUORESCENCE ANALYSIS

The dry powder was subjected to fluorescence analysis with different reagents in normal light, short UV and long UV. The colour changes are summarized (Table 1)

Table 1: fluorescent analysis of *Rivina humilis*

Powder+reagent	Visible (400- 800nm)	UV short (254nm)	UV long (366nm)
Powder such as	Green	Black	Green
Powder+1N NaOH in H ₂ O	Black	Black	Green
Powder+1N HCL	Black	Black	Green
Powder+50% HNO ₃	Dark black	Black	Green
Powder+iodine solution	Dark black	Black	Green
Powder+acetic acid	Yellow	Black	Dark green
Powder+ 5% FeCl ₃	Dark brown	Black	Bright green
Powder+ Con. H ₂ SO ₄	Brown	Black	Bright green
Powder+Con.Hcl	Brown	Black	Green
Powder+95% alcohol	Yellow	Brown	Dark green

C. PHYSICOCHEMICAL CHARACTERIZATION

A total of five physicochemical parameters were evaluated in *Rivina humilis* (Table 2). The plant moisture content was reported in moderate amounts. Forming index was more than 100 ml. The pH was found to be 7.8 Foreign matter was found to be 1.86. Swelling index was not observed.

Table 2: Physicochemical character of *Rivina humilis*

Parameters	VALUES
Loss on drying	7.42%
Foaming index	>100ml
Swelling index	Nil

Foreign matter	1.86%
pH	7.8

II. PHYTOCHEMICAL SCREENING

A YIELD OF EXTRACT

The methanol extract was prepared by Solvent extraction. The yield of the methanol extract was 3.8%.

B. QUALITATIVE ANALYSIS

A total of 15 phytochemicals were qualitatively analyzed in methanol extract of the plant. Most of the compounds were present in the extract. Acids, gums and mucilage were absent in the plant extract. The phytochemical screening tests are provided (Table 3 and plate 2&3)

Table 3: phytochemicals tested in *Rivina humilis*

Sl No.	PHYTOCHEMICALS	PRESENT OR ABSENT
1	Tannin	+
2	Saponins	+
3	Flavonoids	+
4	Alkaloids	+
5	Terpenoids	+
6	Phlobatanins	+
7	Glycosides	+
8	Simple phenolics	+
9	Coumarins	+
10	Quinones	+
11	Acids	-
12	Flavanols	+
13	Lignin	+
14	Steroids	+
15	Gums and mucilage	-

C. QUANTITATIVE ANALYSIS

The quantitative analysis of two phytochemicals was done in the methanol extract of *Rivina humilis* (Table 4) by standard procedures. The amount of flavonoids was higher than the amount of alkaloids.

Table 4: Quantitative estimation of *Rivina humilis*

SL.NO	PHYTOCHEMICALS	AMOUNT(mg/g)
1	Alkaloids	22.6
2	Flavonoids	38.1

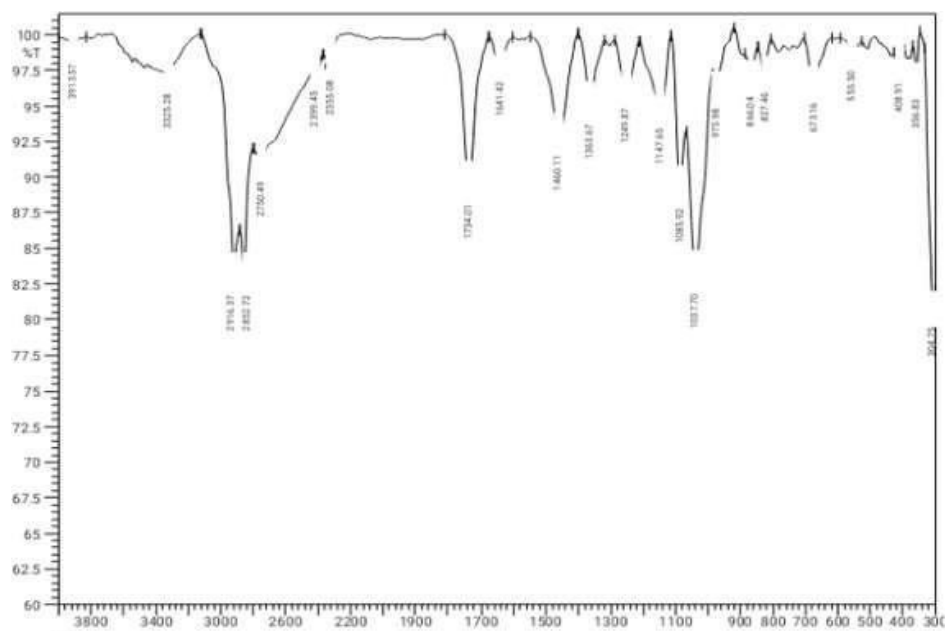
D. FUNCTIONAL GROUP ANALYSIS BY FT-IR SPECTROSCOPY

FT-IR spectrum was used to identify the functional groups of the active components in *Rivina humilis* based on the peak values in the region of infrared radiation. The FT-IR spectra (4000-400 cm⁻¹) of *Rivina humilis* methanol extract were registered and specific wave numbers and intensities considered. The results of FT-IR peak values and functional groups were represented (Table 5) and the FT-IR spectrum profile illustrated (Fig. 1).

Table 5: FTIR spectroscopy in *Rivina humilis* extract

SL. NO	PEAKS	FUNCTIONAL GROUPS
1	3913	Alcohol, phenol
2	3325.28	Amine, amide
3	2916.37	C-H stretch, alkane
4	2852.72	C-H alkanes
5	2750.49	Carboxylic acid
6	2399.45	Aldehydes
7	2355.08	Aldehydes
8	1734.01	Ester group
9	164.42	Aromatic C=C group
10	1460.11	Methylene group
11	1363.67	OH group alcohol

12	1249.87	C=S stretch Sulphur compound
13	1147.65	Ester, ether, alcohol
14	1085.92	Aliphatic amines Sulphur compound
15	1037.70	Aliphatic amines
16	975.98	C-H bent alkenes
17	866.04	S-O stretch, Sulphonates
18	827.46	C-Cl stretch, alkyl halide
19	673.16	Sulphur compound
20	555.50	Disulphides
21	408.91	Alkyl halides
22	356.83	Unknown
23	304.75	Unknown



FT-IR spectrum of *Rivina humilis*

DISCUSSION

The major source of herbs for local people and the herbal drug industry is wild source. Adulteration is often found in the raw materials when purchased from the market (Fazal et al., 2013). It is also reported that herbal industry and local residents face the problems of adulteration and substitution at a raw material stage (Ahmad et al., Citation 2010). As emphasized by WHO general regulations for conducting research and

investigation on traditional medicine, accurate identification is the major step that should be taken prior to guaranteeing the quality, safety, and effectiveness of traditional medicines (Fiaz and Qazi, 2015). It has been reported that misidentification of the medicinal plant species and adulteration of medicinal product are mostly the main obstacles for their marketing and consumption.

Authenticated raw material is the basic starting point in developing a botanical product. In addition, each step of harvest, storage, processing and formulation may dramatically alter the quality and consistency of final product. Therefore methods to ensure quality control in manufacturing and storage are requisite tools to ensure optimal efficacy and safety of these products. Furthermore, such controls are critical for the evaluation of pharmacological, toxicological or clinical studies involving botanical products. Authentication is especially useful in cases of drugs that are frequently substituted or adulterated with other varieties which are morphologically and chemically indistinguishable. Several herbal drugs in the market still cannot be identified or authenticated based on their morphological or histological characteristics. Use of wrong drugs may be ineffective or it may worsen the condition (Revathy et al., 2012).

The pharmacognostic techniques used for standardization of crude plant drugs involve morphological or organoleptic, microscopic, physical, chemical, chromatographic, spectrophotometric and biological evaluations (Kokate et al., 2012). The study of plant material with desired traits by means of the morphological characterization is an essential step for effective utilization of a plant and it is essential to search for solutions accessing all existing genetic variation both inside and outside the species (Verpoorte, 2000). The pharmacognostic standardization of *Rivina humilis* was carried out by the organoleptic analysis. It is from the impression on organs of senses (Kokate et al., 2012). The colour, taste, smell and texture of powder were analysed. *Rivina humilis* plant powder has fade green colour, pungent smell, bitter taste and soft texture. The pungent smell is one of the identifying organoleptic character of plant.

Fluorescence analysis can be used for the identification of the drug and for checking adulteration (Pande et al., 2018). The fluorescence colour is specific for each compound (Kala et al., 2011). The ultraviolet light produces fluorescence in several natural products (e.g. alkaloids like berberine), which do not noticeably fluoresce in daylight. If the substances themselves are not fluorescent, they may often be rehabilitated into fluorescent derivatives or decomposition products by applying diverse reagents. Therefore, crude drugs are often assessed qualitatively in this technique and it is an imperative parameter of pharmacological evaluation (Malathi et al., 2017).

The fluorescent study of *Rivina humilis* was attempted. The crude powder was treated with many chemical reagents and it showed characteristic inflorescence at 254nm to 366nm (Table 1) The fluorescence analysis

is adequately sensitive and enables the precise and accurate determination over a satisfactory concentration range without several time-consuming dilution steps prior to analysis of pharmaceutical samples (Garra 1964).

The physical or physicochemical parameters are useful in establishing quality profile of a crude drug and constitute an important component of qualitative evaluation (Kokate et al., 2012). They help in detecting adulteration or improper handling of drugs (Pande et al., 2018). The physico chemical parameters of *Rivina humilis* were analysed (Table 2). The drying process affects the quality of the medical herbal preparation by altering its chemical composition, active principal content and bioactivity (Jamal and Yousef 2018). However, this drying process is slow and allows the innate metabolic processes of the plant to continue after harvest, which may lead to an adverse effect on the medicinal plants, such as colour changes or loss of active ingredients (Gaurav et al., 2011). The moisture content of *Rivina humilis* were determined with reference to the air dried sample. The moisture content of the medicinal plants at the end of the herbs drying must be 10–14%. With this moisture content most of the drugs can be storage for a longer period of time without damage of the plants. Low moisture content discourages the growth of microbes such as mites, bacteria, fungi or yeast (Pande et al., 2018). The moisture content of *Rivina humilis* plant was 7.42% (Table 2).

The foaming index of *Rivina humilis* was found to be > 100ml indicating the presence of saponins in the plant. The swelling index was absent in the plant indicating the absence of gums and mucilage. The pH of 5% aqueous solution of the plant was 7.8 (Table 2). The presence or absence of foreign matter is another physicochemical parameter determine the purity of drug. Foreign matter includes dead pests (e.g. flies, mice etc.), hair, fingernails, band aids, coins or jewellery, bits of cleaning cloth, razor blades, nuts, bolts, plastic and cardboard, stones, twigs, glass, metal shards, etc. Animal matters such as insects and microbial contaminants, which can produce toxicity, are also among the potential contaminants of herbal medicines. Macroscopic examination can easily detect the presence of foreign matter (Ahmad et al., 2006). The plant powder has 1.86% foreign matter (Table 2). It is less than that of the permissible limit of 2% (WHO).

The phytochemical screening is a part of chemical evaluation. It is useful in detection of adulteration and establishing the chemical profile of the crude drug. The purity of the crude drug is ascertained by quantitative estimation of active chemical constituents present in them (Kokate et al., 2012). One of the major steps in phytochemical screening is extraction. This technique which involves the use of different solvents is commonly employed for separation of active substance from crude drug. Dried powdered

material is commonly used for extraction. The extraction can be performed by repeated maceration with agitation, percolation or by continuous extraction using Soxhlet extractor (Kokate et al., 2012).

The factors affecting the choice of the solvent are the quantity and diversity of compounds extracted, number of inhibitors extracted, rate of extraction, toxicity in a bioassay, ease of removal of solvent and potential health hazards (Eloff, 1998). Since the end product will contain traces of residual solvent, the solvent should be non-toxic and should not interfere with the bioassay (Tiwari et al., 2011). In the present study, powdered leaf part of *Rivina humilis* was subjected to solvent extraction using methanol.

The plant contains many chemical compounds that has therapeutic effects. A small portion of the dry extract was used for the phytochemical tests for compounds which include tannins, flavonoids, alkaloids, saponins, and steroids in accordance with the methods of (Jegade et al., 2011) little modifications. The search for effective and safe solvents for the extraction of bioactive compounds from plant materials is a challenging task due to the increasingly strict control requirements for solvent residues in the final products and the need for environmental friendliness. In addition to the used solvent, the extraction efficiency can be significantly influenced by the used extraction technique.

The methanolic extract of *Rivina humilis* was subjected to preliminary phytochemical screening. The chemicals present were tannins, saponins, flavonoids, terpenoids, alkaloids, simple phenolics, coumarins, quinones, phlobatanins, glycosides, flavanols, lignin and steroids. (Table 3). The quantitative estimation of alkaloids and flavonoids were carried out using standard methods. The compounds were found to be considerable amounts. (Table 4).

Alkaloids are produced by a large variety of organisms including fungi, plants, and animals (Stepp and Moerman, 2001); Matsuura and Fett-Netto. They can be purified from crude extracts of these organisms by acid-base extraction, or solvent extractions followed by silica-gel column chromatography (Chandrasekara and Shahidi, 2018). Alkaloids have a wide range of pharmacological activities including antimalarial (e.g. quinine), antiasthma (e.g. ephedrine), anticancer (Gurib-Fakim, 2006).

Flavonoids are secondary metabolites that are very abundant in plants, fruits, and seeds, responsible for the color, fragrance, and flavor characteristics. In plants, flavonoids perform many functions like regulating cell growth, attracting pollinators insects, and protecting against biotic and abiotic stresses (Cushnie and Lamb 2005). Tannins are water-soluble high molecular weight polyphenolic compounds with an ability to bind to proteins, sugars, and starches forming strong chemical complexes stable at pH 3.5 to 7 (Leal et al., 2000).

Saponins are compounds formed by triterpenoids or steroidal aglycones and a carbohydrate moiety by ester or ether linkages (Shi et al,2004; Gurib-Fakim, 2006). Saponins are a large family of amphiphilic glycosides of steroids and triterpenes found in plants and some marine organisms. By expressing a large diversity of structures on both sugar chains and aglycones, saponins exhibit a wide range of biological and pharmacological properties and serve as major active principles in folk medicines, especially in traditional Chinese medicines. Terpenoids represent a highly diverse group of natural products with wide applications. (Harborne 1973). Engineering approaches have been used to increase titers of many value-added terpenoids, such as farnesene, taxadiene, lycopene, and astaxanthin. Phytocannabinoid–terpenoid interactions could produce synergy with respect to treatment of pain, inflammation, depression, anxiety, addiction, epilepsy, cancer, and fungal and bacterial infections (Bently 2010).

Glycosides are a heterogeneous and diverse group of secondary metabolites with significant bioactivity, which include phenol, alcohol, or sulfur-related compounds (Gopi 2000). They are characterized by one or more sugar components combined with a bioactive nonsugar component (a lipophilic aglycone joined with a hydrophilic glycone unit) through a glycosidic bond (Namitha et al., 2011). Coumarins are well known for their antithrombotic, anti-inflammatory and vasodilatory activities. They also have anticoagulant, rodenticide, antiviral and antimicrobial effects. The quinones have limited pharmaceutical significance; however, they exhibit antimicrobial and cytotoxic properties (Gurib-Fakim, 2006; Cowan, 1999).

The pharmacological importance of lignin is antitumor, anti-allergic and anti-rheumatic activities (Gurib-Fakim, 2006). Terpenoids have antitumor and anticancer, anti-inflammatory, analgesic, antiviral and antibacterial activities. It is useful in the treatment of arthritis, skin allergies and gastric ulcers (Motaleb et al.,2011).

FTIR spectroscopy appears to be a good tool for analysing variations in the proportions of main organic compounds not only between species, but also between specimens (of the same species) growing in different environmental conditions. In FTIR analysis, the quality of the obtained spectra depends on the proper selection of sampling techniques and methods of sample preparation, where preparation of a sample also includes the method of material storage.

Infra-Red spectroscopy is basically a vibrational spectrum and involves the measurement of wavelength and intensity of absorption of mid infrared light by a sample. The principle value of this technique relates to the detection of the bands present in organic molecules. The FT-IR spectrum was used to identify the functional groups of active components based on the peak value in the region of infrared radiation. The sample was passed into the FT-IR and the functional groups of the components were separated based on



their peak ratio. The spectrum shows substantial overlap between the absorption spectra of various components, each band representing an overall overlap of some characteristic absorption peaks of functional groups in the sample.

The present FT-IR results confirmed the presence of alcohols, phenols, amines amides, alkanes, alkenes, alkynes, nitrites, carboxyls, aromatics, aliphatic amines, carboxylic acids, esters, ethers, sulphonates, alkyl halides and aryl compounds in the methanolic extract of *Rivina humilis*.

Aromatic amines are used in rubber, textile and dye industries. Many amine-rich proteins are bound to DNA and some neurotransmitters are amines including epinephrine, dopamine. They are used industrially for removing carbon dioxide and hydrogen sulphide from natural gas and refinery process streams. Alkynes have been isolated from a wide variety of plant species, fungi, corals, bacteria, marine sponges. Some acids like tartaric acid contain alkynes. Alkynes are highly bioactive nematocides. They possess antifungal, antitumor and antiviral properties. Phenols are of great importance as they protect the human body from the oxidative stress, which cause many diseases including cancer, cardiovascular problems and ageing (Robards et al.,1990). They are of great importance as cellular support material as they form the integral part of the cell wall structure. They exhibit antimicrobial, antihelmintic, antiapoptotic and anti-diarrheal activities (Cowan et al.,1999). They are antiseptic and reduce inflammation when taken externally. They regulate nitric oxide, decrease leukocyte immobilization and exhibit phytoestrogenic activity. They have been found to be useful in the preparation of some antimicrobial compounds such as Dettol and cresol.

The alkanes are found in the plant cuticle and epicuticular wax of many species. They protect the plant against water loss, prevent the leaching of important minerals by rain and protect against microorganisms and harmful insects (Baker EA et al.,1982). Alkenes are important in the manufacture of plastics, e.g. polythene and as fuel and illuminant. They serve as raw materials for the manufacture of alcohols and aldehydes. They are used for artificial ripening of fruits, a general anaesthetic, making poisonous mustard gas and ethylene-oxygen flame. Amines and amides are the main groups of protein synthesis.

Carboxylic acids are biologically very important in the formation of fat in the body and act as strong antibacterial agents. They serve as main pharmaceutical products in curing ulcers, jaundice, headache, fever, pain in liver, wound in cattle, treatment of edema and rheumatic joint pains. Aldehydes are used in the production of resins when combined with phenols (Reuss G et al., 2005). Esters in combination with volatile oils produce the pleasant aroma of fruits.

Pharmacognostic studies and phytochemical screening can serve as a basis for proper identification and investigation of a plant. Before any drug can be included in the pharmacopoeia, these standards must be established. The present study attempted to record the pharmacognostical standards and chemical constituents in *Rivina humilis*. The study also confirms the presence of many functional groups and phytochemical markers in the plant material. The results thus infer that this plant can be used for medicinal, cosmetics and pharmaceutical industries for the betterment of human health care.

SUMMARY AND CONCLUSION

The information transferred from traditional medicinal practices help the modern men to create and synthesize most effective clinical drugs. These practices are not based on the structural elucidation of bioactive components but formed a basic foundation for modern pharmacology. The present study dealt with the pharmacognostic standardization and phytochemical evaluation of *Rivina humilis*.

Pharmacognostic studies serve as a basis for proper identification and investigation of a plant. Standardization is a pre-requisite for the clinical evaluation of drugs. It must be done prior to the drug inclusion in pharmacopoeia. The current study helps us to elucidate the medicinal potential of *Rivina humilis*. The dried plant powder was subjected to powder analysis. Organoleptic analysis is a superficial study on morphology and will be useful in the proper identification, collection and investigation of the plant. Fluorescent analysis was carried out in the plant powder. The plant powder showed characteristics fluorescent pattern using different reagents. Fluorescence is an important phenomenon exhibited by various chemical constituents show fluorescence in the visible range in day light. UV light produces fluorescence in many natural products (e.g. alkaloids like berberine) which do not visibly fluoresce in day light. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives by applying different reagents. This phenomenon can be used in pharmacology for checking the adulteration.

The physical parameters are almost constant for a plant therefore these are helpful in setting standards for a crude drug. The physiochemical characters investigated will play a crucial role in detecting adulteration.

The plant extract studied could be an answer to the need for better therapeutic agents from natural resources which having more efficiency with less side effects in comparison to common synthetic therapeutic agents. The present study could successfully evolve important standardization parameters for the species. The data obtained would be of immense help in authenticating the plant and will serve as a standard for the quality control of any drug preparation containing this plant in the future. The phytochemical screening of the methanolic extract of *Axonopus compressus* detected the quality and quantity of chemical composition. It is

very important in the identification of useful bioactive components present in the sample. This helped us to confirm the presence of alkaloids, Tannins, saponins, flavanols, flavonoids etc. in the crude extract. Preliminary qualitative tests are useful in detection of bioactive compounds and may subsequently lead to drug discovery and development. Bioactive extracts should be standardized on the basis of phytochemicals.

The FT-IR studies uses the vibrations of the bonds in the compounds. The peaks obtained were evaluated and estimated for identifying the functional groups. It also authenticated the presence of amines, alkanes, alkenes, alkynes, amides, acids etc. The results of FT-IR study revealed phytochemical markers for the identification of *Rivina humilis*. The FT-IR spectrum reflects the panorama of chemical constituents in complex systems thereby validating and identifying the mix-substance systems such as traditional and herbal medicines. The data generated from the present investigation provides evidence for the isolation of phytochemicals thereby laying the foundation for drug development.

ACKNOWLEDGEMENT

The authors gratefully acknowledge Dr. Anjana J., Principal, N.S.S College, Pandalam, Pathanamthitta for the invaluable support. The authors would like to thank Dr. Sandhya P., HOD, Research and Post Graduate Department of Botany, N.S.S College, Pandalam, Pathanamthitta for providing the required facilities to complete the work.

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