

Research on Analytical Activity Cordia Gharaf

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ABSTRACT:

Herbal Medicine sometimes referred to as Herbalism or Botanical Medicine is the use of herbs for their therapeutic or medicinal value. Herb plants produce and contain a variety of chemical substances that act upon the body. The World Health Organization (WHO) estimated that 80% of the population of developing countries relies on traditional medicines, mostly plant drugs, for their primary health care needs. When the whole plant is used rather than the extracted constituents, the different parts interact, producing a greater therapeutic effect than the equivalent dosage of isolated active constituents. Cordia is a genus of flowering plants in the borage family, Boraginaceae. It contains about 300 species of shrubs and trees that are found worldwide, mostly in warmer regions. Many of the species are commonly called man jack, while bocote may refer to several Central American species in Spanish. Antioxidants are radical scavengers, which protect the human body against free Radicals. They may offer resistance against oxidative stress by scavenging free Radicals, inhibiting lipid peroxidation and by other mechanisms and thus prevent Disease (Miller et al. 1997). They are used to counteract deleterious effects of free Radicals in oxidative stress. In the modern medicine, plants occupy significant berth as raw materials for some important drug preparations. The traditional Indian medicinal plants act as antiradicals and DNA cleavage protectors.

Key Words: DNA, Cordia, Antioxidants, scavenging, peroxidation etc.

I. INTRODUCTION

Herbal Medicine sometimes referred to as Herbalism or Botanical Medicine is the use of herbs for their therapeutic or medicinal value. An herb is a plant or plant part valued for its medicinal, aromatic or savory qualities. Herb plants produce and contain a variety of chemical substances that act upon the body.

The World Health Organization (WHO) estimated that 80% of the population of developing countries relies on traditional medicines, mostly plant drugs, for their primary health care needs. Also, modern pharmacopoeia still contains at least 25% drugs derived from plants and many others which are synthetic analogues built on prototype compounds isolated from plants. Demand for medicinal plant is increasing in both developing and developed countries due to growing recognition of natural products, being non-narcotic, having no side-effects, easily available at affordable prices and sometime the only source of health care available to the poor. Medicinal plant sector has traditionally occupied an important position in the socio cultural, spiritual and medicinal arena of rural and tribal lives of India.

Medicinal plants as a group comprise approximately 8000 species and account for around 50% of all the higher flowering plant species of India. Millions of rural households use medicinal plants in a self-help mode. Over one and a half million practitioners of the Indian System of Medicine in the oral and codified streams use medicinal plants in preventive, promotive and curative applications. There are estimated to be over 7800 manufacturing units in India. In recent years, the growing demand for herbal product has led to a quantum jump in volume of plant materials

traded within and across the countries. According to an all India ethno biological survey carried out by the Ministry of Environment & Forests, Government of India, there are over 8000 species of plants being used by the people of India.

Herbal medicine is the oldest form of healthcare known to mankind. Herbs had been used by all cultures throughout history. It was an integral part of the development of modern civilization. Primitive man observed and appreciated the great diversity of plants available to him. The plants provided food, clothing, shelter, and medicine. Much of the medicinal use of plants seems to have been developed through observations of wild animals, and by trial and error. As time went on, each tribe added the medicinal power of herbs in their area to its knowledgebase. They methodically collected information on herbs and developed well-defined herbal pharmacopoeias. Many drugs commonly used today are of herbal origin. Indeed, about 25 percent of the prescription drugs dispensed in the United States contain at least one active ingredient derived from plant material. Some are made from plant extracts; others are synthesized to mimic a natural plant compound.

Popularity of Herbal Medicine

The traditional medicine is largely gaining popularity over allopathic medicine because of the following reasons favorable to it

- Risking costs of medical care.

- As these are from natural origin, so free from side effects.

- Goes to root cause and removes it, so the disease does not occur again.

- Freedom from approaching various specialists.

- Cure for many obstinate diseases.

- Easy availability of drug from natural sources.

- Cure of diseases used by life style changes and social pathology.

- Problems with modern drugs

- High cost and long-time taken in development of new drug.

- Toxicity

- Non-renewable source of basic raw materials. Most synthetic drugs utilize

- Fossil resources like petrochemicals.

Advantages of Plant Based Drugs

- Long history of use and better patient tolerance as well as public acceptance.

- Renewable source.

- Cultivation and processing-environmental friendly.

- Local availability, especially in developing countries

- Several important reason outcomes.

Plants constitute to be a major source of new lead generation.

Organizations like the World Health Organization (WHO) and United Nations Children Educational Fund (UNICEF) are very much interested in plants to be used for the treatment of various diseases of children. In the “Inter Country Meeting on Use of Medicinal Plants at Primary Health Care Level” held in Kuwait, the representatives of different countries, all using herbal medicine, identified twelve conditions encountered at the primary health level for which herbal medicines could be used. The conditions are as listed below:

Based on the strong traditional knowledge on the use of plants and therapeutic agents, a rational approach is being developed to use the medicinal plants as lead for the discovery of active molecules with one of the largest reservoirs of bio resources. The criteria for the selection of plants for herbal drug research for various human ailments are as follows:

- Use of medicinal plants for therapeutic purposes in countries outside the region.

- To evaluate plants with possible therapeutic effects, the first World Congress of Clinical Pharmacology and Therapeutics was held in London in 1980. The traditional approach on herbal drug research consists of the following steps.
- Identification of the plant reportedly in use
- Collection of the plant

- Transport of the plant to the research laboratory

- Storage of the plant

- Preparation of the extracts.

- Toxicity studies of the plant extracts in animals.

- Evaluation of therapeutic efficacy of the extract in animal models.

- Identification of the extracts which is having more activities.

- Further fractionation of the active molecule.

- Structural elucidation of the bio-active molecule.

- Synthesis of bio-active molecule.

Requirements and method for research and evaluation is complex. For example, it can be difficult to assess the quality of finished herbal medicine products depend upon on the quality of their source material (which can include hundreds of natural constitute) and how elements are handled through production process. WHO & it members state cooperate to promote the use of traditional medicine for health care. The collaboration aims to:

- Support and integrate traditional medicine into national health system in combination with national policy and regulations for product, practice and providers to ensure that safety and quality.

Ensure the use of safe, effective and quality products and practices, based available evidence.

Extraction

Extraction is the crucial first step in the analysis of medicinal plants, because it is necessary to extract the desired chemical components from the plant materials for further separation and characterization. The basic operation included steps, such as pre-washing, drying of plant materials or freeze drying, grinding to obtain a homogenous sample and often improving the kinetics of analytic extraction and also increasing the contact of sample surface with the solvent system. Proper actions must be taken to assure that potential active constituents are not lost, distorted or destroyed during the preparation of the extract from plant samples. If the plant was selected on the basis of traditional uses then it is needed to prepare the extract as described by the traditional healer in order to mimic as closely as possible the traditional 'herbal' drug. The selection of solvent system largely depends on the specific nature of the bioactive compound being targeted.

Identification and characterization

Due to the fact that plant extracts usually occur as a combination of various type of bioactive compounds or phytochemicals with different polarities, their separation still remains a big challenge for the process of identification and characterization of bioactive compounds. It is a common practice in isolation of these bioactive compounds that a number of different separation techniques such as TLC, column chromatography, flash chromatography, Sephadex chromatography and HPLC, should be used to obtain pure compounds. The pure compounds are then used for the determination of structure and biological activity. Beside that, non-chromatographic techniques such as immunoassay, which use monoclonal antibodies (MAbs), phytochemical screening assay, Fourier-transform infrared spectroscopy (FTIR), can also be used to obtain and facilitate the identification of the bioactive compounds.

Chromatographic techniques

Thin-layer chromatography

TLC is a simple, quick, and inexpensive procedure that gives the researcher a quick answer as to how many components are in a mixture. TLC is also used to support the identity of a compound in a mixture when the R_f of a compound is compared with the R_f of a known compound. Additional tests involve the spraying of phytochemical screening reagents, which cause

color changes according to the phytochemicals existing in a plants extract; or by viewing the plate under the UV light. This has also been used for confirmation of purity and identity of isolated compounds.

Bio-autography is a useful technique to determine bioactive compound with antimicrobial activity from plant extract. TLC bioautographic methods combine chromatographic separation and in situ activity determination facilitating the localization and target-directed isolation of active constituents in a mixture. Traditionally, bioautographic technique has used the growth inhibition of microorganisms to detect anti-microbial components of extracts chromatographed on a TLC layer. This methodology has been considered as the most efficacious assay for the detection of anti-microbial compounds (Shahverdi, 2007). Bio-autography localizes antimicrobial activity on a chromatogram using three approaches: (i) direct bio-autography, where the micro-organism grows directly on the thin-layer chromatographic (TLC) plate, (ii) contact bio-autography, where the antimicrobial compounds are transferred from the TLC plate to an inoculated agar plate through direct contact and (iii) agar overlay bio-autography, where a seeded agar medium is applied directly onto the TLC plate. The inhibition zones produced on TLC plates by one of the above bioautographic technique will be use to visualize the position of the bioactive compound with antimicrobial activity in the TLC fingerprint with reference to R_f values .

UV-VIS Spectroscopy

UV-visible spectrometers can be used to measure the absorbance of ultra violet or visible light by a sample, either at a single wavelength or perform a scan over a range in the spectrum. The UV region ranges from 190 to 400 nm and the visible region from 400 to 800 nm. The technique can be used both quantitatively and qualitatively.

The light source (a combination of tungsten/halogen and deuterium lamps) provides the visible and near ultraviolet radiation covering the 200 – 800 nm. The output from the light source is focused onto the diffraction grating which splits the incoming light into its component colours of different wavelengths, like a prism (shown below) but more efficiently.

High performance liquid chromatography

High performance liquid chromatography (HPLC) is a versatile, robust, and widely used technique for the isolation of natural products (Cannell, 1998). Currently, this technique is

gaining popularity among various analytical techniques as the main choice for fingerprinting study for the quality control of herbal plants (Fan et al., 2006). Natural products are frequently isolated following the evaluation of a relatively crude extract in a biological assay in order to fully characterize the active entity. The biologically active entity is often present only as minor component in the extract and the resolving power of HPLC is ideally suited to the rapid processing of such multicomponent samples on both an analytical and preparative scale.

Fourier-transform infrared spectroscopy (FTIR)

FTIR has proven to be a valuable tool for the characterization and identification of compounds or functional groups (chemical bonds) present in an unknown mixture of plants extract (Eberhardt et al., 2007; Hazra et al., 2007). In addition, FTIR spectra of pure compounds are usually so unique that they are like a molecular "fingerprint". For most common plant compounds, the spectrum of an unknown compound can be identified by comparison to a library of known compounds. Samples for FTIR can be prepared in a number of ways. For liquid samples, the easiest is to place one drop of sample between two plates of sodium chloride.

Non-chromatographic techniques

Phytochemical screening assay

Phytochemicals are chemicals derived from plants and the term is often used to describe the large number of secondary metabolic compounds found in plants. Phytochemical screening assay is a simple, quick, and inexpensive procedure that gives the researcher a quick answer to the various types of phytochemicals in a mixture and an important tool in bioactive compound analyses. A brief summary of the experimental procedures for the various phytochemical screening methods for the secondary metabolites is shown in .
Cordia

Cordia is a genus of flowering plants in the borage family, Boraginaceae. It contains about 300 species of shrubs and trees that are found worldwide, mostly in warmer regions. Many of the species are commonly called manjack, while bocote may refer to several Central American species in Spanish. The generic name honours German botanist and pharmacist Valerius Cordus. Like most other Boraginaceae, the majority have trichomes (hairs) on the leaves.

Cordia Gharaf

Cordia Gharaf belongs to the family Boraginaceae. It includes a variety of shrubs, Trees

and herbs. It is a Small tree, to 10 m high, bark grey or brownish-grey with deep longitudinal furrows, smooth, peeling off in thin linear strips; young branches brown tomentose.

Cordia Macleodii

Cordia macleodii belongs to the family Boraginaceae. It includes a variety of shrubs, Trees, and herbs, totalling about 2,000 species in 100 genera found worldwide. A Number of familiar plants belong to this family. In India, the fruits of local species are used as a vegetable, raw, cooked, or pickled, and are known by many names, including lasora in Hindi. One such species is Cordia dichotoma (fragrant manjack), This is called gunda in Hindi and lasura in Nepali. Cordia macleodii (Griff) Hook. F. & Thomas is commonly known as Dahiphalas or Dahivan in Hindi and Bhoti in Marathi.

Antioxidant activity

Antioxidants are radical scavengers, which protect the human body against free Radicals. They may offer resistance against oxidative stress by scavenging free Radicals, inhibiting lipid peroxidation and by other mechanisms and thus prevent Disease (Miller et al. 1997). They are used to counteract deleterious effects of free Radicals in oxidative stress.

Antimicrobial activity

Infections diseases are the second leading cause of death world wide. The emergence Of multidrug-resistant bacteria has created a situation in which there are few or no Treatment options for infections with certain microorganisms (Wenzel et al., 2000). Fungal infections remain a significant cause of morbidity and mortality despite Advances in medicine and the emergence of new antifungal agents. Many plants have been reported to have antifungal activity.

Hepatoprotective activity

In the modern medicine, plants occupy significant berth as raw materials for some important drug preparations. The traditional Indian medicinal plants act as antiradicals and DNA cleavage protectors. Moringa oleifera, Eclipt alba, Phyllanthus niruri, Picrorhiza kurroa etc. possess hepatoprotective property against toxins and drugs induced hepatotoxicity.

Anti-inflammatory activity

Inflammation, though a defense mechanism, owing to its tendency to induce, maintain and even aggravates several diseases, has always been a matter of concern for the physicians. It is a complex pathophysiological response of tissue to injury leading to local accumulation of

plasmic fluid and blood cells. Although scores of anti-inflammatory agents are available, the search for better anti-inflammatory agents continues to avert the side effects of the available agents.

Analgesic activity

The process of nociception describes the normal processing of pain and the responses to noxious stimuli that are damaging or potentially damaging to normal tissue. Chronic pain can be a major problem for some people and affect their quality of life. It can be caused by alterations in nociception, injury or disease and may result from current or past damage to the peripheral nervous system (PNS), CNS, or may have no organic cause.

II. MATERIALS AND METHODS

Authentication

The barks of *CordiaGharafbark* were collected from local area of Otur (Pune, Maharashtra). It is then authenticated by Dr. P.G. Diwakar (Joint Director of Botanical Survey of India Western regional Centre Koregaon Road, Pune, Maharashtra; voucher No.COGBIJ3

Pharmacognostic study of *CordiaGharafbark*

These bark pieces were cleaned and shade dried at room temperature then subjected to physical evaluation with different parameters then these selected bark pieces were subjected to size reduction to get coarse powder by using mechanical grinder to get a desired particle size and store in a well closed air tight jars. This uniform powder was subjected to standardisation with different parameters

Macroscopic study

For these study fresh as well as dried *CordiaGharafbark* was taken and observed for its morphological characters.

Microscopic study

For this purpose transverse section of freshly obtained *CordiaGharafbark* was taken and observed under compound microscope. Their histological characters were observed and were noted down.

Total ash

Placed about 2-4 gm. of ground air dried material accurately like weighed in a previously ignited and tarred crucible spread the material in an even layer and ignite it by gradually increasing the heat to 500 to 600 0c until it is white, indicating the absence of carbon. Cooled in a desiccator and weighed. If carbon free ash cannot be obtained in this manner, cool the crucible and moistened the residue with about 2 ml of water or saturated solution of ammonium nitrate reagent dry on a

water bath, then on a hot plate and ignite to constant weight.

Acid insoluble ash

To the crucible containing the total ash add 25 ml of hydrochloric acid (~70g/1TS), cover with a watch glass and boil gently for five minutes. Rinse the watch glass with 5 ml of hot water and add this liquid to the Crucible. Collect the insoluble matter on an ash less filter paper and wash with hot water until the filtrate is neutral. Transfer the filter paper containing the insoluble matter to the original crucible, dry on a hot plate and ignite to constant weight.

Water soluble ash

To the crucible containing the total ash, add 25 ml of water and boil for five minutes. Collect the insoluble matter in a sintered glass crucible or on an ash less filter paper. Wash with hot water and ignite in a crucible for 15 minutes at a temperature not exceeding 450 0c. Subtract the weight of this residue in mg from the weight of total ash. Calculate the content of water soluble ash in mg per gram of air dried material.

Extractive values

These are useful for the evaluation of crude drug. It gives idea about the nature of the chemical constitution present in a crude drug and is useful for the estimation of specific constitute soluble in that particular solvent used for the extraction.

Phytochemical Screening

Extraction of drug:

CordiaGharafbarks were dried and powdered. The powder obtained was weighed, and then used for extraction. The powder was extracted with methanol (40_60⁰C) for 8 hrs. Till exhaust by Soxhlet extraction method. The resulting extract was concentrated transferred in petri dishes and allowed to dry in hot air oven at 60 0c for 24 hrs. The amount of extract were weighed and stored in an airtight self-sellable pouch with aluminium foil wrapping. The percentage yield, colours and nature of the extract was



Figure No. 7.1 Extraction of CordiaGharafbark by Soxhlet Apparatus

Preliminary phytochemical test:

The methanolic extract of the crude drug were subjected to qualitative chemical test in order to identify class compound present as per procedures K.R. Khandelwal, 2005. Following chemical tests were performed for identifying the different chemical constituents present in the extract.

Test for sterol

Salkowaski test

A quantity of 5 mg of the residue of each extract was taken in a test tube having 2 ml of chloroform. To this concentrated sulphuric acid 2 ml was added from the side of the test tube and was shaken for 5 min. Development of red colour in the chloroform layers indicated the presence of sterol.

Test for Alkaloids

Dragendorff's test

Dragendorff's reagent was sprayed on Whitman No.11 filter paper and paper was dried. The previously obtained test filtrate after basification with dilute ammonia was extracted with chloroform. The chloroform extract was applied on the filter paper, impregnated with Dragendorff's reagent, with the help of a capillary tube. Development of an orange red colour on the paper indicated the presence of alkaloids.

Wagner's test

When 2 drop of Wagner's reagent was added to the filtrate, formation of brown precipitate indicates the presence of alkaloids.

Test for Saponins

Foam test

A quantity of 5 mg of the residue was taken in a test tube and shake vigorously with a small amount of water .formation of stable, characteristics honeycombs like froth indicated the presence of Saponins.

Molisch's test

A quantity of 5 mg of the residue was placed in a test tube containing .5 ml of water, and it was mixed with 2 drop of Molish reagent. To this solution, 1 ml of concentrated sulphuric acid was added from the side of the inclined test tube so that acid formed layer beneath the aqueous solution without mixing with it. Presence of red brown ring at the junction of two layers indicated the presence of sugars.

Test for Tannins

Ferric chloride test

Ferric chloride solution (2_3 drops) was added to 1 ml of the above filtrate. Presence of dark green or deep blue colour indicated the presence of tannins.

Test for protein

Biuret test

A quantity of 5 mg of the residue was taken in water and 1 ml of 4% of copper sulphate was added to it. Formation of Violet or pink colour indicated the presence of protein.

Million tests

Aqueous solution of the residue was taken and to it 2 to 3 ml of millions reagent was added. Presence of white precipitate which slowly turns to pink indicated the presence of protein.

Khandelwal, 2005)

Isolation of C.G.B.I.F.

Column chromatography

The methanolic extract of CordiaGharafbark were subjected to column chromatography over silica gel, Pet ether gradient , elution using Pet ether: acetone (85:15) & Ethyl acetate: Methanol (80:20) respectively, for the total 35 elutes collected in a vial were collected and combined into basis of their TLC similarities. All the collected fractions were subjected for TLC; and fraction with similar Rf value combined together and evaporated under reduced pressure to get active constitutes.



Figure No. 7.2 Column Chromatography

Ultra Violet Spectroscopy of C. G. B. I. F.

Perform by an Absorbance Spectrum

UV-visible spectrophotometric analysis was conducted on the CordiaGharafbark isolated methanolic extract using a UV-visible spectrophotometer (Perkin Elmer, USA Model: Lambda 950) with a slit width of 2nm, using a 10-mm cell at room temperature. The extract was examined under visible and UV light in the wavelength ranging from 300-800nm for proximate analysis. For UV-VIS spectrophotometer analysis, the extract was centrifuged at 3000 rpm for 10 min and filtered through Whitman No. 1 filter paper. The sample is diluted to 1:10 with the same solvent To make a calibration curve, collect the UV-Vis spectrum of samples.

Fourier Transform Infrared Spectroscopy of C.G.B.I.F

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 light absorbed is characteristic of the chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. CordiaGharafbarks isolated methanolic extract used for FTIR analysis. 10 mg of the CGBI methanolic extract was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The sample of CordiaGharafbark isolated methanolic extract was loaded in FTIR spectroscope (Shimadzu, IR Affinity 1, Japan), with a Scan range from 400 to 4000 cm⁻¹ with a resolution of 4 cm⁻¹.

These absorbance peaks indicate functional). Different types of bonds, and thus different functional groups, absorb infrared radiation of different wavelengths.

Mass Spectroscopy:

To identify the presence of active constituents and the chemical composition of CordiaGharafbark isolated methanolic extract was characterized by the use of mass spectrometry (MS). The MS analysis of CordiaGharafbarks isolated methanolic extract was performed by using MS Shimadzu QP-2010 plus with thermal desorption system TD 20. The column used was Rtx-5 of 30m X 0.25mm X 0.25µm size. The initial column temperature was 1000C rising 2800C at a rate of 50C/min and the temperature was

maintained for 3 minutes. The temperature was further increased to 2800C at a rate of 150C/min with a hold time of 35 minutes. The ion source of mass spectrometer was held at 2300C with an interface temperature of 2700C .

High Performance Liquid Chromatography

The HPLC analysis of methanolic extract was carried out with Chromatographic system (YL 9100, Korea) consist of autosampler (YL 9150) with 100 µl fixed loop and an YL9120 UV-Visible detector. The separation was performed on a SGE Protecol PC18GP120 (250mm×4.6 mm, 5µm) column at ambient temperature. The mobile phase consists of methanol to water (70:30 v/v) and the separations were performed by using isocratic mode, elution performed at a flow rate of 0.8 ml/min. The samples were run for 12.23min. And detection was done at 276 nm by UV detector. All chromatographic data were recorded and processed using autochro-3000 software.

PLANT PROFILE



Figure No. 6.1 Plant of CordiaGharaf

General Information

Scientific Name: CordiaGharaf (Forsskal) Ehrenb.ex.Asch.

Synonyms

Synonym: Cordiarothii, CordiaGharaf
Common Name: Cordiarothii, Gondhani, Gundi
Botanical Name: Cordiarothi
English: Grey Leaved Saucerberry, Narrow Leaved Sepistan

Hindi: Lasora, Gondi

Malayalam: Narivirayan, Verasham, Veri

Tamil: Sirunaruvuli

Marathi: Gondani

Punjab: Gondi

Telugu: Chinnabotuku, Chinnavirigi

Sanskrit: Laghusleshamataka.

Scientific classification of CordiaGharaf

Species: CordiaGharaf

Kingdom: Plantae

Subkingdom: Tracheobionta (Vascular Plant)

Division: Magnoliophyta (Seed Plant)

Class: Magnoliopsida

Subclass: Asteridae

Family: Boraginaceae

Genus: Cordia

Plant Description

Plant Type: Tree

Height: 6-12m; high

Plant form: Large imposing

Bark: Grey Furrowed

Habitat

CordiaGharaf Roem and Schult (Boraginaceae) is a small tree growing in mostly tropical and temperate India, Sri Lanka and Abyssinia. In India it is mostly found in Gujarat, Maharashtra and Punjab.

Morphology

CordiaGharaf (Boraginaceae) is a small tree characterized by small trunk, and bunch of fruits. Fruits are organic coloured having gummy mass.

Fruit

Drupe usually one seeded ovoid, acute, mucronate 1- reddish brown when ripe with a gelatinous; pellucid edible pulp.



Fig. 6.2 CordiaGharaf Fruit

Leaves

Leaves are sub-opposite, 6.3 cm in length, 2-3cm in width oblanceolateoblong, rounded at the apex, rough at above more or less pubescent beneath, feather nerved, base tapering at petiole.



Figure no. 6.3 CordiaGharaf Leaves

Bark

Bark is curved to channelled in shape; young bark 10-20 cm in length, 12-20mm in width and 1-2 mm in thickness with smooth greenish brown outer surface transverse by transversely elongated whitish lenticles. Older barks are thicker 3-5mm and with are very rough external surface due to presence of rhytidoma. Inner surface is longitudinally striated, whitish in colour when fresh.

III. RESULT

Pharmacognostic Study

Macroscopic Study

The dried bark is curved to channel in shape; young bark, 10-20cm in length, 12-20mm in width and 1-2 mm in thickness with smooth greenish brown outer surface transverse by transversely elongated whitish lenticels. Older barks are thicker (3-5mm)

The diagrammatic transverse section shows narrow cork, cortex and pericyclic zone containing stone cell as well as group of fibres and wide phloem with multiseriate medullar rays and tangentially running bands of fibres. Cortex is narrow, 5-6 layered parenchymatous and is transverse by small and big sized stone cell isolated or in groups of 2-3. Smaller tone cells are few in number.

Table No. 8.2 Physicochemical content of CordiaGharaf Bark

Sr. No.	Types of Ash Value	Result Obtained (%w/w)
1.	Total ash	8
2.	Acid insoluble ash	0.14
3.	Water soluble ash	0.056

Phytochemical Studies

Isolation of C.G.B.I.F.

Methanolic extract was subjected for Column Chromatography. Eluted with Pet Ether: Acetone (85:15) & ethyl Acetate: Methanol (80:20) respectively. Fractions were collected in glass vials up to 10 ml & closed with rubber closers.

Ultra Violet spectroscopy:

The UV-VIS analysis performed for identification of phytoconstituents present in methanolic extract of CordiaGharaf. The UV-visible spectra were performed to identify the compounds containing σ -bonds, π -bonds and lone pair of electrons, chromophores and aromatic rings. The qualitative UV-VIS profile of methanolic extract of CordiaGharaf was taken at the wavelength of 300 nm to 800 nm due to the sharpness of the peaks and proper baseline.

The FTIR spectrum of CordiaGharafbark isolated of methanolic extracts of CordiaGharaf. The data on the peak values and the probable functional groups (obtained by FTIR analysis) present in the CordiaGharaf bark isolated of methanolic extracts of CordiaGharaf are presented.

The diagrammatic transfer section shows narrow cork, cortex & pericyclic zone containing stone cell as well as group of fibres & wide floem with multiseriate medullary rays & tangentially running bands of fibres. Odour is slight but characteristic. It has disorted epidermis & cuticle, oval to circular parenchymatous cells of the lenticels .cortex is narrow, 5-6 layered, parenchymatous& it's transverse by small & big size stone cells, located in groups.

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