Phytochemical Screening of Secondary Metabolites From Medicinal Plants

A DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE

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Submitted by

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CANDIDATE'S DECLARATION

I Asmita Kumari, Roll Number: 2K20/MSCBIO/03, student of M.Sc. Biotechnology, here by declare that the work which is presented in the Major Project entitled- Phytochemical Screening of Secondary Metabolites From Medicinal Plants in the fulfillment of the requirement for the award of the degree of Master of Science in Biotechnology and submitted to the Department of Biotechnology, Delhi Technological University, Delhi, is an authentic record of my own carried out during the period from January- May 2021,under the supervision of Dr. Navneeta Bharadvaja.

The matter presented in this report has not been submitted by me for the award for any other degree of this or any other Institute/University. The work has been accepted in SCI/SCI expanded/SSCI/Scopus Indexed Journal OR peer-reviewed Scopus Index Conference with the following details:

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CERTIFICATE

I hereby certify that the project dissertation titled "Phytochemical Screening of Secondary Metabolites From Medicinal Plants" which is submitted by, Asmita Kumari, Roll Number: 2K20/MSCBIO/03, Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Science, is a record of the project work carried out by the student under my supervision. To the best of my knowledge this work has not been submitted in part or full for any degree or diploma to this university or elsewhere.

Place: Delhi

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Abstract:

Ongoing research in food supplement sector provides an insightful information regarding algae and plant secondary metabolites as new generation nutritional supplement and is also referred to as superfood. Due to the diverse nutritional components, plant secondary metabolites have documented numerous health benefits like fighting microbial diseases, hypertension, obesity and diabetes. Therefore, plant derived nutraceuticals accounts for a significant rapidly expanding market in the food supplements sector. The concept of plant prebiotics and their role in modulating gut microbiota has also been a chief contributor to this. This project evaluates presence and estimation of secondary metabolites present in plant. Proteins, peptides, polysaccharides, phenolics, vitamins gives an insight into the significance of algae in boosting immune system and improving the body's nutritional makeup. In addition, phytocompounds such as polysachharides and polyphenols, alkaloids are also receiving a lot more interest in cosmeceutical applications for protecting skin from photodamge. Incorporation of plant derived secondary metabolites in diet helps in management and prevention of chronic diseases like cancer, neurodegenerative disease, lung and heart disease.

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CHAPTER 1. Introduction

1.1 General Introduction

Plants have been a source of fulfillment of diverse needs of humans and plant as source of medicine have existed for thousands of years. Phytochemicals present in plants contribute to several therapeutic and nutraceutical values to mankind. In today's world as well plant derived natural products contribute to more than 50% of all drugs that are being applied to clinical use (Petrovska, 2012). Roots of modern allopathic medical science lies in traditional and ancient medicine which used plant derived compounds and its formulations. Though traditional form of plant derived medicine is used to treat chronic illnesses while the allopathic formulations are used to treat acute diseases. Plant derived medicine helps in reducing disease symptoms and improves th quality of life as well (Gurib-Fakim, 2006).

Recent trends in food industry demands for a more sustainable switch in food products. For this plant based food supplement is preferred over animal-derived products and are considered to be more healthy. Terrestrial crops possess more nutritional value further its production is more sustainable. Food and herbs has been used as medicines in traditional asian cultures. Therefore the idea of incorporating phytocompounds have been adopted in the modern approach. This not only meets the current demand for more sustainable and healthy food but also act as therapeutic agents for treatment and prevention of certain deadly diseases (Shannon and Abu-Ghannam, 2019)(Katiyar and Arora, 2020). In comparison to modern treatment methods such as radiotherapy or chemotherapy that have several side effects on the body these phytocompounds

are being used as substitute by the pharmaceutical industries. Furthermore, worldwide availability, diversity and productivity makes it the best substitute for integral utilization where a specific refined phytocompounds are used or even whole plant can be used as target product. Improvements in technical conditions and development of advanced biorefineries has made it possible to exploit every fraction of plant biomass to beneficial byproducts. In addition, intensive research and current studies are making use of genetic engineering tools to increase the value of these phytocompounds (Domínguez, 2013)(Martins *et al.*, 2018). Owing to its bioactive molecules and its effect on human health, plants are extensively desired product for use in nutraceuticals. Protein, carbohydrates, and lipids are among the important substances derived from these algae for pharmacological and nutraceutical purposes.

Medicinal plants contains phytochemicals in potential amounts that have numerous benefits in control and treatment of several diseases such as cancer, diabetes, cardiovascular diseases, neurodegenerative diseases, gastrointestinal diseases, viral and bacterial diseases. Moreover due to the surge in demand for organic products has led to promotion of research in medicinal properties of plants and discovery of new plants with therapeutic potential and nutraceutical values. In India Ayurveda has existed for over 5000 years as medical science and to increase longitivity of life. In today's world there is a shift in demand from allopathic food supplements and medicines to herbal based products (Raina *et al.*, 2014). They are not only safer alternatives that doesn't have any side effects but are also less expensive. "Phyto-compounds are recognized to play a key part in plant adaptation to their surroundings, but they are also a valuable source of medications. The utilization of plants and their extracts in the manufacture of herbal medicines laid the groundwork for current therapeutic sciences" (Chitra Jain; Shivani Khatana; Rekha Vijayvergia, 2019).

1.2 Secondary Metabolites:

The intermediates and products of metabolism are known as metabolites. These secondary metabolites are non essential natural products that are not required for growth and development of an organism but rather serve to meet the secondary requirements. “Metabolites serve a variety of purposes, including structure, sending signals, enzyme activation and inhibition, catalytic activity (typically as a cofactor), contact and defense from microorganisms. Secondary metabolites form a major component of pharmaceutical and nutraceutical products (Calabrò, 2015). Plants create a wide range of organic chemicals, the overwhelming majority of which does not have a direct role in growth and development. Secondary metabolites are chemicals that are often distributed differently throughout limited taxonomic groupings within the plant kingdom. The increasing economic value of secondary metabolites has sparked a lot of interest in the prospect of modifying the synthesis of bioactive plant metabolites utilizing tissue culture technologies in recent years” (Chitra Jain; Shivani Khatana; Rekha Vijayvergia, 2019). Explants such as stems, meristems, leaves and roots can be used to construct plant cell and tissue culture technologies under sterile conditions for both multiplication and secondary metabolite extraction. Commercial and therapeutic plants have been reported to produce secondary metabolites in vitro in plant cell suspension cultures (Srivastava and Srivastava, 2007). These secondary metabolites have shown to have therapeutics effects on diseases such as viral diseass, bacterial diseases, cancer and have also shown to posses antioxidant potential, immunosuppressant activity, antileishmanian effects, antibiotic activity (Vaishnav and Demain, 2011).

1.3 Classification:

On the basis of chemical structure these secondary metabolites can be classified into following molecular families: Phenols, steroids, Alkaloids, Flavonoids, carotenoids (Calabrò, 2015).

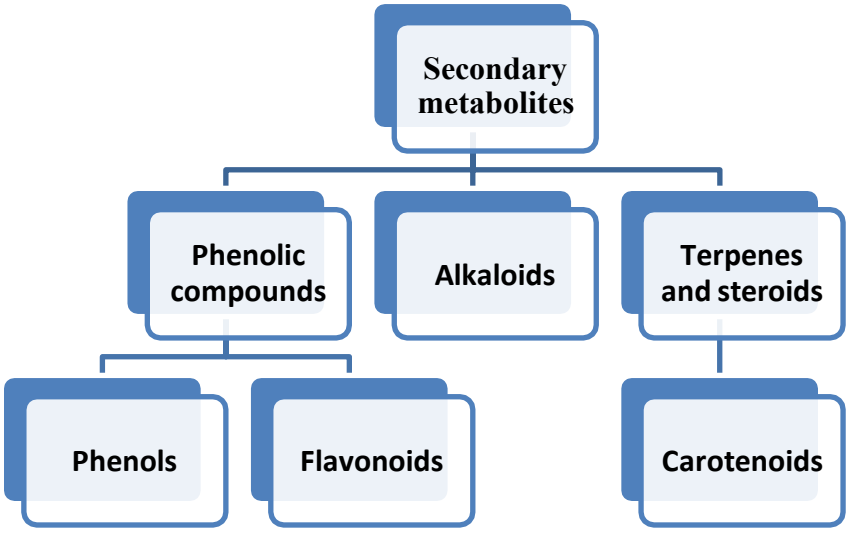


Figure 1: Classification of Secondary Metabolites

1.4 Objective:

Objective 1: To perform qualitative test for Phenol and Alkaloid in *Kalanchoe pinnata*, *Catharanthus roseus, Murraya koenigii, Moringa oleifera, Hibiscus rosa-sinensis* using different solvents.

Objective 2: To perform quantitive test for Phenol in *Catharanthus roseus, Murraya koenigii, Moringa oleifera, Hibiscus rosa-sinensis.*

Objective 3: Estimation of β- Carotene in *Daucus carota using Petroleum ether and Hexane as solvent.*

1.5 ORGANIZATION OF THESIS

The following thesis title as "Phytochemical analysis of alkaloid, phenol and β- Carotene from Plants and Algae" is a reviewed information gathered from various research and review article. The thesis focuses on qualitative analysis of secondary metabolites- Phenols and Alkaloids in following plants: *Kalanchoe pinnata*, *Catharanthus roseus, Murraya koenigii, Moringa oleifera, Hibiscus rosa-sinensis.* Further it also describes quantitative estimation of Phenol in *Catharanthus roseus, Murraya koenigii, Moringa oleifera, Hibiscus rosa-sinensis* and estimation of β- Carotene in *Daucus carota.*

The chapter 1 represents general introduction of medicinal plant and importance of secondary metabolites.

The chapter 2 is a review of literature which contains a broad knowledge about the overview of secondary metabolites phenols and alkaloids and carotenoids. It also covers five medicinal plants *Kalanchoe pinnata*, *Catharanthus roseus, Murraya koenigii, Moringa oleifera, Hibiscus rosa-sinensis.*

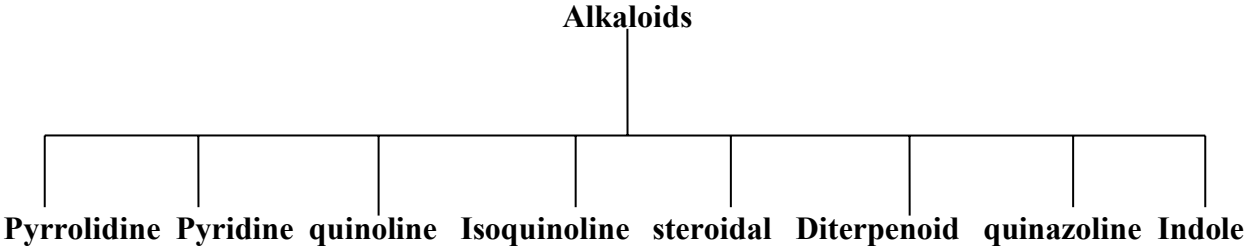
The chapter 3 is a proposed methodology for the project and briefs about the protocols for preliminary tests for secondary metabolites and their quantitative estimation.

The chapter 4 contains result, discussion and conclusion part.

CHAPTER 2. Review Of Literature:

2.1 Alkaloids:

Alkaloids comprises of a diverse group of chemical compounds and are considered as on of the largest group of plant natural product. The most essential feature of an alkaloid group is the presence of nitrogen atom at any position in the molecule. The nitrogen atom present is not included in an amide or peptide bond. The term alkaloid was isolated by W. Meisner from opium poppy *Papaver somniferum* and it was then introduced into a number of medicines that were alkaloid containing drugs (Kaur and Arora, 2015). In addition to carbon, nitrogen and hydrogen most of the alkaloids also contain oxygen. Basic character of an alkaloid is that they form salt with mineral acids which are soluble in water and dilute alcohols but in some very few cases are not soluble in organic solvents. Alkaloids are classified into following groups on the basis of C-N skeleton.



Benzylisoquinoline, an alkaloid exhibit inhibitory effect on viral replication in humans and indoquinoline isolated from *Cryptolepsis sanguinolenta* have shown to posses antimicrobial activity as it acts against certain gram-negative bacteria. Another popular alkaloid Quinine, is known for its antimalarial action(Uzor, 2020). Moreover, alkaloids present in plants provides protection against plant pathogens. There are approximately 11000 alkaloids known till date which are being used in pharmaceutical industries. These alkaloid containing compounds are

being used in dyes, drugs and herbicides (Chitra Jain; Shivani Khatana; Rekha Vijayvergia, 2019).

2.2 Phenols:

Medicinal and aromatic compounds are rich sources of phenolic compounds which exhibit several health effects. These compounds are mostly produced by two pathways: (i) shikimic acid and (ii) acetic acid. When estimated quantitatively total phenolic content is found in larger quantity in dark vegetables such as black beans, kidney beans, black gram. Phenolic acid contain a benzene ring, one or more hydroxyl group, a carboxylic group (Pinto *et al.*, 2021). Depending on their carbon structure they are divided into (i) benzoic acid derivatives, (ii) cinnamic acid derivatives. Hydroxybenzenes are common phenols that is not present in higher plants. Quinol and catechol are other popular phenols where quinol is widely distributed in plant kingdom than catechol(Pinto *et al.*, 2021). Polyphenol rich diet provide protection against deadly diseases such as cancer, cardiovascular, alzhimer's and parkinson's disease. They also possess antidiabetic, hypotensive and anti-inflammatory properties (Hollman, 2001). Phenolic acids are the most prominent available polyphenol in plant and comprises of hydroxycinnamic acids. The subtypes of hydroxycinnamic acids present in plants are ferulic acid, caffeic acid, coumaric acid, chlorogenic acid and sinapic acid. Among all these droxycinnamic acids, caffeic acid is most commonly widespread. Hydroxycinnamic acids are present as simple esters with glucose or hydroxyl carboxylic acids whereas the hydroxybenzoic acids are mainly present as glucosides. Phenolic acids are potent antioxidants not only in terms of their ability to scavenge free radicals but also in strengthening the endogenous antioxidant defense system of body (Pridham, 1965).

Types of polyphenols

Polyphenols are classified on the basis of number of phenolic ring present and the attachment of structural element that hind to these rings. They are broadly classified as phenolic acids, flavonoids, stibenes, tannin, lignan and these classes are further subdivided. Flavonoids comprises of approximately half the number of polyphenols (Espín, González-Sarrías and Tomás-Barberán, 2017)(Pinto *et al.*, 2021).

2.3 Carotenoids:

Carotenoids are natural pigments present in plants and their characteristic color lies in the range of yellow and red spectrum. according to their function carotenoids are divided into two groups: xanthophylls and carotenes. Xanthophylls comprises of lutein and zeaxanthin, while carotenes include β-carotene, α-carotene and lycopene (Langi *et al.*, 2018). Since these compounds cannot be synthesized inside human body therefore they are essential nutrients that should be consumed through diet. Carotenoids exhihibit high anti-inflammatory and antioxidant effects and play an important role in maintain good health (Zakynthinos and Varzakas, 2016). They possess free radical scavenging capacity and provides protection against premature aging and cancer. Yellow-orange fruits and vegetables are rich in β-carotene and α-carotene, whereas green vegetables are rich in lutein and tomatoes are rich in lycopene. Till date over 600 carotenoids have been studied out of which ~40 are present in human diet. β-carotene are precursors of vitamin A and intake of carotenoids rich diet help to combat malnourishmient (Langi *et al.*, 2018). Recently astaxanthin, a carotenoid has gained much popularity in pharmaceutical industry because of its therapeutic effect in treating cancer, cardiovascular diseases and diabetes. Moreover, carotenoids have also gained value in cosmaceutical industry because of its scavenging effect on reactive oxygen species anti-inflammatory effects. These effects protect skin from UV damage and thus provide protection against skin aging (Cazzonelli, 2011) (Langi *et al.*, 2018).

2.4 Medicinal plants:

2.4.1 Botanical name: *Catharanthus roseus*

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Gentianales

Family: Apocynaceae

Genus: Catharanthus

Species: *C. roseus*

This plant is commonly known as Sada bahar in Hindi and is dicotyledonous angiosperm . It is an evergreen herbaceous plant native to Indian Ocean Island of Madagascar. *C. roseus* are rich in carbohydrate, saponin, flavonoid and alkaloid. Vinblastin and vincristine are the two indole alkaloids found in large amounts in this plant (Nayak, Mishra and Verma, 2017). Alkaloids derived from leaf and stem of this plant exhibit anti-cancerous activity. Vinblastine have been used for the treatment of neoplasms, leukemia in children and Hodgkins disease. Methanolic extracts of *C. roseus* exhibit anticancerous activity on numerous drug resistant tumor types. Ethanolic extracts prepared from leaves and flowers exhibit anti-diabetic effect in dose-dependent manner (Nayak, Mishra and Verma, 2017). It increases the utilization of glucose inside the liver. Vinculin is the pharmacological marketed product that contains alkaloids isolated from *C. roseus.* Crude extracts from leaves show significant anti-bacterial activity. Recent studies have been done to test wound healing activity, and it was found that treatment with *C. roseus* 100 mg/kg/day in rats shows significant increased rate in wound contraction.

Studies have also shown significant decrease in serum levels of low density and high density cholesterol and triglycerides (Das, Krishi Viswavidyalaya and Sharangi, 2017).

2.4.2 Botanical name: *Hibiscus rosa-sinensis*

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Malvales

Family: Malvaceae

Genus: *Hibiscus*

Species: *Hibiscus rosa-sinensis*

It is commonly known as China-rose in English and belongs to tropical Asia in origin. In traditional use roots and leaves were used as medicines. It was used to improve blood circulation during menstruation and was also used to stimulate parturition during child birth. Early phytochemical analysis showed that the plant contains quinines, tannins, phenols, terpenoids, flavonoids, alkaloids. Besides this it also contains saponins, steroids, reducing sugars (Al-Snafi, 2018). In rats thereproductive effects was tested and it was found that chloroform and alcoholic extract of flowers lowered the sperm count in males by decreasing the spermatogenic elements. Ethyl acetate fraction of *Hibiscus rosa-sinensis* exhibits anti-diabetic as well as hypolipidemic effects. Experiments were carried out in a dose dependent manner and was compared with metformin and it was found that there was a significant decrease in glycated haemoglobin and serum glucose level (Al-Snafi, 2018) (Maganha *et al.*, 2010). Also there was decrease in levels of total cholesterol and triglycerides. Furthermore, extracts of *Hibiscus rosa-sinensis* also have therapeutic effect on chronic diseases such as cancer and protects against progression of tumor.

Pretreatment with its extract prevents against oxidative damage and also restores cells protective enzymes. There was also a strong antimicrobial effect exerted by the plant effect and is maximum against *Bacillus subtillis* and *Escherichia coli.* Antiparasitic effect was shown against *Hymenolepis diminuta* (Al-Snafi, 2018).

2.4.3 Botanical name: *Murraya koenigii*

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Sapindales

Family: Rutaceae

Genus: *Murraya*

Species: *Murraya koenigii*

Murraya koenigii is an aromatic plant used in Indian medicine. It is a shrub that is found throughout India. This plant contains a characteristic strong odour and is widely used to enhance food flavor. It is rich in alkaloids such as murrayanine, murrayacine, koenidine, koenine, isomahanine, O-methylmurrayamine. Furthermore, the leaves are rich sources of calcium and vitamin A, the dry leaves are rich in monoterpenes.. Major amino acids found in this plant are glycine, serine, alanine, tryptophan, proline, tyrosine, glutamic acid (Iyer and Devi, 2008). Pharmacological activity include its antibacterial activity against *P.vulgaris, Staphylococcus aureus, Pasteurella multicida, B.subtilis.* It is alaso effective against *M. tuberculosis*. Its antifungal activity are against *C. albicans, A. niger, Microsporum gypseum*. Oral administration of aqueous extract resulted in hypoglycemic and anti-hyperglycemic effect on rats. It lead to decrease in blood glucose and glycosylated hemoglobin as well as creatinine in diabetic animals. Several studies has been done to test the antioxidant potential of this plant and it has been

reported that it successfully protects against oxidative stress and damage, chemical carcinogen, beta-cell damage as well chronic diseases such as arthritis, atherosclerosis (Iyer and Devi, 2008). Another unique pharmacological activity exhibited by this plant is anti-lipid peroxidase activity. Oral administration of plant extract led to decrease in levels of glutathione in heart, liver and kidney (Gahlawat, Jakhar and Dahiya, 2014).

2.4.4 Botanical name: *Moringa oleifera*

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Capparales

Family: Moringaceae

Genus: *Moringa*

Species: *Moringa oleifera*

Moringa oleifera has been put to medicinal use since ages. This pant is quick developing deciduous tree native to sub-Himalayan tracts. It is rich in vitamins and minerals and proteins and various secondary metabolites such as alkaloids, terpenoiids, flavonoids, steroids, saponins, anthroquinones and carbohydrates (Lakshmana Prabu, Umamaheswari and Puratchikody, 2019). Its pharmacological activity include anti-diabetic potential. Ethanolic extract and aqueous extractled to significant decrease in blood glucose levels normalized insulin levels in body. Extracts of *Moringa oleifera* was studied for its anti-ulcer activity and was found that when administered in dose dependent manner it led to reduction in ulcer index such as decrease in gastric volume and acidity (Sugunabai *et al.*, 2014). Extracts of its roots and bark also shows anti-ulcer activity. It also possess anthelmintic activity. Chloroform and methanolic extracts were potent source for killing of worms and also destroys larvae of worms such as *Pheretima*

posthuma and *Haemonchus contortus*. Ethanolic and aqueous extracts possess anti-inflammatory effects and leads to significant decrease in edema volume and also exhibit antipyretic effects (Lakshmana Prabu, Umamaheswari and Puratchikody, 2019). Another unique pharmacological effect include anti-epileptic effects that prevents hind limb extension stimulated by maximal electro shock and also lead in reduction of convulsion duration. Its cardioprotective effects include decrease in levels of cholesterol levels thus exhibiting hypolipidemic and antiatherosclerotic activities (Lakshmana Prabu, Umamaheswari and Puratchikody, 2019) (Mwanga, Mvungi and Tibuhwa, 2019).

2.4.5 Botanical name: *Kalanchoe pinnata*

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Saxifragales

Family: Crassulaceae

Genus: *Kalanchoe*

Species: *Kalanchoe pinnata*

Kalanchoe pinnata is a glabrous herb with elliptic leaves and bears reddish purple flowers. It is distributed throughout India and is widely used in folk medicines. Leaves of *Kalanchoe pinnata* are rich in phenolic compounds, alkaloids, flavonoids and macro and micro nutrients such as calcium, potassium, sodium, vitamins, iron and zinc (Quazi Majaz A.1*, A.U. Tatiya2, Molvi Khurshid1, Sayyed Nazim1, 2011). Three very rare flavonoids that are present in plants are also present in leaves of *Kalanchoe pinnata* , these are kaempherol, 3-O-α-Larabinopyranosyl and Quercetin 3- O- α-Larabinopyranosyl(1→2) α-L-rhamnopyranoside. Its pharmacological activity

include- anticonvulsant activity, antidiabetic activity, anticancer activity, antileishmanial activity. It also exhibit anti-nociceptive activity against nociceptive pain stimuli. Methanolic and aqueous extract possess mild antiproliferative effect on sarcoma cell lines (Quazi Majaz A.1*, A.U. Tatiya2, Molvi Khurshid1, Sayyed Nazim1, 2011). Antiurolithic activity is one of the unusual effect exhibited by this plant and helps in treatment of kidney dysfunction and kidney stones. It helps in reduction of hepatotoxicity thus exhibiting hepatoprotective activity. Under immunomodulatory effect exerted by this plant, it leads to increase in T cell proliferation, Histamine release and cytokine production. Dose dependent introduction of saline leaf extract result in neurosedative and muscle relaxant activity. Wound healing and deadspace wound in albino is also one of the significant effect exerted by this plant (Quazi Majaz A.1*, A.U. Tatiya2, Molvi Khurshid1, Sayyed Nazim1, 2011).

CHAPTER 3. Methodology:

3.1 Preparation of Plant Extract:

3.1.1 Aqueous Extract of Shade Dried leaves:

Leaves of *Kalanchoe pinnata, Catharanthus roseus, Murraya koenigii, Moringa oleifera, Hibiscus rosa-sinensis* were plucked from the plant. The petioles from the leaves were removed. The leaves were then washed with running tap water to remove all the dust particles, further it was washed with milli-Q water twice to make it germ free. The leaves were then spread individually on a clean surface and kept in shade for five days. After completion of drying process the leaves were weighed and then crushed into fine powder using mortar and pestle. For preparation of plant extract, 1gm of leaf powder was added to 100 ml of milli-Q water in a 200ml flask and then it was boiled for 5 min at temperature 50°C for 10 min using a heating mantle. The flask was then left to cool down at room temperature and then the content was transferred to 50ml falcon tube. The extract was then centrifuged at 3000 rpm for 5 min. The supernatant was separated from the leaf pellets using a whatman filter paper. The collected supernatant is the aqueous extract of the leaf.

3.1.2 Aqueous Extract of Sun Dried Leaves:

Leaves of *Kalanchoe pinnata, Catharanthus roseus, Murraya koenigii, Moringa oleifera, Hibiscus rosa-sinensis* were plucked from the plant. The petioles from the leaves were removed. The leaves were then washed with running tap water to remove all the dust particles, further it was washed with milli-Q water twice to make it germ free. The leaves were then spread individually on a clean surface and kept in sun for three days. After completion of drying process

the leaves were weighed and then crushed into fine powder using mortar and pestle. For preparation of plant extract, 1gm of leaf powder was added to 100 ml of milli-Q water in a 200ml flask and then it was boiled for 5 min at temperature 50˚C for 10 min using a heating mantle. The flask was then left to cool down at room temperature and then the content was transferred to 50ml falcon tube. The extract was then centrifuged at 3000 rpm for 5 min. The supernatant was separated from the leaf pellets using a whatman filter paper. The collected supernatant is the aqueous extract of the leaf.

3.1.3 Aqueous Extract of Fresh Leaves:

Leaves of *Kalanchoe pinnata, Catharanthus roseus, Murraya koenigii, Moringa oleifera, Hibiscus rosa-sinensis* were plucked from the plant. The petioles from the leaves were removed. The leaves were then washed with running tap water to remove all the dust particles, further it was washed with milli-Q water twice to make it germ free. Mixer grinder was then used to make fine paste of the leaf. For preparation of plant extract, 1gm of leaf paste was added to 100 ml of milli-Q water in a 200ml flask and then it was boiled for 5 min at temperature 50˚C for 10 min using a heating mantle. The flask was then left to cool down at room temperature and then the content was transferred to 50ml falcon tube. The extract was then centrifuged at 3000 rpm for 5 min. The supernatant was separated from the leaf pellets using a whatman filter paper. The collected supernatant is the aqueous extract of the leaf.

3.1.4 Petroleum Ether Extract of shade dried leaves:

Leaves of *Kalanchoe pinnata, Catharanthus roseus, Murraya koenigii, Moringa oleifera, Hibiscus rosa-sinensis* were plucked from the plant. Procedure for shade drying was followed. After completion of drying process the leaves were weighed and then crushed into fine powder using mortar and pestle. For preparation of plant extract, 0.2 gm of leaf powder was added to 15 ml of Petroleum Ether in a 100ml flask and then it was kept in a BOD Incubator for 48 hrs. The content was then transferred to 50ml falcon tube. The extract was then centrifuged at 3000 rpm for 5 min. The supernatant was separated from the leaf pellets using a whatman filter paper. The collected supernatant is the petroleum ether extract of the leaf.

3.1.5 Chloroform Extract of shade dried leaves:

Leaves of *Kalanchoe pinnata, Catharanthus roseus, Murraya koenigii, Moringa oleifera, Hibiscus rosa-sinensis* were plucked from the plant. Procedure for shade drying was followed. After completion of drying process the leaves were weighed and then crushed into fine powder using mortar and pestle. For preparation of plant extract, 0.5 gm of leaf powder was added to 50ml of chloroform in a 100ml flask and then it was kept in a BOD Incubator for 48 hrs. The content was then transferred to 50ml falcon tube. The extract was then centrifuged at 3000 rpm for 5 min. The supernatant was separated from the leaf pellets using a whatman filter paper. The collected supernatant is the chloroform extract of the leaf.

3.1.6 Methanolic Extract of shade dried leaves:

Leaves of *Kalanchoe pinnata, Catharanthus roseus, Murraya koenigii, Moringa oleifera, Hibiscus rosa-sinensis* were plucked from the plant. Procedure for shade drying was followed.

After completion of drying process the leaves were weighed and then crushed into fine powder using mortar and pestle. For preparation of plant extract, 0.5 gm of leaf powder was added to 50ml of Methanol in a 100ml flask and then it was kept in a BOD Incubator for 48 hrs. The content was then transferred to 50ml falcon tube. The extract was then centrifuged at 3000 rpm for 5 min. The supernatant was separated from the leaf pellets using a whatman filter paper. The collected supernatant is the Methanol extract of the leaf.

3.1.7 Hexane Extract of shade dried leaves:

Leaves of *Kalanchoe pinnata, Catharanthus roseus, Murraya koenigii, Moringa oleifera, Hibiscus rosa-sinensis* were plucked from the plant. Procedure for shade drying was followed. After completion of drying process the leaves were weighed and then crushed into fine powder using mortar and pestle. For preparation of plant extract, 0.5 gm of leaf powder was added to 50 ml of Hexane in a 100ml flask and then it was kept in a BOD Incubator for 48 hrs. The content was then transferred to 50ml falcon tube. The extract was then centrifuged at 3000 rpm for 5 min. The supernatant was separated from the leaf pellets using a whatman filter paper. The collected supernatant is the hexane extract of the leaf.

3.2 Qualitative test:

3.2.1 Test for alkaloid:

Wagner's Test was followed for qualitative test for alkaloids. For preparation of Wagner's reagent, 2.25 gm of iodine and 12.5 gm of Potassium Iodide was measured using weighing balance. Iodine and Potassium Iodide was added to 250 ml milli-Q water. Then 1ml of prepared plant extract was pipetted and 1ml of prepared Wagner's Reagent was added.

3.2.2 Test for Phenol:

Ferric Chloride test was followed to test presence of phenol in the plant. To prepare 5% ferric chloride solution 1gm of ferric Chloride salt was measured and transferred into a flask. 20ml of milli-Q water was added to the flask. Then 1ml of prepared plant extract was pipetted and 1ml of ferric chloride solution was added to it.

Table 1: Protocol for Qualitative Test

Sl. No.	Preliminary Test	Procedure	Expected Result
1.	Alkaloid	1ml Plant Extract + 1ml Wagner's Reagent	Reddish Brown Precipitate
2.	Phenol	1ml Plant Extract + 1ml Ferric Chloride Solution	Bluish Black ring

3.3 Test for β- Carotene:

3.3.1 Preparation of Carrot Extract:

3 samples of *Daucus carota* taken and peeled. It was then washed to remove all the impurities. Each of the 3 carrots were then cut into small pieces and grated separately using cheese grater. The grated carrot was then added in 3 separate flasks. Then 240 ml of ethanol was then added to each beaker. Further 10 ml of water added to each flask and then it was covered with a lid. The flask was then kept in BOD Incubator for 1 hr. after incubation 10 ml of extract was filtered using whatman filter paper.

3.4 Quantitive Test:

3.4.1 Estimation of Total Phenolic Content:

"Determination of total phenolic content (TPC) in the extract was done using Folin-Ciocalteu (FC) assay as described by Singleton et al (1999) slight modifications. For this 100 µl of the extract was added to 900 µl, of milli-Q water. Then 200 µl of 1 N FC reagent was added and 2.0 ml. of sodium carbonate (7% w/v) was added to the soilution. To prepare 7% sodium carbonate solution 7gm of anhydrous sodium carbonate was added to 100 ml of milli-Q water. Further, the contents were mixed and allowed to stand for 30 min at room temperature (25°C). The absorbance was measured at 750 nm using UV-Vis spectrophotomer (LAMBDA 25 UV/Vis Systems, PerkinElmer, Inc. MA 02451, USA)". Total phenolic content was expressed as µg/g GAE (Gallic acid equivalent).

Table 2: Preparation of Standard solution

Gallic acid dilutions	FC reagent	Sodium Carbonate
1mg/ml	5ml	4ml
0.5mg/ml	5ml	4ml
0.25mg/ml	5ml	4ml
0.125mg/ml	5ml	4ml

3.4.2 Estimation of β- Carotene:

Amount of β- Carotene present in 3 samples of *Daucus carota* was calculated using two organic solvents separately in all the three samples. For this after the separation carrot extract in a test tube, 10 ml of petroleum ether, Hexane was added separately into all the three samples of *Daucus carota.* The solution was allowed to stand for 15 min and then the supernatant was collected in a cuvette and absorbance was measured at 450 nm using UV-Vis spectrophotomer (LAMBDA 25 UV/Vis Systems, PerkinElmer, Inc. MA 02451, USA). Total β- Carotene was expressed in terms of β- Carotene equivalent.

Table 3: Preparation of Standard solution with Petroleum Ether as solvent

β- Carotene Dilutions	Petroleum Ether
0.5mg/ml	6ml
0.25mg/ml	3ml
0.125mg/ml	2ml
0.062mg/ml	1.5ml
0.031mg/ml	1ml

Table 4: Preparation of Standard solution with Hexane as solvent

β- Carotene Dilutions	**Hexane**
0.5mg/ml	6ml
0.25mg/ml	3ml
0.125mg/ml	2ml
0.062mg/ml	1.5ml
0.031mg/ml	1ml

CHAPTER 4. Result:

4.1 Qualitative Test:

Plant leaves extract was prepared and protocols qualitative for test of alkaloids and phenols was performed. It was observed that in test for phenols a bluish black ring appeared and in test for alkaloids reddish brown precipitate appeared.

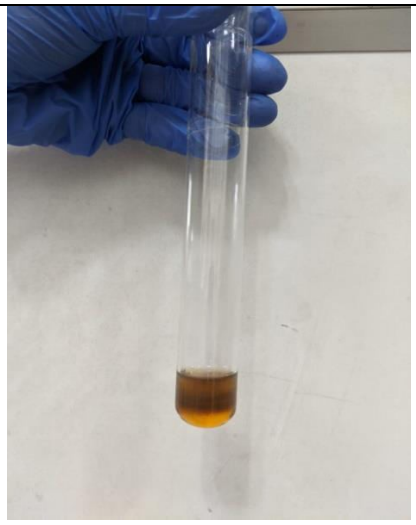
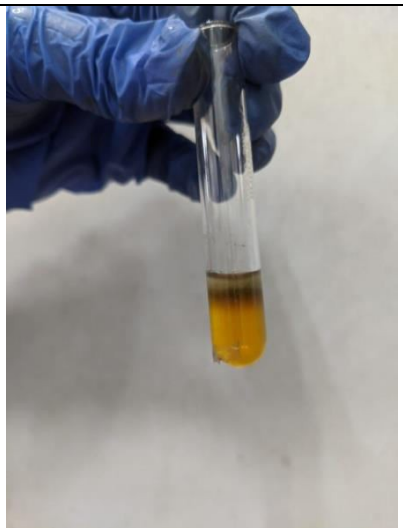
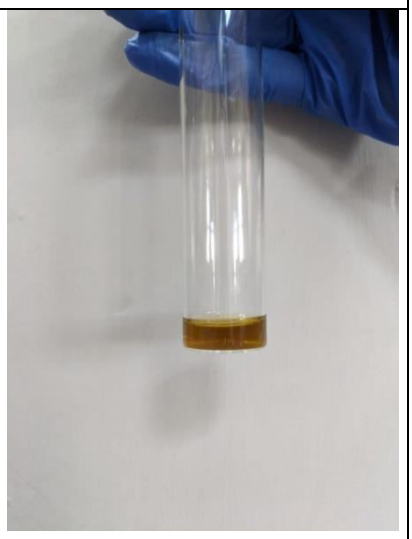
Table 5. Aqueous extract of Shade Dried Leaves:

Sl.No.	Plant	Alkaloid	Phenol
1.	*Kalanchoe pinnata*	+	+
2.	*Hibiscus rosa-sinensis*	+	-
3.	*Moringa oleifera*	+	++
4.	*Catharanthus roseus*	+	+
5.	*Murraya koenigii*	+	++

Wagner's Test was followed for qualitative test for alkaloids. For preparation of Wagner's reagent, 2.25 gm of iodine and 12.5 gm of Potassium Iodide was measured using weighing balance. Iodine and Potassium Iodide was added to 250 ml milli-Q water. Then 1ml of prepared plant extract was pipetted and 1ml of prepared Wagner's Reagent was added. Reddish brown precipitate was observed.

Ferric Chloride test was followed to test presence of phenol in the plant. To prepare 5% ferric chloride solution 1gm of ferric Chloride salt was measured and transferred into a flask. 20ml of milli-Q water was added to the flask. Then 1ml of prepared plant extract was pipetted and 1ml of ferric chloride solution was added to it. Bluish black ring was observed.

Figure 2: Qualitative test for phenol and alkaloid in shade dried leaves

Moringa oleifera	*Catharanthus roseus*	*Hibiscus*
		
Positive Test for Phenol	Positive Test for Phenol	Negative Test for Phenol

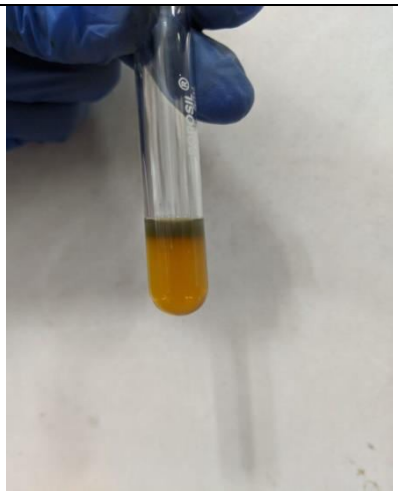
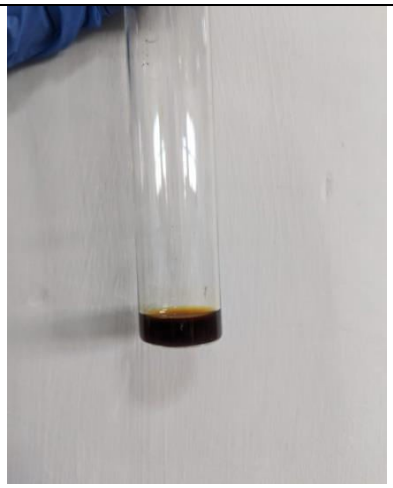
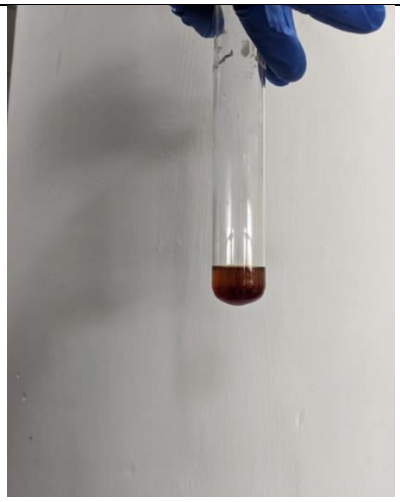
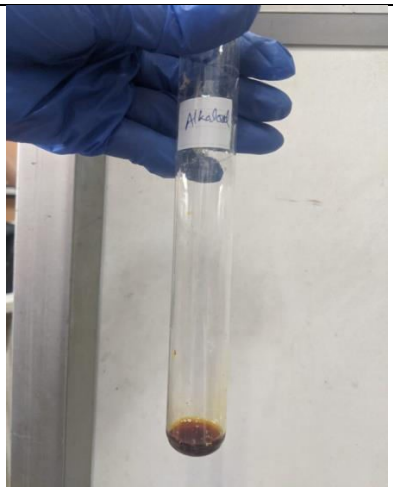
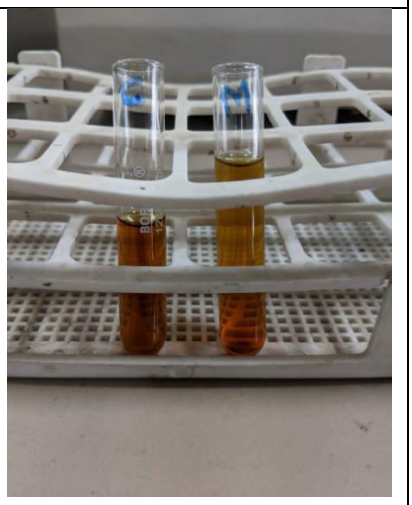
Kalanchoe pinnata	*Murraya koenigii*	*Hibiscus rosa-sinensis*
		
Positive Test for Phenol	Positive test for phenol	Positive test for Alakloid
Catharanthus roseus	*Kalanchoe pinnata*	*Murraya koenigii, Moringa oleifera*
		
Positive Test for Alkaloid	Positive Test for Alkaloid	Positive Test for Alkaloid

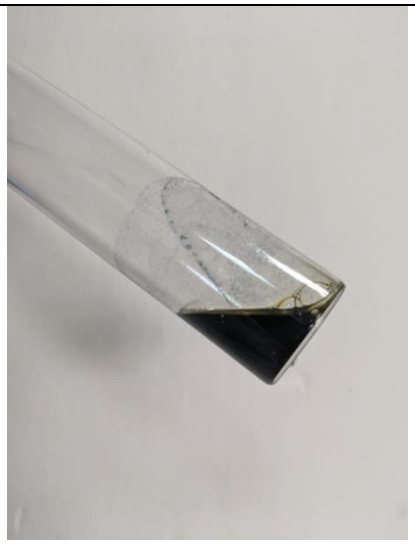
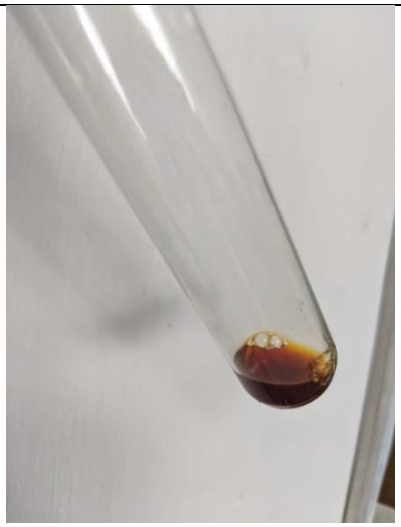
Table 6. Aqueous extract of Fresh Leaves:

Sl.No.	Plant	Alkaloid	Phenol
1.	*Kalanchoe pinnata*	++	+++
2.	*Hibiscus rosa-sinensis*	++	+++
3.	*Moringa oleifera*	+	+++
4.	*Catharanthus roseus*	++	+
5.	*Murraya koenigii*	+++	++

Wagner's Test was followed for qualitative test for alkaloids. For preparation of Wagner's reagent, 2.25 gm of iodine and 12.5 gm of Potassium Iodide was measured using weighing balance. Iodine and Potassium Iodide was added to 250 ml milli-Q water. Then 1ml of prepared plant extract was pipetted and 1ml of prepared Wagner's Reagent was added. Reddish brown precipitate was observed.

Ferric Chloride test was followed to test presence of phenol in the plant. To prepare 5% ferric chloride solution 1gm of ferric Chloride salt was measured and transferred into a flask. 20ml of milli-Q water was added to the flask. Then 1ml of prepared plant extract was pipetted and 1ml of ferric chloride solution was added to it. Bluish black ring was observed.

Figure 3. Qualitative test for phenol and alkaloid in Fresh leaves

Murraya koenigii	*Catharanthus roseus*	*Kalanchoe pinnata*
		
Positive Test for Alkaloid	Positive Test for Alkaloid	Positive Test for Alkaloid
Hibiscus rosa-sinensis	*Moringa oleifera*	*Murraya koenigii*
		
Positive Test for Alkaloid	Positive Test for Alkaloid	Positive Test for Phenol

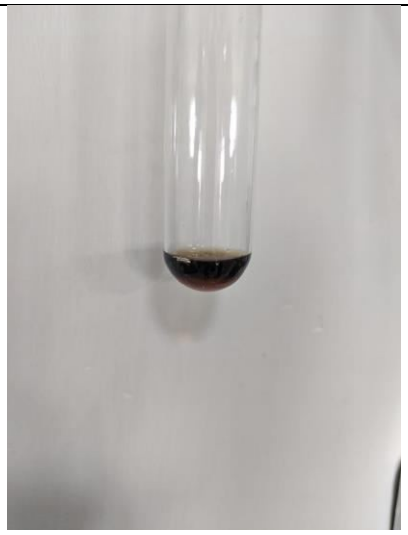
Moringa oleifera	*Hibiscus rosa-sinensis*	*Catharanthus roseus*
		
Positive Test for Phenol	Positive Test for Phenol	Positive Test for Phenol

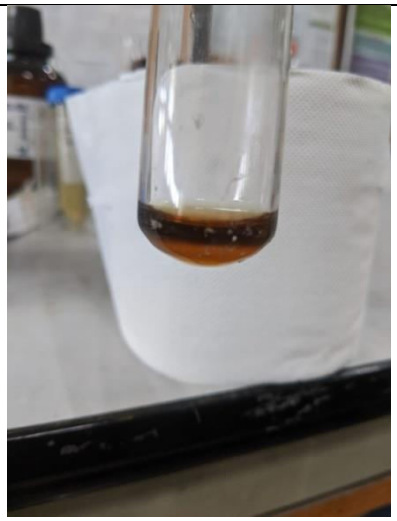
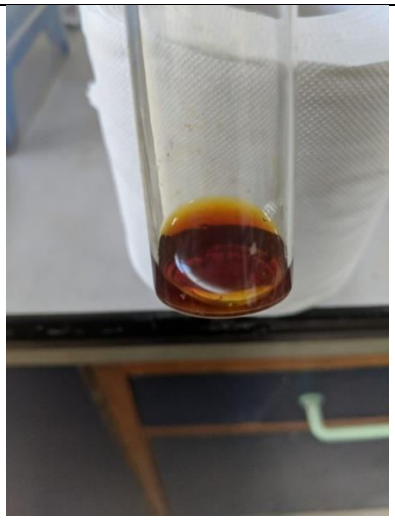
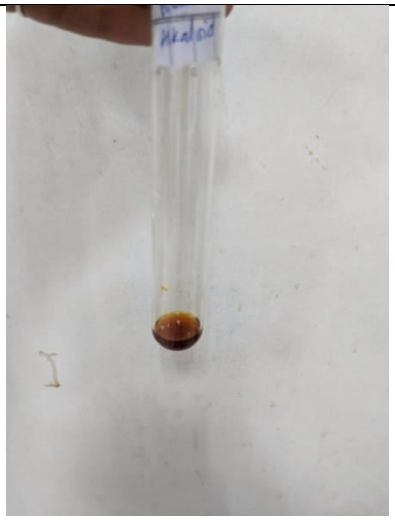
Table 7. Aqueous extract of Sun Dried Leaves:

Sl.No.	Plant	Alkaloid	Phenol
1.	*Kalanchoe pinnata*	+	-
2.	*Hibiscus rosa-sinensis*	+	-
3.	*Moringa oleifera*	+	+
4.	*Catharanthus roseus*	-	+
5.	*Murraya koenigii*	+	+

Wagner's Test was followed for qualitative test for alkaloids. For preparation of Wagner's reagent, 2.25 gm of iodine and 12.5 gm of Potassium Iodide was measured using weighing balance. Iodine and Potassium Iodide was added to 250 ml milli-Q water. Then 1ml of prepared plant extract was pipetted and 1ml of prepared Wagner's Reagent was added. Reddish brown precipitate was observed.

Ferric Chloride test was followed to test presence of phenol in the plant. To prepare 5% ferric chloride solution 1gm of ferric Chloride salt was measured and transferred into a flask. 20ml of milli-Q water was added to the flask. Then 1ml of prepared plant extract was pipetted and 1ml of ferric chloride solution was added to it. Bluish black ring was observed.

Figure 4: Qualitative test for phenol and alkaloid in sun dried leaves

Murraya koenigii	*Moringa oleifera*	*Hibiscus rosa-sinensis*
		
Positive test for Alkaloid	Positive test for Alkaloid	Positive test for Alkaloid

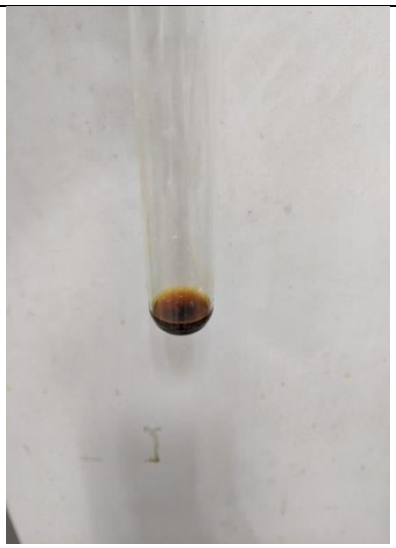
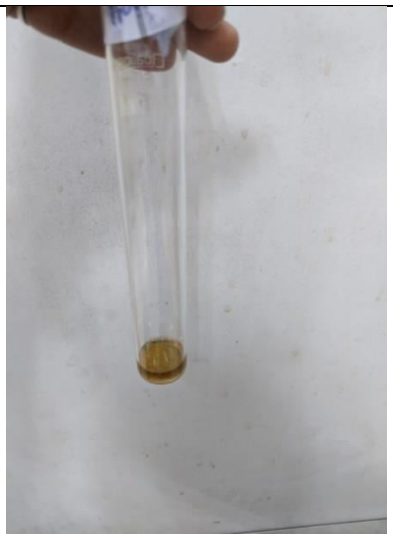
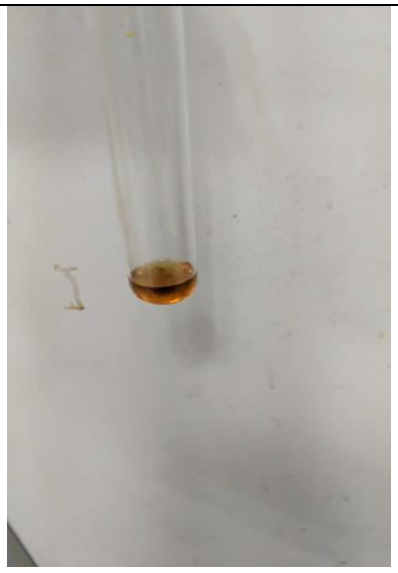
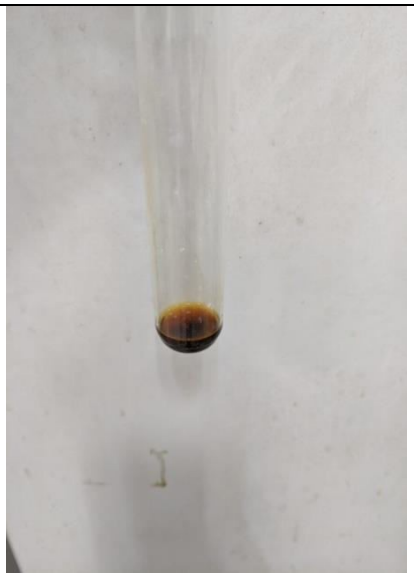
		
Negative test for Alkaloid *Catharanthus roseus*	Positive test for Alkaloid *Kalanchoe pinnata*	Negative test for Phenol *Kalanchoe pinnata*
Moringa oleifera	*Catharanthus roseus*	*Murraya koenigii*
		
Positive test for Phenol	Positive test for Phenol	Positive test for Phenol

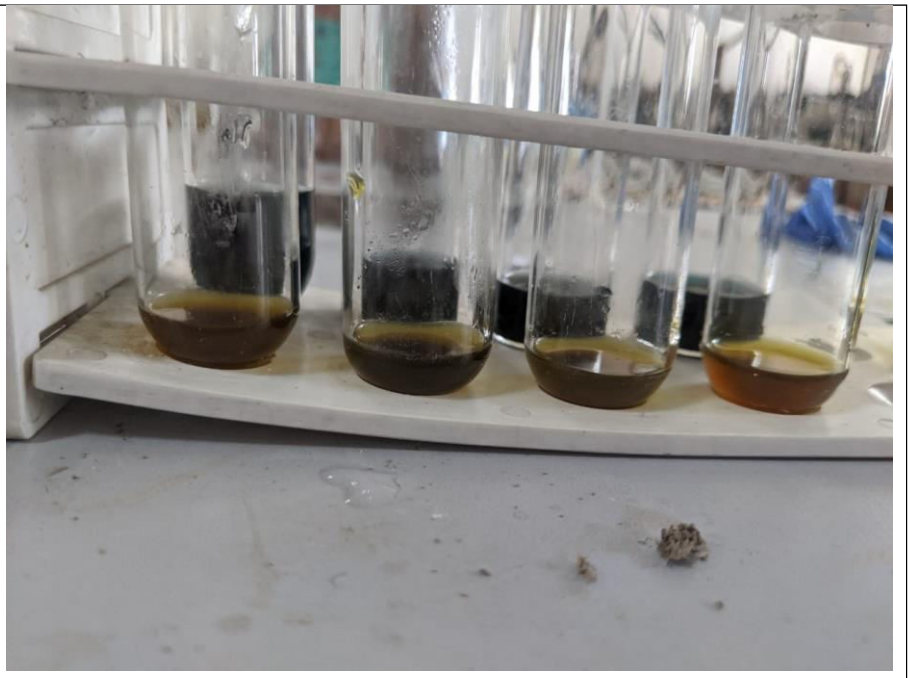
Table 8. Petroleum Ether Extract of Shade dried Leaves:

Sl.No.	Plant	Alkaloid	Phenol
1.	*Kalanchoe pinnata*	+	**-**
2.	*Hibiscus rosa-sinensis*	++	**-**
3.	*Moringa oleifera*	+++	**-**
4.	*Catharanthus roseus*	+++	**-**
5.	*Murraya koenigii*	++	**-**

Table 9. Methanol Extract of Shade Dried Leaves:

Sl.No.	Plant	Alkaloid	Phenol
1.	*Kalanchoe pinnata*	+	+
2.	*Hibiscus rosa-sinensis*	++	+
3.	*Moringa oleifera*	+	+
4.	*Catharanthus roseus*	-	+
5.	*Murraya koenigii*	+	+

Figure 5. Qualitative test for phenol and alkaloid in methanolic extract

Positive Test for Alkaloid for the following plants: *K. pinnata* *Hibiscus* *M.olifera* *C. roseus* *M. koenigii*	

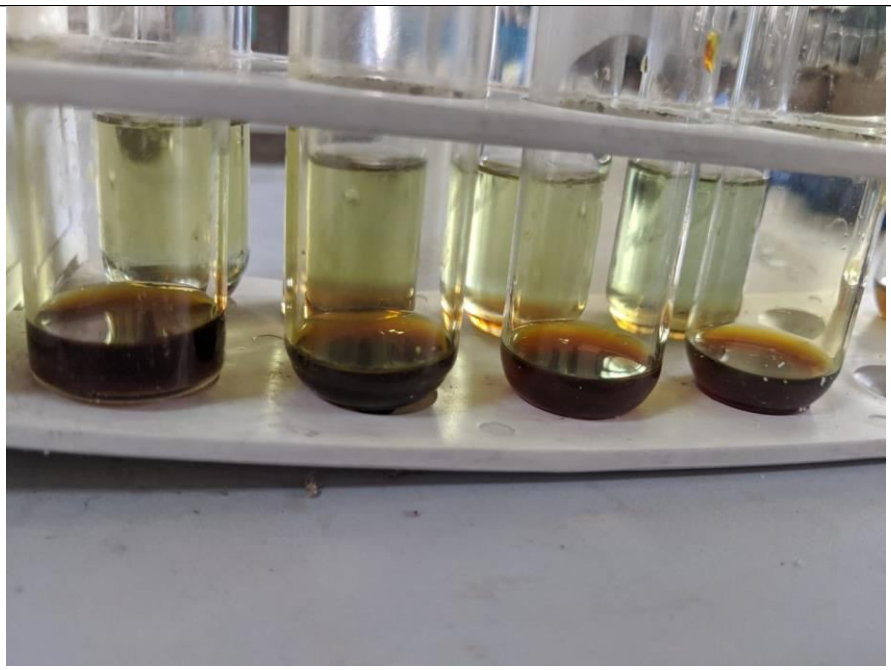
Positive Test for Phenol for the following plants: *K. pinnata* *Hibiscus* *M.olifera* *C. roseus* *M. koenigii*	

Table 10. Hexane Extract of Shade Dried Leaves:

Sl.No.	Plant	Alkaloid	Phenol
1.	*Kalanchoe pinnata*	+	+
2.	*Hibiscus rosa-sinensis*	+	+
3.	*Moringa oleifera*	+	+
4.	*Catharanthus roseus*	+	+
5.	*Murraya koenigii*	+	+

Wagner's Test was followed for qualitative test for alkaloids. For preparation of Wagner's reagent, 2.25 gm of iodine and 12.5 gm of Potassium Iodide was measured using weighing balance. Iodine and Potassium Iodide was added to 250 ml milli-Q water. Then 1ml of prepared plant extract was pipetted and 1ml of prepared Wagner's Reagent was added. Reddish brown precipitate was observed. Reddish brown precipitate was observed.

Ferric Chloride test was followed to test presence of phenol in the plant. To prepare 5% ferric chloride solution 1gm of ferric Chloride salt was measured and transferred into a flask. 20ml of milli-Q water was added to the flask. Then 1ml of prepared plant extract was pipetted and 1ml of ferric chloride solution was added to it. Bluish black ring was observed.

4.2 Quantitative Test: Estimation of Total Phenolic Content

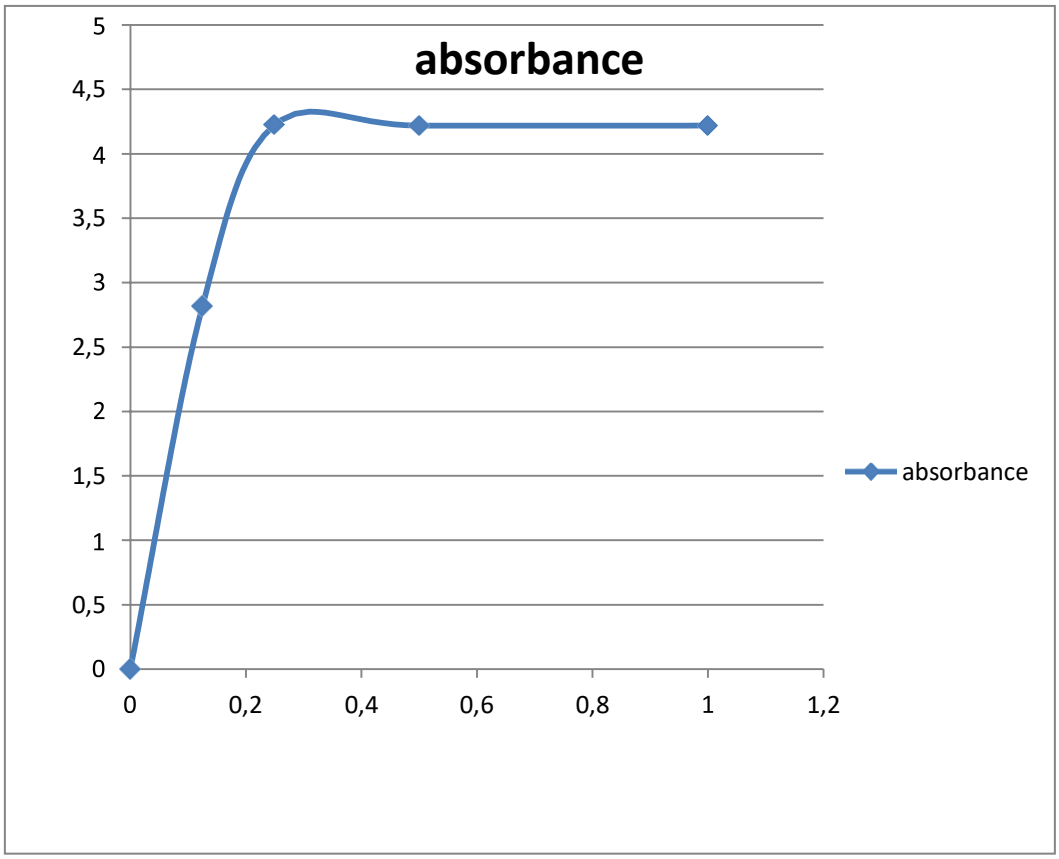
Gallic acid dilutions were prepared using FC reagent and Sodium Carbonate and absorbance was taken using UV spectroscopy. Folin-Ciocalteu (FC) assay was followed for calculation of TPC in different plants.

Table 11. Absorbance of standard dilutions:

Gallic acid dilutions	FC reagent	Sodium Carbonate	Absorbance at 750nm
1mg/ml	5ml	4ml	4.22
0.5mg/ml	5ml	4ml	4.22
0.25mg/ml	5ml	4ml	4.2253
0.125mg/ml	5ml	4ml	2.8181

Table 12: TPC for different plants:

Plant	Absorbance at 750 nm	Concentration of Phenol
Hibiscus rosa-sinensis	2.8262	0.18 mg/ml
Moringa oleifera	1.2651	0.08 mg/ml
Catharanthus roseus	2.5254	0.10 mg /ml
Murraya koenigii	3.8406	0.20 mg/ml



Y-axis: Absorbance

X-axis: Concentration

Figure 6. Absorbance graph for total phenolic content

4.3 Estimation of β- Carotene in *Daucus carota*

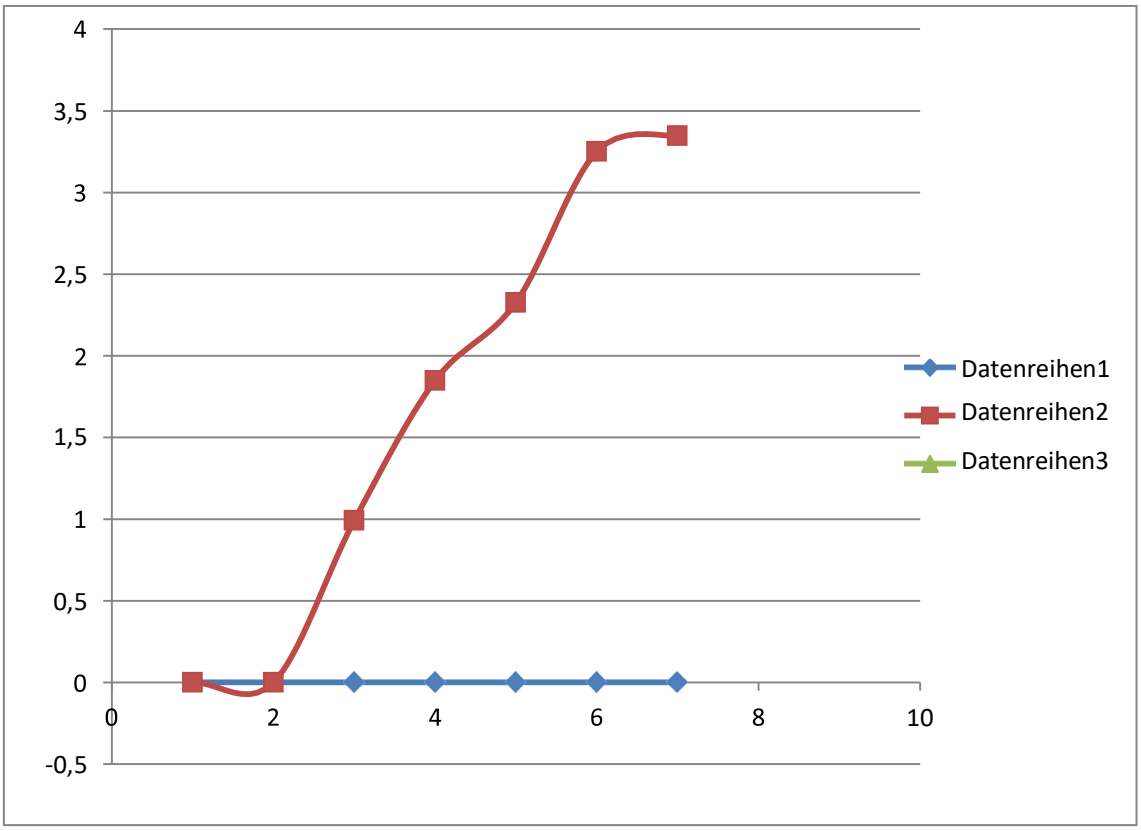
Amount of β- Carotene present in 3 samples of *Daucus carota* was calculated using two organic solvents separately in all the three samples. For this after the separation carrot extract in a test tube, 10 ml of petroleum ether, Hexane was added separately into all the three samples of *Daucus carota.*

Table 13. Absorbance of standard dilutions with petroleum ether as solvent

β- Carotene Dilutions	**Petroleum Ether**	**Absorbance at 450nm**
0.5mg/ml	6ml	3.348
0.25mg/ml	3ml	3.2508
0.125mg/ml	2ml	2.3261
0.062mg/ml	1.5ml	1.8487
0.031mg/ml	1ml	0.992

Table 14. Absorbance of samples with petroleum ether as solvent

Daucus carota	Petroleum Ether solvent Absorbance at 450 nm	Concentration of β- Carotene
Sample I	0.2173	2.16 mg/ml
Sample II	0.2315	2.32 mg/ml
Sample III	0.2143	2.09 mg/ml



X-axis: Concentration

Y-axis: Absorbance

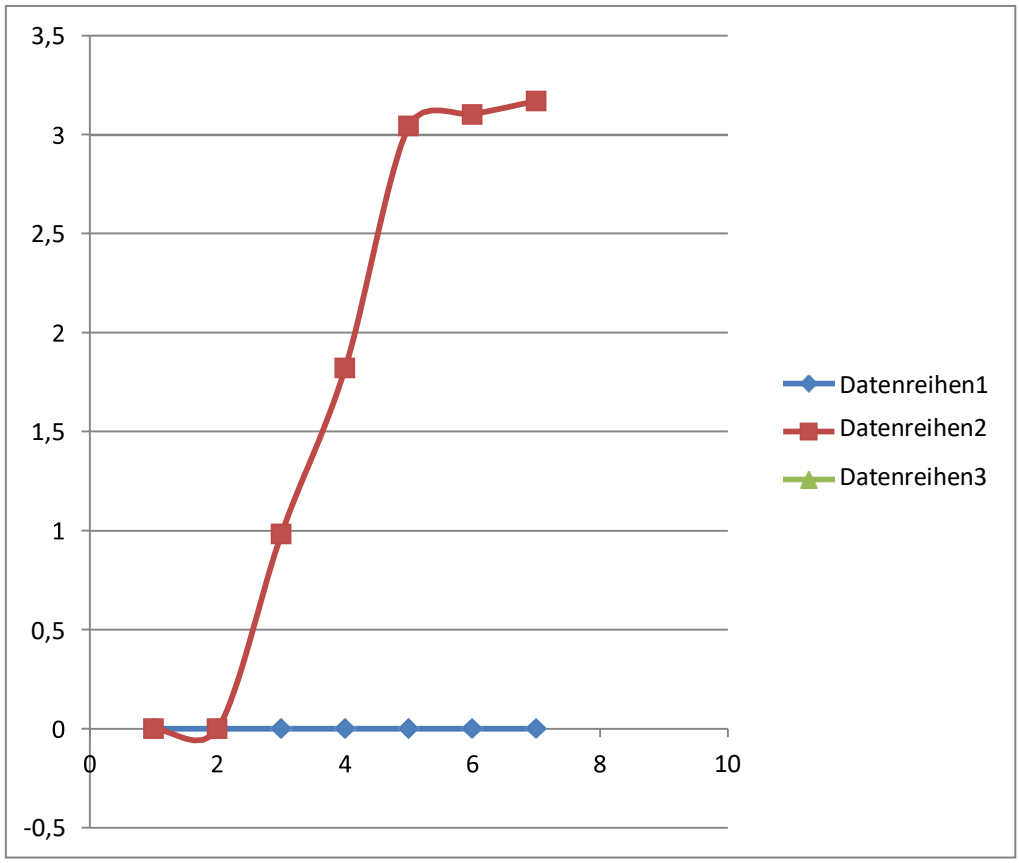
Figure 7. Absorbance graph for β- Carotene with petroleum ether as solvent

Table 15: Absorbance of Standard Dilutions with Hexane as Solvent

β- Carotene Dilutions	**Hexane**	**Absorbance at 450nm**
0.5mg/ml	6ml	3.1692
0.25mg/ml	3ml	3.1017
0.125mg/ml	2ml	3.0425
0.062mg/ml	1.5ml	1.8208
0.031mg/ml	1ml	0.9824

Table 16: Absorbance of Samples with Hexane as Solvent

Daucus carota	Hexane Solvent Absorbance at 450 nm	Concentration of β- Carotene
Sample I	0.2767	2.72 mg/ml
Sample II	0.2233	2.28 mg/ml
Sample III	0.2354	2.41 mg/ml



X-axis: concentration

Y-axis: Absorbance

Figure 8. Absorbance graph for β- Carotene with petroleum ether as solvent

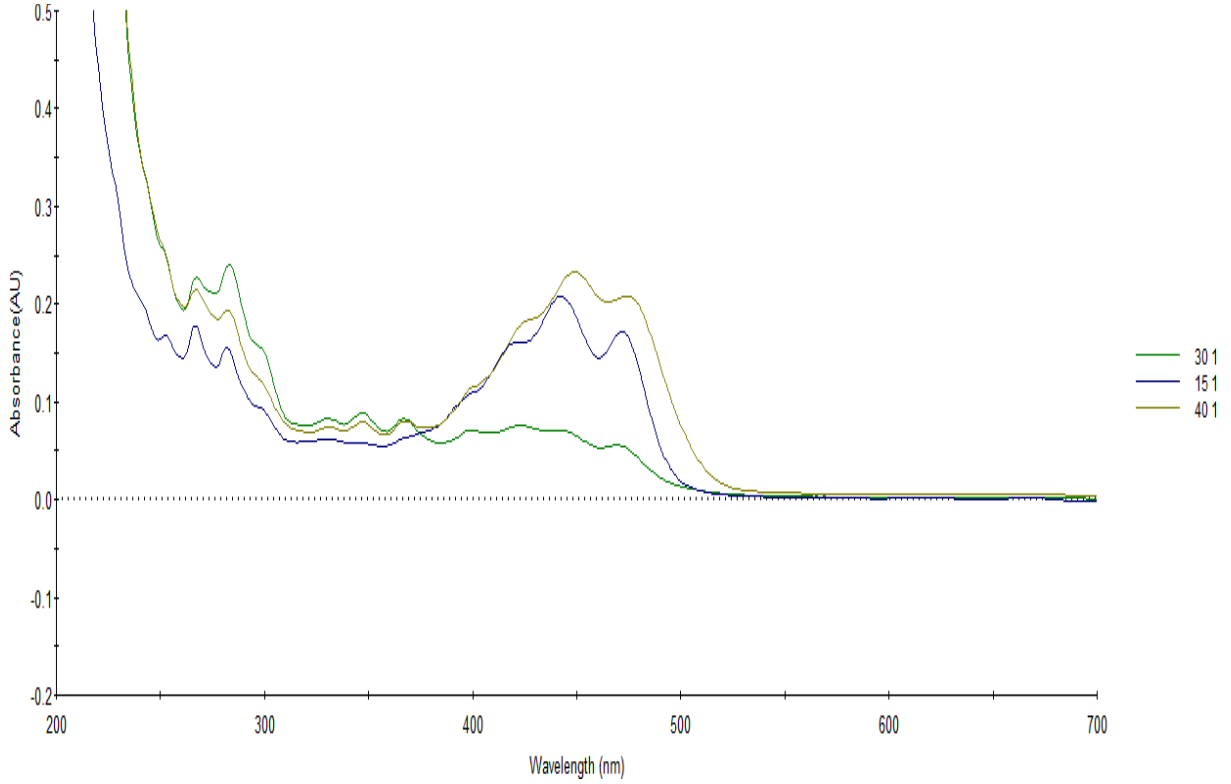


Figure 9. Scanned Graph of *Daucus carota* samples obtained from UV visible Spectroscopy

Discussion:

Plant extracts prepared using water, chloroform, methanol, petroleum ether, hexane shows varying degree of presence of secondary metabolites in leaves. This reveals that certain secondary metabolite can be extracted in maximum amounts using appropriate solvent. A positive qualitative test for phenol is confirmed by appearance of Bluish Black ring at top of the solution. The maximum intensity this ring was found in fresh leaf extract while no ring was formed in petroleum ether extract. While the other solvents confirmed the presence of phenol in the extract but the quantity is very less as there appeared a faint ring at the top of the testtube. In the qualitative test for alkaloid, formation of reddish brown precipitate confirmed its presence. Aqueous extract of fresh leaves gave the positive result for alkaloids in with highest possibility of its quantity of occurrence. In fresh leaf extract there was formation of distinct reddish brown precipitate, while in aqueous extract of shade dried leaves the formation of reddish brown precipitate was very less and faintly visible depicting that very less amount of alkaloid can be extracted through aqueous extract. The quantitative estimation of phenols was done by taking Gallic acid as standard and the maximum amount of phenol was estimated in *Murraya koenigii* with the concentration 0.20 mg/ml while the minimum amount of phenol was present in *Moringa oleifera* with a concentration of 0.08 mg/ml. In the quantitative estimation of β- Carotene from *Daucus carota,* β- Carotene was taken as standard and two solvents hexane and petroleum ether were. With hexane as solvent sample I contained highest amount of β- Carotene, 2.72 mg/ml. with petroleum ether as solvent sample II contained highest amount of β- Carotene, 2.32 mg/ml.

A number of preclinical studies suggest that berberine and related compounds possess significant antiinflammatory activity in models of colitis (Yesilada and Kupeli, 2002; Zhou and Mineshita,

2000), rheumatoid arthritis (Wang et al., 2014a,b), and metabolic syndrome (Li et al., 2015). Another important isoquinoline alkaloid with antiinflammatory activity is colchicine. Found in crocus (Colchicum), colchicine was traditionally used for treating gout (Wallace and Roberts, 1953). This traditional use was supported by preclinical studies showing colchicine inhibits histamine release in mast cells (Gillespie et al., 1968) and expression of LPS-induced TNF-α (Li et al., 1996). In fact, colchicine was even found effective at reducing inflammation in a clinical trial of osteoarthritis (Das et al., 2002) and is currently approved by the FDA for treating gout. Another group of alkaloids with significant antiinflammatory activity is capsaicin and the related capsaicinoids. However, the related compound, brucine, possesses much lower toxicity and shows promising therapeutic activity in animal models (Chen et al., 2012; Yin et al., 2003). According to Khadem and Marles, gallic acid has antineoplastic and bacteriostatic activities, and salicylic acid exerts anti-inflammatory, analgesic, antipyretic, antifungal, and antiseptic properties. Protocatechuic acid has also been described as having several biotivities such as anti-inflammatory, antifungal, and antioxidant ones. The four most common hydroxycinnamic acids (type of Phenol) are ferulic acid, caffeic acid, coumaric acid, and sinapic acid. These acids are frequently present in plants in the combined forms such as glycosylated derivatives or esters of tartaric acid, shikimic acid, and quinic acid rather than in the free form. Hydroxycinnamic acids are recognised as powerful antioxidants playing an essential role in protecting the body from free radicals.

Conclusion:

The secondary metabolites is an emerging source of herbal medicines. Being natural in origin and having very less side effects they have gain enormous popularity among consumers as natural cosmetics.The bioactive compounds are not only potentially effective but also ecofriendly and safe. Furthermore, the cultivation process is sustainable as it has the ability for carbon dioxide sequestration. These plant and algal bioactive compounds are being employed in food. Medicine, nutraceuticals. They serve in fulfilling various human dietary requirements and also a significant role in combating serious health problems like cardiovascular, neurodegenerative diseases, immune disorders, atherosclerosis, stroke and carcinomas. Owing to the greater diversity in biochemical composition of algae, it is now considered as a potential functional food or superfood. Multinational food companies and pharmaceutical industries are bringing these nutritional innovations to customer consumption products (like breakfast cereals, probiotic, soft drinks) by incorporating these plant secondary metabolites. Due to increasing rate of inflation in recent years, future seems more promising for commercial application of algae and plant in food, comsectic, pharmaceutical industries as well as feedstock and energy.

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