AN INNOVATIVE ASSIMILATION ON PHARMACOGNOSTICAL, PHYTOCHEMICAL AND CONTENDING AGAINST *Malassezia Furfur* OF *Spinacia oleracea* L. LEAVES AND ITS BIOIACTIVE COMPONENT

A Dissertation submitted to

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MASTER OF PHARMACY

IN

BRANCH III-PHARMACOGNOSY

Submitted by

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UNDER THE GUIDANCE OF

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MADURAI - 625 020

MAY-2018

CERTIFICATE

This is to certify that the dissertation entitled **" AN INNOVATIVE ASSIMILATION ON PHARMACOGNOSTICAL, PHYTOCHEMICAL AND CONTENDING AGAINST *Malassezia Furfur* OF *Spinacia oleracea* L. LEAVES AND ITS BIOACTIVE COMPONENT"** Submitted by **Mr.M.MOHANRAJ (Reg.No.261620704), DEPARTMENT OF PHARMACOGNOSY, COLLEGE OF PHARMACY, MADURAI MEDICAL COLLEGE, MADURAI-625020** in partial fulfillment of the Tamil Nadu Dr. M.G.R. Medical University rules and regulations for award of **MASTER OF PHARMACY IN PHARMACOGNOSY** under my guidance and supervision during the academic year 2017-2018.

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CERTIFICATE

This is to certify that the specimen brought by Mr.M.Mohanraj, II M.Pharm, Department of Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai is identified as *Spinacia oleracea Linn.* belonging to the family Chenopodiaceae.

Station : Madurai.

Date : 11.08.2017.

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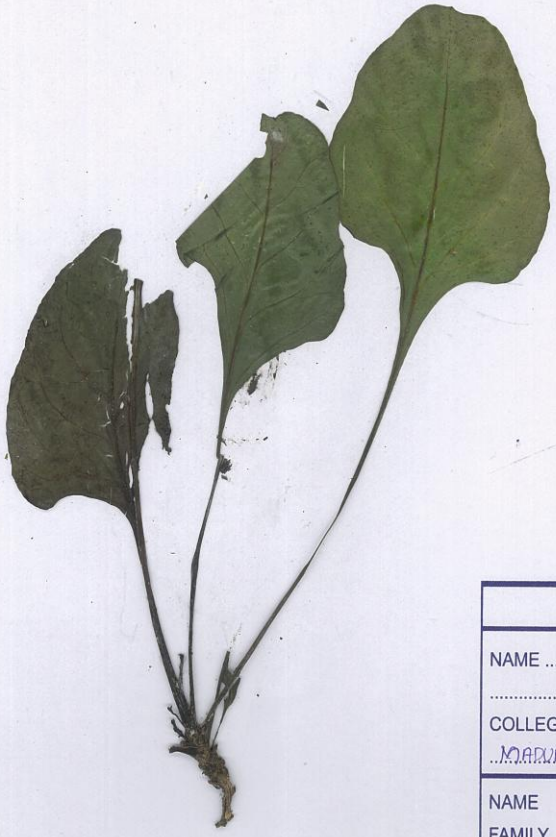

HERBARIUM

NAME ... M. MOHAN RAJ

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COLLEGE/SCHOOL ..COLLEGE OF PHARMACY

MADURAI MEDICAL COLLEGE, MADURAI

NAME : Spinacia oleracea Linn.

FAMILY : Chenopodiaceae

GENUS : Spinacia

SPECIES : oleracea

LOCALITY : Nagamalai Pudukottai, Madurai (D.T)

DATE : 01-09-2017

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CHAPTER-I

CHAPTER I

INTRODUCTION

Herbal medicines have been widely utilized as effective remedies for the prevention and treatment of multiple health conditions for centuries by almost every known culture. The first documented records of herbal medicine use date back 5,000 years in China. Similarly, India's Ayurvedic medicine tradition is thought to be more than 5,000 years old and herbal medicines remain an essential component of its practice (**Garodia P *et al*., 2007**).Today, the populations of certain countries still depend on herbal medicines to address their healthcare needs. In the U.S. the use of herbal medicines continues to grow since conducted the first national study of complementary and alternative medicine use.

Additionally, as a general rule, older adult populations are more likely to use both conventional drug therapy and herbal medicines. This population is also more likely to have a higher incidence of chronic disease, which more often than not requires the use of increasingly complex conventional drug therapy. As such, the potential for herb-disease and herb-drug interactions increases with older adult populations. At present, there is a death of research evaluating the use of herbal medicines, especially clinical trials. This, together with the ongoing development of new conventional drug therapies, further compounds the number of unknown out comes when using elements of these two treatment approaches together. In many countries, including the U.S., herbal medicines are not regulated as extensively as conventional drug therapy. Also, globalization has greatly increased accessibility of herbal medicines from all parts of the world to any single consumer. Clearly there is a great need for coordinated efforts to conduct the necessary

clinical trials to study the efficacy and safety of herbal medicines, both alone and in conjunction with conventional drug therapies. **(Eisenberg *et al.,* 1998)**

HERBAL MEDICINE

The World Health Organization (WHO) has recently defined traditional medicine (including herbal drugs) as comprising therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine and are still in use today. Traditional medicine is the synthesis of therapeutic experience of generations of practicing physicians of indigenous systems of medicine. The traditional preparations comprise medicinal plants, minerals, organic matter, etc. Herbal drugs constitute only those traditional medicines which primarily use medicinal plant preparations for therapy. The earliest recorded evidence of their use in Indian, Chinese, Egyptian, Greek, Roman and Syrian texts dates back to about 5000 years. The classical Indian texts include *Rigveda*, *Atherveda*, *Charak Samhita* and *Sushruta Samhita*. The herbal medicines/traditional medicaments have, therefore, been derived from rich traditions of ancient civilizations and scientific heritage (**World Health Organisation, 1991**).Plants had been used for medicinal purposes long before recorded history. Ancient Chinese and Egyptian papyrus writings describe medicinal uses for plants as early as 3,000 BC. Indigenous cultures (such as African and Native American) used herbs in their healing rituals, while others developed traditional medical systems (such as Siddha, Ayurveda, Unani) in which herbal therapies were used.(**Kamboj V P.,2000**)

Herbal medicine was also an effective method, but was viewed less enthusiastically. Herbal products were discarded from conventional medical use in the mid-20th century, not necessarily because they were ineffective but because they were not as economically profitable as the newer synthetic drugs.

In the early 19th century, scientific methods become more advanced and preferred, and the practice of botanical healing was dismissed as quackery. In the 1960s, with concerns over the important effects of conventional medicine and desire for more self-reliance, interest in "natural health" and the use of herbal products increased. Recognition of the rising use of herbal medicines and other non-traditional remedies led to the establishment of the office of Alternative Medicine by the National Institute of Health USA, in 1992. Worldwide, herbal medicine received a boost when the WHO encouraged developing countries to use traditional plant medicine to fulfill needs unmet by modern systems. **(Bassam Abdul Rasool Hassan, 2012)**

ETHICS FOR USAGE HERBAL DRUGS

In 1992, the WHO Regional Office for the Western Pacific invited a group of experts to develop criteria and general principles to guide research work on evaluating herbal medicines This group recognized the importance of herbal medicines to the health of many people throughout the world, stating: 'A few herbal medicines have withstood scientific testing, but others are used simply for traditional reasons to protect, restore, or improve health. Most herbal medicines still need to be studied scientifically, although the experience obtained from their traditional use over the years should not be ignored. As there is not enough evidence produced by common scientific approaches to answer

questions of safety and efficacy about most of the herbal medicines now in use, the rational use and further development of herbal medicines will be supported by further appropriate scientific studies of these products, and thus the development of criteria for such studies' **(World Health Organization, 1993).**

NOVEL RESEARCH ON HERBAL DRUGS

There are limited clinical trials to determine efficacy and safety of traditional herbal medicines. This lack of research does not impede most from using them, given that these remedies are often grounded in long standing cultural traditions. When trials are conducted, the Western-defined disease classification may not be appropriate to measure efficacy and safety in relation to the use of herbs in other countries **(Tylburt JC and Kaptchuk TJ, 2008).**recently published an ethical analysis of global herbal medicine research. They pose multiple scientific questions that shed light on the difficulties of conducting research with herbal medicines worldwide. Finding appropriate ways to conduct this type of research is an ongoing challenge. (**Rivera J O,et al.,2013**).

INCLINATION ON THE HERBAL DRUGS

Worldwide it is estimated that 80% of the population uses herbs; in the developing world rates could be as high as 95% .The U.S. continues to see an increase in the use of herbs. The most recent national survey conducted in 2007 by the National Center for Complementary and Alternative Medicine (NCCAM) showed that 17.7 % of adults have used natural products (primarily herbs) in a one year period. Complementary and alternative medicine (CAM) was used most commonly by whites (43.1%) followed by Hispanics (23.7%) (**Barnes PM and Bloom B, 2008**) In the El Paso region studies, use of

herbs by Hispanics, including older adults was much higher (between 59-70%) It is possible that certain methodologies may underestimate rates of use. In most studies, disclosure rates of herb use to providers are very low (a major concern). We found two main reasons for such low rates: providers did not ask about herb use or they showed displeasure with use of herbs. Our experience indicates that in some countries, herbs more commonly used vary depending on which products are marketed and on regional practices. Another recent trend in Western countries involves adding herbs to energy drinks and weight loss and nutritional products **(Robinson M M and Zhang X, 2011).**

GLOBAL HERBAL MARKET

It is extremely difficult to calculate sales data regarding the use of herbs worldwide; these calculations are likely underestimated. This is due in part to the varied ways in which herbs are used (e.g. food products, energy drinks, multivitamins, raw form). The World Health Organization (WHO) estimates that the global market is approximately US $83 billion annually. In some countries, marketing and sales of some herbs is driven primarily by profits. However, in other countries, herbs can serve as a major way of treating certain conditions or diseases more cost effectively, especially if the herb can be grown locally or regionally **(Rivera JO *et al*.,2013)**.

ROLE OF THE PHARMACIST IN THE USE OF HERBAL MEDICINE

The practice of pharmacy has evolved into a role that includes an expanded clinical application of pharmacotherapy knowledge as a member of the healthcare team. In many settings the pharmacist is in an ideal position to advise/monitor the use of herbs, especially in older adults. Recognizing this expanded role, in 1998 the WHO provided a technical

document entitled “the role of the pharmacists in self-care and self -medication”. This document explains the role of the pharmacist in self-care and self-treatment of patients, one of the four elements of good pharmacy practice. **(World Health Organization., 1998)**

STRATEGIES FOR THE SAFE USE OF HERBS

Perhaps the most important strategy for the safe use of herbs is to integrate evidence-based herbal medicine knowledge into the Western medicine healthcare curriculum. Several strategies may help with the management of herbs. They include: educating providers and patients about the possible benefits and risks of herbs, encouraging providers to ask their patients about their use of herbs without being judgmental, ensuring open communication with patients. Patients should also be careful when claims are made for a particular herb and should only purchase herbs from a reputable provider, company, or internet site. **(Rivera JO *et al*., 2013)**

GLOBAL BURDEN DISEASES:

In day to life human has suffered from communicable & non communicable diseases. They are cancer, TB, dengue, diabetes, CVS related diseases and many infectious diseases. From the above cancer type of melanoma, non-melanoma skin cancer, skin cancer are emerging one now days.

Skin diseases:

The skin care has exposed tremendous growth and emerging one in recent years. In recent studies 10 most emerged and burden skin diseases globally affect the life. These are dermatitis, acne, hives, psoriasis, dandruff, seborrheic dermatitis, viral skin disease, fungal

skin diseases, scabies, melanoma, pyoderma, cellulitis, non-melanoma skin cancer, decubitus, and alopecia areata. (**IJD Syposium 2017**)

. The skin diseases in India 10-12% of total population affected with eczema, psoriasis being major problem. Due to dust and pollutions, ultraviolet radiations, and global warming, photosensitive skin disorder like danning, pigment darkening, sunburn, skin cancer.one percent of damage in ozone leads to four percent of skin tumours rate.

DANDRUFF

Dandruff (also called as Pityriasis capitis) means scaliness of the scalp skin without signs of inflammation. Dandruff is so common that it can be considered physiological. It represents desquamation of the skin surface, due to separation of layers of stratum corneum, which is a continuous process, in the form of scales (**Amit kumar diwari et al., 2011)**

In general, dandruff occurs after puberty and mainly affects males more than the females. The most common symptoms are hair falling, light brown or white batches on the skin, redness, itching, seborrheic. **(Anitha A *et al.*, 2013).** Dandruff may improve in summer as an ultra violet ray from sun light counteracts fungus species and may get worse in winter (**Regupathi and chitra, 2015**). Dandruff results from at least three etiologic factors, *Malassezia* fungi, sebaceous secretions, and individual sensitivity. *Malassezia* species are involved in the etiology of pityriasis versicolor, folliculitis, seborrhoeic dermatitis and dandruff. They are normally found in areas rich in sebaceous glands as they are lipid dependent **(Sibi.G *et al.*, 2012).** The *Malassezia* species is a lipophilic fungus belonging to a dimorphic fungi group and is well known for its scalp diseases like

seborrheic eczema and dandruff on the human skin. The genus *Malasseiza* include 10 anthropophilic and obligatory lipophilic species *M.globosa, M.restricta, M.slooffiae, M.obtusa, M.furfur, M.sympodialis, M.jabonica, M.yamatoensis, M.dermatis, M.nana,* and three zoophilic species *M.packidermatis, M.cabrae, M.equime. Malassezia* species have been associated with a number of diseases of human skin such as pityriasis versicolor, seborrhoeic dermatitis, dardruff, folliculitis, atopic dermatitis and psoriasis **(Amit kumar diwari *et al.*, 2011).**

MALASSEZIA

The microbial origin of dandruff centers on the causal role of yeasts of the genus *Malassezia*. The vast majority of recent data supports a direct causal link between *Malassezia* fungi and dandruff. First, effective treatment of the condition can occur with a wide range of material types, from zinc and selenium salts to highly specific azoles, with the only known functional link between these materials being antifungal activity (**D Saint-Leger *et al.*, 1990**) The second supporting factor is that improvement in dandruff correlates considerably with reduction in scalp *Malassezia* level. While the absolute level of *Malassezia* correlates less well with dandruff, its reduction amongst those individuals that express the symptoms strongly supports its role. Originally named *Malassezia* by *Malassezia* in 1898.(**YM DeAngelis, *et al.*,2007**) this genus was renamed and referred to as *Pityrosporum* during the second half of the 20th century.35-36 At one time, members of *Malassezia* were classified into two species: a lipid-dependent species, *M. furfur*, and a non-lipid-dependent species, *M. pachydermatis*. In the mid-1990s studies of the morphological, ultra structural, physiologic and genomic differences in *Malassezia* led to

the identification of multiple lipid-dependent species(including *M.globosa, M.restricta, M.furfur, M.obtusa, M.slooffiae, M.sympodialis, M. japonica, M.nana, M. dermatis,* and *M. yamatoensis*), in addition to the nonlipid-dependent, primarily zoophilic, species, *M. pachydermatis*. Use of molecular markers is generally required to correctly differentiate between the various lipid-dependent species. **(E Gueho *et al.*, 1996)** A detailed model for the metabolic pathways involved in dandruff genesis has been formally proposed (**Fig: 1**). *Malassezia globosa* reside on the surface of the scalp and in the follicular infundibulum. These cells secrete hydrolytic enzymes, including lipase, into the extracellular milieu.

The lipase enzymes cleave sebaceous triglycerides into free fatty acids and glycerol. The *Malassezia* consume the saturated fatty acids necessary for their proliferation and leave behind an increased amount of irritating unsaturated free fatty acids. These unsaturated fatty acids penetrate into the epidermis, and in susceptible individuals (discussed below) induce a breach of the skin's barrier function, inducing either directly or indirectly irritation and a subsequent hyperproliferation and flaking. Lipases have been shown to play a key role in the lifestyle of *Malassezia* species on skin.45 In order to better understand this role, we isolated a lipase from *M. globosa*. One highly expressed lipase was sequenced and the corresponding lipase gene (*LIP1)* cloned and sequenced. This work was a first step toward a molecular description of lipid metabolism on the scalp and a more complete understanding of the role of microbial metabolism in the etiology of dandruff. Based on the limited activity of LIP1, it was postulated that additional lipases were present in *Malassezia*, and sequencing of the *Malassezia* genomes has revealed that to be the case.

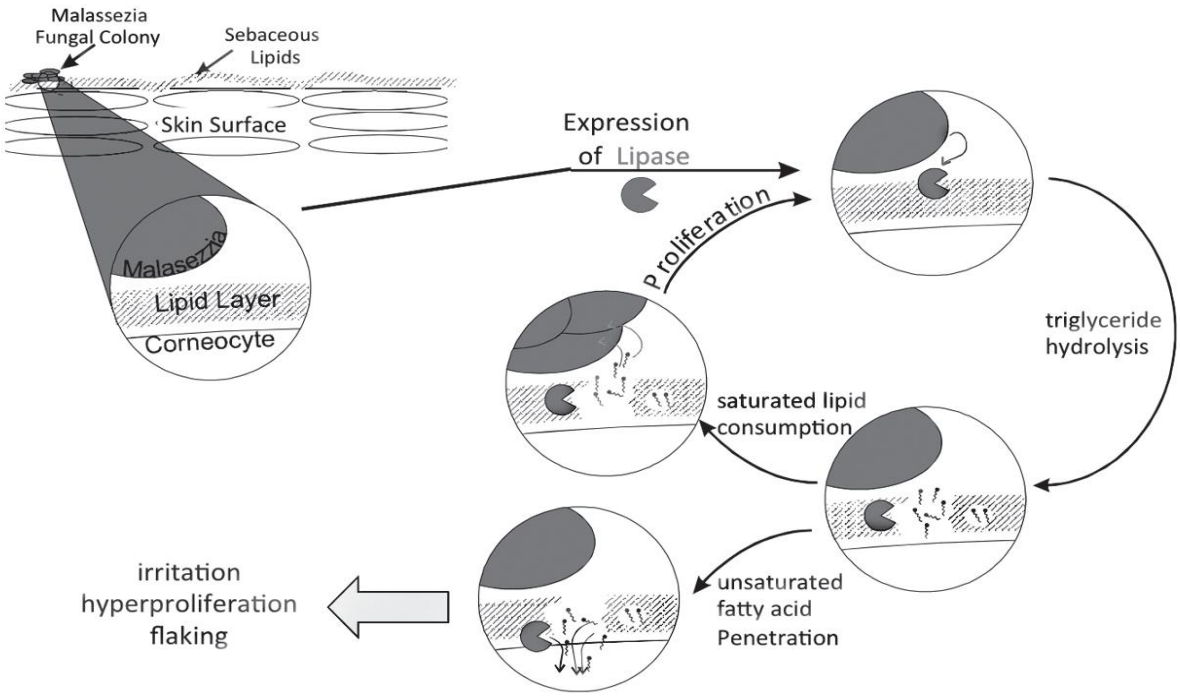


Figure: 1. The role of *Malassezia* lipid metabolism in dandruff genesis (**YM De Angelis, *et al.*, 2007**).

ETIOLOGY

Based upon the most recent evidence, the etiology of dandruff and seborrheic dermatitis appears to be dependent upon three factors: sebaceous gland secretions, microfloral metabolism, and individual susceptibility. This chapter will describe recent advances in the understanding of these factors, especially the role of the yeast *Malassezia*

ROLE OF INDIVIDUAL SUSCEPTIBILITY

It is well known and often cited as a confounding fact that while *Malassezia globosa* is present on almost all humans only one-half to three-quarters of people suffer from dandruff. One hypothetical explanation of this phenomenon is the possibility that

there exists a fundamental difference between dandruff suffers and non-dandruff individuals. To test this hypothesis we applied a fatty acid *Malassezia* metabolite, oleic acid, to the scalp of human volunteers who were clinically assessed for the effect of *malassezia* species. In this experiment, oleic acid dosed at physiologically relevant concentration was able to induce a flaking response which was indistinguishable from dandruff by visual observation or electron microscopy in dandruff susceptible patients but not in non-susceptible patients **(BI Ro and TL Dawson, 2005**). This finding provides evidence for a direct role of these fatty acid metabolites in dandruff and suggests an underlying difference amongst individuals that predisposes some to the development of dandruff or seborrheic dermatitis.

The difference between dandruff susceptible and non-susceptible individuals remains unclear. Multiple possibilities exist, including innate differences in stratum corneum barrier function, skin permeability, and immune response to free fatty acids or proteins and polysaccharides from *Malassezia*. Further work will be necessary to fully understand the susceptibility response. It will be necessary to conduct significantly more research into *Malassezia* biology and its interaction with human skin to understand the fundamentals of the interactions. The sequencing of these genomes, in conjunction with the already sequenced human genome, will allow a detailed investigation of the metabolic interactions between human skin and *Malassezia*. As new pathways are elucidated, new intervention targets will arise. This new, groundbreaking research will enable development of new technologies to interrupt dandruff, which may not be dependent on and complimentary to existing antifungal treatments.

TRADITIONALLY USED HERBS IN DANDRUFF

Ancient literature revealed the effective herbal medicines are available for aging disorders and other diseases like, osteoporosis, diabetic, cancer and infectious diseases like Tuberculosis, other viral diseases, immune disorders etc. for which no effective modern medicine are available. Among these diseases, immune disorders, asthma and tuberculosis are of great concern worldwide. The leaves of *Spinacia oleracea* is cooling ,emollient, wholesome, antipyretic, diuretic, laxative, digestible, anthelmintic, useful in urinary concentration, inflammation of lungs, and the bowels, sore throat, pain in joints, thirst, lumbago, cold and sneezing, sore eye , ringworm, scabies, leucoderma, arrest vomiting , biliousness, flatulence and treatment of febrile conditions.

SOME MEDICINAL PLANTS FOR ANTI-DANDRUFF ACTIVITY

Under this category, numerous plant extracts and essential oils have been reported to have beneficial effects on hair and are commonly used in shampoos for dandruff treatment. Some plant extracts such as Aloe vera, *Eucalyptus globulus, Phyllanthus emblica, Wrightia tinctoria. Zingiber officinale, Wrightia tinctoria, Cassia alata, Azadirachtaindica,* etc. have been reported for their good activity against the *M. furfur fungus.*

PHYTOCONSTITUENT USED FOR ANTIDANDRUFF ACTIVITY

In shampoo formulations there is also an increasing trend of natural surfactant (saponin) based shampoos instead of synthetic surfactant based ones. Saponins are also known for their antimicrobial, antioxidant and anti-dandruff activity, and as a result, they are used alone or in combination with other plant extracts. For instance, saponins from

Asparagus racemosus, Sapindus mukorossi, eclipta alba, lippia nodiflora, Vernonia cinerea, Ricinus communis, and *Acacia concinna* are used as herbal shampoo because of their surface active properties and additionally they may also have some antidandruff activity (**Santanu Paria & Jagajjanani Rao.K , 2016).**

PARTAKING OF HERBAL DRUGS IN TREATMENT OF DANDRUFF

***Spinacia oleracea* L. (SPINACH)**

Spinacia oleracea L. is an annual plant having medicinal property native to central and southwestern asia. In different traditional medicinal system it is known by different names. It's ayurvedic name is 'Paalankikaa', in 'Unani' it is called as 'Paalak', where as in 'Siddha' it is known by 'Vasaiyila-keerai. Spinach has a high nutritional value and is extremely rich in antioxidants, especially when fresh, steamed, or quickly boiled .it is a rich source of vitamin A (lutein), vitamin C, vitamin E, vitamin K, magnesium, manganese, folate, iron. *Spinacia oleracea* L. is edible Flowering plant in the family of chenopodiacea.

TRADITIONAL USES:

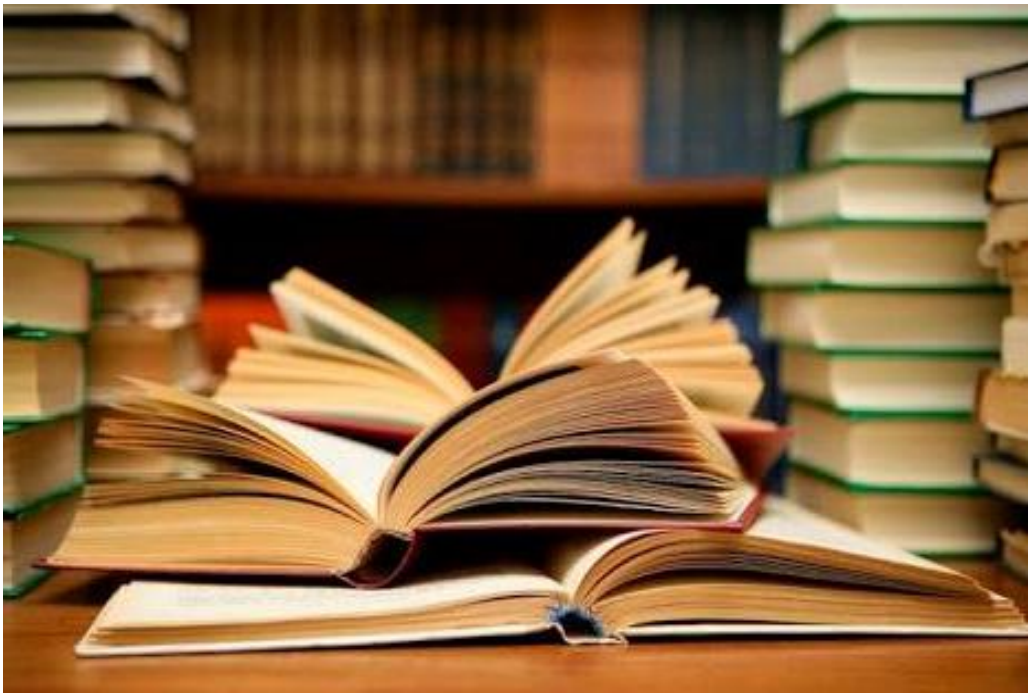
Spinacia oleracea L. (spinach) has been reportedly used widely in traditional herbal medicine. The **seeds** is used as a fever, lucorrhoea, urinary discharges lumbago,and disease of the brain and of the heart (Yunani).

The **leaves** of *Spinacia oleracea* is cooling ,emollient, wholesome, antipyretic, diuretic, maturant, laxative, digestible, anthelmintic, useful in urinary concentration, inflammation of lungs, and the bowels, sore throat, pain in joints, thirst, lumbago,cold and

sneezing, sore eye , ringworm, scabies, leucoderma, arrest vomiting, biliousness, flatulence and treatment of febrile conditions.

The plant *Spinacia oleracea* has been selected (specially the leaves) for the present investigation on the basis of the ethnomedical information and the review of literature as the plant is widely cultivated throughout India. (*Namratha singh et al.,* 2015)

CHAPTER-II



LITERATURE REVIEW

CHAPTER II

LITERATURE REVIEW

PLANT DESCRIPTION:

Spinacia oleracea is an edible flowering plant in the family of Chenopodiaceae, common name is spinach or in Hindi known as Palak. It is annual herb, which grows to a height of up to 30 cm. Spinach may survive over winter in temperate regions. The leaves are alternate, simple, and ovate to triangular-based, very variable in size from about 2-30 cm long and 1-15 cm broad, with larger leaves at the base of the plant and small leaves higher on the flowering stem. The flowers are inconspicuous, yellow-green, 3-4 mm diameter, maturing into a small, hard, dry, lumpy fruit cluster 5-10 mm across containing several seeds.

Stem:

Erect from 30-60 cm high, round, smooth, piped, succulent, sometime reddish.

Leaves:

Alternative, the lower ones very long petiole, variously lobed with lobes of anacute triangular shape, smooth on both the side.

Flowers Male:

Flowers on long terminal glomerate spikes and on shorter ones from the axial, very numerous, sessile, calyx 4-parted, Stamen 4, Anthers twin, very large.

Flowers Female:

Flowers axillary, sessile, crowded. Calyx 2- tipped with a projecting horn in each side, growing into spines when the seed is ripe. Styles are generally 4, white tapering.

Capsule 1-celled, 1-valved, armed, with 2 opposite short horns,and crowned with the small remaining calyx **(Deven Metha & Sateessh Belemkar, 2014).**

SCIENTIFIC CLASSIFICATION (otari K V *et al.*, 2010).

Kingdom	:	Plantae.
Sub Kindom	:	Traccheobiota.
Super Division	:	Spermatophyta.
Division	:	Magnoliophyta.
Class	:	Magnoliophyta.
Subclass	:	Caryophyllidae.
Order	:	Caryophyllidae.
Family	:	Chenopodiaceae.
Genus	:	*Spinacia*
Species	:	*oleracea*
Botanical Name	:	*Spinacia oleracea Linn.,*
Common Name	:	Spinach.

VERNACULAR NAME:

English	:	Spinach.
Hindi	:	isfanak, palak.
Punjabi	:	palak, isfanak ,valayatisag.
Gujarat	:	palak.
Telungu	:	Dumpabachhali.
Assam	:	palangsag.
Bengali	:	palang, pinnis.
Tamil	:	vasayleykiray.

Habit:

Annual-plant (rarely biennial)

Habitate:

Subtropical temperate

GEOGRAPHICAL DISTRIBUTION

Spinach is not known in a wild state. It probably originated in northern Iran, Afghanistan and Turkmenistan where related wild species such as *Spinacia tetrandra* Steven ex M.Bieb. And *Spinacia turkestanica* can be found. It spread to china in the 7th century. Spinach is now cultivated worldwide, mostly in temperate regions, but also in the cooler parts of the tropics. In tropical Africa it is grown to a limited extent in the highland areas of east and South- west Asia; cultivated throughout India.

CULTIVATION OF SPINACH

Spinach is popular because of its high yield, wide adaptability to varying soil and climatic conditions and high nutritional value. Spinach is a cold-season crop.it can be grown pure or as a mixed crop with peas, cabbage and other comparatively longer duration vegetables. It is sown during September-November in the plains and during feburary-april in the hills.

CLIMATE AND SOIL:

Spinach prepare the average montly temperature of 15-19^{0}c, but growth is good even at 10-15^{0}c. It withstands temperature as low as-7^{0}c and also acute frosts. the cool and short days with the onset of long and hot days. It starts flowering and friable soil,and shady situations. It yields best when grown on a heavy loam or silt. When grown on sandy soils, it needs a substantial supply of humus and nitrogen. The plant can thrive even saline soils. Growth is, however, restricted on acidic soils.

PH:

6.0 to 7.0. Spinach is extremely susceptible to water-logging. (**The wealth of India, 2005)**

CHEMICAL CONSTITUENTS:

Spinach has a high nutritional value and is extremely rich in antioxidants, especially when fresh, steamed, or quickly boiled. It is a rich source of vitamin A, vitamin C, vitamin E, vitamin K, magnesium, manganese, folate, betaine, iron, calcium, vitamin B6, folic acid, copper, protein, phosphorus, zinc, niacin, and selenium and omega-3 fatty acids. Recently, opioid peptides called rubiscolins have also been found in spinach. It is a

source of folic acid (Vitamin B9), and this vitamin was first purified from spinach. It contain ascorbic acid, dehydroascorbic acid, two spinach flavonoids; 5,3',4' -trihydroxy-3-methoxy-6:7-methylenedioxyflavone and spinacetin and carotenoid; β-caroteneand, lutein.

FLAVONOIDS:

Spinacia oleracea is very rich in the flavonoids. Various flvonoids reported to be present are querecetin, myricetin, kampeferol, apigenin, luteolin, patuletin, spinacetin, jaceidin, 4- glu-curonide, 5,3,4-trihydroxy-3-methoxy-6:methylenedioxyflavone-4-glucuronide, 5,4-dihydroxy-3.3-dimethoxy-6.7 methylene dioxyflavone-4-glucuronide, 5,4-dihydroxi-3,3-dimethoxi-6,7-methylene-dioxi-flavone,3,5,7,3,4-pentahydroxi-6-methoxiflavone.

PHENOLIC COMPOUNDS:

The polyphenols isolated from the plant are *para*-coumaric acid, ferulic acid, *ortho*-coumaric acid.

CAROTINODS:

Spinach shows presence of different carotinoids like lutein, β-carotene, violaxanthin and 9'-(Z)-neoxanhin.

VITAMINS:

Spinacia oleracea contains high concentration of vitamin A, E, C, and K. and also folic acid, oxalic acid.

MINERALS:

Magnesium, Manganese, Calcium, Phosphorus, Iron, Zinc, Copper and Potash.

GLYCOLIPIDS:

It also contains mainly three glycolipids: monogalactosyl diacylglycerol, digalactosyl diacylglycerol, and sulfoquinovosyl diacylglycerol.

TWO ANTIFUNGAL PEPTIDES:

Designated alpha and beta-basrubrins. **(Namrata singh *et al*., 2016)**

ETHNOMEDICINAL USES

WHOLE PLANT:

It is sweet, cooling, carminative, laxative, alexipharmic; useful in diseases of blood and brain, asthma, leprosy, biliousness; causes‟ kapha″ (Ayurveda). It has been used in the treatment of urinary calculi. In experiments it has been shown to have hypoglycemic properties.

LEAVES:

These are cooling, emollient, wholesome, antipyretic, diuretic, maturant, laxative, digestiblle, anthelmentic, useful in urinary concretion, inflammation of the lungs and the bowels, sore throat, pain in joints, thirst, lumbago, cold and sneezing, sore eye, ring worm scabies, leucoderma, soalding urine, arrest vomiting , biliousness, flatulence.

SEEDS:

Seeds are useful in fevers, leucorrhoea, urinary discharges, lumbago, and diseases of the brain and of the heart (Yunani). Seeds are laxative and cooling. They have been used in the treatment of difficulty in breathing, inflammation of the liver and jaundice. The green plant is given for the urinary calculi. **(Richa Shri *et al*., 2016)**

Used as astringent, anemia, antivirus, antibacterial, bowel, carminative, demulcent, depurative, diuretic, Fatigue, fever, inflammation, laxative, refrigerant, thirst, tuberculosis and tumor. Pharmacological activities like anticancer (**Maeda *et al*., 2008**), antifungal activity antioxidant neuroprotective activity **(YU Wang *et al*., 2005)** has been reported.

PHARMACOGNOSY REVIEW:

Maryian R Patel., *et al* (2017) had investigated Pharmacognostical evaluation and quality parameters of leaves on *Spinacia oleracea* L. was carried out to determine its macro-and microscopical characters and also total ash, insoluble ash, alcohol and water-soluble extractive values were determined for spinach leaves. The Proximate analysis of powder was also carried out in which extractive value, ash value, foreign matter, loss on drying were determined.

Payal R. Dande *et al*., (2015) had investigated Pharmacognostical studies of leaves of Spinacia oleracea L. fresh leaves were washed and used for study of organoleptic and microscopic characteristics. The powder of shade dried leaves was used for the determination of ash values, extractive values and phytochemical investigations. All chemicals and reagents used for testing were of analytical grade. Percentage of total ash, acid-insoluble ash, water soluble ash and sulphated ash were calculated as per the Indian Pharmacopoeia.. Different extracts of the leaves were prepared for the study of extractive values. Fluorescence analysis of powdered leaf was carried out by standard methods.

Scholz miklas *et al*., (2015) had investigated toxicity and bioaccumulation of heavy metals in spinach (*spinacia oleracea* L.) Grow in a controlled environment. It shows

result is revealed that cd and Pb treatment even at low concentration and Zn at high concentration induces a significant ($p<0.05$) reduction in all growth parameters (shoots and root lengths biomass) and number of leaves as well as total protein content, fiber, moisture content and minerals (Na, K, Ca, Fe,Mg,Mn and Cu).

Naser sabaghnia *et al.*, (2013) had studied genetic diversity of spinach (*Spinacia oleracea* L.) Landraces collected in Iran using some morphological traits. Fifty-four spinach landraces collected from diverse geographical regions of Iran were evaluated for several qualitative and quantitative traits. Results of this investigation also can aid to define strategies for further collection. Since our results show that the pattern of observed variation is governed by morphological traits.

Anitha S and Karthiga Gandhi P, (2012) had reported allelopathic effect of *Spinacia oleracea* L. Var. KM-2 andVamban-2. In the present study, the interaction of spinach (*Spinacia oleracea* L.) was studied using leaf extract. Two common varieties of green gram viz., KM-2 and Vamban-2 were used to study the interactions at sub-species level. Among the two tested plants, spinach was most inhibitory on seed germination..

Beignan Mou., *et al* (2008) had investigated leafminer resistance in spinach (*Spinacia oleracea* L.). The purposes of the present experiments were to evaluate differences in leafminer damage among spinach genotypes, to compare results obtained from insect cage and field experiments, and to study the association among different resistant trait.

Markus Bechmann, (1996) reported the inhibitor protein of phosphorylated nitrate reductase from spinach (*spinacia oleracea* L. Leaves is a 14-3-3 protein. The inhibitory protein that inactivates spinach leaf NADH: Nitrate reductase (NR) has been identified for first time as a number of eukaryotic 14-3-3 protein family based on three lines of evidence.

Akira Watanabe and Carl A. Price , (1982) had investigated Trancslation of mRNAs for subunits of chloroplast coupling factor 1 in *spinacia oleracea* L.Our present studies with cell-free translation of mRNAs clearly show that poly (A)+ RNA from spinach leaves directs the synthesis of polypeptides immunochemically related to the y and subunits, whereas RNA from does not purified chloroplasts.

Muneo ilzuka *et al.*, (1962) had investigated sex chromosome translocation in *spinacia oleracea* L. The standard chromosome1 is heterobrachial with one arm about twice as long as the other. A homobrachial variant of this chromosome was found in accession.which is due to an added segment on the short arm. The present report describes a radiation-induced translocation on the long arm of chromosome 1.

PHYTOCHEMICAL REVIEW:

Anil kumar sah *et al.*, (2017) had investigated Methanolic leaf extract of *Spinacia oleracea* L. was analyzed by HPTLC using mobile phase as chloroform: Isopropyl alcohol: Acetic acid (12:8:1). Nine different compounds were detected with retention factor (Rf) 0.10, 0.13, 0.14, 0.25, 0.38, 0.44, 0.52, 0.60, and 0.69 Among them, peak 5 with Rf 0.38 was identified as 20-hydroxyecdysone which is an important chemical constituent in spinach as reported.

Mariyan R Patel *et al.*, (2017) had reported powder of *Spinacia oleracea* leaves were extracted with ethanol by soxhlet apparatus. The aqueous extract was prepared by maceration method. The various extracts showed the presence of triterpenoids, saponins, flavonoids, phenolic compounds and tannins. Identification of phytoconstituents Thin layer chromatography by using different reagents were using and reported.

Narmatha sing *et al.*, (2016) had evaluated preliminary phytochemical analysis of different (n-hexane , dichloromethane, methanolic, aqueous) extract of *spinacia oleracea* L. Methanolic extract showed the presence of carbohydrate,glycoside, alkaloid, phytosterol and triterpenoids, protein and amino acid, phenolic and tannins, flavonoids, and absence of fixed oil and fat, saponin, gum and mucilage. Dichloromethane extract showed the presence of carbohydrate,glycoside, alkaloid, phytosterol and triterpenoids, protein and amino acid, flavonoids, and absence of fixed oil and fat, phenolic and tannins saponin , gum and mucilage.aqueous extract showed the presence of carbohydrate,glycoside, phenolic and tannins, flavonoids, and absence of alkaloid, phytosterol and triterpenoids, protein and amino acid, and absence of fixed oil and fat,

saponin , gum and mucilage. n-hexane extract did not shows any positive reaction in phytochemical analysis.

Namrata sing *et al*., (2016) had investigated isolation and characterization of new triterpenoid compound (α-amyrin) from dichloromethane extract of *Spinacia oleracea* L. In this study, extracted dichloromethane extract by soxhlet extractor. Then dichloromethane extract purified by TLC, isolated by HPTLC analysis and characterized with spectroscopy methods.

Rao K.V.N *et al*., (2015) had studied preliminary phytochemical screening of aqueous and alcoholic extract of dried and fresh leaves of *Spinacia oleracea* L. Aqueous and Alcoholic extract of dried leaves showed the presence of carbohydrate, glycosides, flavonoids, steroids, phenols and tannins, saponins, fat.and absence of protein, amino acid, alkaloids. Aqueous and Alcoholic extract of fresh leaves showed the presence of carbohydrate, glycosides, flavonoids, steroids, phenols and tannins, saponins, fat and absence of protein, amino acid, alkaloids.and absence of protein, amino acid, alkaloids.

Ravindra D. chauudhari *et al*., (2015) had investigated phytochemical investigation of *spinacia oleracea* L. The presence of phytochemicals including phytosterols, saponins, alkaloids, phenolic compounds and tannins, proteins, glycosides, flavonoids, carbohydrates,quinones, coumerin, terpenoids, anthocyanins and emodins were determined in the *Spinacia oleracea*. It was concluded that the extracts of *Spinacia oleracea* consists of important constituents for pharmacological activities.

Macro Malferrai and Francesco Francia (2014) had investigated isolation of plastoquinone from *Spinacia Oleracea* L. by using HPLC method. Extraction of Plastoquinone was achieved using partition of chloroplast suspension with methanol:petroleum ether. This procedure removed large amounts of green pigments from the extract and thus facilitates the subsequent chromatographic isolation of Plastoquinone. The reported methodology represents a valuable tool for the fast production of small amounts of Plastoquinone, which there are no commercial standards available.

Immanuel selvaraj C *et al.*, (2013) had reported different solvent (acetone,ethanol,butanol)using extraction and quantification of Lutein from *Spinacia oleracea* L. to detected lutein content thin layer chromatography (TLC) and spectrophometric method was performed. The relative amount of lutein was measured using UV Spectrophotometer at wavelength of 446 nm. The absorbance and concentration of lutein in different extracts at different standing times were mentioned. Results obtained from different sources showed Rf values for acetone to be around 0.42, ethanol to be around 0.30 and butanol to be around 0.55.

Nilesh Kumar Jain and Abhay K. Singhai., (2012) had investigated the seeds of *Spinacia oleracea* using various extract of (Aqueous, Pet. ether and ethanol) and fractions derived from ethanol extract (i.e. chloroform, ethyl acetate and n-butanol).

Rozita Osman *et al.*, (2009) had investigated simultaneous extraction and cleanup of chloropyrifos from *Spinacia Oleracea* L. using pressurized liquid extraction (PLE). The extraction is carried out using n-hexane and performed using these PLE condition. Extraction temperature of 100 _C, pressure of 1500 psi and static extraction time of 10

min. it was found that 1.0 g Florisil was able to absorb 92.0% chlorophyll a without affecting the recovery of chlorpyrifos.

Enas Jawad Kadeem *et al*., (2009) had investigated by identification and quantitative estimation of lutein in Iraqi *Spinacia oleracea* L. by using chromatographic (TLC, HPTLC, HPLC) methods. Identified by thin layer chromatography using two different solvents system: (petroleum ether: diethyl ether:acetic acid) and (petroleum ether: acetonitrile: methanol) compared with standard , melting point, mixed melting point and high performance liquid chromatography (HPLC).50gm of Iraqi spinach gives about 32mg of lutein.

Isabirye D A and Dikio E D, (2008) had investigated isolation of chlorophyll a from spinach leaves. An efficient method for separating chlorophyll *a* from spinach leaves by colum chromatography and solvent extraction techniques has. The purity and identity of the chlorophyll *a* have been confirmed by UV-Vis, IR and mass spectrometry. Yields from 100 g of freeze-dried spinach were 23 – 24 mg of chlorophyll *a*.

Naoki maeda *et al*., (2007) had investigated isolation and purification of glycolipid fraction of *Spinacia oleracea* L. containing majar glycolipid fraction of monogalactosyl diacylglycerol (MGDG), digalactosyl diacylglycerol (DGDG), and sulfoquinovosyl diacylglycerol (SQDG) present in spinach. Each of these compounds was completely purified by silica gel column chromatography, and their chemical structures were determined by 1H-, 13C-, and DEPT (Distortionless Enhancement by Polarization Transfer) NMR spectroscopic analyses. These compounds were glycolipids such as monogalactosyl diacylglycerol (MGDG), digalactosyl diacylglycerol DGDG), and SQDG.

The weight percents of MGDG, DGDG, and SQDG in the glycolipids fraction were 72.0%,2.8%, and 25.2%, respectively, and no other glycolipids were detected.

PHARMACOLOGY REVIEW:

LEAVES

ANTICANCER ACTIVITY:

Umamahesheswari G and Aiyasamy Nishanthini, (2017) had investigated anticancer activity on methanolic extract of *spinacia oleracea* L., on *invitro* by using MTT ([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]) assay. It shows significant anticancer activity in comparision with doxorubicin used as standard drug.

Preethi sagar *et al*., (2015) had investigated evaluation of anticancer activity of ethanolic extract of *spinacia oleracea* L.by high throughput screening on *invitro* by using MTT ([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]) assay. It shows significant anticancer activity against lung and bone cancer when test different cell line (A 549 cell line 236µg and K562 cell lines 226 µg) to determine IC.

Naoki maeda *et al*., (2007) had Reported Anti-Tumor Effects of the Glycolipids Fraction of *Spinacia oleracea* L. which Inhibited DNA Polymerase activity by using *in vivo* animal model of male mice. This glycolipids fraction inhibited the activities of replicative DNA polymerases (pols) such as α, δ, and ε, and mitochondrial pol γ with IC50 values of 44.0–46.2 µg/ml, but had no influence on the activity of repair-related pol β. The fraction also inhibited the proliferation of human cervix carcinoma (HeLa) cells with LD50values of 57.2µg/ml. In an in vivo anti-tumor assay on nude mice bearing solid

tumors of HeLa cells, the fraction was shown to be a promising suppressor of solid tumors. Histopathological examination revealed that tumor necrosis with hemorrhage was significantly enhanced with the glycolipids fraction *in vivo*.

IMMUNOMODULATORY ACTIVITY:

Narmatha singh, S.C *et al.*, (2017) had investigated immunomodulatory Activity of different (n-hexane, dichloromethane, methanol, water) extract of *spinacia oleracea* L. Leaves on albino wister rat, it shows significant immunomodulatory activity in comparision with levamisole 50mg/kg was used as standard drug.

ANTIMICROBIAL ACTIVITY:

Umamahesheswari G and Aiyasamy Nishanthini, (2017) had investigated antimicrobial potential of metthanolic extract of *spinacia oleracea* L.leaves on Invitro antimicrobial activity against selective pathogens (*staphylococcus aureus, klebsiella pneumonia, Candida albicans* using agar well diffusion method. It shows significant antimicrobial activity in compared with azithromycin (bacteria) 30µg/well and clortrimazole (fungai) 30µg /well.

Jayasimha rayalu daddam *et al.*, (2015) had studied Biosynthesis and characterization of Silver and Iron Nanoparticles from Aqueous leaf extract of *Spinacia oleracea* L. and by using *invitro* Antimicrobial Studies. The Screening of ethanolic extracts of *Spinacia oleracea* resulted in moderate antibacterial activities against different bacteria. The silver and iron nanoparticles of average size have been synthesized using dried leaves of plant *spinancia oleracea*. Characterizations from UV-Vis, SEM, and EDX support the stability of biosynthesized nanoparticles. The silver and iron nanoparticles

using spinacia proved excellent antibacterial activity.it shows significant antibacterial activity in comparision with amoxyclav disc were used as standard drug.

Amaranth kanchana *et al.*, (2011) had investigated biogenic of silver nano particle of antimicrobial activity of aqueous leaf extract of *Spinacia oleracea* L by using *in vitro* agar disc diffusion method. In this study was report the synthesis of silver nanoparticles from *Spinacia oleracea* leaves by 'exploiting' the reduction capabilities of varied phytochemicals present in it, as confirmed by the FTIR characterization analysis technique. The biogenic nanoparticles synthesized using spinach leaves exhibited variety of shapes, exposed by SEM and TEM. Due to increasing development of the resistance to the existing antibiotic and various drugs, we extended our research to utilize the efficacy of these synthesized nanoparticles as antimicrobial agents, against *B.subtilis, S.aureus, K.pneumonia* and *E. faecalis* strains.

Fatih Fidan *et al.*, (2008) had Reported Radioproductive potential of methanolic leaf extract of *Spinacia oleracea* L. against oxidative tissue damage induced by radiation by using *in vivo* animal wister albino rat. At the end of experimental period the animals sacrificed by anesthetizing at 1 and 15 days post irradiation. Malondialdehyde and reduced Glutathione levels in tissue and *in vitro* antimicrobial activity in the plant extracts were determined. The results indicate that Spinach treatment decreases the tissue oxidative stress in irradiation-induced oxidative tissue damage by maintaining the GSH recycling activity and free radical scavenging potential. The result demonstrate that, in animals exposed to irradiation, spinach extract could provide great advantages against to systemic infection from endogenous and exogenous organisms increased after exposure to ionizing radiation.

ANTIOXIDANT ACTIVITY:

Anil kumae sah *et al*., (2017) had investigated antioxidant activity of methanolic leaf extract of *Spinacia oleracea* L. by using *invitro* OH-scavenging and DPPH scavenging assay methods. Further, methanolic extract of spinach showed the antioxidant activity inhibitory concentration of 3.03 µg/mL, 6.03 µg/mL for OH- scavenging,DPPH inhibition respectively.it shown antioxidant activity in compared with ascorbic acid used as standard drug.

Ali muhamadi sani, *et al.*, (2013) had investigated antioxidant activity of *Spinacia oleracea* L.leaves extract on physicochemical, phenolic content, antioxidant activity and microbial properties of yogurt. *invitro* antioxidant activity by using DPPH (1,1-diphenyl-2-picrylhydrazyl radical) inhibition.

Maryam tazarv and farhad hatamjafari, (2013) had investigated antioxidant activity of methanolic leaf extract of Iranian *Spinacia oleracea* L. between Babol and Varamin regionswas examined. In addition, total amount of DPPH (1,1-diphenyl-2-picryl hydrazyl) radical scavenging activities and reductive power of crude extracted its different fractions were determined. This research has shown Spinach has antioxidant activity.

HYPOGLYCEMIC ACTIVITY:

Anil kumar sah *et al*., 2017 had Reported antidaibetic activity of methanolic leaf extract of *Spinacia oleracea* L. by using *in vitro* α-amylase inhibition assay method. The results suggested the good nutritional values such as total crude fiber 4.55 ± 0.244% w/w, proteins 0.052 ± 0.0068% w/w, oils and fats 0.72 ± 0.036% w/w, carbohydrate 61.95 ± 0.382% w/w, Vitamins A 26.85 ± 0.154 µg, and Vitamins C 19.66 ± 0.21 µg. Further,

methanolic extract of spinach showed the antidiabetic effect with an inhibitory concentration of 3.046µg/ml for α-amylase inhibition, respectively.

Gomathi V *et al.*, 2010 had investigated Antidiabetic activity of ethanol and aqueous leaf extract of *Spinacia oleracea* L. by using *in vivo* model of alloxan induced diabetic rats. Ethanolic and aqueous extract of *Spinacia oleraceae* produced a significant reduction in fasting blood glucose levels in the normal and significant reduction in fasting blood glucose levels in the alloxan-induced diabetic rats significant differences were observed in serum lipid profiles (Cholesterol and triglyceride) and changes in body weight by both ethanolic and aqueous treated diabetic animals concurrent histopathological studies of the pancreas of these animals showed comparable regeneration by extract which were earlier necrosed by alloxan.

Jai kumar N and loganathan P , 2010 had investigated hypoglycemic effect of 70% etanolic *Spinacia oleracea* L. leaves extract were studied in normal alloxan induced diabetic rats. Animal treated with extract showed much lowered serum glucose level;54%($p<0.001$).the level of serum triglyceride and cholesterol increased significantly in diabetic rat as compared to normal rats. It shows significant hypoglycemic activity in compared with metformin 100mg/kg body weight 1ml water orally (once daily) used as standard drug.

ANTIDEPRRESANT ACTIVITY:

Rajya laxmi Gudepu, *et al.*, (2017) reported evaluation of antidepressant activity of ethanolic leaf extract of *Spinacia oleracea* L. by using i*nvivo* albino rats model. Treatment with (200mg/kg body wt) decreases the immobility time against forced swim

test and tail suspension test. Fluoxetine (30mg/kg, i.p) was selected as standard and it showed significant antidepressant activity in rodents.

PITUTARY-GONADAL AXIS ACTIVITY:

Mehrdad modaresi and Fatemeh matboo, 2016 had investigated pituitary-gonadal axis activity of hydroalcoholic leaf extract of *Spinacia oleracea* L. by using male mice. At the end of period, blood samples were taken and testosterone, LH and FSH amounts were measured using Eliza test. Results showed that FSH and LH amounts were increased by 200 mg/kg group. Testosterone was increased in 50 and 100mg/kg groups but not in 200 mg/kg group. Obtained data were analyzed using SPSS (Statistical Package for the Social Sciences) program and one way analysis of variance ($P<0.05$).

ANTI EPILEPSY ACTIVITY:

Mondal M *et al.*, (2015) studied antiepileptic activity of aqueous extract of *Spinacia oleracea* L.on DNA fragmentation in pentylenatetrazole induced experimental epileptic rat model. From the behavioral study it was clear that *Spinacia oleracea* L. leaf extract at the dose of 400mg/kg significantly decreased the seizure activity, ictal phases and increased the interical phase.

HEPATOPRODUCTIVE ACTIVITY:

Maximas H Rose HR, *et al*, (2014) had investigated hepatoproductive activity of methanolic extract of *spinacia oleracea* L. on *invivo* studies of carbon tetrachloride induced hepatotoxicity in wister albino rat model.the result is showed carbon tetrachloride administration was associated with considerable increase in the activities of alanine amino

transferase, aspartate amino transferase and bilirubin ($p<0.05$) in comparision with the respective mean values of the control.

Farah K *et al*, (2012) had reported study of Iraqi *spinacia oleracea* L. aqueous leaf extract (phytochemical and productive effect against methotrexate induced hepatotoxicity in rats). The rats were divided into three groups as control, MTX group following a single dose of MTX (20 mg/kg, i.p) saline was administered for 5 days and the MTX+aqueous spinach extract group were rats received 200mg/kg orally of aqueous spinach extract 7days before and 5 days after MTX treatment. MTX administration increased the MDA and decreased GSH, ALP while these changes were reversed in aqueous spinach extract treated group. Histological changes observed in MTX treated group was improved by aqueous spinach extract treatment.

Gupta R.S. and dharmendra singh, *(*2006) investigated *Spinacia oleracea* L. leaves extract against amelioration of carbon tetrachloride (ccl_4) induced hepato suppression in wister albino rats model. Which was evaluated in terms of serum marked enzymes like GGT(Gama Glutamyl Transferase),AST,ALT(Aspartate and Alanine Transaminase),LHD(Lactate Dehydrogenase),SDH(Sorbitol Dehydrogenase),GDH (Glutamate Dehydrogenase)ALP(Alkaline Phosphate) and serum total bilirubin ,total protein levels along with concomitant hepatic antioxidant like SOD (Superoxide dismutase),CAT(catalase),GST (Glutathione reductase), GST(glutathione–s-transferase),ascorbic acid (vit-c),β-carotene and cytochrome p-450 enzyme whereas LPO (Lipid peroxidation)was monitored in both serum and liver contents.it shows anti-hepatosuppresion compared with silymarin (100mg/kg) was used as standard drug.

ANTI-ANEMIC ACTIVITY:

Tijjani H *et al.*, (2014) had investigated anti-anemic potential activity of aqueous extract of *Spinacia oleracea* L. leaf in phenylhydrazine treated rats.it shows Aqueous extract of *Spinacia oleracea* leaf at 100 mg/kg body weight significantly increase ($p<0.05$) only the haemoglobin concentration of normal treated rats . it shows significant anti-anemic activity in compared with that of the anemia untreated group.

ANTITHROMBOLYTIC ACTIVITY:

Ramakrishnan papa ammal and Amirtha lingam pushpa, (2014) had investigated aqueous extract of *in vitro* thrombolytic property of curry leaves palak (*Spinacia oleracea*) was determined by using human blood. The plant extracts, when given in combination showed clot lysis of 33.22% at 20mg/ml. The thrombolytic activity was correlated with serum cholesterol and the findings suggested that the plant extract could possibly lyse a clot regardless of the level of cholesterol. Membrane stabilizing potential of the selected plants was analysed and both the plant extracts moderately protected the human RBC membrane which is an essential quality for thrombolysis.Aqueous extract of *in vivo* thrombolytic property of curry leaves palak (*Spinacia oleracea*) was determined by using *in vivo* models of FeCl3 - induced thrombosis was developed experimental animal model. Haematological and biochemical parameters were determined in experimental animals. Histopathological examination was also carried out after the experimental period.

URINARY TRACK INFECTION:

Merina Paul Das and Souvik Chatterjee, (**2013)** had reported the effect of different solvent extract of *Spinacia oleracea* L. on some urinary pathogens *(E.coli, E.faecalis, K.pneumoniae) in-vitro* well diffusion method. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were evaluated for all solvent extracts of *Spinacia oleracea* against each pathogen that recorded antibacterial activity and found the significant bactericidal MIC ranged between 1 to 40 mg/ml and MBC ranged between10 to 50 mg/ml against UTI causing bacteria.

HYPOLIPIDEMIC ACTIVITY:

Rangan kumar Giri *et al.*, (2012) had investigated by hypolipidemic activity of *Spinacia oleracea* L. dried leaves Powder (200mg/kg, 400mg/kg) was dissolved in 9ml salin. *In vivo* studies of atherogenic diet induced hyperlipidemia in rats.spinacia oleracea produced a significant decrease in the serum level of lipids in atherogenic diet induced hyperlipidemia in rats. It shows significant hypolipidemic activity is compared in Gemfibrozil (50mg/kg) used as standard drug.

ANTI-ULCER ACTIVITY:

Kore kakasaheb *et al.*, (2011) studied antiulcer activity of aqueous extract of *spinacia oleracea* L. by using albino rat model. The anti-ulcer activity was assessed by determined and compared the Gastric total acid output and pepsin activity were estimated in the pylorus ligated rats.it shows significant antiulcer activity in compared to Ranitidine (30mg/kg) used as standard drug.

ANTI-ASTHMA ACTIVITY:

Sang Han Lee *et al.*, (2010) had investigated amelioration of asthmatic inflammation by an aqueous extract of *Spinacia oleracea* L. by using *invivo* oval albumin induced animal model .The data showed that the number of eosinophil was significantly lower than that of eosinophil was significantly lower than that of the *Spinacia oleracea* L treated group,indicating that *Spinacia oleracea* L treatment decreased BAL´s eosinophil expression.

ANTI-INFLAMMATORY ACTIVITY:

Vipin KR Garg *et al.*, (2010) had investigated anti-inflammatory activity of ethanolic and aqueous extract of *Spinacia oleracea* L. on by carrageenan induced rat paw oedema method for acute inflammation and cotton pellet granuloma method for chronic inflammation. It shows significant anti-inflammatory activity in comparision with indomethacin (20mg/kg) used as standard drug.

ANTHELMINTIC ACTIVITY:

Pathil U K and shuchi dave., (2009) studied anthelmintic activity of different leaf extract (fresh juice and methanolic) of *Spinacia oleracea* L. by using *Pheretima posthuma* as test worms. Different concentrations 10 mg/ml, 20 mg/ml, 30 mg/ml, 40 mg/ml and 50 mg/ml of fresh juice extract and methanolic extract of *Spinacia oleracea* Linn (MSO) were studied to determine the time of paralysis and time of death of worms. Both the extract performed *invitro* anthelmintic activity. Albendazole was used as standard reference and saline water as control. The result was revealed that the fresh juice extract may show more potent anthelmintic activity than MSO.

CNS DEPRESSION:

debjani guha and Sutapa (2008) had investigated CNS depressive of aqueous extract of *Spinacia oleracea* it shows L,leaves by using *invivo* adult albino rats. To evaluated *Spinacia oleracea* L. induced alteration in behavioral activities including locomotor activity, PB (Phenobarbitone) induced sleeping time,grip strength, PTZ-induced seizure status with subsequent modulation in brain monoamines such as 5-HT, DA ,and NE in various region of brain were examined.

RADIATION INDUCED BIOCHEMICAL CHANGES IN MICE TESTIS:

Rashmi sisodia *et al*, (2008) had investigated radioprotective efficacy of methanolic leaf extract of *Spinacia oleracea* L. against radiation induced oxidative stress by using biochemical changes in mice testis. Testis was removed for various biochemical estimations viz. LPO, Protein, cholesterol, and glycogen. Radiation induced augmentation in lipid peroxidation, glycogen, and cholesterol, values were significantly ameliorated by supplementation of spinach extract whereas radiation induced deficit in protein content could be elevated. This indicates that spinach extract pre-treatment renders protection against various biochemical changes in the mice testis to some extent if taken continuously which might be due to synergistic effect of antioxidant constituents present in the spinach.

ANTI-ARSENIC POISONING ACTIVITY:

Badar Uddin Umar *et al*., (2007) had investigated Effect of hexane extract of spinach in the removal of arsenic from arsenic treated rat. Hexane extract of spinach significantly decreased accumulated arsenic from rat liver, spleen, kidney, intestine, lungs, and skin of all the rats were measured by SDDC method by spectrophotometer. It shows

the hexane extract is decreased both arsenic level and (MDA) malondialdehye Level in rat in tissues.

ISCHEMIC BRAIN DAMAGE:

Yu Wang., *et al* (2005) had studied dietry supplementation with, Spinach (*Spinacia oleracea* L.) reduces ischemic brain damage by using adult male Sprague-Dawley rats. Animals were sacrificed and brains were removed for caspase-3 enzymatic assays and triphenyltetrazolium chloride staining at 8 to 48 hrs after onset of reperfusion. A subgroup of animals was used for locomotor behavior and biochemical assays. After received spinach, enriched diets had a significant reduction in the volume of infarction in the cerebral cortex and an increase in post-stroke locomotor activity. Animals treated with spinach, had significantly lower caspase-3 activity in the ischemic hemisphere. In conculsion our data suggest that chronic treatment with blueberry, spinach reduces ischemia/ reperfusion-induced apoptosis and cerebral infraction.

PRODUCTION AGAINST GAMMA RADIATION:

Bhatia AL and Jain M., (2003) had investigated methanolic leaf extract of *Spinacia oleracea* L against radiation induced oxidative stress were evaluated in terms of lipid peroxidation (LPO) and tissue levels of glutathione by using mice *invivo* animal model. The animals were exposed to gamma radiation at the rate of 1.07Gy/min with a source to surface distance of 77.5cm. LPO values were significantly lower in the methanolic extract of *Spinacia oleracea* pretreated irradiated mice as compared to respective untreated-irradiated mice at all intervals, which reached normal values from day 7 onward. It was found that radiation induced augmentation in malondialdehyde content s

and depletion in glutathione changes in liver can altered by methanolic extract of *Spinacia oleracea* L.

SULPHITE OXIDASE ACTIVITY:

Jolivet P *et al*., (1995) had investigated The spinach chloroplasts possess a sulphite oxidase activity coupled with oxygen consumption and reduction of ferricyanide. This activity is associated with thylakoids and solubilized by non-ionic biological detergents. The pH and temperature dependencies of sulphite oxidase activity solubilized by Triton X-100 from spinach thylakoids were consistent with those of an intrinsic membrane protein. Thus, observed sulphite oxidation was not induced through the photosynthetic electron transport system, but achieved via a thylakoid membrane enzymic system showing a sulphite oxidase activity. Kinetic parameters of thylakoid sulphite oxidase were measured and compared with those of other sulphite oxidases.

SEEDS:

ANTISPASMODIC AND BRONCHODILATOR ACTIVITY:

Asifa S and Safur RM *et al*., (2017) had investigated pharmacological evaluation of antispasmodic and bronchodilator effects of 70% aqueous ethanolic extract of *Spinacia oleracea* L. by using in-vitro isolated tissues of rabbit jejunum and tracheal preparations and in-vivo castor oil induced diarrhoeal mice model were used to test the antispasmodic,antidiarrhoeal,and bronchodilatory. It shows significant antispasmodic, antidiarrhoeal, and bronchodilatory activity is compared in dicyclomine (antispasmodic), and carbachol (anticholinergic) used as standard drug.

HEPATOPRODUCTIVE ACTIVITY:

Nilesh kumar jain and abhay K.singhai, (2012) had investigated In-vitro and In-vivo hepatoproductive activity of *Spinacia oleracea* L.Seeds on ccl_4(carbon tertrachloride) induced hepatic toxicity.the result of present study indicated significant invitro and invivo activity hepatoproductive activity of n-butanol fraction of *Spinacia oleracea* on ccl4 induced hepatotoxicity.and hence suggests its use as potential therapeutic agent in liver disease. .it shows hepatoproductive activity is compared with silymarin (100mg/kg.) was used as standard drug.

CHAPTER-III

AIM AND SCOPE

CHAPTER III

AIM OF THE STUDY

Plants are the main source of medicine the largest users of medicinal plants are china and India. Traditional chinese medicines user overs 5000n plant species; India uses about 7000. All the major herbal based pharmaceutical companies are showing a constant growth of about 15 percent. Traditional medicine has served as a food sources of alternative medicine, new pharmaceuticals and herbal care products. Medicinal plants are important for pharmacological research and drug development, not only when plant constituents are used directly as therapeutic agents, but also as starting materials for the synthesis of drugs or as models for pharmacologically active compounds. The derivatives of medicinal plants are non-narcotic with little or no side effects.

The plant Spinacia oleracea belongs to chenopodiacea family provide the major contribution for economically important domesticated species and are cultivated for medicinal and nutritional value.

Traditionally *Spinacia oleracea* L. Leaves has been cooling, emollient, wholesome, antipyretic, diuretic, laxative, digestible, anthelmintic, useful in urinary concretion, inflammation of the lungs and the bowels, sore throat, pain in joints, thirst, lumbago, cold and sneezing, sore eye, ring worm scabies, leucoderma, soalding urine, arrest vomiting , biliousness, flatulence.

Seeds are useful in fevers, leucorrhoea, urinary discharges, lumbago, and diseases of the brain and of the heart (Yunani). Seeds are laxative and cooling. They have been used in the treatment of difficulty in breathing, inflammation of the liver and jaundice. The green plant is given for the urinary calculi.

The phytochemical review of *Spinacia oleracea* L. reveals the presence tannin, saponins, flavonoids, terpenoids, steroids, glycosides, and phenols.

The pharmacological review of Spinacia oleracea L. has anticancer, diabetics, asthma, Hepatoproductive effects, Antiulcer, immunomodulatory effect antibacterial and antifungal effect, antioxidant effects.

Based on the literature survey and ethno medical information, the antidandruff activity of Spinacia oleracea L. in leaf part was not reported. Keeping the above information. The present study was designed to evaluate the antidandruff activity of *Spinacia oleracea* L. leaf. The aim of this work is to study the herbal drug with lesser side effect and effective to treat dandruff.

AIM:

The aim of the present research is to study "An Innovative Assimilation on Pharmacognostical, Phytochemical and Contending against *Malassezia furfur* of *Spinacia oleracea* L. Leaves and Its Bioactive Component".

OBJECTIVE:

The objective of the study was divided into three parts.

PART 1: PHARMACOGNOSTICAL STUDY

- Collection and authentication of plant
- Morphological study of plant
- Microscopy of the leaves
- Anatomical study using light microscope
- Powder microscopy
- Microscopic schedules

PHYSIO-CHEMICAL PARAMETER

- Ash value
- Loss on drying
- Extractive values

PART 2: PRELIMINARY PHYTOCHEMICAL SCREENING

QUALITATIVE ANALYSIS

- Qualitative analysis of the leaves for the presence of various phytoconstituents.
- Preparation of hydroalcoholic extract
- Identification of R_f value by TLC method

QUANTITATIVE ANALYSIS

- Determination of flavonoids content
- Determination of phenolic content
- Determination of chlorophyll "a" chlorophyll "b", Total chlorophyll and total carotenoids.

ISOLATION AND CHARATERISTATION OF PHYTOCONSTITUENT

- Isolation of compound
- TLC of Isolated compound
- Spectral studies of isolated compound
    - ✓ UV Spectroscopy
    - ✓ IR Spectroscopy

PHARMACOLOGICAL STUDIES

To Evaluate the Anti-dandruff efficacy on the leaves of *Spinacia oleracea*

- In vitro Well diffusion
- Hair strand method

CHAPTER-IV

MATERIALS AND METHODS

CHAPTER IV

MATERIAL AND METHODS

Nowadays there is a renewed interest in drugs of natural origin simply, because they were consider as green medicine and is always supposed to be safe. Another factor is the incidences of harmful nature of synthetic drugs, which were regarded as harmful to human beings and environment.. Therapeutic efficacy of medicinal plants depends upon the quality and quantity of chemical constituents. The misuse of herbal medicine or natural products starts with wrong identification. The most common error is one common vernacular name is given to two or more entirely different species **(Dineshkumar C, 2007)**. All these problems can be solved by pharmacognostic studies of medicinal plants.

Pharmacognosy is the study of medicines derived from natural sources, mainly from plants. It basical deals with standardization, authentication and study of natural drugs. Most of the research in pharmacognosy has been done in identifying controversial species of plants, authentication of commonly used traditional medicinal plants through morphological, phytochemical and physicochemical analysis. Pharmacognostic studies ensure plant identity, lays down standardization parameters, which will help and prevents adulterations The Pharmacognostical standardization parameters were generally described below. After decades of serious obsession with the modern medicinal system, people have started looking at the ancient healing systems like Ayurveda, Siddha and Unani to treat the various types of ailments. This is because of the adverse effects associated with synthetic drugs. Herbal traditional medicines have gained considerable momentum worldwide

during the past decade and play a paramount role in health care programs especially in developing countries.

Ancient Indian literature incorporates a remarkable broad definition of medicinal plants and considers all plant parts to be potential sources of medicinal substances. **(Darshan S *et al.*, 2003)** However, a key obstacle, which has hindered the acceptance of the alternative medicines in the developed countries, is the lack of documentation and non-compliance of GMP guidelines basically due to poor standardization statusAdding to this variability it is the fact that in herbal medicine several plants may be used together in the same preparation. These factors corroborate basic need of standardized quality control tests for herbal preparations to ensure quality of the product.

There is an internationally increasing demand for documentation of research work carried out on traditional medicines (**Dahanukar S.A *et al*., 2000).** With this backdrop, it becomes extremely important to make an effort towards standardization of the plant material used as traditional medicine for proper market in authorization and approval. Morphological authentication is not sufficient to ensure quantitative consistency of bioactive or marker compounds responsible for the therapeutic effects. Advances in chemical and instrumental techniques have made it easier to estimate phytochemical parameters of crude drugs. The process of standardization can be achieved by stepwise pharmacognostic studies. Keeping in view the above-mentioned problems, an attempt has been made to standardize the ethno pharmacologically useful whole plants of *Spinacia oleracea* L.

4.1. COLLECTION AND IDENTIFICATION OF PLANT MATERIALS

The whole plant of *Spinacia oleracea* L. were collected from Madurai, Tamilnadu India, during the months of September and October 2017 and all the primary work done (washing, drying…etc.).The plant materials were identified and authenticated by Dr.stepen Research officer-botany, Central Council for Research in Ayurveda and Siddha (C.C.R.A.S).Govt.of India, Tiruneveli. The herbarium of this specimen was kept in the department for further reference.

Organoleptic characters

Organoleptic evaluation can be done, by means of sense organs, which provide the simplest as well as quickest means to establish the identity and purity to ensure quality of a particular drug. Organoleptic characters (External appearance) such as shape, size, colour, odour, taste and fracture of stem bark, leaf structure like margin, apex, base surface, venation and inflorescence, etc. are evaluated. (**Siddiqui *et al*., 1995**)

Siddiqui & M.A. Hakim, Format for the pharmacopoeial analytical standards of compound formulation, workshop on standardization of Unani drugs, January, Central Council for Research in Unani Medicine (CCRUM), New Delhi, (appendix), 1995, 24-25.

Spinacia oleracea Linn.were examine for their organoleptic character and external appearance like colour, odour, taste, shape and surface.

PREPARATION OF LEAF POWDER

The leaves were collected and shade dried. It was powdered in a mixer. The powder was sieved in a No.60 sieve and kept in a well closed container in a dry place.

4.2. PHARMACOGNOSTICAL STUDIES

4.2.1. MICROSCOPIC STUDIES ON THE LEAF OF *Spinacia oleracea* L.

COLLECTION OF SPECIMEN

Care was taken to select healthy plants and for normal organs. Leaf, Petiole specimens were collected from a healthy plant by making a cut with petioles. The materials were cut into pieces and immediately immersed in fixative fluid FAA (Formalin – 5ml + Acetic acid – 5ml +70% Ethyl alcohol – 90ml).

INFILTRATION WITH PARAFFIN WAX

After dehydration, the shavings of paraffin wax were added to the vial containing the plant material with pure TBA. The paraffin shavings are added every 30mts at about 40-45ºC four or five times. Then the vials were filled with wax without damaging the tissues. The vial filled with wax is kept open in warm condition to evaporate all TBA, leaving the specimen in pure molten wax. The specimen filled with pure molten wax for 2 or 3 times by decanting the old wax every time.

CASTING TO MOLD

A boat made out of chart board, by folding the margin, is used to prepare a mold of wax containing specimens. The paraffin along with the leaf and petiole specimen was poured into the boat. With the help of heated needles, the specimens were arranged in parallel rows with enough space in between the specimens. The block was then immersed in chilled water and allowed to cool for few hours.

4.2.2. MICROSCOPY OF LEAF

SECTIONING

The paraffin-embedded specimen was sectioned with the help of rotary microtome. The thickness of the sections was 10-12µm, de-waxing of the sections were carried out by customary procedure (**Johansen, 1940**). The sections were stained with toluidine blue as per the method published by **(O'Brien *et al.* 1964)** since toludine blue is a polychromatic stain, the staining results were remarkably good, and some phytochemical reactions were obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. The sections were, also stained with saffron in and fast green and iodine wherever necessary.

For studying the stomata morphology, venation pattern, and, para dermal sections were used (sections taken parallel to the surface of leaf).The clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid (Sass,1940) were also prepared for studying stomatal morphology. Glycerin mounted temporary preparations were made for macerated/ cleared material. The powdered materials of different parts were cleared with sodium hydroxide and mounted in glycerin medium after staining. Studied and measured the different cell components.

For studying the stomata morphology, venation pattern, and para dermal sections were used (sections taken parallel to the surface of leaf).The clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid (Sass,1940) were also prepared for studying stomatal morphology. Glycerin mounted temporary preparations were made for macerated/ cleared material. The

powdered materials of different parts were cleared with sodium hydroxide and mounted in glycerin medium after staining. Studied and measured the different cell components.

PHOTOMICROGRAPHS

Photographs of different magnifications were taken with Nikon lab photo 2 microscopic units. For normal observation bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have bierfringiest property, under polarized light they appear bright against dark background. The scale bars on the photomicrographs indicated the magnification of the figures. The microscopic features observed for the leaves of the plant are presented.

QUANTITATIVE MEASUREMENTS OF *Spinacia oleracea* L.

Determination of Leaf Constants

The stomatal number, stomatal index, vein islet number and vein termination number were determined on fresh leaves by using standard procedures.

DETERMINATION OF STOMATAL NUMBER

The **Stomatal number** may be defined as the "average number of stomata per square mm area of epidermis of the leaf".

Small pieces of upper and lower epidermal peelings of the leaves were mounted onto a slide. The camera Lucida and stage micrometer were used to draw 1mm square on a paper. The stage micrometer was replaced by the preparation slide. The stomata were marked in that unit area were observed under microscope and. The number of stomata present in unit area was calculated. Ten such readings were taken and the average of stomatal number was calculated and presented in the(**Table-1)** for both upper and lower epidermis.

DETERMINATION OF STOMATAL INDEX

The **stomotal index** is the percentage of the ratio of the numbers of stomata to the total number of epidermal cells where each stoma also being counted as one cell. It is calculated using the following formula **S.I** $= \frac{S}{E+S} \times 100$**; w**here S is the number of stomata per unit area and E is the number of epidermal cells in the same unit area

The procedure adopted for the determination of stomatal number was followed and the preparation was observed under high power. The epidermal cells and the stomata were counted. From these values the stomatal index was calculated using the above formula and was given in **table 1.**

DETERMINATION OF VEIN ISLET NUMBER AND VEIN TERMINATION NUMBER

The term vein islet is used to denote the minute area of photo synthetic tissue encircled by the ultimate division of the conducting strands. The **vein islet number** may be defined as the" number of vein islets per square mm area".

The term **vein termination number** may be defined as the "number of vein terminations present in one square mm area of the photosynthetic tissue".

Small pieces of leaves were cut on the lamina between midrib and the margin, cleared in chloral hydrate and mounted on a slide. The camera Lucida and drawing board were arranged. With the help of a stage micrometer, camera Lucida and microscope, 1mm square was drawn on the paper. Then the stage micrometer was replaced by the sample slides and the veins were traced over the square. The vein islets and vein terminations were

counted in the square. Ten such readings were taken, the average was calculated, and the results were presented in (**Table-1)**

4.2.3. POWDER MICROSCOPY SCHEDULES

PREPARATION OF POWDER

The collected whole plant samples were washed thoroughly with water to free from foreign organic matters. Then the plant materials were cut in to small pieces and dried for few days. These dried whole materials were pulverized mechanically and passed through 40 mess sieve to obtain coarse powder and store in an air tight container. These coarse powdered materials were used for further Pharmacognostical physiochemical phytochemical, fluorescent analysis and preparation of extracts for pharmacological evaluation.

PHYSIOCHEMICAL EVALUATION of *Spinacia oleracea* Linn.

Determination of Colour

The untreated part of the drug was taken and colour of the drug was examined under sunlight.

Determination of Odour

A small portion of the drug was taken, slowly and repeatedly inhaled the air over the material and examined the odor.

Determination of Taste

For taste, a small portion of drug was taken on the tongue and find out the taste of drug.

The parameters which are studied are moisture content, loss on drying, total ash, acid-insoluble ash, alcohol and water-soluble, extractive values, petroleum ether soluble

extractive value, ethyl acetate soluble extractive value, acetone soluble extractive value, etc.

DETERMINATION OF ASH

The residue remaining/left after incineration of the crude drug is designated as ash. The ash remaining following the ignition of medicinal plants is determined by three different methods which measures, total ash, acid-insoluble and water soluble ash. Ash values are used to determine quality and purity of crude drug. It indicates presence of various impurities like carbonate, oxalate and silicate. The water soluble ash is used to estimate the amount of inorganic compound present in drugs. The acid insoluble ash consist mainly silica and indicate contamination with earth material. Moisture content of drugs should be at minimal level to discourage the growth of bacteria, yeast or fungi during storage. Estimation of extractive values determines the amount of the active constituents in a given amount of plant material when extracted with a particular solvent. The extractions of any crude drug with a particular solvent yield a solution containing different phytoconstituents. The compositions of these phytoconstituents depend upon the nature of the drug and the solvent used. It also gives an indication whether the crude drug is exhausted or not **(Bele A *et al.*, 2011)**. The procedure recommended in Indian Pharmacopoeia (Anonymous, 1966;1985;1996;2007 and WHO.) were followed for the determination of total ash, water-soluble ash, acid- insoluble ash, sulphated ash and loss on drying.

DETERMINATION OF TOTAL ASH

About 2 g of the powdered drug was accurately weighed in silica crucible, which was previously ignited and weighed. The powdered drug was spread as a fine even layer on

the bottom of the crucible. The crucible was incinerated at a temperature not exeeding450°C, until free from carbon. The procedure was repeated to get the constant weight. The percentage of the total ash was calculated with reference to the air-dried drug and the values are recorded in (**Table-2**)

DETERMINATION OF WATER SOLUBLE ASH

The ash obtained as described in the determination of total ash was boiled for five minutes with 25 ml of water. The insoluble matter was collected on an ash less filter paper and washed with hot water. The insoluble ash was transferred into a pre-weighed silica crucible and ignited for 15 minutes at a temperature not exceeding 450°C.

The procedure was repeated to get constant weight. The weight of insoluble matter was subtracted from the weight of total ash. The difference in weight was considered as water soluble ash. The percentage of water soluble ash was calculated with reference to the air-dried drug (**table-2**)

DETERMINATION OF ACID INSOLUBLE ASH

The ash obtained as described in the determination of total ash was boiled with 25 ml of 2M Hydrochloric acid for 5 minutes. The insoluble ash was collected on an ash less filter paper and was washed with hot water. The insoluble ash was transferred into a pre weighed silica crucible, was ignited, cooled in desiccator and weighed. The procedure was repeated to get constant weight. The percentage of acid insoluble ash was calculated with reference to air-dried drug (**Table-2)**

DETERMINATION OF SULPHATE ASH

A silica crucible was heated to redness for ten minutes and allowed to cool in a desiccator and weighed. About 1 g of the powdered drug was accurately weighed and was taken in the previously weighed empty crucible. The crucible was ignited, gently at first until the drug was thoroughly charred. The crucible was cooled and the residue was moistened with 1 ml of 2M sulphuric acid, heated gently until the white fumes were no longer evolved and ignited at 800°C ± 25°C until all black particles had disappeared. The ignition was conducted in a place protected from air currents. The crucible was allow and weighed. The operation was repeated until two successive weighing did not differ by more than 0.5 mg. The percentage of sulphate ash was calculated with reference to the air-dried drug (**Table-2)**

4.3. PHYSIOCHEMICAL PARAMETERS

Extractive Value (KOKATE, 1994)

Extractive value of crude drug is useful for the evaluation especially when the constituent of a drug cannot be readily estimated by any other means. Further these values are the indicatives of the approximate measures of their chemical constituents and the nature of the constituent present in the crude drug. Taking into consideration the diversity in chemical nature and the properties of content of drugs, various solvents are used for determination of extractives. The solvent used for extraction is in a position to dissolve appreciable quantities of substance desired.

- Ethanol soluble extractive
- Methanol soluble extractive
- Water soluble extractive

- Chloroform extractive
- Ethyl acetate extractive
- Petroleum ether extractive

DETERMINATION OF EXTRACTIVE VALUES

Petroleum ether soluble extractive value

An accurately weighed 5g of coarsely powdered air dried drug was macerated in 100mL of petroleum ether in a closed flask for 24h, shaking frequently during 6h and allowed to stand for 18h. It was filtered rapidly taking precautions against loss of solvent. 25mL of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish and dried at 105˚C to constant weight. The percentage of the petroleum ether soluble extractive with reference to the air dried drug was calculated and presented in **(table-2)**

Ethanol soluble extractive

An accurately weighed 5g of coarsely powdered air dried drug was macerated in 100mL of ethanol in a closed flask for 24h, shaking frequently during 6h and allowed to stand for 18h. It was filtered rapidly taking precautions against loss of solvent. 25mL of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish and dried at 105˚C to constant weight. The percentage of the petroleum ether soluble extractive with reference to the air dried drug was calculated and presented in **(table 2).**

Water-Soluble Extractive

An accurately weighed 5g of coarsely powdered air dried drug was macerated in 100mL of chloroform water in a closed flask for 24h, shaking frequently during 6h and allowed to stand for 18h. It was filtered rapidly taking precautions against loss of solvent.

25mL of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish and dried at 105˚C to constant weight. The percentage of the petroleum ether soluble extractive with reference to the air dried drug was calculated and presented in **(table-2).**

Determination of chloroform, ethyl acetate, soluble extractives

The procedure followed for the determination of alcohol soluble extractive value was adopted for the determination of chloroform soluble extractive, ethyl acetate soluble extractive, and benzene soluble extractive. Instead of ethanol respective solvents were used for the determination of their extractive values.

The percentage of chloroform, ethyl acetate, and benzene soluble extractives were calculated and presented in **(table-2)**

POWDER ANALYSIS

The behavior of the powder with different chemical reagents was carried out as mentioned by Kay (1938) and Johansen (1940). The observations are presented in **(table 6)** .The powder showed the presence of phytosterols, tannins, proteins, flavonoids and phenolic compounds.

LOSS ON DRYING

It is used for determination of moisture content. The percentage of active chemical constituents in crude drugs is mentioned on air-dried basis. Hence, the moisture content of the drug should be determined and should also be controlled. The moisture content of a drug should be minimized in order to prevent decomposition of crude drugs either due to chemical change or microbial contamination.

About 2gm powdered drug was accurately weighed in a tarred dish and dried in an oven at 105°C for one hour. It was cooled in a desiccator and again weighed. The loss on drying was calculated with reference to amount of air-dried drug and the values are recorded in (**Table-2).**

4.6. EXTRACTION

According to the World Health Organization (WHO), nearly 20,000 medicinal plants exist in 91 countries including 12 mega biodiversity countries. The premier steps to utilize the biologically active compound from plant resources are extraction, pharmacological screening, isolation and characterization of bioactive compound, toxicological evaluation and clinical evaluation.

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 (**Fabricant and Farnsworth, 2001**), 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. Different solvent systems are available to extract the bioactive compound from natural products. The extraction of hydrophilic compounds uses

polar solvents such as methanol, ethanol or ethyl-acetate. For extraction of more lipophilic compounds, dichloromethane or a mixture of dichloromethane/methanol in ratio of 1:1 are used. In some instances, extraction with hexane is used to remove chlorophyll (**Cos *et al.*, 2006**). As the target compounds may be non-polar to polar and thermally labile, the appropriateness of the methods of extraction must be considered. Various methods, such as sonification, heating under reflux, soxhlet extraction and others are commonly used (United States Pharmacopeia and National Formulary, 2002; Pharmacopoeia of the People's Republic of China, 2000; The Japanese Pharmacopeia, 2001) for the plant samples extraction. In addition, plant extracts are also prepared by maceration or percolation of fresh green plants or dried powdered plant material in water and/or organic solvent systems.

4.4. PHYTOCHEMICAL STUDIES

PREPARATION OF HYDROALCOHOLIC EXTRACT OF (HAESO)

PROCEDURE

The shade dried and coarsely powdered leaf of *Spinacia oleracea* L. (Leaf) was defatted with petroleum ether (60-80˚c). The residue was dried and extracted with maceration method by hydroalcohol (70%) extraction until the complete exhaustion of the material and filtered. The extract was concentrated under reduced pressure to obtain a solid residue (dark green).

The above extract was subjected to physical analysis such as colour, consistency, wt/ ml, refractive index and brix. The results obtained are presented in **Table-4**

4.4.1. PRELIMINARY PHYTOCHEMICAL ANALYSIS

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 Table. After obtaining the crude extract or active fraction from plant material, phytochemical screening can be performed with the appropriate tests as shown in the Table to get an idea regarding the type of phytochemicals existing in the extract mixture or fraction.

Preliminary phytochemical screening of the whole plant powder and/or crude drugs extracted in different solvents has been performed to detect the phytoconstituents like; alkaloid, aminoacid, carbohydrate, glycoside, mucilage, tannin, saponins, steroid, triterpenoid, Gums, fixed oils, fat, phenol and flavonoid were qualitatively analyzed by using the standard procedures. (**Harbone J.B 1998, Nagani K *et al*., (2012) and Kokate C. K., (2000).**

TEST FOR ALKALOIDS

Mayer's test:

A small quantity of the extract was treated with Mayer's reagent. Cream colour precipitate indicates the presence of alkaloids.

Dragendorff's test:

A small quantity of the extract was treated with Dragendorff's reagent. Orange brown precipitate indicates the presence of alkaloids.

Wagner's test:

A small quantity of extract was treated with Wagner's reagent. Reddish brown precipitate indicates the presence of alkaloids.

Hager's test:

A small quantity of extract was treated with Hager's reagent. Yellow precipitate indicates the presence of alkaloids.

TEST FOR CARBOHYDRATES

Molisch's test

The extract of the powdered drug was treated with 2-3 drops of 1% alcoholic α-naphthol and 2ml of concentrated sulphuric acid was added along the sides of the test tube. A purple colour indicates the presence of carbohydrates.

Fehling's test

The extract of the powdered leaf was treated with Fehling's solution I and II and heated on a boiling water bath for half an hour. Red precipitate was obtained indicating the presence of free reducing sugars.

Benedict's test

The extract of the powdered leaf was treated with equal volume of Benedict's reagent. A red precipitate was formed indicating the presence of reducing sugar.

TEST FOR ANTHRAQUINONE GLYCOSIDES

Borntrager's test

The powdered drug was boiled with dilute sulphuric acid, filtered and to the filtrate benzene was added and shaken well. The organic layer was separated to which ammonia solution was added slowly. No pink color was observed in ammoniacal layer showing the presence of anthraquinone glycosides.

Modified borntrager's test

About 0.1 g of the powdered drug was boiled for 2 minutes with dil.HCl and few drops of $FeCl_3$ solution, filtered while hot and cooled. The filtrate was then extracted with benzene and the benzene layer was separated. Equal volume of dil.NH_3 solution was added to the benzene extract. No pink color was observed in ammonia layer showing the presence of glycosides.

TEST FOR CARDIAC GLYCOSIDES (FOR DEOXYSUGAR)

Keller kiliani test

About 1 g of the powdered leaf was boiled with 10 ml of 70 % alcohol for 2 minutes, cooled and filtered. To the filtrate 10 mL of water and 5 drops of solution of lead subacetate were added and filtered, evaporated to dryness. The residue was dissolved in 3 mL of glacial acetic acid. To these 2 drops of ferric chloride solution was added. Then 3 mL of concentrated H_2SO_4 was added to the sides of the test tube carefully and observed. No reddish brown layer was observed indicating the absence of deoxysugars.

Raymond test

Test solution treated with dinitrobenezene in hot methanolic alkali gives violet colour.

Legal's test

Test solution when treated with pyridine made alkaline by sodium nitroprusside solution gives pink to red colour.

TEST FOR TANNINS

Ferric chloride

Small quantity of the powdered drug was extracted with water. To the aqueous extract few drops of ferric chloride solution was added. Bluish black color was produced indicating the presence of tannins.

Gold beater's skin test

Add 2 % hydrochloric acid to all small piece of g old beater's skin, rinses it with distilled water and place in the solution to be tested for five minutes. Then give wash of distilled water and transfer to a 1% ferrous sulphate solution. A brown or black color on the skin indicates presence of tannin.

TEST FOR PHENOLIC COMPOUNDS

Ferric chloride

A small quantity of the powdered drug was extracted with water. To the alcoholic extract few drops of ferric chloride solution was added. Bluish black color was produced indicating the presence of tannins.

TEST FOR FLAVONOIDS

Shinoda's test

Little of the powdered drug was heated with alcohol and filtered. To the test solution magnesium turnings and few drops of concentrated hydrochloric acid were added. Boiled for five minutes. Red colour was obtained indicating the presence of flavonoids.

Alkali test

To the small quantity of test solution 10% aqueous sodium hydroxide solution was added. Yellow orange color was produced indicating the presence of flavonoids.

Lead acetate

To the test solution add a mixture of 10 % lead acetate in few drops added. It gives white precipitate.

TEST FOR TERPENOIDS

Little of the powdered drug was extracted with chloroform and filtered. The filtrate was warmed gently with tin and thionylchloride. Pink color solution appeared which indicated the presence of terpenoids.

TEST FOR TRITERPENOIDS

Salkowaski test:

To 2ml of extract 5 drops of con.H_2SO_4 was added, shaken and allowed to stand. Apperance of greenish blue colour indicates the prescence of triterpenoids.

Libermann burchard test:

To 2ml of test solution, 10 drops of acetic anhydride was added and mixed well. To this 5ml of concentrated sulphuric acid was added from the sides of the test tube,appearance of greenish blue colour indicates the prescene of triterpenoids.

TEST FOR PROTEIN AND AMINO ACIDS

Millon's test

Small quantity of acidulous – alcoholic extract of the powdered drug was heated with Millon's reagent. White precipitate turned red on heating indicates the presence of proteins.

Biuret test

To one portion of acidulous – alcoholic extract of the powdered drug one ml of 10% sodium hydroxide solution and one drop of dilute copper sulphate solution were added. Violet color was obtained indicating the presence of proteins.

Ninhydrin test

To the test solution add Ninhydrin solution, boil, violet colour indictes presence of amino acid.

TEST FOR VOLATILE OIL

Weighted quantity (250 gm) of fresh leaves were extracted and subjected to hydro distillation using volatile oil estimation apparatus.

TEST FOR FIXED OIL

A small amount of the powder was pressed in between in the filter paper and the paper was heated in an oven at 105^0 C for 10 minutes. A translucent greasy spot appeared indicating the papers.

TEST FOR SAPONINS

About 1 ml of the extract was dissolved in 20 ml of water and shake in graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

GUM AND MUCILAGE

About 10 ml of the extract was slowly added to 25 ml of absolute alcohol under constant string. Precipitation indicates the presence of gum and mucilage.

TEST FOR GUM

The small quantity of extract was added with few drops of alcohol to form white precipitate which indicates the presence of gum.

4.4.2. FLUORESCENT ANALYSIS

A small quantity of dry plant powder is placed on grease free clean microscopic slide and 1-2 drops of freshly prepared reagent solution is added, mixed by gentle tilting the slide and wait for few minutes. Then the slide is placed inside the UV chamber and observe the colour in visible light, short (254 nm) and long (365nm) ultra violet radiations. The colour observed by application of different reagents in different radiations is recorded.

Generally the colour change is noted in reagents like Powder + 1 N NaOH(aq), Powder + $AlCl_3$ (alc), Powder + NH_3, Powder + Acetic anhydride, Powder + methanol, Powder + HCl, powder+ 50% H2SO4, etc.

Some constituents show fluorescence in the visible range in daylight. The ultra violet light produces fluorescence in many natural products which do not visibly fluoresce in daylight. Substance themselves are not fluorescent; they may often be converted into fluorescent derivatives or decomposition products by applying different reagents. .Thus the process of standardization can be achieved by stepwise. Pharmacognostical studies as stated above. These studies help in identification and authentication of the plant material. Such information can act as reference information for correct identification of particular plant and also will be useful in making a monograph of the plant. Further, it will act as a tool to detect adulterants and substituent and will help in maintaining the quality, reproducibility and efficacy of natural drugs.

Fluorescent analysis was carried out by using the method of Chase and Pratt, (1949). Behavior was different reagents was carried out as mentioned by Johansen,(1940) and (Kokoski *et al*., 1958).

Thus the process of standardization can be achieved by step wise Pharmacognostical studies as stated above. These studies help in identification and authentication of the plant material. Such information can act as reference information for correct identification of particular plant and also will be useful in making a monograph of the plant. Further, it will act as a tool to detect adulterants and substituent and will help in maintaining the quality, reproducibility and efficacy of natural drugs.

QUANTITATIVE ESTIMATION OF PHYTO CONSTITUENTS

4.4.3. DETERMINATION OF GALLIC ACID EQUIVALENT IN (HAESO) (Singleton *et al*., 1999)

Principle

Total phenolic content of the various concentrations of HAESO was determined by Folin-ciocalteu reagent method. The hydroxyl group (OH) of phenolic compounds reduces the phosphomolybdic acid to molybdenum blue in the presence of alkaline medium (present in Folin reagent). The blue coloured complex was then spectrophotometrically measured at 760nm.

Instrument

UV visible spectrophotometer, (Shimadzu -Model 1800)

Reagents required

- Folin-Ciocalteu Reagent (1N)

- Sodium carbonate solution (10%)
- Standard Gallic acid solution

Procedure

About 1 mL (1mg/ml and 0.5 mg/mL) of Hydroalcoholic extract of *Spinacia oleracea* L.(Leaf) (HAESO), 0.5 mL of Folin-ciocalteu reagent (1N) were added and allowed to stand for 15 minutes. Then 1 mL of 10% sodium carbonate solution was added to the above solution. Finally the mixtures were made up to 10 mL with distilled water and allowed to stand for 30 minutes at room temperature and total phenolic content was determined spectrophotometrically at 760nm wavelength.

The calibration curve was generated by preparing gallic acid at different concentration (10, 20, 30, 40 and 50 µg/mL). The reaction mixture without sample was used as blank. Total phenolic content of HAESO extract is expressed in terms of mg of Gallic acid equivalent per gm of extract (mg GAE/g). The results are tabulated in **Table: 6** and the calibration graph was presented at **Fig: 2**

4.4.4. DETERMINATION OF RUTIN (FLAVONOID) EQUIVALENT IN (HAESO)

Principle

Flavonoids present in the extract form, a charge transfer complex with several heavy metals to give a characteristic colour. In this reaction, the high electron positive nature of aluminium attracts the atomic nuclei of the aromatic rings in the flavonoids. Then it will react with potassium acetate in alkaline medium to form a pink coloured complex that is measured spectrophotometrically at 415 nm.

Instrument

UV Visible spectrophotometer, Shimadzu (Model 1800).

Reagents required

- 10% aluminium chloride
- 1M potassium acetate
- Standard rutin

Procedure

1mL of hydroalcoholic extract of *Spinacia oleracea* L (Leaf), 0.1 mL of aluminium chloride solution, 0.1 mL of potassium acetate solution and 2.8 mL of ethanol were added and the final volume was then made up to 5 mL with distilled water. After 20 min the absorbance was measured at 415 nm.

A calibration curve was constructed by plotting absorbance reading of rutin at different concentrations (10, 20, 30, 40 and 50 µg/mL). The sample without aluminium chloride was used as a blank. The total flavonoid content in the extract was expressed as milligrams of rutin equivalent per gram of extract. **(Zhishen *et al*., 1999)**

The results are tabulated in **Table: 7** and the calibration graph is presented at **Fig: 3**

4.4.5. DETERMINATION OF CHLOROPHYLL "a", CHLOROPHYLL "b", TOTAL CHLOROPHYLL AND TOTAL CAROTENOID

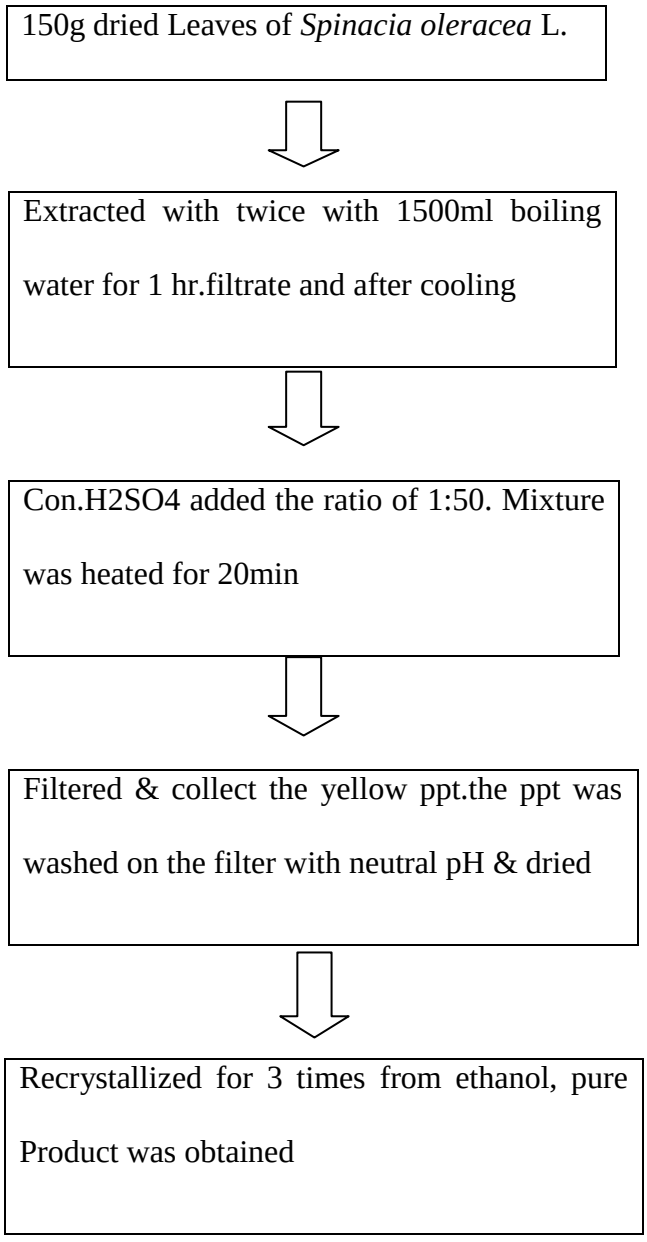
Total chlorophyll, Chlorophyll "a", chlorophyll "b" and total carotenoids were estimated as described by Hiscox and Israelstam (1979). 50 mg fresh leaves were cut into small pieces and kept in 10ml dimethyl sulfoxide (DMSO) containing tube overnight. The extract was filtered through Whatman No.1 filter paper. Absorbance was measured in spectrophotometer at 645 nm and 663 nm for determination of total chlorophyll and

absorbance was measured in spectrophotometer at 453nm for determination of total carotenoids. (J.**J.Dhurve *et al.*, 2015**) . The results are depicted in **Table: 8**

THIN LAYER CHROMATOGRAPHY OF HAESO

As soon as the fractions were eluted, it was analyzed by using readymade TLC plate with suitable mobile solvent (Ethyl acetate: Formic acid: Glacial acetic acid: Water) and using ratio (100:11:11:26) according to the polarity of elute. The development chromatography was observed on 5% ethanolic ferric chloride used as spraying agent and dried at hot air oven at 105^0 c few min to observed colour of spot. The TLC plate with suitable mobile phase (Toluene: Ethyl acetate: Formic acid: Methanol (3:6:1.6:0.4) according to polarity of elute. The developed chromatogram was observed under UV-365nm.

4.4.6. ISOLATION OF APIGENIN FROM *Spinacia oleracea* L.



Identification test of isolated compound:

The isolated compound is identified sample mixed with small amount of Aqueous NaOH solution. The compound produced yellow colour. Add with Con.H_2SO_4 produced yellowish orange colour (**OP Agarwal, 2003)**

IDENTIFICATION AND CHARACTERIZATIONS OF ISOLATED COMPOUND

Thin Layer Chromatography of Isolated Compound

As soon as the fractions were eluted, it was analyzed by using readymade TLC plate with suitable mobile solvent (Benzene: acetic acid: water) and using ratio (100:11:11:26) according to the polarity of elute. The mobile phase of solvent is saturated with 45 min and development chromatography was observed on UV365nm and 5% ethanolic ferric chloride used as spraying agent and dried at hot air oven at 105^0 c few min to observed colour of spot.

UV SPECTRAL STUDIES (SHIMADZU 1800)

The isolated compound was dissolved in ethanol and transferred to cuvette (quartz) and was scanned under UV range from 200 - 400 nm in the UV-Visible spectrophotometer. The main flavonoid obtained in this study match the standard UV/VIS spectra of apigenin (λ/nm): (MeOH) 267, 296nm (**Benguo Lino *et al.*, 2008**). The results are tabulated in (**Table: 13)** and the calibration graph were presented in **Figure: 3.**

INTERPRETATION OF INFRARED SPECTRUM OF ISOLATED COMPOUND

The isolated compound was sample handling by pressed pellet technique in which solid sample are mixed with potassium bromide and compressed in to a thin transparent pellet using a hydraulic press and it is carried out for analysis in infrared spectrophotometer. Group frequency region was 4000cm^{-1} to 1500cm^{-1} & finger print

region was $4000cm^{-1}$- $400cm^{-1}$. Interpretation of infra-red spectrum of isolated compound (apigenin). The results are tabulated in (**Table: 14)** and the calibration graph was presented in (**Figure: 4)**

4.5. PHARMACOLOGICAL STUDIES:

Pharmacological screening procedures are important and necessary in order to estimate the harmful or therapeutic potential of useful drug. Molecular procedures are used nowadays to screen the herbal compounds and extracts. The classical method of pharmacological screening involves sequential testing of any new chemical compounds or extracts from herbal sources by i*n -vitro* and *in vi*vo experiments. Most of the extracts or drugs used in therapy have been found and evaluated with these methods.

4.5.1. IN VITRO ANTI-DANDRUFF ACTIVITY:

Collection and maintance of the culture:

Pure culture of *Malassezia furfur* (MTCC: 1374) was obtained from institute of microbial type of culture collection, chandigarh, india. The culture was maintained in SDA medium.

Inoculum preparation:

The peptone was added to the liquid SDM in the concentration of 5, 10, 15 and 20 g/lr. Pure culture of *M.furfur* grown in liquid medium was inoculated and incubated at 30 ± 2^0 c for 7 days.

Preparation of the medium:

2g of SDA medium and 1g of Agar was dissolved in 50ml of distilled water heat to boiling to dissolve the medium completely sterilize by autoclaving at 15Ibs pressure (121^0 c) For 15min P^H is adjusted to (5.6 ± 2^0 C). the medium was poured into the sterile

petridishes to get a thickness of 5-6mm. The medium was allowed to solidify and petridish was inverted and were dried at 37^0 C just before inoculation.

In vitro Anti-dandruff activity:

The broth culture of *M.furfur* was swabbed over the sabouraud dextrose agar by using sterile cotton buds. Sterile 6mm diameters well are punched and added in plant extracts and ketoconazole (Standard drug 10µg/disc) and control DMSO well were placed Equidistantly (3cm apart) round the margin of the plate (plate No-11 & 12).at 30 ± 2^0 c and zone of inhibition was observed after 3days. (**M.Vijayalakshmi and K.Periyanayagam , 2014) and (sekar M *et al*.,2012)**

4.5.2. HAIR STRAND TEST

***Malassezia* Species**

Hair specimens were taken from ten volunteer so different hair color (six female, four Male: mean2 8.2 years, 5 -53 years), who did not use antidandruff preparations or hair dyes. By means of scissortsh e strands were cut neart he scalps Surface (hair roots were not included in the sample). Two different concentrations were used.

STRUCTURE OF THE TRIAL

Sterile glass Petri dishes (3 cm in diameter) were filled with 4 ml of selective agar for Pathogenic fungi (SDA). Cold sterile olive oil was inoculated with different *Malassezia* strains were cultured for four days on SPF (Specific pathogen Free) overlaid earlier with olive oil and adjusted to an inoculation density of 5×10^3 CFU/µl using Neubauer Chamber from each voluenteer, hair strand approximately 5cm in length were incubated with one of the five test substances at 30^0 c for 5min in sterile petri dishes. The hairs were then

transferred to a sieve with filter paper, rinsed for 1min in running water (30^0 c), and dried at room temperature. By means of sterile scissors, 1-cm pieces were cut from the dried hair and distributed in the center of the different test dishes. To approximate natural scalp conditions, 200 hairs/ cm^2 were inoculated. (**Peter mayser, and Frank rippke., 2003**).

CHAPTER-V

PLATE 1

HABIT AND HABITAT OF *Spinacia oleracea* L.

CHAPTER V

RESULT AND DISCUSSION

5.1 PHARMACOGNOSTICAL STUDIES

5.1.1 MORPHOLOGICAL FEATURES OF *Spinacia oleracea* L.

Spinacia oleracea L.is a small to medium size evergreen plant growing to a height of about 20-60cm (1ft).spinach may survive over winter in temperate regions. The leaves are alternative, simple, ovate to triangular and very variable size from about 2-30cm (1-2) long and 1-15cm (0.4-5.9in) broad with larger leaves at the bases of the plant and small leaves higher on the flowering stem.

LEAVES (PLATE 2, 3 &4)

ARRANGEMENT AND SHAPE	**:**	Leaves are simple, no stipules; Leaf –blade angular- ovate or arrow –head shaped with round to Sharp pointed basal lobes, 9-30 cm×7-20cm, smooth Surface
SIZE	**:**	25-50cm in diameter and 10-20 cm high of 12-20 leaves Cluster at ground level.
COLOUR	**:**	Light to dark green.
MARGIN	**:**	Serrate
BASE	**:**	Oval or Triangular
APEX	**:**	Acute
PETIOLE	**:**	Green long Petiole
VENATION	**:**	Pinnately reticulate lower surfaces prominent
SURFACE	**:**	Both the surfaces are very smooth

PLATE -2

LEAF ARRANGEMENT OF *Spinacia oleracea* L.

PLATE -3

DORSAL VIEW OF *Spinacia oleracea* L.

PLATE- 4

VENTRAL VEIW OF *Spinacia oleracea* L.

The cytomorphological studies perhaps the role of crude drug authentication. This study illustrate the morphological characteristics based on different parameters of the plant organs like Colour, size, shape, margin, texture, arrangement were observed and compared with previous data. From the results of these illustrations give a guideline for the diagnosis of the original plant and its adultrants.

Morphological features of *Spinacia oleracea* L. shows diagnostic characters. In *Spinacia oleracea* L. presence of stem triangular shape, apex acute, base oval and triangulate and venation is pinnately reticulate and lower surfaces are prominent , margin is serrate are the diagnostis characters. **(Plate -2, 3 & 4).**

PLATE-5

T.S OF LEAF *Spinacia oleracea* L. THROUGH THE MIDRIB

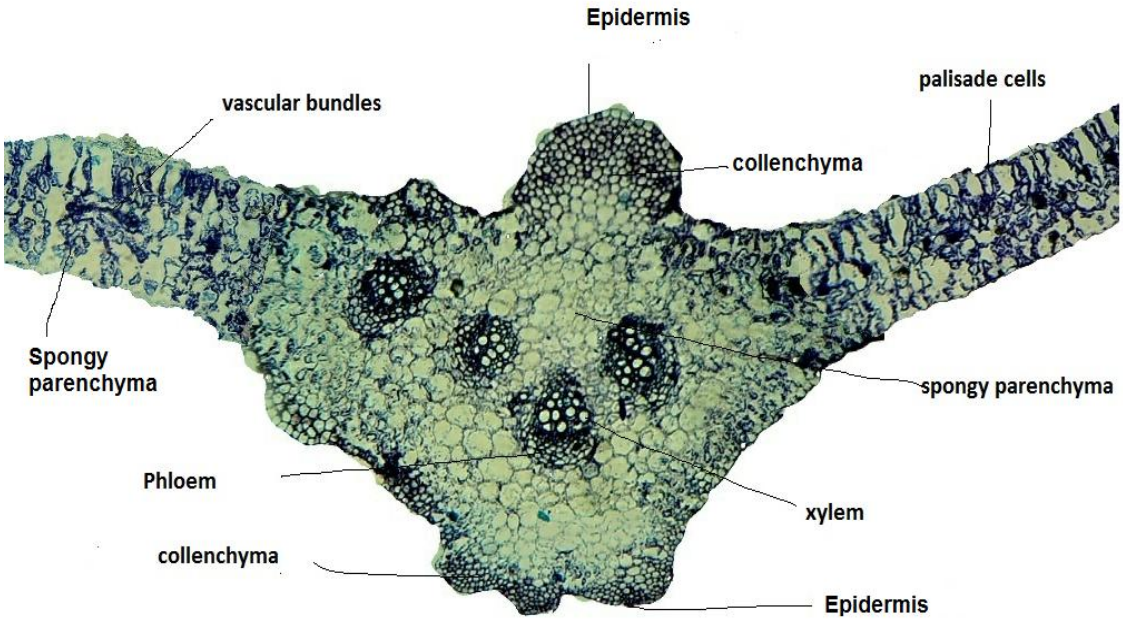


PLATE-6

T.S OF LAMINA OF *Spinacia oleracea* L.

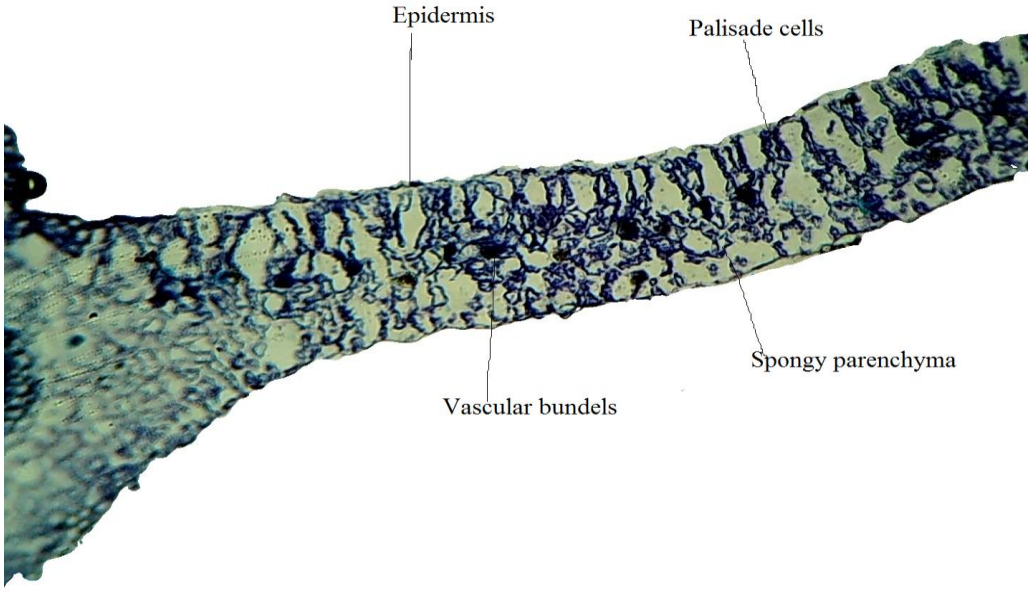


PLATE-7

T.S OF PETOLE OF *Spinacia oleracea* Linn.

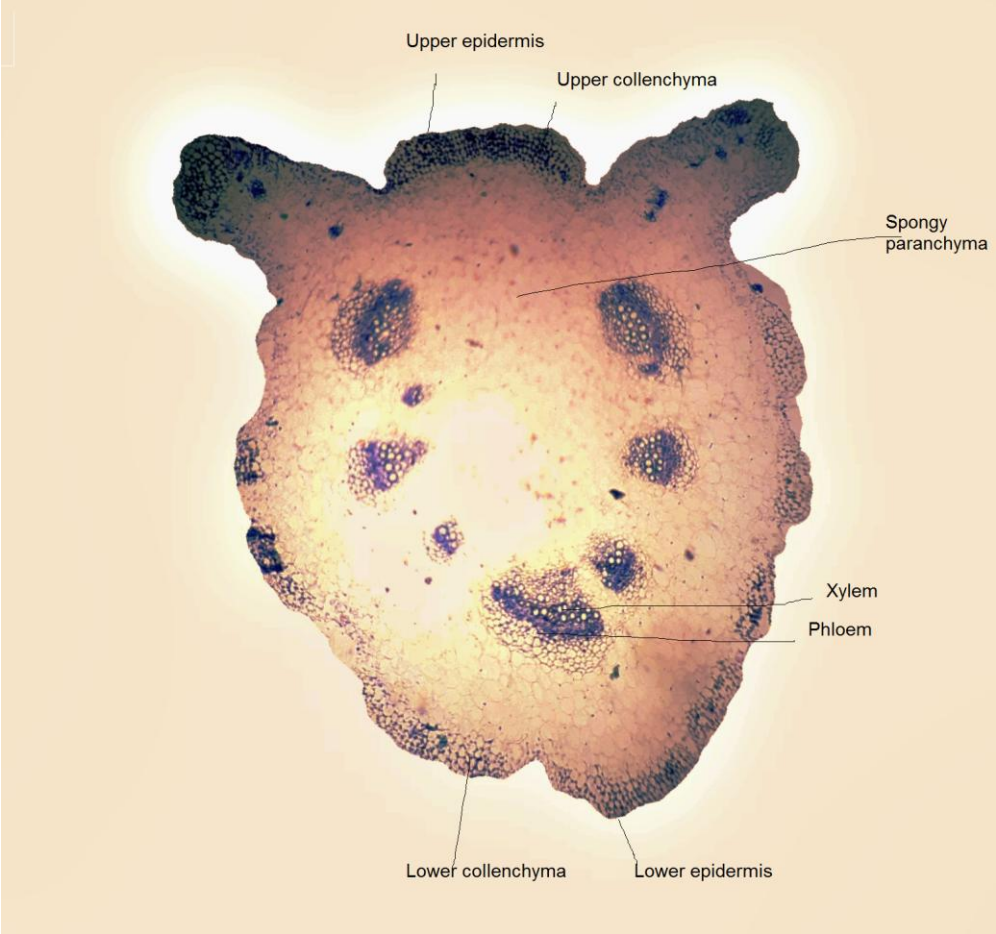
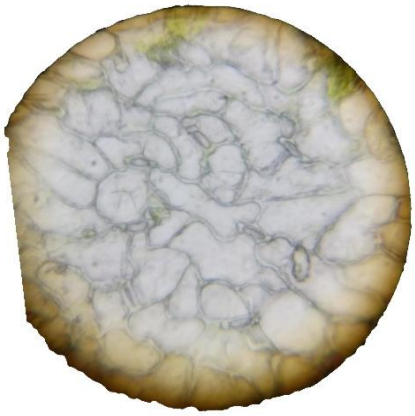
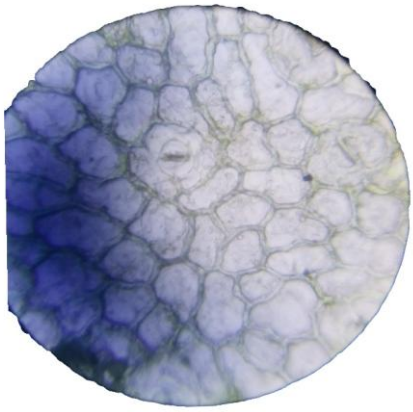


PLATE 8

LOWER LAYER STOMATA OF *Spinacia oleracea* L.



UPPER LAYER STOMATA OF *Spinacia oleracea* L.



5.1.2 MICROSCOPY OF THE LEAF

Microscopical studies of whole plant of *Spinacia oleracea* L.

Leaf: Leaf is a narrow and thin midrib and wing like lamina **(Plate-5)**. The midrib is planoconvex with flat adaxial side and convex aboxial side. There are four vascular bundle of the midrib exhibiting an arc and collateral. There are few xylem elements which are narrow, circular with diffuse distribution. Phloem occurs in this segment beneath the xylem strand **(Plate-5).** The adaxial side of the midrib shows three small projetions. Below the hump and abaxial projections Three to four layer of collenchyma present. In the addax Ground tissue made up of 2-8 layers of thin walled rounded parenchyma cells.

Epidermal cells and Stomata: (Plate-5 & 8).

The epidermal cells are small with highly wavy anticlinal walls. The cells appear amoeboid in outline due to the wavy walls. The stomata are abundant; they are oriented transversely to the longitudinal axis. The stomata are anomocytic type with three subsidiary cells lying on either side of the stomata and being transverse to the guard cells. The stomata are cylindrical with prominent stomata pores. Trichomes were absent.

Venation of the Lamina: (Plate-5)

The leaf consist of thin , less prominent lateral veins forming less reticulations .The vein-islets are wide, rectangular in outline and the vein boundaries are fairly distinct. Vein terminations are present in most of the islets. The vein terminations may be simple (unbranched) or branched once. **(Plate-5)**

Lamina: (Plate-6)

The lamina is distinctly dorsiventral with smooth and even surfaces. The adaxial epidermis is thick and the cells dilated, in shape oval and thin walled. The abaxial side epidermis is thin with small rectangular cells. Both adaxial and abaxial side epidermis are stomatiferous (Amphistomatic). The mesopyll consists of single rows of palisade cells. The spongy parenchyma zone includes five layers of small loosely arranged reticulate cells.

PETIOLE: (Plate-7)

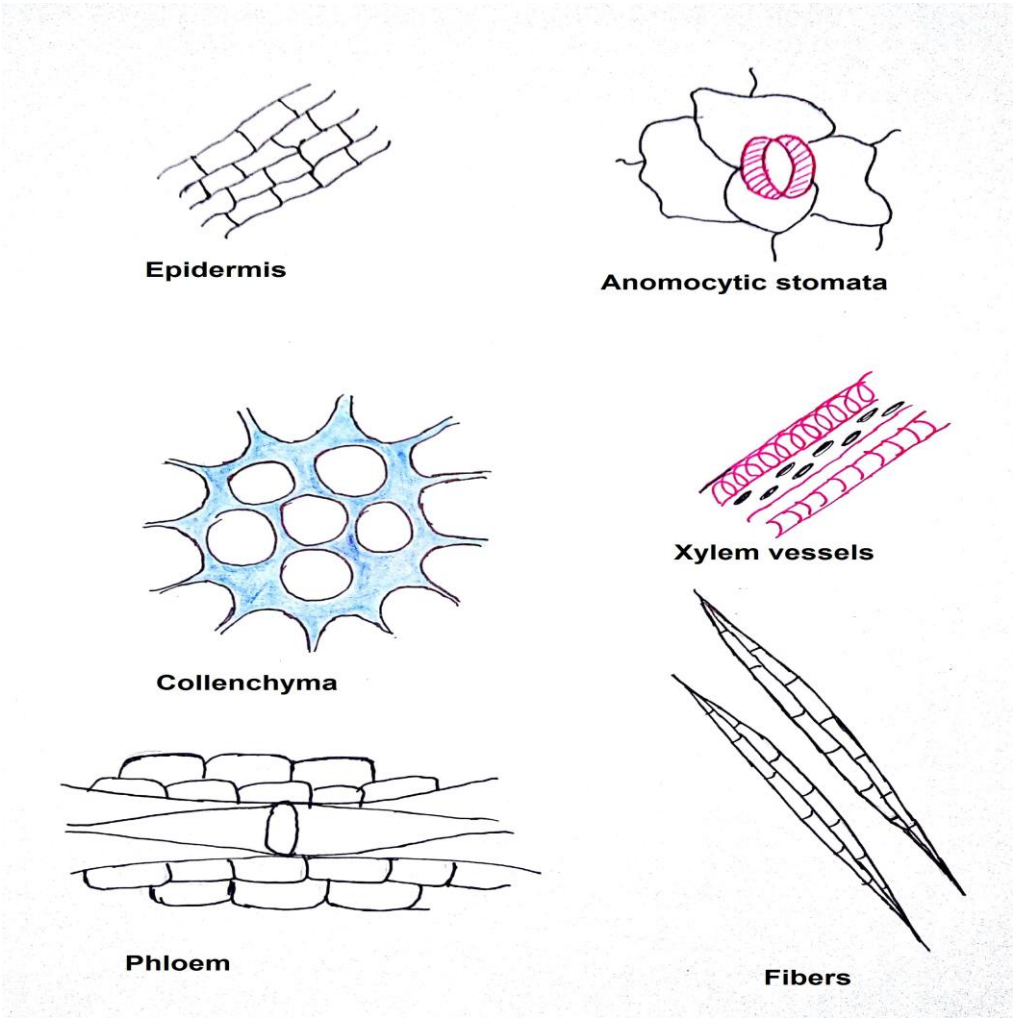
In transverse section through the distal end exhibiting More or less round with small projections all over the periphery, Collenchyma present below the projections. Single layer of epidermis without hypodermis having collenchyma cells below the projections. 6 or 7 vascular bunles arranged in a ring (**Plate-7).**

Ground tissue made of parenchymatous cell. Trichromes and secretory glands were absents.

Microscopic features of *Spinacia oleracea* L. shows T.S of leaf is generally ovate to triangular based and midrib of leaf consist of both upper and lower epidermis is a single layered containing polygonal thick walled cells covered by thick cuticle. Four number small in size and collateral. Xylem is lignified radiating from the center surrounded by phloem. Adaxial layer of lamina consist of single layered palisade cells are present. Abaxial sides of epidermis consist of spongy parenchyma cells are present. Stomata are present in both epidermis and they are anomocytic. Crystals and secretary cavities are absent. Trichomes were absent.

FIG-1

POWDER MICROSCOPY OF *Spinacia oleracea* L.



5.2 POWDER MICROSCOPY

ORGANOLEPTIC CHARACTERS

- NATURE : Coarse powder.
- COLOUR : Green
- ODOUR : Odourless.
- TASTE : Palatable taste.

Pressed in between two filter paper, No oil mark on the paper.

We have observed the following microscopical cell structures, the powder microscopy of *Spinacia oleracea* L. results were showed on **(fig-1)**

- Epidermis
- Stomata
- Fibers
- Collenchyma
- Xylem vessels
- Phloem.

5.2.1 MICROSCOPIC SCHEDULES

QUANTITATIVE MICROSCOPY OF *Spinacia oleracea* L.

The parameters such as vein termination, vein islet and stomatal numbers, stomatal index and palisade ratio of the leaf of *Spinacia oleracea* L. were observed and recorded. The observations and results are summarized in the **(Table-1)**

VEIN ISLET AND VEIN TERMINATION NUMBER AND STOMATAL NUMBER AND INDEX OF *Spinacia oleracea* L.

TABLE-1

S.NO	PARAMETERS	AVERAGE
1	Vein Islet Number	9
2	Vein termination number	11
3	upper epidermis of stomata Number	6
4	Lower epidermis of stomata Number	23
5	Upper epidermis of stomata index	9.2
6	Lower epidermis of stomata index	51

Vein islet and vein termination number are another simple technique for distinguishing fragmentary specimens at specific levels. It is used as the distinguishing character for the leaf of the same species or different one. Vein islet number is = 9 and vein termination number is =11.vein islet number and termination value range is above the **(table-1).**

Quantitative microscopy includes certain measurements to distinguish some closely related species which are not easily differentiated by general microscopy. The upper epidermis of stomatal number and index is 6 & 9.2 the oldest technique but a simple method of diagnosis of fragmentary leaf parts. The lower epidermis of stomatal index and number is 51 & 23 the percentage of stomata in relation to the epidermal cells. Both are very specific criteria for the identification and characterization of leafy drugs. The compared to upper stomatal number and index value is higher than lower stomatal number and index values are mentioned (**Table-1).**

5.2.3 PHYSIOCHEMICAL PARAMETERS

As per the methods described in materials and methods, physic chemical parameters were carried out and the results were tabulated in (**Table-2)**

TABLE-2

S.No	PHYSIOCHEMICAL CONSTANT	REPORTS % (w/w)
1	Total ash	23.17
2	Water soluble ash	4.7
3	Acid insoluble ash	9.4
4	Loss on drying	0.54
5	Petroleum ether extractive value	8.0
6	Chloroform extractive value	6.04
7	Ethyl acetate extractive value	9.45
8	Methanol extractive value	23.4
9	Ethanol extractive value	21.2
10	Aqueous extractive value	18.74
11	Hydro-alcoholic extractive value	22.08

The percentage of total ash was found to be 21.17(w/w) and the percentage of water soluble ash was found to be 4.7(w/w) while the acid insoluble ash was 9.4(w/w). The determination of ash values helps to find out where the powdered material was adulterated with sand and other inorganic material. The water soluble ash helps us to find the amount of inorganic material present in the crude drug, while acid insoluble ash helps us to find the

amount of sand and other debris in the crude material. The various extractive values with different solvents have been determined. A maximum extractive value was found with methanol 23.4% (w/w) ethanol 21.2% (w/w) and water 18.74% (w/w) and followed by hydro-alcoholic extractive value22.08% (w/w). The extractive value helps us to decide what solvent will be useful for extraction of maximum active principle and also helps to decide whether the crude material has already seen exhausted or not. The physiochemical parameters of *Spinacia oleracea* L. was reported on (**table-2**)

The percentage of Loss on drying value was found to be 0.54% (w/w) at 105^{o}C is determined as the presence of excess moisture is Loss conductive to the promotion of mold and bacterial growth, and subsequently to deterioration and spoilage of the drug.

5.3. FLUORESCENCE ANALYSIS OF POWDERED LEAF

The fluorescence analysis of the leaf powder of *Spinacia oleracea* L. was studied. The results were as follows **(Table-3).**

FLUORESCENCE ANALYSIS

TABLE-3

S.NO	TREATMENT	VISIBLE LIGHT	UV254nm	UV365nm
1	POWDER	Green	Green	Purple
2	POWDER+ con.HCl	Dark green	Dark green	Greenish brown
3	POWDER+ con.H_2SO_4	Dark green	Brown	Purple
4	POWDER+ con.HNO_3	Dark green	Dark green	Fluorescent red
5	POWDER+Acetic anhydride	Redish Brown	Dark green	Dark brown
6	POWDER+ NaOH	Dark brown	Dark green	Brown
7	POWDER+ Methanol	Green	Green	Violet
8	POWDER+$AlCl_3$	Yellow	Light Green	Green

The fluorescence analysis of the powdered drug of *Spinacia oleracea* L. leaf in different chemial reagents were performed under visible light, UV light in short wavelength (254nm) and longer wavelength (365nm) to detect the fluorescent compounds.

The coloured fluorescence obtained from powdered drug of *Spinacia oleracea* L. leaf are presented in **(Table-3).**

The *Spinacia oleracea* L. leaf powder Visible UV and UV (254nm) produced green colour and UV (365nm) produce purple colour. Powder + con.HCl visible UV and UV (254nm) produced dark green colour. Powder + con.HNO_3 visible UV & UV (254nm) produced dark green colour and UV (365nm) produces fluorescent red. Powder + acetic unhydride Visible UV produced reddish brown colour & UV (254nm) & UV (365nm) produces dark green & dark brown. Powder + methanol visible UV & UV (254nm) produced green colour and UV (365nm) produces violet colour. Powder + $AlCl_3$ produced Visible UV & UV (254nm) produces yellow colour & light green and UV (365nm) produced green colour. The fluorescence analysis of powder drug was reported on **(Table-3)**

In organic molecules absorb light usually over a specific range of wavelength and many of them emit such radiations. So if the powder is treated with different chemical reaction and seen in the UV chamber, different colours will be produced. The results of fluorescence analysis revealed the purity of this plant material.

5.4 PHYTOCHEMICAL STUDIES

Extraction of Hydroalcoholic Leaf Extract of *Spinacia oleracea*

Hydro-alcoholic extract leaf of *Spinacia oleracea* L. by using 70% ethanol and distilled water.

DETERMINATION OF PHYSICAL PARAMETERS OF (HAESO)

Table-4

S. NO	PARAMETERS	REPORTS
1	Refractive Index	1.370 ± 0.003
2	Weight /Ml	0.897 ± 0.007
3	Consistency	Liquid
4	Colour	Dark green

The physical parameters of hydro alcoholic extract of *Spinacia oleracea* L. (Leaf) such as refractive index, weight per ml, consistency and colour was determined. It was found to be refractive index (1.370 ± 0.003), weight per mL (0.897 ± 0.007), and Dark green in colour with liquid consistency (**Table-4**).

QUALITATIVE PHYTOCHEMICAL TEST

5.4.1. PRELIMINARY PHYTOCHEMICAL SCREENING

The results obtained for the preliminary phytochemical screening of the different extract (ethanol, aqueous, ethyl acetate, petroleum ether, chloroform) of *Spinacia oleracea* L.leaves was presented in (**Table-5)**.

TABLE-5

RESULTS OF PRELIMINARY PHYTOCHEMICAL SCREENING OF LEAVES EXTRACT OF *Spinacia oleracea* L.

S.NO	Test	Powder	Pet.ether	$CHCl_3$	Ethyl acetate	Ethanol	Water
I	**ALKALOIDS**						
	Mayer's reagent	-	-	-	-	-	-
	Dragondroff's reagent	-	-	-	-	-	-
	Hager's reagent	-	-	-	-	-	-
	Wagner's reagent	-	-	-	-	-	-

II	**CARBOHYDRATES**						
	Molisch's test	+	+	+	+	+	+
	Fehling's test	+	+	+	+	+	+
	Benedict's test	+	+	+	+	+	+
III	**GLYCOSIDES**						
	Anthroquinone glycosides	+	+	+	-	+	+
	Borntrager's test	+	+	+	-	+	+
	Modified Borntrager's test	+	+	+	-	+	+
	Cardiac Glycosides	+	+	+	-	+	+
	Keller killiani test	+	+	+	-	+	+
	Raymond test	+	+	+	-	+	-

	Legal test	+	+	+	-	+	-
IV	**STEROLS**						
	Salkowski test	+	+	-	-	+	+
	Libberman burchard's test	+	+	-	-	+	+
V	**SAPONINS**	+	+	+	+	+	+
VI	**PHENOLS AND TANNINS**						
	Ferric chloride test	+	+	+	+	+	+
	$Kmno_4$ test	+	+	+	+	+	+
	Gold beater's skin test	+	-	_	+	+	+
VII	**PROTEINS AND FREE AMINO ACIDS**						
	Millon's test	+	-	-	-	+	+

	Biuret test	+	-	-	-	+	+
	Ninhydrin test	+	-	-	-	+	+
VIII	**MUCILAGE**	-	-	-	-	-	-
IX	**TERPENOIDS**	+	-	-	-	+	+
	TRITERPENOIDS	+	-	-	-	+	+
X	**FLAVONOIDS**						
	Shinoda test	+	-	+	+	+	+
	Alkalii test	+	-	+	+	+	+
	Acid test	+	-	+	+	+	+
	Zinc/Hcl test	+	-	+	+	+	+
XI	**VOLATILE OIL**	-	-	-	-	-	-
XII	**FIXED OIL**	-	-	-	-	-	-

(+) indicates positive reaction (-) indicate negative reaction

The preliminary phytochemical screening procedure of the different extract of *Spinacia oleracea* L.leaf showed the presence of carbohydrates, phytosterols, carotenoids,cardiac glycoside, protein and amino acids, flavanoids, terpenoids, phenoli compounds, tannins, saponins.

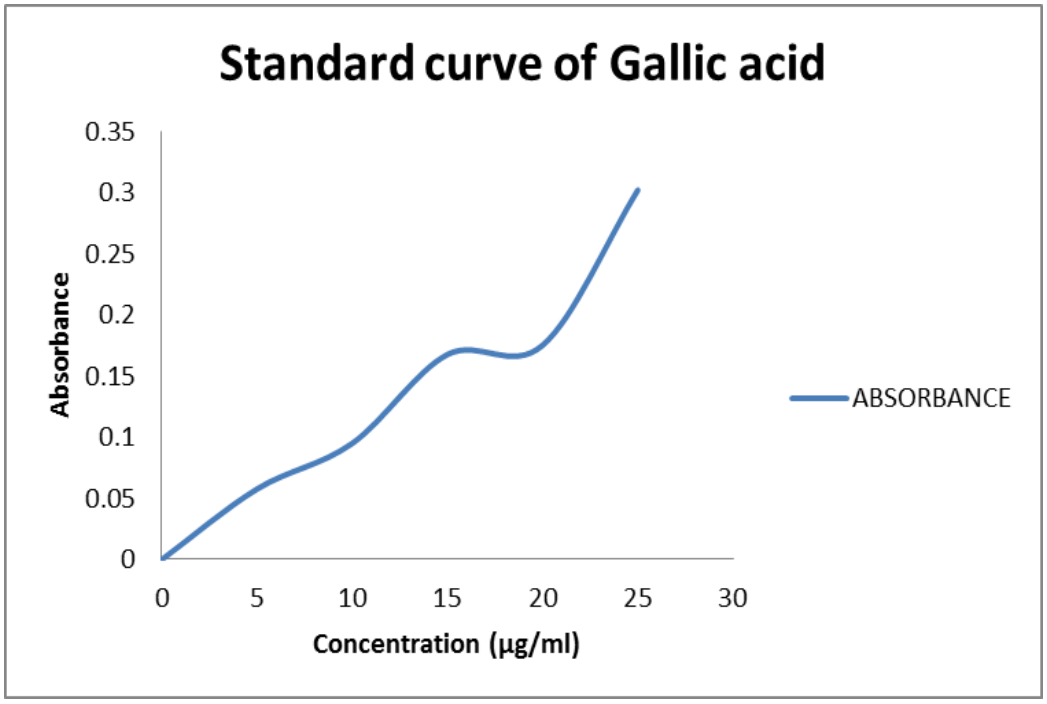
The information obtained from preliminary phytochemical screening will be useful in finding out the genuine of the drug. The result of phytochemical screening of powder drug, alcohol, aqueous extract of *Spinacia oleracea* L. leaf are presented in the phytochemical screening indicated varying quality of alkaloids, carbohydrate, glycoside, sterol, saponins, protein, mucilage, terpenoid, flavonoid, phenol, protein and amino acids in the leaf extract.the phytochemical profile reavealed that the ethanolic extract contained carbohydrate, glycoside, sterol, saponins, protein, mucilage, tannins, terpenoids and phenols. Aqueous extract conained carbohydrate, glycoside, saponins, mucilage, tannins, terpenoids and phenols. Ethylacetate extract contained carbohydrates, flavonoids, saponins, phenols and tannins. Pet.ether extract contained saponins terpenoids, phenols, glycosides, steroids. Chloroform extract contained steroids, saponins, carbohydrates, flavonoids, phenols, alkaloids, glycosides. Volatile oil and fixed oil were not detected in any of the extract. (**Table-5**)

QUANTITAVIE PHYTOCHEMICAL STUDIES

5.4.2. DETERMINATION OF GALLIC ACID EQUIVALENT IN (HAESO)

The phenolic content of HAESO was recorded for calibration curve bellow the **Fig:4.**

Fig-2



DETERMINATION OF GALLIC ACID EQUIVALENT OF HAESO

The gallic acid equivallent of HAESO were recorded absorbance and weight equivalent of extract is mentioned the **(Table-6)**.

TABLE - 6

S. NO	Concentration		Absorbance		Weight of Gallic Acid equivalent (GAE) of (HAESO) (µg/ml)
	Gallic Acid	HAESO	Gallic Acid Mean ± SEM	HAESO Mean ± SEM	
1	5	10	0.0577 ± 0.008	0.0067 ± 0.0006	1.06
2	10	20	0.095 ± 0.0011	0.0163 ± 0.0008	1.94
3	15	30	0.1677 ±0.0008	0.0357 ± 0.0008	3.7
			GAE		111.5 mg/g

The linear regression equation was found to be **y = 0.0111×0.0054** while the correlation Coefficient was found to be **0.9998.** The amount of phenol content present in the extract in terms mg GAE/g of extract was found to be **111.5 mg/g** by using the above linear regression equation **(Table-6).**

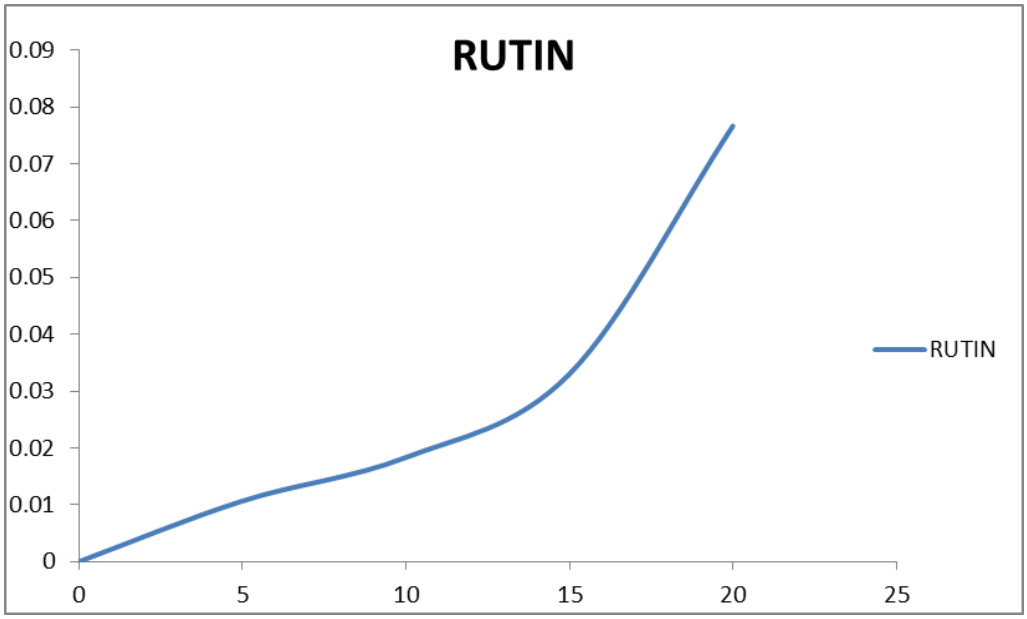
Qualitative phytochemical screening of whole plant powder and extracts of *Spinacia oleracea* L. revealed the similar results .The presence of biologically active compounds like terpene, glycoside, flavanoid and phenols are attributed to antibacterial and antifungal, anti-oxidant, anti-inflammatory, antitumor and in treatment of respiratory complications.

Quantitative estimation of biological compounds showed that *Spinacia oleracea* has more of flavonoids, phenols and carotenoids. This could be used as diagnosis the nature and amount of phytoconstituents.

5.4.2. DETERMINATION OF FLAVONOID CONTENT

The flavonoid content of HAESO was recorded for calibration graph is mentioned

Fig-3



DETERMINATION OF RUTIN EQUIVALLENT IN HAESO

The rutin equivalent of HAESO were recorded absorbance and weight equivalent of extract is mentioned (**Table-7).**

TABLE-7

S. NO	Concentration		Absorbance		Weight Rutin equivalent (RE) of (HAESO) (µg/ml)
	Rutin	HAESO	Rutin	HAESO	
			Mean ± SEM	Mean ± SEM	
1	5	5	0.0106± 0.0008	0.0267 ± 0.0008	4.27
2	10	10	0.0183± 0.0014	0.0583 ± 0.0011	9.04
3	15	15	0.033 ± 0.0015	0.032±0.00208	13.82
4	20	20	0.0766 ±0.0017	0.064± 0.0026	18.91
			RE		910mg/gm

The linear regression equation was found to be **y=0.0035×0.0074** while the Correlation was found to be **0.9974**. The amount of flavonoid content present in the extract in terms mg rutin equivalent/g of extract was found to be **910.mg/g** by using the above linear regression equation (**Table-7**). Qualitative phytochemical screening of whole plant powder and extracts of *Spinacia oleracea* L. revealed the similar results .The presence of biologically active compounds like terpene, glycoside, flavanoid and phenols are attributed to antibacterial and antifungal, anti-oxidant, anti-inflammatory, antitumor and in treatment of respiratory complications.

Quantitative estimation of biological compounds showed that *Spinacia oleracea* has more of flavonoids, phenols and carotenoids. This could be used as diagnosis the nature and amount of phytoconstituents.

5.4.3. DETERMINATION OF CHLOROPHYLL‟ a" & CHLOROPHYLL „b" and TOTAL CHLOROPHYLL AND TOTAL CAROTENOIDS CONTENT

The fresh leaf contains chlorophyll „a" and chlorophyll „b" and total chlorophyll and total carotenoids content were reported on **(Table-8).**

TABLE-8

S.no	Constituent	Wavelength (nm)	Absorbance (λmax)	Amount present in (mg/gm) of leaves
1	Chlorophyll a	663nm	0.20	94mg/gm
		645nm	0.07	
2	Chlorophyll b	645nm	0.07	26mg/gm
		663nm	0.20	
3	Total chlorophyll	645nm	0.07	120mg/gm
		663nm	0.20	
4	Total carotenoids	663nm	0.20	45mg/gm
		645nm	0.07	
		453nm	1.25	

Determination of chlorophyll a & chlorophyll b and total chlorophyll and total carotenoids content of *Spinacia oleracea* L. leaves are measured and data presented in

(**Table-8)** chlorophyll a was found to be 94mg/gm while chlorophyll b was found to be 26mg/gm and total chlorophyll content was found to be (120mg/gm) and total carotenoids of Leafy vegetable was found to be (45mg/gm). Leafy vegetables contain several types of photosynthetic pigments that are chlorophylls and carotenoids (**Table-8).**

Quantitative estimation of biological compounds showed that *Spinacia oleracea* has more of flavonoids, phenols and carotenoids. This could be used as diagnosis the nature and amount of phytoconstituents.

5.4.4 THIN LAYER CHROMATOGRAPHY

Separation of phytoconstituents in hydroalcholic extract of *Spinacia oleracea* L**.** leaf carried out by thin layer chromatography.

Among the above 2 mobile phase i) Toluene: ethyl acetate: formic acid: methanol in the ratio is (3:6:1.6:0.4) and ethyl acetate: formic acid: glacial acetic acid: water in the ratio is (100: 11: 11: 26) gave better elution for hydroalcoholic extract of *Spinacia oleracea* L. Leaf, and hence it was used as mobile phase for the detection of constituents in the extracts. The optimized chamber saturation time for mobile phase is 45 min at room temperature (25 ± 1^{o}c).

PLATE-9

TLC PROFILE OF HYDROALCOHOLIC EXTRACT OF *Spinacia oleracea* L.

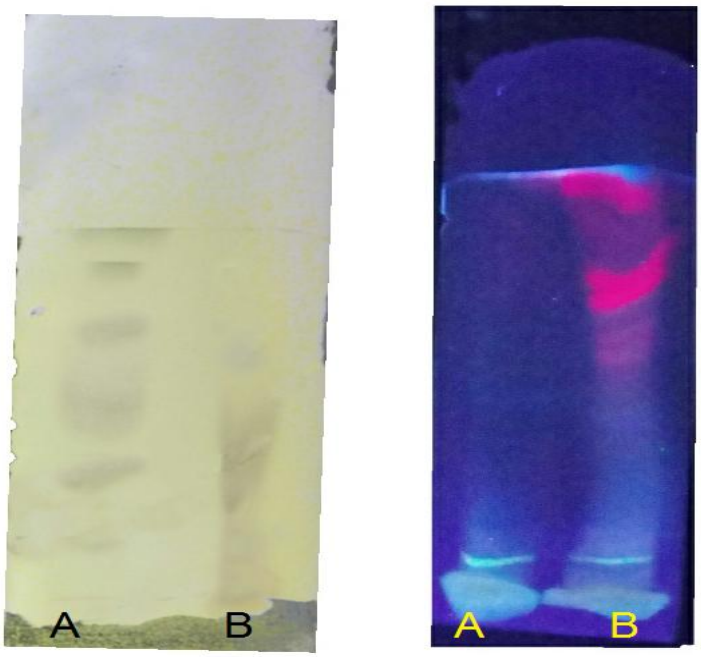


PLATE-I PLATE-2

PLATE-I:

A= Sample dissolved in methanol, **B=** Sample dissolved in ethyl acetate

PLATE-II:

A= Sample dissolved in methanol, **B=** Sample dissolved in ethyl acetate

TLC PROFILE OF HYDROALCOHOLIC EXTRACT OF *Spinacia oleracea* L.

TABLE-9

S. NO	SOLVENT SYSTEM	DETECTING AGENT	NO.OF SPOTS	COLOUR OF SPOTS	Rf Value
1	Ethylacetate: formic acid:glacial acetic acid:water (100:11:11:26)	5% ethanolic ferric chloride	1 2 3 4 5	violet Yellow Yellowish brown Brown colour Dark brown colur	0.23 0.31 0.34 0.67 0.86
2	Toluene:ethyl acetate: formic acid: Methanol (3:6:1.6:0.4)	UV-365	1 2 3 4	Violet colour Yellow colour Pale yellow colour Red colour	0.26 0.34 0.37 0.68

Thin layer chromatography (TLC) of the hydroalcoholic extract of *Spinacia oleracea*(leaf) showed the R_f value 0.23,0.31,0.34 may indicate the presence of rutin, quercetin, apigenin the solvent system used Ethylacetate: formic acid: glacial acetic acid: water (100:11:11:26).and Rf value 0.26,0.34,0.37 may indicate the presence of rutithe solvent system used Toluene:ethyl acetate: formic acid: Methanol (3:6:1.6:0.4). Rf value 0.62 may indicates the presence of the solvent system used Benzene: acetic acid: water (12.5:7.2:0.3) (**Table-9**)

5.5. ISOLATION OF PHYTOCONSTITUENT FROM *Spinacia oleracea* L.

The preliminary phytochemical screening of *Spinacia oleracea* L. accentuate the presence of biologically important phytoconstituents such as tannin , Steroids carbohydrates , flavonoids , and phenols .Quantitative estimation highlight the amount of flavonoid was rich in *Spinacia oleracea* L the nature and type of flavonoid was identified by means of chemical method confirms the presence of flavonones. The compound was isolated, using method introduced by (**Bengu Liu *et al.*, 2008)**. The results of physical characters identification and amount were given in (**Table-10)**

5.5.1 IDENTIFICATION AND CHARACTERISATION OF ISOLATED COMPOUND FROM *Spinacia oleracea* L.

IDENTIFICATION TEST FOR ISOLATED COMPOUND

A. Determination of physical parameters

TABLE-10

S.NO	PARAMETERS	REPORT
1.	Solubility	Ethanol, DMSO,
2.	Colour	Yellow colour
3.	Consistancy	Powder
4.	Refractive Index	1.734

Isolated compound of ***Spinacia oleracea*** L. was determined some physical parameters Solubility of isolated compound is freely soluble in ethanol & DMSO. The refractive index is 1.734 Range was observed the isolated compound **(table-10).** The several reports on isolated compound was determined some physical parameters is compared with previous data.

b) Chemical test for isolated compound

The isolated compounds of flavones give rise to characteristic colour reactions which are summarized in the following (**Table-11)**

TABLE- 11

S.NO	Compound	Colour with aq.NaOH	Colour with conc. H_2SO_4
1	Flavones	Yellow colour	Yellowish orange

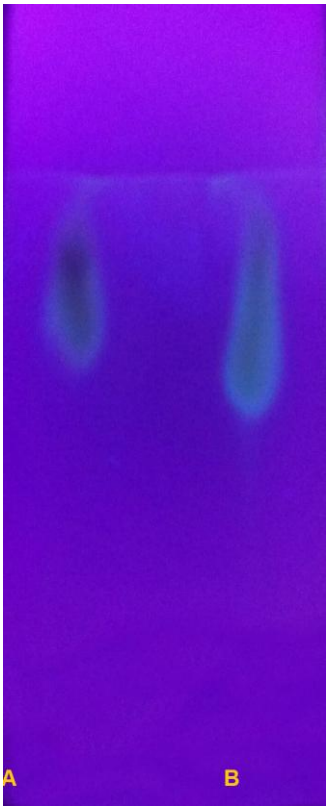
From the above finding identification test of isolated compound results were tabulated (**table-11).** Identification test of isolated compound was compared with textbook **(O.P. AGRAWAL, 2003)**

C).THIN LAYER CHROMATOGRAPHY OF ISOLATED COMPOUND

In this study results obtained in isolated compound of *Spinacia oleracea* L. leaf by thin layer chromatography with mobile phase (benzene: acetic acid: water (12.5:7.2:0.3) were tabulated. gave better elution for isolated compound from *Spinacia oleracea* L. Leaf, and hence it was used as mobile phase for the detection of constituents were showed on **(PLATE-12).**

PLATE-10

TLC PROFILE OF ISOLATED COMPOUND FROM *Spinacia oleracea* L.



A- Sample dissolved in methanol

B- Sample dissolved in ethanol

TLC PROFILE OF ISOLATED COMPOUND FROM *Spinacia oleracea* L.

The isolated compound Rf value and colour of compound was mentioned (**Table - 12).**

TABLE-11

S.NO	SOLVENT SYSTEM	DETECTING AGENT	NO.OF SPOTS	COLOUR	R_f value
1.	(benzene : acetic acid : water) (12.5:7.2:0.3)	UV-365nm	1	Pale yellow	0.63

The results were obtained in isolated compound of *Spinacia oleracea* L. leaf by thin layer chromatography with mobile phase (benzene: acetic acid: water (12.5:7.2:0.3) elution for isolated compound the Rf =0.63 value was obtained the colour of the spots under the detection of UV-365nm were tabulated gave better elution for isolated compound from *Spinacia oleracea* L. **(Table-12).**

From the above findings the Rf values of isolated compound was compared with previous data it may be present in flavones (Apigenin) (**benguo linu *et al.*, 2008**)**.** The results of this study the compared to the previous datas, reports and standards were ascribed the isolated compound was may be flavone (apigenin).

5.5.2 SPECTRAL STUDIES OF ISOLATED COMPOUND

A) UV SPECTROSCCOPY OF ISOLATED COMPOUND

The UV spectral study of isolated compound calibration graph at UV range is recorded for 267.80(nm) the graph were reported **(Fig-4)**

Figure-4

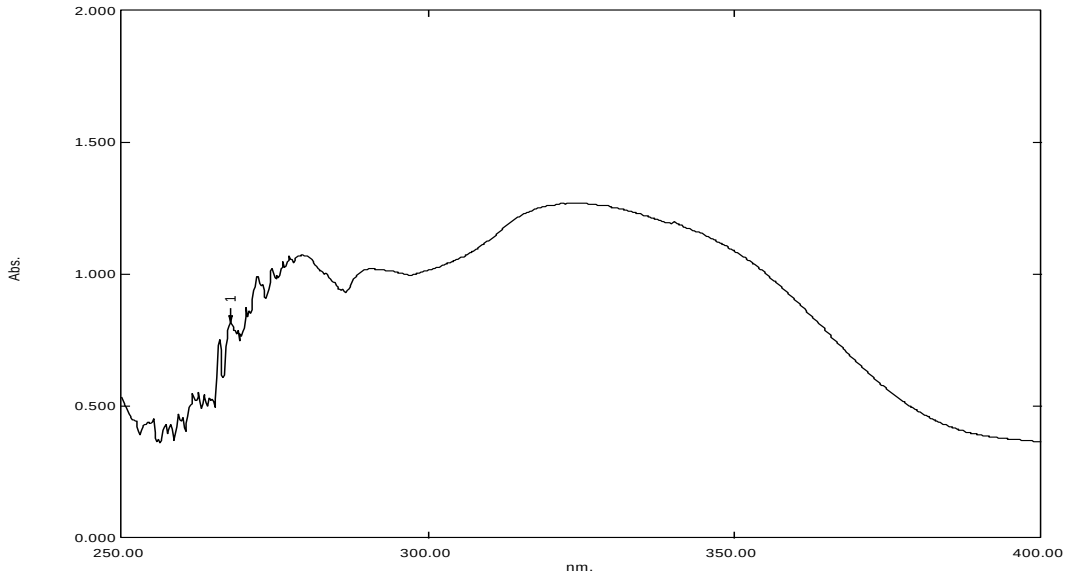


Table-13

S.No	Wave length (nm)	Maximum absorbance maxima
1	267.80nm	0.819

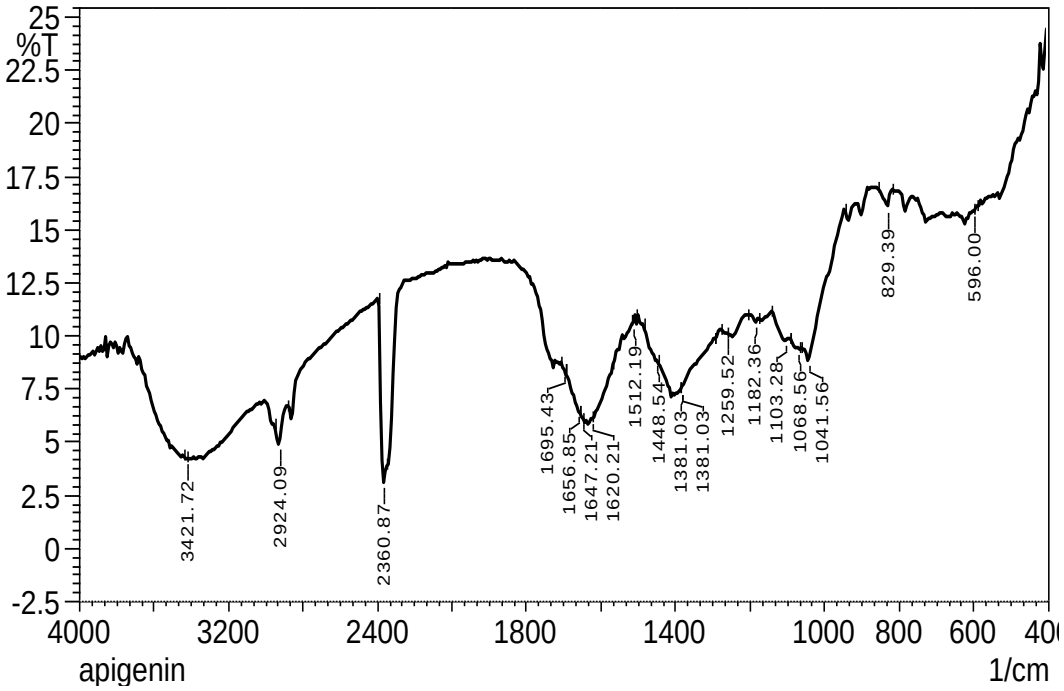
The isolated compound of *Spinacia oleracea* L. shows absorbance maxima (λ max) at 267.80nm (**Table-13**). From the above findings the UV spectroscopy of isolated compound was compared with previous data it may be present in flavones (Apigenin) (**benguo linu *et al.*, 2008**).

B) INFRARED SPECTROSCOPY OF ISOLATED COMPOUND FROM

***Spinacia oleracea* L.**

The IR spectroscopy of isolated compound was reported on below the (table-10) And (figure -3) recorded for IR

Fig-5



INTERPRETATION OF INFRARED SPECTROSCOPY OF ISOLATED COMPOUND FROM *Spinacia oleracea* L

The isolated compound was observed IR region and type of vibration and functional group were mentioned (**table-14)**

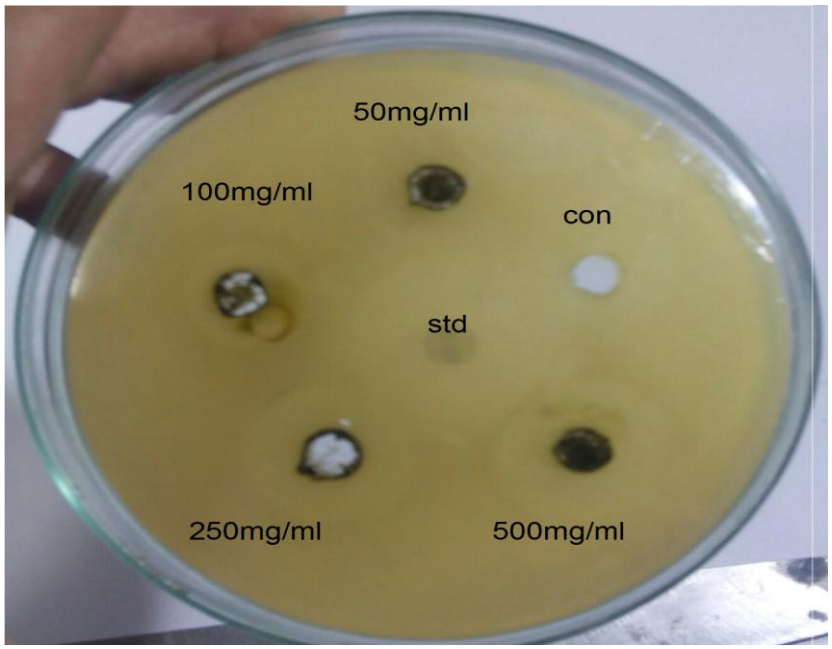
TABLE-14

Standard IR range cm^{-1}	**Observed IR region** cm^{-1}	**Type of Vibrations**	**Group**
1250-1150 cm^{-1}	1182.36cm^{-1}	-OH Str	Tertiary alcohol
840-800 cm^{-1}	829.39cm^{-1}	C-H ben	Para disubstituted benzene
1680-1650cm^{-1}	1656cm^{-1}	C=O Str	Aromatic ketone
2940-2850cm^{-1}	2924.09cm^{-1}	C-H str	Aromatic compound
1300-1050cm^{-1}	1182.36cm^{-1}	C-O-C Str	Aromatic ether
1640-1700cm^{-1}	1695cm^{-1}	C=C Str	conjucated disubstituted

The isolated compound of *Spinacia oleracea* L. was observed IR range 1182.36 cm^{-1} & type of vibration is C-OH Stretch may be presence of tertiary alcohol, 829.39cm^{-1} & type of vibration is C-H benending may be presence of Para disubstituted benzene, 1656cm^{-1} & type of vibration is C=O Stretch may be presence of Aromatic ketone. 1182.36cm^{-1} & type of vibration is C-O-C Stretch may be presence of aromatic ether. 1695cm^{-1} & type of vibration is C=C Stretch may be presence of conjucated disubstituted (**Table -14).** From the above findings the IR spectroscopy of isolated compound was compared with data it may be present in flavones (Apigenin) (**Beckett A H & Stenlake J B, 1997**)**.** The results of this study the compared to the previous datas, reports and standards were ascribed the isolated compound was may be flavone (apigenin)

PLATE-11

ANTI-DANDRUFF ACTIVITY OF HAESO AGAINST *M.furfur* (MTCC 1374)



Con= control

Std= standard

Control= 70% hydro-alcoholic

Std= ketoconazole 30mg/dis.

5.6 PHARMACOLOGICAL ACTIVITY

5.6.1. ANTIDANDRUFF ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *Spinacia oleracea* L. AGAINTS *Malassezia furfur* (MTCC1374) BY USING WELL DIFFUSION METHOD.

The HAESO is reported at different concentration and Zone of inhibition is mention (**Table-15**)

TABLE NO: 15

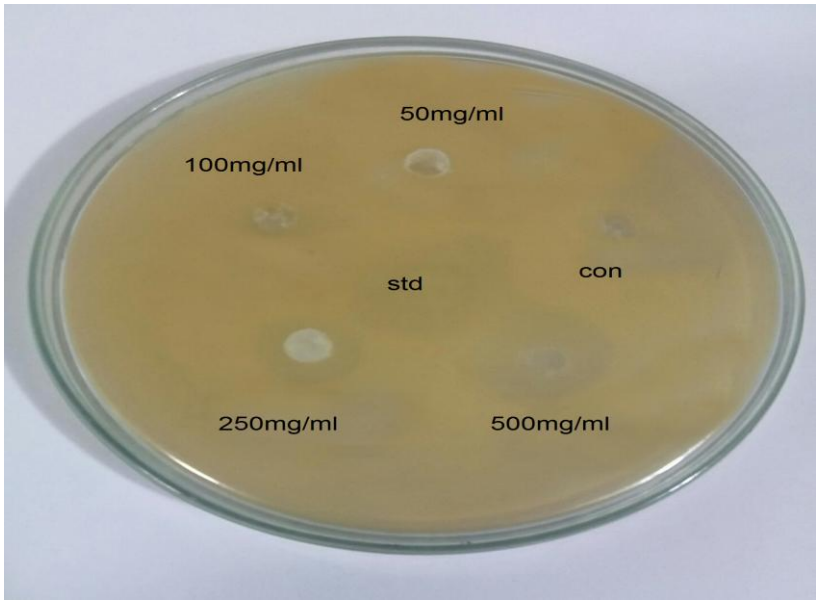
S.NO	DRUG	CONCENTRATION	ZONE OF INHIBITION
1.	Hydro alcoholic Extract of spinach	100mg/ml	12±0.236
		250mg/ml	12.8±0.272
		500mg/ml	14.2±0.286
2.	Ketoconazole(STANDARD)	30mg/disc	16.2±0.313

The hydroalcoholic extract of *Spinacia oleracea* L. was tested for their efficacy against dandruff causing agent *Malassezia furfur* by well diffusion method. The zone of inhibition was clearly visible and the diameter of the zone was measured and shown (**PLATE-11**). *Malassezia furfur* was sensitive to all concentrations tested in hydroalcoholic extract of *Spinacia oleracea* L. of showed the inhibition of 12±0.236mm in 100mg/ml, 12.8±0.272mm in 250mg/ml and 14.2±0.286mm in 500mg/ml respectively **(Table-15).** There are several reports of antidandruff activity against *M.furfur* were compared with reference (balakrishnan *et al*., 2011).

From the above finding the HAESO was significantly inhibiting of the growth of *M.furfur* and then compared with standard 16.2±0.313mm for 30mg/disc had significant growth inhibition was recorded.

PLATE-12

ANTI-DANDRUFF ACTIVITY OF ISOLATED COMPOUND AGAINST *M.furfur* (MTCC1374)



Con = control

Std = Standard

Standard = ketoconazole 30mg/disc

ANTI-DANDRUFF ACTIVITY OF ISOLATED COMPOUND FROM *Spinacia oleracea* L. AGAINST *Malassezia furfur* (MTCC 1374)

The isolated compound is reported at sensitive to two different concentration and zone of inhibition is tabulated (**Table-16**).

TABLE NO: 16

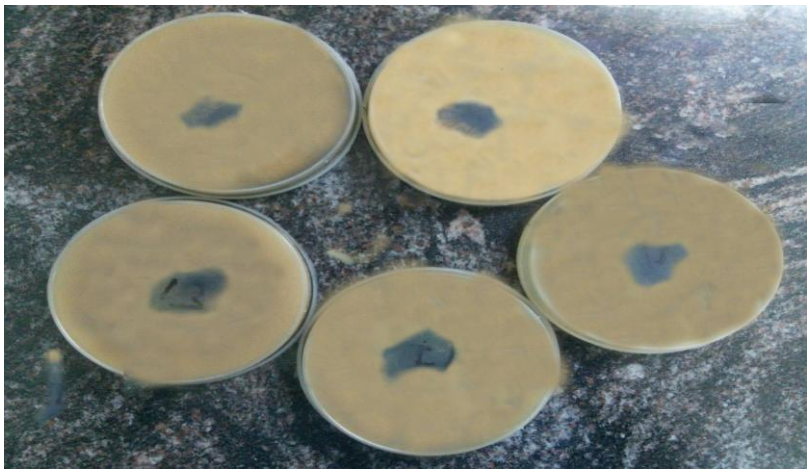
S.NO	DRUG	CONCENTRATION	ZONE OF INHIITION
1.	ISOLATED COMPUND (Flavones)	250mg/ml	12.7±0.144
		500mg/ml	13.8±0.170
2.	Ketoconazole (STANDARD)	30mg/disc	15.8±0.235

The isolated compound from *Spinacia oleracea* L. was tested for their efficacy against dandruff causing agent *Malassezia furfur* by well diffusion method. The zone of inhibition was clearly visible and the diameter of the zone was measured and shown (**Plate-12**). *Malassezia furfur* was sensitive to two concentrations tested in isolated compound from *Spinacia oleracea* L. of showed the inhibition of 12.7±0.144mm in 250mg/ml, 13.8±0.170mm in 500mg/ml. **(Table-16).** The results of isolated compound were compared with previous data (**Geetha singh *et al*., 2011**).

From the above finding the isolated compound of apigenin was significantly inhibiting of the growth of *M.furfur* and then compared with standard 15.8±0.235 for 30mg/disc had significant growth inhibition was recorded.

PLATE-13

Growth of *Malassezia furfur*

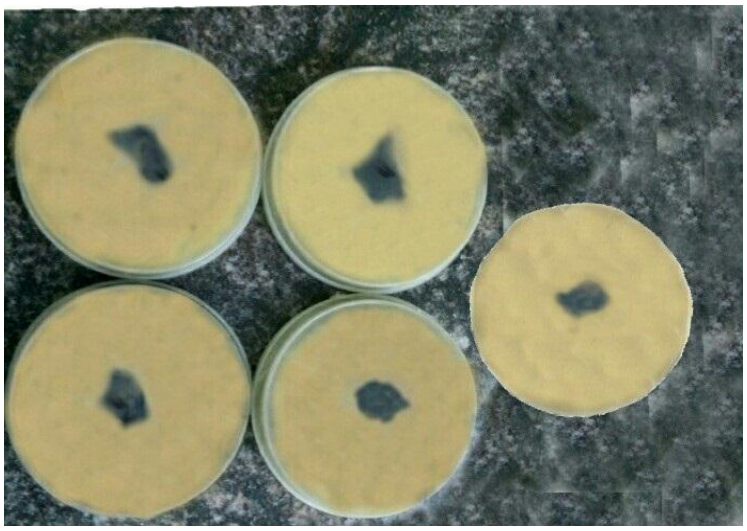


Inhibition of *Malassezia furfur* growth by HAESO (500mg/ml)

HAIR STRAND TEST FOR FEMALE VOLUNTEERS

PLATE-14

Growth of *Malassezia furfur*

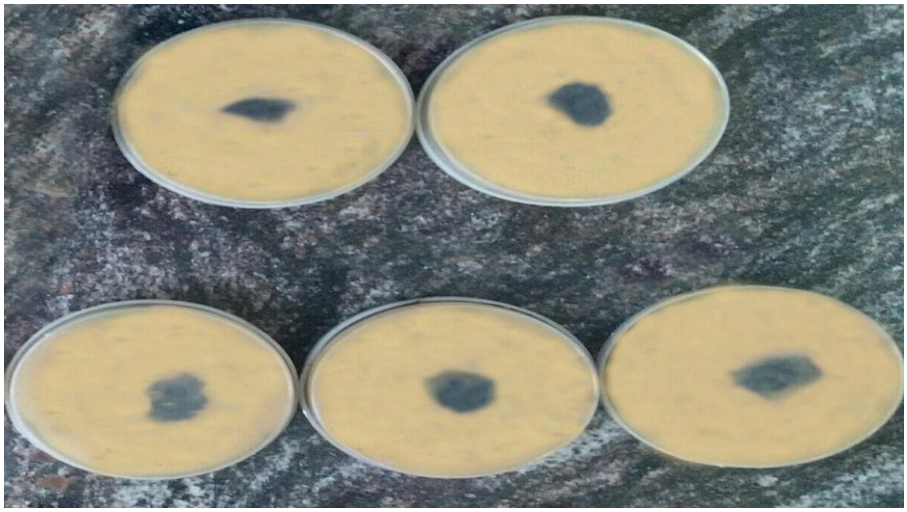


Inhibition of *Malassezia furfur* by HAESO (250mg/ml)

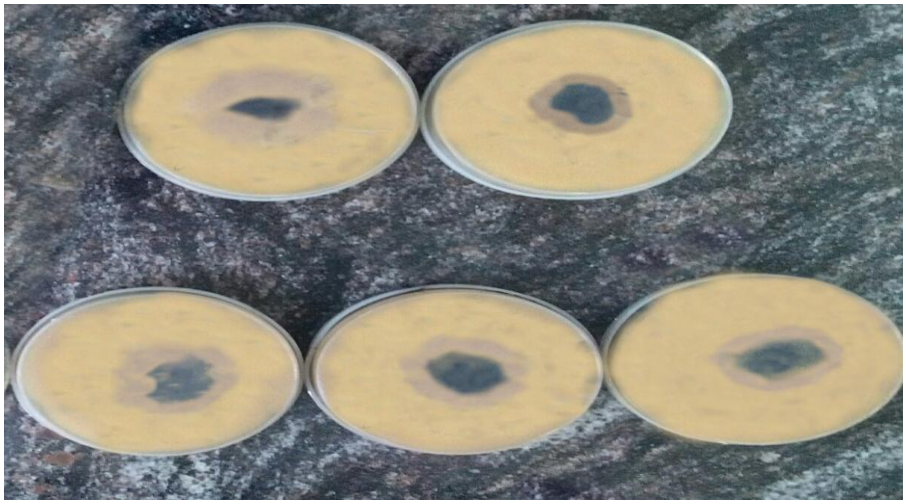
HAIR STRAND TEST FOR MALE VOLUENTEERS

PLATE-15

Growth of *Malassezia furfur*



Inhibition of *Malassezia furfur* by HAESO (500mg/ml)

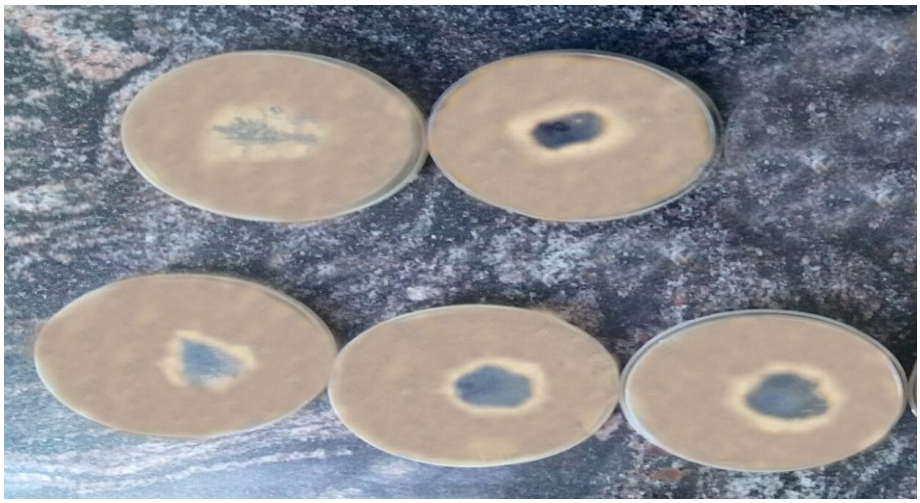


HAIR STRAND TEST FOR MALE VOLUENTEERS

PLATE-16

Growth of *Malassezia furfur*

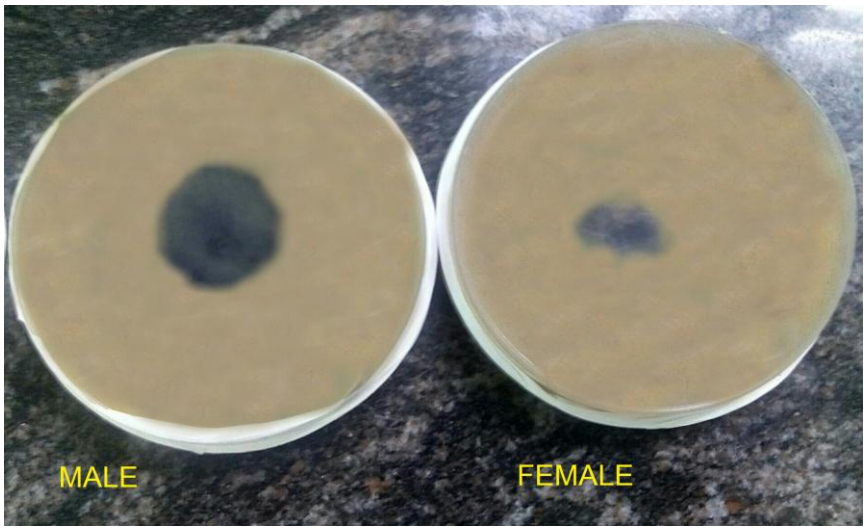
Inhibition of *Malassezia furfur* by HAESO (250mg/ml)



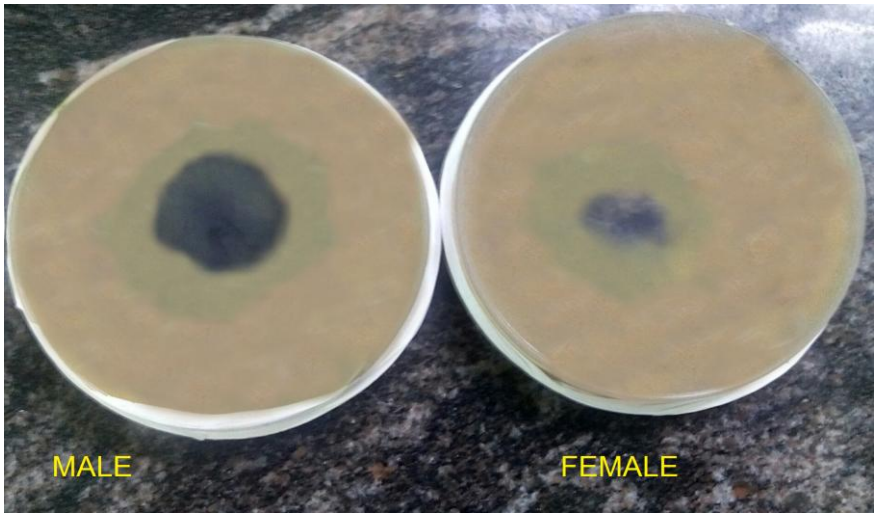
HAIR STRAND TEST FOR BOTH MALE AND FEMALE VOLUENTEERS

PLATE-17

Growth of *Malassezia furfur*



Inhibition of *Malassezia furfur* by KETOCONAZOLE (30mg/ml)



5.6.2. HAIR STRAND TEST

RESULT OF HAIR STRAND TEST

TABLE-17

Hair from volunteer	HAESO Female		HAESO Male		Ketoconazole (STD) 30mg/ml	
	250mg	500mg	250mg	500mg	Male	Female
1	0	+	+	0	0	0
2	+	0	0	0	0	0
3	0	0	+	0	0	0
4	0	+	+	+	0	0
5	+	0	0	0	0	0
Growth/total number	3/5	2/5	3/5	1/5	0/1	0/1

0= no growth, + = growth, HAESO = Hydroalcoholic extract of *Spinacia oleracea* L.

The antidandruff efficacy of *Spinacia oleracea* L. was tested against *M.furfur* revealed that the inhibition of HAESO 250mg/ml & 500mg/ml and standard drug ketoconazole 30mg/ml. all ten hair specimens(male and female) that had been treated with growth of *M.furfur* after four days. Growth was only observed in the directly contact with inoculated hairs. There was no direct contact with the marginal region increasing incubation time, however, homogenous growth was observed. Results of the hair strand test for *Malassezia furfur* after 18 days are shown in **(Table-17)** and the **(plate-13 to 17**) was observed with *M.furfur*. The hair strand test of HAESO 250mg/ml & 500mg/ml had a significant growth-inhibiting effect respectively. Similarly standard antifungal drug ketoconazole 30mg/ml was recorded the growth –inhibiting effects.

RESULT OF HAIR STRAND TEST FOR ZONE OF INHIBITION:

TABLE-18

Hair From volunteer	HAESO Female		HAESO Male		KETOCONAZOLE 30mg/ml	
	250mg/ml	500mg/ml	250mg/ml	500mg/ml	Male	Female
1	12.7±0.148	14.2±0.389	12.9±0.047	14.4±0.07	15.4±0.6	15.7±0.9
2	12.6±0.211	14.7±0.215	12.5±0.166	13.8±0.424	15.8±0.2	16.1±0.1
3	12.9±0.089	14.1±0.156	12.1±0.272	14.7±0.29	15.7±0.0	15.9±0.4
4	13.1±0.176	13.9±0.316	12.7±0.341	14.6±0.367	15.9±0.3	15.4±0.7
5	12.9±0.246	14.5±0.277	12.3±0.109	13.9±0.131	15.7±0.8	15.5±0.3

The antidandruff efficacy of *Spinacia oleracea* L. was tested against *Malassezia furfur* revealed that the inhibition substances HAESO 250mg/ml & 500mg/ml and standard drug ketoconazole 30mg/ml. Results of the hair strand test for *Malassezia furfur* after 18 days are shown in **(Table-18)** and the **(Plate 13to 17**). The HAESO was inhibiting growth for inoculated hairs area only the zone of inhibition range of 12.8mm for 250mg/ml & 14.7mm for 500mg/ml had a significant zone of inhibiting effect of both male and female volunteers.

From the above finding the HAESO was significant inhibiting of the growth of *Malassezia furfur* and then compared with standard ketoconazole zone of inhibition range 15.9mm for 30mg/ml had significant growth inhibition was recorded.

CHAPTER-VI

SUMMARY AND CONCLUSION

CHAPTER VI

SUMMARY AND CONCLUSION

The present investigation highlights "An Innovative Assimilation on Pharmacognostical, Phytochemical and Contending against *Malassezia furfur Spinacia oleracea* L. Leaves and Its Bioactive Component". The family chenopodiaceae, a widely available plant commonly called as Spinach.

Ethno medical information revealed that it was used in various ailments for long time all over the world. It is traditionally known to be useful for the treatment of wide panel of diseases like antipyretic, diuretic, laxative, anthelmintic, useful in urinary concretion, inflammation of the lungs and the bowels, sore throat, pain in joints, thirst, lumbago, cold and sneezing, sore eye, ring worm scabies, leucoderma, arrest vomiting , biliousness, flatulence. And have been used in the treatment of febrile conditions.

Pharmacognostical studies:

Pharmacognostical studies have been determined on the leaf of *Spinacia oleracea* L. Order to substantiate and identify the plant for future work. This study established pharmacognostical parameters including morphological, microscopical and physiochemical parameters. These characteristic can be used further as identification and authentication parameters of leaves.

Physiochemical studies:

The leaf extract of *Spinacia oleracea* L. is subjected for phytochemical studies including preliminary phytochemical screening, quantitative estimation of phytoconstituents and chromatography studies.

The preliminary phytochemical screening on leaf extract of *Spinacia oleracea* L. revealed the presence of carbohydrate, glycosides, sterols, saponins, protein, terpenoids, proteins, mucilage, flavonoids, tannins and phenols.

The quantitative estimation of phytoconstituents like flavonoids and phenols were done by using Aluminum chloride methods for flavonoids, flolin-ciocalteau methods for phenols. The results were found to be **111.5 mg/g** and **910.mg/g** respectively for flavonoids, phenols. These studies revealed that hydro-alcoholic extract of Spinacia oleracea L. leaf consist of significant phenols and flavonoids contents. It conform the momentous concentration of these phytoconstituents in hydro-alcoholic extract of *Spinacia oleracea* L. leaf which satisfy our aim and standing proof the modern approach for analysis and production of secondary metabolites for effective treatment of ailments for the herbal drug development in near future.

The fresh leaf of *Spinacia oleracea* L. were determined chlorophyll ‟a” and chlorophyll ‟b” and total chlorophyll content and total carotenoids were done by using DMSO solvents. The results were found to be 120mg/ml & 45mg/ml respectively for total chlorophyll and total carotenoids contents.

TLC studies were performed the hydro-alcoholic extract of *Spinacia oleracea* L. leaf to identify the Phytoconstituents present in this plant.

Isolation of Apigenin from *Spinacia oleracea* L. were identified (Chemical test and TLC) and characterized (UV & IR Spectral studies).

Pharmacological studies:

Pharmacological screening of hydroalcoholic extract of *Spinacia oleracea* L. showed *in vitro* antidandruff activity which was evaluated by well diffusion method and hair strand test. The results were revealed that hydroalcoholic extract of *Spinacia oleracea*

L. leaf showed significant antidandruff activity for two methods. It was evident from it was evident from the phytochemical studies of the plant, that essential amount of flavonoids and phenolic contents were present in these extracts which exhibited significant *in vitro* antidandruff activity.

Pharmacological screening of isolated compound from *Spinacia oleracea* L. Leaf showed antidandruff activity was evaluated by well diffusion methods. The result exposed that isolated compound (apigenin) showed the zone of inhibition at 250mg/ml & 500mg/ml significant antidandruff activity.

Hair strand test was found to be an interesting and reliable new test model for Evaluation of the antifungal activity especially with regard to a possible depot effect 250mg/ml & 500mg/ml proved to be effective. Similarly standard antifungal drug ketoconazole zone of inhibition range 15.9mm for 30mg/ml had significant growth inhibition was recorded.

In present study, it concludes that further innovative studies on the clinical trials and find out new compounds for other therapeutic efficacy.

FUTURE SCOPE

Future Studies are needed for the formulation and new methods develop technique. The synthetic treatment options available have certain limitations, which may be either due to poor efficacies or due to compliance issues. Furthermore, synthetic drugs are unable to prevent recurrence, the common problem associated with them. So the herbal drugs prove the alternative for synthetic drugs prove to be an alternative for synthetic drugs.

CHAPTER-VI

REFERENCES

CHAPTER-VII

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Thank
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