

***Extraction and Characterization of Antifungal Compounds from
Poly Herbal***

Submitted in partial fulfillment of the requirements for the award of
Bachelors of Technology degree in Biotechnology

by

Gokul S (38230013)



**DEPARTMENT OF BIOTECHNOLOGY
SCHOOL OF BIO AND CHEMICAL ENGINEERING**

SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

Accredited with Grade “A” by NAAC

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APRIL 2020



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DEPARTMENT OF BIOTECHNOLOGY

BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **Gokul S (38230013)** who carried out the project entitled “***Extraction and Characterization of Antifungal Compounds from Poly Herbal***” under our supervision from October 2021 to March 2022.

Internal Guide

(Mr. P.Prakash)

Head of the Department

Dr. Ramesh Kumar. V

_ Submitted for Viva voice Examination held on _____ 2020

Internal Examiner External Examiner

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DECLARATION

I, **Gokul S (38230013)** hereby declare that the Project Report entitled “***Extraction and Characterization of Antifungal Compounds from Poly Herbal***” done by us under the guidance of **Dr. P.Prakash** is submitted in partial fulfillment of the requirements for the award of Bachelors of Technology degree in Biotechnology.

DATE:

PLACE:

SIGNATURE OF THE CANDIDATE 1.

ACKNOWLEDGEMENT

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ABSTRACT

Tinea is a common chronic fungal disease characterized by destruction of the skin and surrounding tissues. It has been observed that, in the past decade there is a steady increase in the number of fungal cases around 20-25%. The present study is to evaluate the bioactive compounds from *Butea monosperma* O, *Laurus nobilis* L., Nutmeg, Turmeric, Garlic, Neam, Kuppaimeni. It contains seven ingredients, each ingredient's phytochemical compounds were extracted by aqueous solution. Various studies such as qualitative and quantitative analysis, GCMS analysis were carried out on the sample. The crude showed presence of phytochemicals such terpenoids, alkaloids, carbohydrates, fixed oils and fats and steroids. The gel formulation was also analyzed for GCMS, MTT assay, anti-fungal activity showed positive results.

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INTRODUCTION

1.1. Overview

Mycosis a common name for skin infections caused by fungus. There are over a million varieties of fungi which when comes in contact with human skin causes skin problems like rashes or bumps. It has been observed that, in the past decade there is a steady increase in the number of fungal cases around 20-25% (Alok Kumar Sahoo 2016), this is mainly due to the changes in epidemiology of fungi, increasing number of immunocompromised patients and growing popularity of health clubs and wimming pools which facilitates the spread of infection. Fungal infections occurs at different parts of body like skin, hair, mucus membrane and these are called Superficial fungal infection as they grow on outer most layer of the above-mentioned body parts.

Common cutaneous mycoses include tinea caused by dermatophytes of the genera *Trichophyton*, *Epidermophyton*, and *Microsporum*. In the medical world, tinea refers to a group of diseases caused by fungi, a highly contagious fungal infection of the skin or scalp. It is a growing health problem and its prevalence is dramatically increasing. Types of tinea include *Tinea cruris* (jock itch), *Tinea corporis* (ringworm), *Tinea capitis* (ringworm), *Tinea barbae* (barber's itch). These infections are usually not serious, but they can be uncomfortable. If left untreated, it can affect the toenails, skin, and the scalp — which may thicken, become discolored or crumble — and even spread to your hands or groin. Additionally, these diseases can makes us more vulnerable to bacterial infections, such as cellulitis.

Treatment depends on the cause of the infection and the severity. Some types of viral skin infections may improve on their own within days or weeks (Am Fam Physician, 2002 May). Fungal infections are often treated with topical antibiotics applied directly to the skin like Ketoconazole, Ciclopirox or with oral antibiotics like Fluconazole, Itraconazole or antifungal sprays like terrasil, ting and anti-inflammatory drugs to reduce discomfort like itching and scratching .

But most importantly, the efficacy of antifungal drugs has decreased over the years,

and now the drug requires larger doses and longer treatment durations. This suggests that these drugs are no longer the treatment of choice in superficial cutaneous fungal infections.

All serious fungal infections need appropriate antifungal therapy for successful patient outcome. Only a few classes of antifungal drugs are available, so the emergence of resistance to single drug classes and now multidrug resistance greatly hampers patient management. The molecular mechanisms that cause drug resistance are naturally occurring in less susceptible species and are acquired in strains of susceptible organisms. Drug resistance mechanisms include altered drug-target interactions, reduced cellular drug concentrations mediated by drug efflux transporters, and permeability barriers associated with biofilms. Drug exposure also drives the emergence of resistance(ref 3).

Resistance to antimicrobial agents, which was recognized more than 50 years, continues to be a major cause of increased morbidity, mortality and health care cost. Overuse of antibiotics is considered the major contributing factor; however, poor implementation of infection control measures, prolonged hospitalization, admission to intensive care units and the use of invasive procedures are other contributing factors. The number of antifungal classes is small, and resistance is becoming a much more frequent problem. Much greater emphasis needs to be placed on susceptibility testing and antifungal stewardship. Such efforts demonstrably improve survival and overall clinical outcomes. Positively diagnosing a fungal infection with laboratory markers often allows antibacterial therapy to be stopped (ie, anti-tuberculous therapy in chronic pulmonary aspergillosis or antibiotics other than cotrimoxazole in *Pneumocystis pneumonia*), contributing to antimicrobial resistance control generally. Non-culture based diagnostics for fungal disease are transformational in terms of sensitivity and speed, but only occasionally identify antifungal resistance.

Tinea capitis, cruris, corporis and pedis are common infections across the world, affecting ~1 billion people. They are caused by a variety of filamentous fungi including *Trichophyton*, *Microsporum* and *Epidermophyton* species. Tinea capitis is especially common in children in Africa, affecting an estimated 138 million. *Microsporum canis* infections are more difficult to treat and are refractory to terbinafine. In recent years, increasing resistance to terbinafine in *Trichophyton interdigitale* is increasingly recognised, especially in India. Multiple strains of *T.*

interdigitale causing tinea corporis or tinea cruris are terbinafine resistant.(ref 5)

1.2. DESCRIPTION OF SELECTED PLANTS

1.2.1.Turmeric/Curcuma longa

Kingdom:Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Monocots

Clade: Commelinids

Order: Zingiberales

Family: Zingiberaceae

Genus: Curcuma

Species: C. longa

General Description:

Turmeric is a perennial herbaceous plant that reaches up to 1 m (3 ft 3 in) tall. Highly branched, yellow to orange, cylindrical, aromatic rhizomes are found. The leaves are alternate and arranged in two rows. They are divided into leaf sheath, petiole, and leaf blade. From the leaf sheaths, a false stem is formed. The petiole is 50 to 115 cm (20–45 in) long. The simple leaf blades are usually 76 to 115 cm (30–45 in) long and rarely up to 230 cm (7 ft 7 in). They have a width of 38 to 45 cm (15 to 17+1/2 in) and are oblong to elliptical, narrowing at the tip.

Geographical distribution

The greatest diversity of Curcuma species by number alone is in India, at around 40 to 45 species. Thailand has a comparable 30 to 40 species. Other countries in tropical Asia also have numerous wild species of Curcuma. Recent studies

have also shown that the taxonomy of *Curcuma longa* is problematic, with only the specimens from South India being identifiable as *C. longa*. The phylogeny, relationships, intraspecific and interspecific variation, and even identity of other species and cultivars in other parts of the world still need to be established and validated. Various species currently utilized and sold as "turmeric" in other parts of Asia have been shown to belong to several physically similar taxa, with overlapping local names.

Medicinal benefits

Turmeric is used as an herbal medicine for rheumatoid arthritis, chronic anterior uveitis, conjunctivitis, skin cancer, small pox, chicken pox, wound healing, urinary tract infections, and liver ailments.

1.2.2.*Acalypha indica/Kuppaimeni*

Taxonomical classification

Kingdom: Plantae
Clade: Tracheophytes
Clade: Angiosperms
Clade: Eudicots
Clade: Rosids
Order: Malpighiales
Family: Euphorbiaceae
Subtribe: Acalyphinae
Genus: *Acalypha*
Species: *A. indica*

General information

An erect annual herb that can be easily distinguished by the cup-shaped involucre that surrounds the small flowers in the catkin-like inflorescence. It can

grow up to 1.2 m (3.9 ft) tall in favorable circumstances, but is usually smaller. The tiny male flowers are white-green, located on the upper part of the flower spikes, and are ebracteate, minute, and clustered with vermiculiform anthers. The pollens are roughly round and approximately 10–12 microns in diameter. The green female flowers are located lower on the spikes, and are subtended by 3–7 mm (0.12–0.28 in) long suborbicular-cuneiform, many-nerved, toothed bracts that are foliaceous. The ovary is hispid, 3-lobed. Styles are 3, each 2-fid. Capsules are hispid, 3-valved and concealed by a bract. The stem is striate (longitudinally ribbed) and pubescent. The fruit is 1.5–2 mm (0.059–0.079 in), 3-lobed, tuberculate and pubescent.

Geographical distribution

Acalypha indica occurs widely throughout the Old World tropics. In Africa, it occurs in Nigeria in West Africa and further widely throughout tropical Africa and the Indian Ocean islands. It also occurs in India, South East Asia, Yemen, and Oceania. It has been introduced to the New World Tropics.

Medicinal benefits

Kuppaimeni is a good herbal remedy for Skin problems like Acne, Eczema, Cuts, Scraps. It is effective in curing Burns and Wounds. It is effective in getting rid of Hair troubles like Dandruff, Hair fall, Baldness. It adds shine and promotes growth of the Hair.

1.2.3. *Allium sativum*/Garlic

Taxonomical classification

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Monocots

Order: Asparagales

Family: Amaryllidaceae

Subfamily: Alliioideae

Genus: Allium

Species: A. sativum

General information

Garlic (*Allium sativum*) is a species of bulbous flowering plant in the genus *Allium*. Its close relatives include the onion, shallot, leek, chive, Welsh onion and Chinese onion. It is native to Central Asia and northeastern Iran and has long been used as a seasoning worldwide, with a history of several thousand years of human consumption and use. It was known to ancient Egyptians and has been used as both a food flavoring and a traditional medicine. China produces 76% of the world's supply of garlic.

Geographical distribution

Garlic is a fundamental component in many or most dishes of various regions, including eastern Asia, South Asia, Southeast Asia, the Middle East, northern Africa, southern Europe, and parts of Latin America.[53] Latin American seasonings, particularly, use garlic in sofritos and mofongos.

Medicinal benefits

As of 2016, clinical research found that consuming garlic produces only a small reduction in blood pressure (4 mmHg), and there is no clear long-term effect on hypertension, cardiovascular morbidity or mortality. A 2016 meta-analysis indicated there was no effect of garlic consumption on blood levels of lipoprotein(a), a biomarker of atherosclerosis. Because garlic might reduce platelet aggregation, people taking anticoagulant medication are cautioned about consuming garlic. A 2016 meta-analysis found no effect of garlic on colorectal cancer. A 2014 meta-analysis found garlic supplements or allium vegetables to have no effect on colorectal cancers.

A 2013 meta-analysis of case-control and cohort studies found limited evidence

for an association between higher garlic consumption and reduced risk of prostate cancer, but the studies were suspected as having publication bias. A 2013 meta-analysis of epidemiological studies found garlic intake to be associated with decreased risk of prostate cancer.

1.2.4. *Azadirachta indica* /Neem

Taxonomical classification

Kingdom: Plantae
Clade: Tracheophytes
Clade: Angiosperms
Clade: Eudicots
Clade: Rosids
Order: Sapindales
Family: Meliaceae
Genus: *Azadirachta*
Species: *A. indica*

General information

Azadirachta indica, commonly known as neem, nimtree or Indian lilac, and in Nigeria called dogoyaro or dogonyaro, is a tree in the mahogany family Meliaceae. It is one of two species in the genus *Azadirachta*, and is native to the Indian subcontinent and most of the countries in Africa. It is typically grown in tropical and semi-tropical regions. Neem trees also grow on islands in southern Iran. Its fruits and seeds are the source of neem oil. Neem is a fast-growing tree that can reach a height of 15–20 metres (49–66 ft), and rarely 35–40 m (115–131 ft). It is deciduous, shedding many of its leaves during the dry winter months. The branches are wide and spreading. The fairly dense crown is roundish and may reach a diameter of 20–25 m (66–82 ft). The neem tree is similar in appearance to its relative, the chinaberry (*Melia azedarach*).

Geographical distribution

Neem is native to the whole Indian subcontinent; others attribute it to dry forest areas throughout all of South and Southeast Asia, including Pakistan, Sri Lanka, Thailand, Malaysia, and Indonesia. It is in India that the tree is most widely used.

Medicinal benefits

Products made from neem trees have been used in the traditional medicine of India for centuries, but there is insufficient clinical evidence to indicate any benefits of using neem for medicinal purposes. In adults, no specific doses have been established, and short-term use of neem appears to be safe, while long-term use may harm the kidneys or liver; in small children, neem oil is toxic and can lead to death. Neem may also cause miscarriages, infertility, and low blood sugar. The neem tree is of great importance for its anti-desertification properties and possibly as a good carbon dioxide sink. It is also used for maintaining soil fertility. And also neem extract is added to fertilizers (urea) as a nitrification inhibitor. Even the neem leaves can be occasionally used as forage for ruminants and rabbits. Neem has also traditionally been used as a type of teeth-cleaning twig.

1.2.5.*Myristica fragrans*/Nutmeg

Taxonomical classification

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Magnoliids

Order: Magnoliales

Family: Myristicaceae

Genus: *Myristica* Gronov

General information

Nutmeg is the seed or ground spice of several species of the genus *Myristica*. *Myristica fragrans* (fragrant nutmeg or true nutmeg) is a dark-leaved evergreen tree cultivated for two spices derived from its fruit: nutmeg, from its seed, and mace, from the seed covering. It is also a commercial source of an essential oil and nutmeg butter. The California nutmeg, *Torreya californica*, has a seed of similar appearance, but is not closely related to *Myristica fragrans*, and is not used as a spice. Indonesia is the main producer of nutmeg and mace.

If consumed in amounts exceeding its typical use as a spice, nutmeg powder may produce allergic reactions, cause contact dermatitis, or have psychoactive effects.

Geographical information

Nutmeg is only known from cultivation but it most probably originated in Indonesia from the southern Moluccan Islands, especially Ambon and Banda. Nutmeg and mace (the dried aril) spread from there and became known throughout South East Asia.

Medicinal benefits

Nutmeg is a rich source of antioxidants, which help protect against the signs of aging and serious conditions such as cancer, heart disease, and liver disease. Nutmeg oil is used in several dental products. Nutmeg was once considered an abortifacient, but may be safe during pregnancy if used only in flavoring amounts. If consumed in large amounts, nutmeg could cause premature labor and miscarriage. Nutmeg may also interact with pain-relievers such as pethidine, so it is recommended that it be avoided during pregnancy.

1.2.6. Laurus nobilis L./Bay Leaf

Taxonomical classification

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Magnoliids

Order: Laurales

Family: Lauraceae

Genus: Laurus

Species: L. nobilis

General information

Bay laurel (*Laurus nobilis*, Lauraceae). Fresh or dried bay leaves are used in cooking for their distinctive flavour and fragrance. The leaves should be removed from the cooked food before eating (see safety section below). The leaves are often used to flavour soups, stews, braises and pâtés in many countries. The fresh leaves are very mild and do not develop their full flavour until several weeks after picking and drying. Indian bay leaf or malabathrum (*Cinnamomum tamala*, Lauraceae) differs from bay laurel leaves, which are shorter and light- to medium-green in colour, with one large vein down the length of the leaf. Indian bay leaves are about twice as long and wider, usually olive green in colour and have three veins running the length of the leaf. Culinarily, Indian bay leaves are quite different, having a fragrance and taste similar to cinnamon (cassia) bark, but milder.

Geographical information

Bay leaf is present in California and wooded grassland of the regions of India, Indonesia, and Mexico.

Medicinal benefits

The humble bay leaf can pack a mighty punch when it comes to health benefits. While adding minimal calories, bay leaf increases the amount of fiber, vitamins, minerals, and antioxidants to a dish. Bay leaf is a good source of vitamin A, vitamin B6, and vitamin C. These vitamins are all known to support a healthy immune system. And bay leaf tea can help ease bouts of upset stomach. The tea is also very aromatic, which can help relieve sinus pressure or stuffy nose, even Reduces Type 2 Diabetes Risk Factors.

1.2.7. *Butea monosperma*/Flame-of-the-forest

Taxonomical classification

Kingdom: Plantae
Clade: Tracheophytes
Clade: Angiosperms
Clade: Eudicots
Clade: Rosids
Order: Fabales
Family: Fabaceae
Subfamily: Faboideae
Genus: *Butea*
Species: *B. monosperma*

General information

It is a small-sized dry-season deciduous tree, growing to 15 m (49 ft) tall. It is a slow-growing tree: young trees have a growth rate of a few feet per year.

Geographical information

Calamus rotang are found commonly in coastal fresh water swamp forests, frequent along fresh water streams of lower hill valleys of India, Sri Lanka and Myanmar.

Medicinal information

The wood of Calamus rotang is used as vermifuge. The leaves are used in the treatment of biliousness and diseases of the blood. The root is used in the treatment of chronic fevers and as an antidote to snake venom. The wood of *C. rotang* is also used medicinally to expel intestinal worms. The leaves are used for biliousness and blood diseases, and the root for fevers and as antidote to venom.

2. REVIEW OF LITERATURE

The plant material was air dried at room temperature and ground to a coarse powder in a blender and added Various solvents- polar, non polar, semi polar.(Arthur F. DiSalvo, M. D., Sep 2018)

The results indicate that scientific studies carried out on medicinal plants having traditional claims of effectiveness might warrant fruitful results (S. Chanda* and Y. Baravalia, 2010).

For cold maceration, the extract yields were 14.3%, 17.4% and 7.6% for *P. niruri*, *O. stamineus* and *L. pumila*, respectively. Whilst for soxhlet method, the same trend was observed where 50% ethanol (v/v) gave the highest extract yield of 21.2%, 14.3% and 6.8% for *P. niruri*, *O. stamineus* and *L. pumila*, respectively. (Nur Aqilah Kamarudin, Masturah Markom* and Jalifah Latip, june 2016).

Studies on the antimicrobial, especially antibacterial, activity of lemon grass oil and its components had been reported (Kokate and Verma, 1971; Moleyer and Narasimham, 1988; Onawunmi, 1989; Onawunmi and Ogunlana, 1986; Owunami et al., 1984). The factors affecting its activity were determined (Bose et al.,1950). In addition to its antibacterial activity, the antidermatophytic activity of this oil against a few dermatophytes had been studied by Hemtanont et al. (1991).

R. N. Yadava & Lata Tiwari (2007) New antifungal flavone glycoside from *Butea monosperma* O. Kuntze, *Journal of Enzyme Inhibition and Medicinal Chemistry*, 22:4, 497-500.

Nadjib Rahmoun, Zahia Boucherit-Otmani, Kebir Boucherit, Mohammed Benabdallah & Noureddine Choukchou-Braham (2013) Antifungal activity of the Algerian *Lawsonia inermis* (henna), *Pharmaceutical Biology*, 51:1, 131-135.

Mohammad A. Alzohairy, "Therapeutics Role of *Azadirachta indica* (Neem) and Their Active Constituents in Diseases Prevention and Treatment", *Evidence-Based Complementary and Alternative Medicine*, vol. 2016, Article ID 7382506, 11 pages, 2016.

Farzad Aala, Umi Kalsom Yusuf, Rosimah Nulit, Sassan Rezaie, *Iran J Basic Med Sci*. 2014 Mar; 17(3): 150–154.

Viorica Maria Corbu, Irina Gheorghe, Ioana Cristina Marinaș, Elisabeta Irina Geană, Maria Iasmina Moza, Ortansa Csutak, Mariana Carmen Chifiriuc *Molecules*.

3. AIM

Find a cure for fungal disease (Tinea) from poly herbal compounds.

OBJECTIVES

- To develop a poly herbal antifungal cream for tinea.
- Eradicate the scars from skin.

4. MATERILAS AND METHODS

4.1.SAMPLE COLLECTION

Sample are collected from the nearby Ayurveda shop and washed thoroughly with water and then it crushed into powder.

4.2.EXTRACT PREPARATION

This mixture contains 20grms from each powdered samples, i.e., Butea monosperma O, Laurus nobilis L., Nutmeg, Turmeric, Garlic, Neam, Kuppaimeni. (Ratio is 1:1:1:1:1:1:1), which makes it a total of 140grms.

Then the compounds extraction is tried out using 3 types of solvents, i.e., Hexane, Ethyl Acetate, and Methanol, with 20grms of polyherbs in 100ml of solvent, and it is kept at room temperature for 4 days. The mixture is then collected and dried using distillation to remove the respective solvents.

After drying the crude is collected in a eppendorf tube.

4.3. PHYTOCHEMICAL QUALITATIVE ANALYSIS

4.3.1. Test for alkaloids

Crude extract was mixed with 2ml of 1% HCl and heated gently. Mayer's And Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

4.3.2. Test for Carbohydrates (Benedict's test)

To 0.5 ml of filtrate, 0.5 ml of Benedict's reagent is added. The mixture is heated on a boiling water bath for 2 minutes. A characteristic coloured precipitate indicates the presence of sugar.

4.3.3. Test for Fixed oils and Fats

Spot test A small quantity of extract is pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oils.

4.3.4. Test for gum and Mucilages

The extract (100 mg) is dissolved in 10 ml of distilled water and to this 2 ml of absolute alcohol is added with constant stirring. White or cloudy precipitate indicates the presence of Gums and Mucilages.

4.3.5. Test for steroid

Crude extract was mixed with 2ml of chloroform and concentrated H₂SO₄ was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids. Another test was performed by mixing crude extract with 2ml of chloroform. Then 2ml of each of concentrated H₂SO₄ and acetic acid were poured into the mixture. The development of a greenish coloration indicated the presence of steroids.

4.3.6. Ninhydrin test

Crude extract when boiled with 2ml of 0.2% solution of Ninhydrin, violet colour appeared suggesting the presence of amino acids and proteins.

4.3.7. Test for glycosides

Salkowski's test Crude extract was mixed with 2ml of chloroform. Then 2ml of concentrated H₂SO₄ was added carefully and shaken gently. A reddish brown colour indicated the presence of steroidal ring, i.e., glycone portion of the glycoside.

4.3.8. Test for Terpenoids

To 0.1g of the extract add 2 ml of chloroform, and then add 1.5 ml concentrated Sulphuric acid carefully added. Formation of reddish brown colour at the interface indicates the presence of Terpenoids.

4.3.9. Test for Tannins

About 0.1 g of the seaweed extract was boiled in 2 ml of distilled water in a test tube and then filtered. 1ml of the leaf extract added with 5 % FeCl₃ (1 ml) was added to the filtrate. Appearance of brownish green coloration showed the presence of tannins.

4.3.10. Test for Saponins

Seaweed extract (0.5 g) was dissolved in 2 ml of boiling water in a test tube, allowed to cool and shaken well to mix thoroughly. The appearance of foam indicates the presence of Saponins.

4.3.11. Test for Cardio Glycosides

One ml of the extract, added with 2 ml of glacial acetic acid and few drops of 5 % ferric chloride were added. This was under layered with 1 ml of concentrated sulphuric acid. Formation of brown ring at interface indicates the presence of cardio glycosides.

4.4. PHYTOCHEMICAL QUANTATIVE ANALYSIS

4.4.1. COLUMN CHROMATOGRAPHY and GC-MS ANALYSIS

The crude is purified by using column chromatography, which consist of the silica gel as a stationary phase and acetone as the mobile phase. The silica gel is heated before use for the activation of the silica, the activated silica gel is then mixed with acetone and made into a slurry. The slurry is then poured from the top of the column and allow to settle. For the preparation of the sample the sample is poured into a separating funnel and equal amount of ethyl acetate is added to it. The mixture is then mixed properly and then allowed to settle. The mixture then forms two layer collect the top layer and pour it in a petri plate and let it dry and elute it with acetone. Pour the sample from the top of the column and collect the fraction in an Eppendorf tube. Then send the sample for GCMS analysis.

4.4.2. MTT Assay

The cytotoxic activity of samples on VERO cells were determined by the MTT assay. Cells (1×10^5 /well) were plated in 0.2 ml of medium/well in 96-well plates. Incubate at 5 % CO₂ incubator for 72 hours. Then, added various concentrations of the samples in 0.1% DMSO

for 24 hrs at 5 % CO₂ incubator. View the images under Inverted microscope 40X and take the photos. After removal of the sample solution and 20µl/well MTT reagent was added. Viable cells were determined by the absorbance at 540nm. 50% inhibition of cell viability (IC₅₀) value was determined graphically. The effect of the samples on the proliferation of & VERO cells was expressed as the % cell viability, using the following formula:

Calculation;

$$\% \text{ cell viability} = \text{A540 of treated cells} / \text{A540 of control cells} \times 100\%$$

4.5. Anti-Fungal Assay

The fungicidal activity of extracts was assessed by the agar well diffusion method using Potato dextrose Agar. The isolated fungal pathogens were inoculated into 10 ml of Potato dextrose Broth and incubated at room temperature for 5 days. Using a sterile cotton swab, fungal spores were swabbed on the sterile Potato dextrose agar plates. Agar wells were prepared with the help of a sterile cork borer with a 7 mm diameter. Using a micropipette, different concentrations of medicinal plant extracts were added to different wells on the plate separately. The plates were incubated in an upright position at room temperature. The diameter of inhibition zones was measured in mm and the results were recorded.

4.6. Gel Formulation

Method for Preparation of Gel Containing Extract 1 g of Carbopol 940 was dispersed in 10 ml of distilled water kept the beaker aside to swell the carbopol 940 for half an hour and then stirring should be done to mix the carbopol 940 to form gel. Take 5 ml of distilled water and required quantity of methyl paraben and propyl paraben were dissolved by heating on water bath. Solution was cooled and Propylene glycol 400 was added. Further required quantity of hexane extract was mixed to the above mixture. Finally full mixed ingredients were mixed properly to the Carbopol 940 gel with continuous stirring and triethanolamine was added drop wise to the formulation for adjustment of required skin pH (6.8-7) and to

obtain the gel at required consistency. The same method was followed for preparation of control sample without adding any betel leaves extract.

5. RESULTS AND DISCUSSION

5.1. Phytochemical analysis

The phytochemical screen of the crude is listed in the table below. From the table +ve represent the presence of the phytochemical whereas –ve represents the absence of the phytochemical.

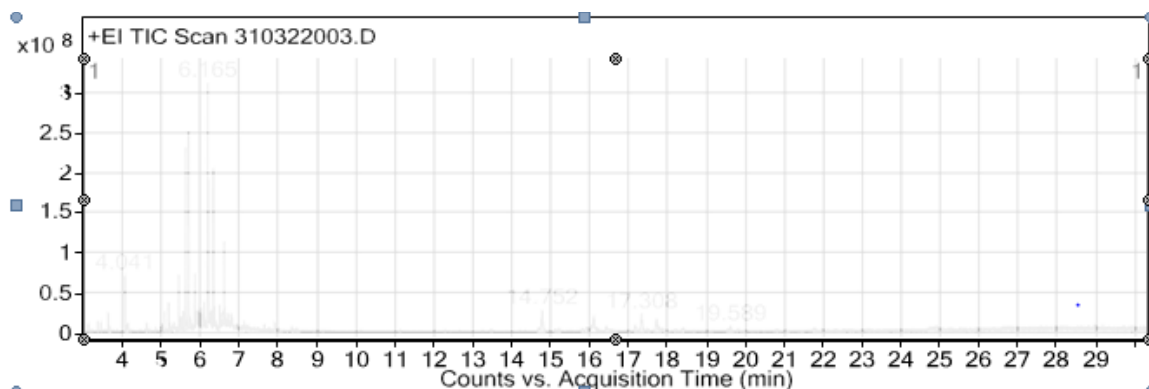
S.no	Test name	Observation
1	Saponins	-ve
2	Tannins	-ve
3	Terpenoids	+ve
4	Glycosides	-ve
5	Alkaloids	+ve
6	Carbohydrates	Trace
7	Protein	-ve
8	Fixed oils and Fats	+ve
9	Steroid	+ve
10	Gum and Mucilages	-ve

Table 5.1 represents the phytochemical analysis of the Curde.

Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups. The phenolic compounds may contribute directly to anti-oxidative action. The total phenolic content of *Terminalia arjuna* was reported by Sundar. S. Mety & Pratima Mathad (2011). It was found to be 1.9543 ± 0.013 in ethanolic extract was reported by Satheesh Kumar et al. (2013). The present study for *Terminalia arjuna* was carried out and it was recorded $630 \mu\text{g/ml}$ for *T. arjuna*. The result was compared with standard Gallic acid ($5\text{-}100 \mu\text{g/ml}$) and was found to contain good amount of phenols in the extract.

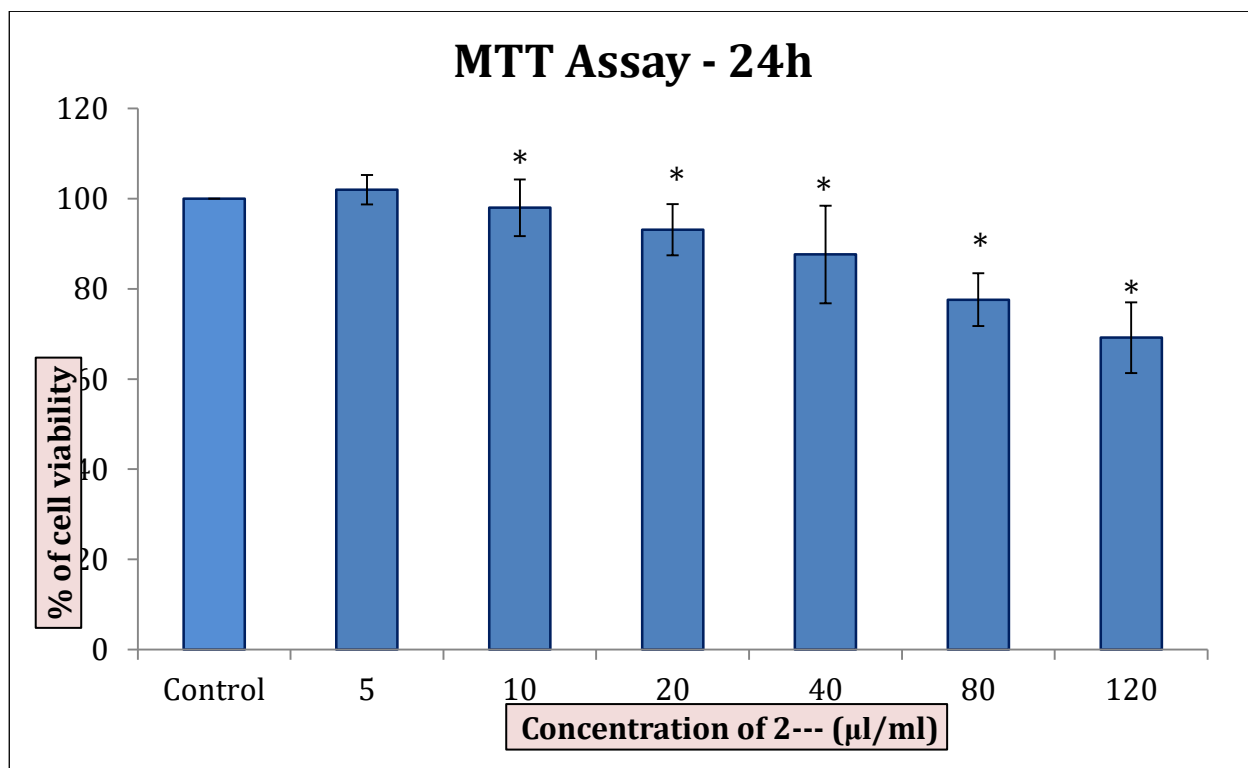
5.2. Column chromatography and GC-MS analysis of Curde

The crude is purified by column chromatography and given for GC-MS analysis. The bio active compounds presents in the sample is found through GC-MS analysis. The GC-MS analysis works on the principle of mass by charge ratio (m/z). GC-MS produces peak based on the molecular weight and mass by charge ratio and compare it with the standard chart which is there in NIST library.

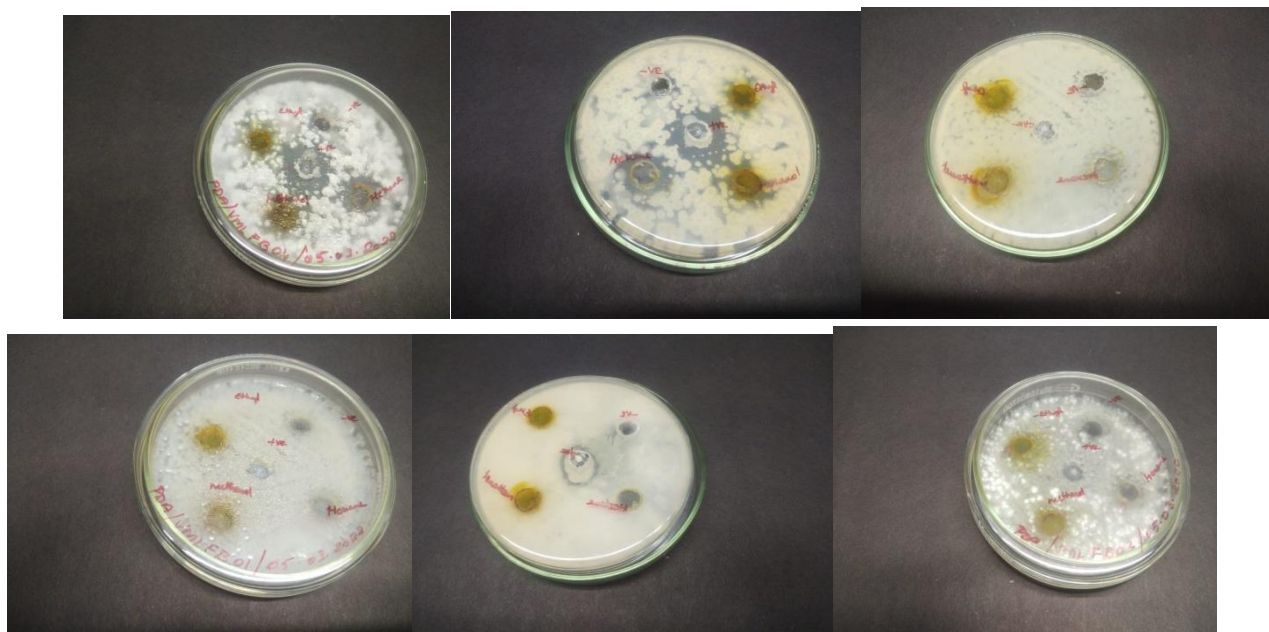


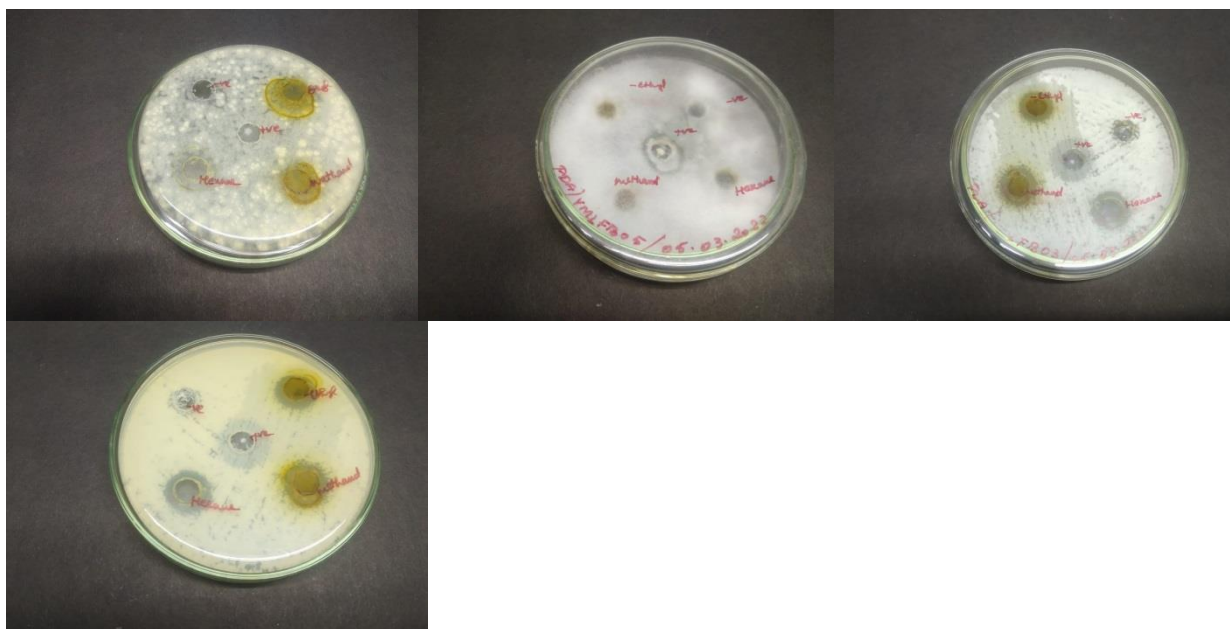
5.3. MTT Assay

MTT assay is used to study about the level of toxicity of the compound to the cells and to find the lethal dose of the drug to human cells. MTT works on the principle of reduction of the yellow colored water soluble Tetrazolium dye 3-[4, 5-dimethylthiazol-2yl]-2, 5-diphenyl tetrazolium bromide (MTT) to formazan crystals. Mitochondrial lactate dehydrogenase produced by live cells reduces MTT to insoluble formazan crystals, which upon dissolution in an appropriate solvent exhibits purple color. The percentage of cell viability is given by the dividing the absorbance of treated cells and the absorbance of control cells. The sample is diluted with DMSO. The compound is diluted in different concentration.



5.4. Anti-Fungal Assay





6. SUMMARY AND CONCLUSION

The seven medicinal plants were collected and their phytochemical study showed the presence of all the essential phytochemicals and were quantified. GC-MS analysis of crude showed the presence of the anti-Fungal anti inflammatory agents present in the plant sample. A per standard industrial procedure the cream was prepared. The antifungal assay shows us that the high inhibition rate in hexane base. Hence, the cream was further developed on the basis of the antifungal result on hexane base. The scratch analysis report makes us known that the wound healing properties are at their minimum, thus the wound caused by itching can be reduced at its best. Further research studies are to be conducted to increase the presence of antifungal properties and to further increase wound healing properties.

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