"IN VITRO LIPOLYTIC ASSAY OF THE PHENOLIC AND FLAVONOID EXTRACTS OF*Curcuma longa* L. AND INCREASING ITS BIOAVAILABILITY USING THE ALKALOIDEXTRACT OF *Piper nigrum* L. TO CURE LIPOMA"

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INTRODUCTION

Ayurveda is an alternative medicine system with historical roots in the Indian subcontinent. Ayurvedic preparations are typically based on complex herbal compounds, minerals and metal substances. Pharmacognosy is the study of herbal medicine and the use of medicinal plants which are a basis of traditional medicine. Plants are used as medicines to cure diseases from ancient times. It is likely that humans have used plants as medicine for as long as we have existed. Archeological excavations dated as early as 60,000 years ago have found remains of medicinal plants. Eventually most scientists believed that there was no need to use plants themselves in drugs because chemists could synthesise compounds that were more potent than the natural products offered by nature. Now, most pharmaceuticals are synthetic compounds. The structure of synthetic pharmaceuticals often resembles natural molecules. And as prominent botanical researcher Joanne Raskin noted in her 2002 study, 11% of the 252 drugs considered essential by the World Health Organization are exclusively derived from flowering plants.

Among ancient civilisations, India has been known to be rich repository of medicinal plants. The forest in India is the principal repository of large number of medicinal and aromatic plants, which are largely collected as raw materials for the manufacturing of drugs and perfumery products. Recently, WHO estimated that 80% of people worldwide rely on herbal medicines for some aspect of their primary healthcare needs. According to WHO, around 21,000 plant species have the potential for being used as medicinal plants. As per data available over three-quarters of the world population relies mainly on plants and plant extracts for their healthcare needs. More than 30% of the entire plant species, at one time or other were used for medicinal purposes. It has been estimated that in developed countries such as United States, plant drugs constitute as much as 25% of the total drugs, while in fast

developing countries such as India and China, the contribution is as much as 80%. Thus, the economic importance of medicinal plants is much more to countries such as India than to rest of the world. These countries provide two third of the plants used in modern system of medicine and the healthcare system of rural population depend on indigenous system of medicine. Treatment with medicinal plants is considered very safe as there is no or minimal side effects. These remedies are in sync with nature, which is the biggest advantage. This is the reason why herbal treatment is growing in popularity across the globe. These herbs that have medicinal quality provide rational means for the treatment of many internal diseases, which are otherwise considered difficult to cure.

Medicinal plants such as *Aloe vera* (L.) Burm. F., *Ocimum tenuiflorum* L., *Azadirachta indica* A.Juss., *Curcuma longa* L., *Zingiber officinale* Roscoe., *Asparagus racemosus* Willd., *Withania somnifera*(L.) Dunal, *Piper nigrum* L., *Piper longum* L., *Acalypha indica* L., *Adhatoda vasica* L., *Andrographis paniculata* (Burm.f.) Nees, *Calotropis gigantea* (L.) Dryand., *Catharanthus roseus* (L.) G.Don, *Rauvolfia serpentina* (L.)Benth. Ex Kurz etc. cure several common ailments. These are considered as home remedies in many parts of the country. Medicinal plants are considered as a rich resources of ingredients which can be used in drug development. Apart from that, these plants play a critical role in the development of human cultures around the whole world. The use of herbal medicines and phytonutrients or nutraceuticals continues to expand rapidly across the world with many people now resorting to these products for treatment of various health challenges in different national healthcare settings(WHO,2004). This past decade has obviously witnessed a tremendous surge in acceptance and public interest in natural therapies both in developing and developed countries, with these herbal remedies being available not only in drug stores, but now also in food stores and supermarkets. It is estimated that upto four billion people (representing 80% of the world's population) living in the developing world rely on herbal medicinal products as a primary source of healthcare and traditional medicinal practice which involves the use of herbs is viewed as an integral part of the culture in those

communities (Mukherjee,2002;Bodeker *et al*.,2005; Bandaranayake, 2006).

The most common reasons for using traditional medicine are that it is more affordable, more closely corresponds to the patient's ideology, allays concerns about the adverse effects of chemical medicines, satisfies a desire for more personalised health care, and allows greater public access to health information. The major use of herbal medicines is for health promotion and therapy for chronic, as opposed to life threatening conditions.Use of traditional remedies increases when conventional medicine is ineffective in the treatment of disease, such as in the advanced cancer and in the face of new infectious diseases. Furthermore, traditional medicines are widely perceived as natural and safe, that is, not toxic. Currently, herbs are applied to the treatment of chronic and acute conditions and various ailments and problems such as cardiovascular diseases, prostate problems, depression, inflammation and to boost the immune system, to name but a few. Herbs and plants can be processed and can be taken in different ways and forms, and they include the whole herb, teas, syrup, essential oils, ointments,salves, rubs, capsules and tablets that contain a ground or powdered form of a raw herb or its dried extract. Plants and herbs extract vary in the solvent used for extraction, temperature and extraction time and include alcoholic extracts, hot water extracts, long-term boiled extract, usually roots or bark (decoctions), and cold infusion of plants. Plants are rich in a variety of compounds. Many are secondary metabolites and include aromatic substances, most of which are phenols or their oxygen-substituted derivatives such as tannins (Hartmann 2007;Jenke-Kodama,Muller and Dittmann 2008). Many of these compounds have antioxidant properties. More than 60% of cancer therapeutics on the market or in testing are based on natural products. It is also estimated that about 25% of the drugs prescribed worldwide are derived from plants,and 121 such active compounds are in use (Sahoo *et al.* 2010). Of the total 252 drugs in the World Health organization's essential medicine list, 11% are exclusively of plant origin (Sahoo *et al.* 2010).Turmeric is a spice derived from the rhizomes of *Curcuma longa* L.which is a member of the ginger family (Zingiberaceae). Also known as 'Golden spice of India'. Turmeric has been used in India for medicinal purposes for centuries. It has been used in

traditional medicine as a household remedy for various diseases including biliary disorders, anorexia,cough, diabetic wounds, hepatic disorders, rheumatism and sinusitis. In addition to its use as a spice and pigment, turmeric and its constituents mainly curcumin and essential oils shows a wide spectrum of biological actions. These include its anti-inflammatory, anti-oxidant, anti-carcinogenic, anti-mutagenic, anti-coagulant, anti-fertility, anti-diabetic, anti-bacterial, anti-fungal, anti-protozoal, anti-viral, anti-fibrotic, anti-venom, anti-ulcer, hypotensive and hypocholesteremic activities. Modern interest on turmeric started in 1970's when researchers found that the herb may possess anti-inflammatory and antioxidant properties. Safety evaluation studies indicate that both turmeric and curcumin are well tolerated at a very high dose without any toxic effects.Thus, turmeric and its constituents have the potential for the development of modern medicine for the treatment of various diseases. More than 100 components have been isolated from turmeric. The main component of the root is a volatile oil, containing turmerone, and there are other colouring agents called curcuminoids in turmeric. Curcuminoids consist of curcumin, demethoxycurcumin, 5'-methoxycurcumin and dihydrocurcumin, which are found to be natural antioxidants. The turmeric anti-oxidant protein(TAP) had been isolated from the aqueous extract of turmeric. The anti-oxidant principle was found to be a heat stable protein. The anti-oxidant principle had an absorbance maximumat 280 nm.(Ruby et al. 1995;Selvam et al. 1995). In a standard form, turmeric contains moisture (>9%), curcumin (5-6.6%) extraneous matter (<0.5%) by weight, mould(<3%) and volatile oils(<3.5%).

Curcuma longa L. is used as a herbal medicine for rheumatoid arthritis, chronic anterior uveitis, conjunctivitis, skin cancer, small pox, chicken pox, wound healing, urinary tract infections, and liver ailments. Fifty percent ethanolic extract of turmeric feedingelevates HDL- cholestrol/total cholestrol ratio. The extracts also caused a significant reduction in the ratio of total cholestrol/phospholipids (Dixit, Jain and Joshi, 1988).It is also used for digestive disorders, to reduce flatus, jaundice, menstrual difficulties and colic;for abdominal pain and distension (Bundy *et al.* 2004);and for dyspeptic conditions including loss of appetite, post prandial feelings of fullness, and liver and gall bladder complaints. The

main clinical targets of turmeric are the digestive organs in the intestine, for the treatment of diseases such as familial adenomatous polyposis (Cruz-Correa et al. 2006); in the bowels, for the treatment of inflammatory bowel disease. Curcumin (diferuloyl methane), is a bioactive substance present in the rhizomes of the herb "*Curcuma longa* L." which has been used for centuries in Asia, both in traditional medicine and in cooking as turmeric which gives food an exotic natural yellow colour. Further, in recent years, a large number of research papers have reported intriguing pharmacologic effects associated with curcumin (Hanai and Sugimoto 2009). In the colon,turmeric is used for the treatment of colon cancer.These findings indicates a possible interaction of both turmeric and curcumin with conjugation reactions in the human intestinal tract and colon.This in turn may affect the bioavailability of therapeutic drugs and toxicity levels of environmental chemicals, particularly procarcinogens (Naganuma *et al*. 2006). In folk medicine turmeric has been used in therapeutic preparations over the centuries in different parts of the world. In ayurvedic practices, turmeric is thought to have many medicinal properties including strengthening the overall energy of the body, relieving gas, dispelling worms, improving digestion, regulating menstruation, dissolving gallstones and relieving arthritis. Many South Asian countries use it as an antiseptic for cuts, burns and bruises, and as an antibacterial agent. In Ayurvedic medicine, turmeric is a well documented treatment for various respiratory conditions (eg;asthma, bronchial hyperactivity and allergy) as well as for liver disorders, anorexia, rheumatism, diabetic wounds, runny nose, cough and sinusitis. In vitro, curcumin exhibits anti-parasitic, antispasmodic, anti-inflammatory and gastrointestinal effects and also inhibits carcinogenesis and cancer growth. In vivo, there are experiments showing the anti-parasitic, anti-inflammatory potency of curcumin and extracts of *C.longa* L. by parenteral and oral application in animal models(Araujo and Leon 2001). Crude organic extracts of turmeric were found to inhibit lipopolysaccharide (LPS)-induced production of tumour necrosis factor. The anticancer activities of turmeric include inhibiting cell proliferation and inducing apoptosis of cancer cells. Arturmerone, which is isolated from turmeric, induced apoptosis in human leukemia Molt 4B and HL-60 cells by fragmenting DNA to oligonucleosome-sized fragments, a known step in the process of apoptosis

(Aratanechemuge *et al*. 2002). Ethanolic turmeric extract was found to have opposing actions on murine lymphocytes and on Ehlrich as citic carcinoma cells.

Curcuma longa L.enhances lymphocyte viability and blastogenesis, but induces formation ofcytoplasmic blebs and plasma membrane disintegration of tumour cells. Thus, it is suggested that turmeric is a conducive agents for lymphocytes and inhibitory as well as apoptosis inducing agent for tumor cells (Chakravarty and Yasmin 2005). A comparative study of edible plants like *C.longa* L. and *Ficus carica L.* and herbaceous plants like *Gossypium barbadense* L. and *Ricinus communis* L. extracts for their antitumor activities showed that the edible plant extracts exhibited higher antitumorigenic activities. Thus, edible plants that show in vivo antitumor activities may be recommended as safe sources of antitumor compounds. Curcumin has been demonstrated to induce multiple cytotoxic effects in tumor cells including cell cycle arrest, apoptosis, autophagy, changes in gene expression, and disruption of molecular signalling. Additionally, curcumin has been shown to potentiate the effect of radiation on cancer cells, while exhibiting a protective effect on normal tissue. Curcumin's positive safety profile and widespread availability make it a promising compound for future clinical trials for high-grade gliomas (Amara, El Masry and Bogdady, 2008). Turmeric showed antioxident potential by lowering oxidative stress in animals.A large number of in vitro and in vivo studies in both animals and man have indicated that curcumin has strong anti-oxidant, anti-carcinogenic, anti-inflammatory, anti-angiogenic, anti-spasmodic, anti-microbial, anti-parasitic and other activities. The mechanisms of some of these actions have recently been intensively investigated. Curcumin inhibits the progression of stage of carcinogenesis by induction of apoptosis and the arrest of cancer cells in the S, G2/M cell cycle phase. The compound inhibits the activity of growth factor receptors. The anti-inflammatory properties of curcumin are mediated through their effects on cytokines, lipid mediators, eicosanoids and proteolytic enzymes. Curcumin is a nutraceutical of low toxicity, which has been used successfully in a number of medical conditions that include cataracts, cystic fibrosis and prostate and colon cancers. Several

studies have shown that curcumin has a strong capability for scavenging superoxide radicals, hydrogen peroxide and nitric oxide from activated macrophages, reducing iron complex and inhibiting lipid peroxidation.This action of curcumin makes the base for our investigation.

Piper nigrum L. is an autoicous and decorous vine cultivated and harvested in tropical regions of Sri Lanka and India. *Piper nigrum* L. is one of the most commonly consumed spices and its pungency is due to the presence of an alkaloid known as piperine, volatile chemical constituents and essential oils. Piperine is found in black pepper (*Piper nigrum* L.), white pepper and long pepper (*Piper longum* L.) belonging to the family Piperaceae. Piperine represents diverse biological activities such as anti-inflammatory, anti-cancer, anti-viral, anti-larvicidal, pesticide, anti-Alzheimer's, anti-depressant and most importantly piperine is known as the bioavailability enhancer. Piperine is used in traditional therapies of Chinese as well as in Indian medicine.Piperine can be used widely in pain management, chills, rheumatism, arthritis, influenza and fever.Piperine is reported to be used for the enhancement of blood circulation, salivation and stimulation of appetite.Piperine has a multifaceted biological profile that includes pain management, hypotension, vascular cell modulation and anti-cancer activity. Piperine has shown various biological activities such as anti-infective, antimicrobial, insecticidal, antiamoebic and antiulcer activities. Different studies on piperine suggest that piperine acts as an excellent bioenhancer to improve the bioavailability of drugs with poor ADMET properties. Some studies also indicate that piperine shows synergistic effects when taken in combination with various classes of drugs (Anshuly Tiwari *et al*., 2020).

The bioavailability of curcumin can be increased through different ways such as through nasal delivery, by using nanocurcumin, through liposomal encapsulation, by the combining effect of piperine along with curcumin etc. Piperine, the major component of black pepper, known as the inhibitor of hepatic and intestinal glucuronidation shown to increase the bioavalability of curcumin. This effect of piperine on the pharmacokinetics of curcumin has been shown to be much greater in humans than in rats. In humans, curcumin bioavailability was increased by 2000% at 45 minutes after

co-administering curcumin with piperine.

A lipoma is a lump of fatty tissue that grows just under the skin. A lipoma is a round or oval-shaped lump of tissue that grows just beneath the skin.It is made of fat, moves easily when you touch it and doesn't usually cause pain. Lipomas can appear anywhere on the body but they are most common on the back, trunk, arms, shoulders and neck. Lipomas are benign soft tissue tumors. They are very common. About 1 of every 1000 people has a lipoma.Lipomas appear most often between ages of 40 and 60, but they can develop at any age. They can even be present at birth. The cause of lipoma is not discovered yet. It is believed that lipomas are inherited. Some conditions cause multiple lipomas to form on the body. Medical treatment includes steroid injections, liposuction and surgical removal. There are many other diseases which are seen in association with lipoma. All these were the results of excessive deposition of fat below the skin.

This project aims to find an alternative remedy for diseases like lipoma, other than surgeries, from herbal medicines. For this, the combined action of curcumin and piperine has been tested. For testing, first we have to isolate phenol and flavonoid from turmeric and alkaloid , piperine from black pepper. After that, we require an in vitro set up to test the efficiency of the medicine. So there is a need to synthesize fat artificially in the laboratory. We all know that human fat is made up of triglycerides and triglycerides are formed by the esterification of fatty acids and glycerol. In human body, seven types of fatty acids are mainly seen. Most important among them are oleic acid, palmitic acid and linoleic acid. They are seen in abundance in human adipose tissue. This project focuses on the synthesis of fat, preparing the combinations of the plant extracts, finding the fat burning ability of the combination and thereby using this combination as a remedy for lipomas and other tumors caused by the excessive deposition of fat under the skin.

AIM AND OBJECTIVES

The aim of this study is to find out the fat burning properties of *Curcuma longa* L. and increasing its efficiency by combining it with the alkaloid present in *Piper nigrum* L.

The present work falls on the following objectives:

- Collection of good quality plant materials
- Preparation of plant extract
- Extraction of phytochemicals

    Extraction of phenol and flavonoid from turmeric using ethanol through soxhlet extraction

    Extraction of alkaloid from black pepper using ethanol through soxhlet extraction

- In vitro synthesis of human adipose tissue/ triglycerides through esterification of fatty acids and glycerol
- Estimation and comparison of the fat burning ability using different combinations of the extract
- Preparation of a suitable formula for using this compound for the treatment of lipomas

REVIEW OF LITERATURE

Lipomas are adipose tumors that are often located in the subcutaneous tissues of the head, neck, shoulders and back. Lipomas have been identified in all age groups but usually first appear between 40 and 60 years of age. These slow growing, nearly always benign, tumors usually present as nonpainful, round, mobile masses with a characteristic soft, doughy feel. Most studies showed that lipomas are best left alone, but rapidly growing or painful lipomas can be treated with a variety of procedures ranging from steroid injections to excision of the tumor (Gohar A Salam, 2002).

Studies conducted by experts defined lipoma as a common subcutaneous tumor composed of adipose cells, often encapsulated by a thin layer of fibrous tissue. Causes of lipomas are found to be a genetic link (Due to genetic abnormalities), obesity, alcohol abuse, liver disease as well as glucose intolerence etc. (Stephanie S. Gardner, 2019. Joana Cavaco Silva, 2020).

Lipomas may occur anywhere with in the body and may even cause death by growth and pressure in vital regions. According to their location, lipomas are classified as subcutaneous, intermuscular and visceral (Frank E. Adair *et al*,1932). The Journal of cancer Research and Experimental Oncology(2009)published an article on the clinicopathologic analysis of 36 cases of head and neck lipomas. The ultrastructure of lipoma was studied and the findings were published through Wiley online library (Kim *et al*, 1982).

Lipomas have been reported to be common in adults, after the fourth decade of life and this is consistence with the findings of this study, with the peak age incidence of fifth decade of life and majority(58.3%)of these patients being older than 40 years (Neville *et al*, 2009.Ndukwe *et al*, 2003.Furlong *et al*, 2004).

A complete treatment for lipoma is not distinguished yet. However a

dermatologist will make the best treatment recommendation based on a variety of factors including the size of the lipoma, the number of skin tumors one have,personal history of skin cancer,patient's family history of skin cancer,whether the lipoma is painful etc. The most common way to treat a lipoma is to remove it through surgery. This procedure is typically done under local anesthesia through a procedure known as an excision(Gilyoma *et al,* 2015). Liposuction is another treatment option which helps to reduce the size of the tumor. It involves a needle attached to a large syringe, and the area is usually numbed before the procedure (Choi *et al*, 2007). Steroid injection may also be used right on the affected area. This treatment can shrink the lipoma, but it doesn't completely remove it (Frank E. Adair *et al*, 1932).

Lipomas are tumors that arises due to the accumulation of fat cells under the skin(Ancel *et al*, 1953). There are studies about the interrelationship of lean body mass and body fat in humans(Forbes *et al,* 1987). Inheritance of the amount and distribution of human body fat is described in the International Journal of Obesity(Bouchard *et al,* 1988). The main types of fat cells are white, brown and beige cells. They can be stored as essential, subcutaneous or visceral fat(Christoph *et al,* 2012). Each type of fat serves a different role. Some promote healthy metabolism and hormone levels, while others contribute to life-threatening diseases like type 2 diabetes, heart disease, high blood pressure, cancer etc (Megan Dix, 2019).

Conventional lipomas are the most common type of lipoma and the fatty lump contains white fat cells. Usually white fat cells helps to store energy and produce hormones that are secreted into the blood stream but increase in its accumulation causes lipoma. Conventional lipomas composed of monolocular adipocytes. The cells contain a large fat droplet, which forces the nucleus to be squeezed into a thin rim at the periphery. There are two types of adipose tissues, that causes lipoma, based on the position of fat deposition. They are Subcutaneous Adipose Tissue which are seen underneath the skin and Intra-Abdominal Adipose Tissue which are present in the intestine and kidney. White adipocytes are the lipid

storage cells of our body(Torres *et al*, 2016. Trayhurn *et al*, 2001). They studied about the physiological role of adipose tissue and the role of white adipose tissue as an endocrine and secretory organ. Studies described on the charcters of fat-containing soft tissue masses through MR imaging spectrum. Based on their findings, there are multiple subtypes of benign and malignant fat-containing tumors,many with overlapping MR imaging features. Lipomas typically have a characteristic MR imaging appearance that is independent of location. Thick irregular septa and nonfatty elements distinguish a WDLPS from a lipoma at MR imaging(Pushpender *et al*,2016). Histologic evaluation is often recommended for lesions that cannot be confidently characterized as lipomas or a benign process suvh as lipomatosis of the nerve or fat necrosis (Shafar *et al*, 1965).

There are many scientifically proven compounds which helps to reduce fat. Antioxidants are one among them which have the ability to burn fat. The major component of antioxidant which helps to reduce fat is the polyphenolic compound(Daniyal *et al*, 2015). Anti- oxidants called flavonoids and phenolic acids may cut fatty buildup in fat cells. A new study shows that, in test tubes, antioxidants called flavonoids and phenolic acids tweak fat cells from mice. Those antioxidants didn't kill fat cells or slash the number of fat cells in the test tubes.Instead, they made fat cells cut their production of triglycerides, which are a heart hazard. The antioxidants did that by curbing an enzyme needed to make triglycerides, according to the study. That particular enzyme was most effectively reduced by the phenolic acid O- coumaric acid and the flavonoid rutin, reported the researchers(Gow-chin Yen *et al*, 2006). Medical News Today, an online journal reported that "Antioxidants are said to help neutralize free radicals in our bodies, and this is thought to boost overall health". Flavonoids, flavones, polyphenols and phytoestrogens are all types of antioxidants and phytonutrients, and are all found in plant-based foods.Each antioxidant serves a different function and is not interchangeable with another. Foods with rich, vibrant colours often contain the most antioxidants(Natalie Olsen, 2018).

Recently natural bioactive phytochemicals present in food have been discovered

for their potential health benefit effects on the prevention of chronic disorders such as cancer, cardiovascular disease,inflammatory and metabolic diseases including obesity. Polyphenols are a class of naturally occurring phytochemicals, of which some such as catechins, anthocyines, resveratrol and curcumin have been shown to modulate physiological and molecular pathways that are involved in energy metabolism, adiposity and obesity(Mohsen *et al*, 2010).

Polyphenols are powerful antioxidants that may help to reduce body weight. They include flavonoids, tannic acid, ellagitannin, phenolic acids, polyphenolic amides and other polyphenols(curcumin in turmeric). Polyphenols are a category of compounds naturally found in plant foods sucha as fruits, vegetables, herbs, spices, tea, dark chocolate and wine. They can act as antioxidants, meaning they can neutralize harmful free radicals that would otherwise damage your cells and increase your risk of conditions like cancer, diabetes and heart diseases(Claudine *et al*, 2004). Polyphenols are also thought to reduce inflammation, which is thought to be the root cause of many chronic illness. Polyphenol's antioxidant and antiinflammatory effects could lower the risk of cancer. Research consistently links diets rich in plant foods to a lower risk of cancer and many experts believe that polyphenols are partly responsible for this. Polyphenols have strong antioxidant and anti-inflammatory effects, both of which can be beneficial for cancer prevention. A recent review of test-tube studies sugg ests that polyphenols may block the growth and development of various cancer cells.Studies gave special emphasize on cancer chemoprevention by dietary polyphenols which will in turn provides a promising role for epigenetics. Epigenetics refers to heritable changes that are not encoded in the DNA sequence itself, but play an important role in the control of gene expression. In mammals, epigenetic mechanisms include changes in DNA methylation,histone modifications and non-coding RNAs. Although epigenetic changes are heritable in somatic cells, these modifications are also potentially reversible,which makes them attractive and promising avenues for tailoring cancer preventive and therapeutic strategies. As a result they came to a conclusion that dietary polyphenols from green tea, turmeric, soybeans, broccoli and others have shown to possess multiple cell-regulatory

activities within cancer cells(Alexander *et al*, 2010). Antioxidant properties of popular turmeric(*Curcuma longa*)was studied and found that turmeric varieties investigated in the study are useful sources of natural antioxidants, which confer significant protection against free radical damage(Tanvir *et al*, 2007).

According to a study conducted at the Tufts University, curcumin can actually suppress fat tissue growth and helps to reduce inflammation. Turmeric or *Curcuma longa* L., is a perennial herb and member of the Zingiberaceae family,and is cultivated extensively in Asian countries. The rhizome, the portion of the plant used medicinally as a yellow powder which is used as a flavour in many cuisines and as a medicine to treat many diseases particularly as an anti-inflammatory and for the treatment of flatulence, jaundice, menstrual difficulties, hematuria, hemorrhage and colic or can be applied as an ointment to treat many skin diseases. The active constituents of turmeric are the flavonoid curcumin(diferuloylmethane) and various volatile oils, including tumerone, atlantone and zingiberone.Water and fat soluble extracts of turmeric and its curcumin component exhibit strong antioxidant activity comparable to vitamins C and E.Turmeric's hepatoprotective effect is mainly a result of its antioxidant properties resulting in enhanced cellular resistance to oxidative damage as well as its ability to decrease the formation of proinflammatory cytokines. Curcumin can be applied topically to counteract inflammation and irritation associated with inflammatory skin conditions and allergies.Curcumin's ability to inhibit carcinogenesis at three stages:tumor promotion, angiogenesis and tumor growth. The review focuses on the medicinal and pharmacological benefits of turmeric in prevention and treatment of diseases (Louay Labban,2014). Turmeric has been used widely in the traditional medicine all over the world. Curcumin, the main yellow bioactive component of turmeric has been shown to have a wide spectrum of biological actions. These include its anti-inflammatory, antioxidant, anticarcinogenic, antimutagenic, anticoagulant, antifertility, antidiabetic, antibacterial, antifungal, antiprotozoal, antiviral, antifibrotic, antivenom, antiulcer, hypotensive and hypocholesteremic activities. For traditional Ayurvedics, turmeric

plant was an excellent natural antiseptic, disinfectant, anti-inflammatory and analgesic while at the same time the plant has been often used to aid digestion, to improve intestinal flora and to treat skin irritations(Rahul *et al*, 2018).

In the Journal of Food Science and Technology, a paper was published on the topic "Chemical constituents and biological activities of turmeric(*Curcuma longa* L.)". The safety evaluation studies indicate that turmeric is well tolerated at very high dose(0.5-1.5g/day/person) without any toxic effects. Turmeric contains 3-6% polyphenolic compounds, collectively known as curcuminoids, which is a mixture of curcumin, demethoxycurcumin and bisdemethoxycurcumin. Curcuminoids are major components responsible for various biological actions. Pure curcumin has more potent superoxide anion scavenging activity than demethoxycurcumin or bisdemethoxycurcumin(Abhishek *et al*, 2008). Curcumin acts as a pro- oxidant in the presence of transition metal ions(Cu and Fe) and is a potent bioprotectant witha potentially wide range of therapeutic applications.curcuminoids belong to the group of diarylheptanoids(or diphenylheptanoids) having an aryl-C7-aryl skeleton. Usually these polyphenols are present in 3-15% of turmeric rhizomes with curcumin as the principal compound(Shiyou *et al*, 2011). Curcumin is a feruloylmethane homodimer consisting of a band of hydroxyl and methoxy(heptadiene with two Michael acceptors) and a b-diketone. Turmeric is capable of influencing the AKT, growth factors, NF-KB and metastatic and angiogenic pathways.It modulates gene expression in human cancer cells in a time and concentration dependent pathway. Curcumin has good therapeutic and preventive potential against several major human conditions such as cardiovascular, inflammation suppression, antimicrobial, obesity, tumorigenesis, chronic tiredness, antidepressant and neurological function, anxiety, muscle and bone loss and neuropathic pain(Muhammad, 2019).

Curcumin is the major, bioactive polyphenol present in the spice turmeric, whichis the ground rhizome of the perennial herb *Curcuma longa* L. In addition to being used as a spice and colourant, turmeric has been used in Asian medicine since the second millennium

BC. Curcumin is a low molecular polyphenol with several biological properties.It has been shown to possess antioxidant, anti-inflammatory, anti-cancer, antiangiogenesis, chemopreventive and chemotherapeutic properties. Recent cell culture and animal studies have explored the impact of curcumin on lipid metabolism, adiposity and inflammation in more details. Curcumin may have a significant effect on adiposity and lipid metabolism through several mechanisms including modulation of energy metabolism, inflammation and suppression of angiogenesis. It has been well established that angiogenesis plays pivotal roles in the growth and expansion of adipose tissue was also reported to reduce weight to suppress angiogenesis, it has mainly been investigated for its effect on cancerous tumor growth. It is known that through down-regulation of several factors including vascular endothelial growth factor(VEGF), basic fibroblast growth factor(EGF),as well as angiopoietin and hypoxia-inducible factors(HIF)-1α, curcumin suppresses angiogenesis and restricts the growth of tumors. Therefore, curcumin may contribute to the prevention of adipogenesis through suppression of angiogenesis into the adipose tissue. The effects of curcumin on energy metabolism were observed both in adipocyte cultures and in adipose tissue of mice fed a high fat diet. The observations of the study are in support of the findings that curcumin supplementation suppressed a high fat diet-induced fatty liver in mice and reduced plasma levels of cholestrol, triglycerides, glucose and free fatty acids. In this study, they found that curcumin supplementation suppressed expression of PPARγ and C/EBPα, transcription factors that are mainly found in adipose tissue and are the key transcription factors in adipogenesis and lipogenesis. Curcumin also suppressed differentiation of pre-adipocytes to adipocytes, which in turn attenuated adipose tissue growth and expansion. This effect of curcumin might have been mediated through suppressing the expression of PPARγ transcription factor because a PPARγ agonist, such as thiazolidinedione, induces differentiation of human pre- adipocytes and increases subcutaneous adiposity(Susan *et al*, 2017) .

Physical, chemical and molecular properties of curcuminoids was described in the

Journal of Traditional and Complementary Medicine(2017) as, two active components of turmeric are the volatile oil and curcuminoids and both are present in oleoresin extracted from the turmeric root. The chemical structures of curcuminoids make them much less soluble in water at acidic and neutral pH, but soluble in methanol, ethanol, dimethyl sulfoxide and acetone. The curcuminoids are a mixture of curcumin, chemically a diferuloylmethane [1,7-bis (hydroxy-3- methoxy-phenyl) -hepta-1,6-diene-3,5-dione] mixed with its two derivatives, demethoxy curcumin [4-hydroxycinnamoyl - (4-hydroxy-3-methoxycinnamoyl) methane] and bis- demethoxy curcumin [bis-(4-hydroxy cinnamoyl)methane], defining the chemical formulae as C21H20O61, C20H18O5 and C19H16O4 respectively.The hydrophobicity of curcuminoids makes them poorly soluble in water. The authors also described about the antitumor activity of curcumin. A series of CUR derivatives were synthesised and evaluated the inhibitory activities on thioredoxin reductase(TrxR) of all analogs by invitro DTNB assay.Most of the analogs inhibited TrxR even in the low micromolar range. Structure-activity relationship analysis revealed that the analogs with furan moiety have an excellent inhibitory effect on TrxR in an irreversible manner,indicated that the furan moiety can serve as a possible pharmacophore during the interaction of curcumin analogs with TrxR.Aldehyde-free-2-hydroxycinnamaldehyde(HCA) analog were synthesised based on the curcumin, which is called as 2-hydroxycurcuminoids for the effect of antitumor activity against various human tumor cells invitro and invivo.2-hydroxycurcuminoids have a strong generator of ROS and strongly inhibited the growth of SW 620 colon tumor cells due to the presence of β-diketone moiety of curcuminoids. These analogs can be used as chemotherapeutic agent against human tumors(Augustine *et al*, 2017).

NATUROPATH(superpharmacy) an online journal, in 2020, published an article on the use of turmeric as a natural remedy for lipomas. They assumed that turmeric, the popular spice used both for culinary and medicinal purposes can be used topically to shrink lipomas. According to their point of view, one teaspoon of powdered turmeric mixed into a

paste using olive oil can be applied to the lipoma and has to be covered with a clean cloth. The active ingredient of turmeric is curcumin. There are numerous studies on the health benefits of curcumin,especially its anti-inflammatory and anti-oxidative role in human health. In 2015, Orlando published an article named “Turmeric a potential natural remedy in preventing and fighting lipoma lumps” through an onine website named dWgPR[SB Wire]. He concluded that lipoma is a non-cancerous soft tissue lump and can also be prevented or remedied through the use of a natural spice. According to his findings, turmeric is a potential natural remedy for preventing and fighting lipoma lumps. This ancient medicinal spice has therapeutic properties that are associated with a range of health benefits. Its most popular therapeutic ingredient is a phytochemical called curcumin. Curcumin has powerful therapeutic substances such as the anti- inflammatory, antibacterial and antioxidant properties.

The studies also discussed about how curcumin can be extracted from turmeric. In the research paper they described that, curcumin is insoluble in water. So an organic solvent has to be used for its isolation.Anderson et al. Developed a technique for isolating curcumin from ground turmeric. They magnetically stirred the ground turmeric in dichloromethane and heated at reflux for 1 hour. The mixture was suction-filtered and the filtrate was concentrated in a hot water bath maintaining at 50^0 C. The reddish-yellow oil residue was collected by suction filtration. Further TLC analysis(3% methanol and 97% dichloromethane)showed the presence of all three components. Bagchi explained extraction of curcumin from turmeric powder with the use of a solvent consisting of a mixture of ethanol and acetone. Chemical analyses have shown that turmeric contains carbohydrates(69.4%), moisture(13.1%) and minerals(3.5%). The essential oil(5.8%)obtained by steam distillation of the rhizomes contains α-phellandrene (1%),sabinene (0.6%), cineol (1%), borneol (0.5%), zingiberene (25%) and sesquiterpines (53%),curcumin (3- 6%) is responsible for the yellow colour (Augustine *et al*, 2017).

Curcumin extracted from turmeric root was found to be anti-angiogenic in a

human tissue-based angiogenesis. As a liposoluble compound, curcumin can be extracted from turmeric with organic solvents such as ethanol or acetone. Curcumin in its pure form has poor solubility in water, potentially limiting its medicinal use for human when it is taken orally or injected. By the end of the experiment they found that a good yield is exhibited by the acetone. Ethanol, methanol, ethyl acetate, isopropanol and hexane were not satisfactory as solvents in the extraction procedure. From the result obtained, they concluded that the optimum time for the extraction is 3 hours, optimum yield is 69.67% and the optimum solvent is acetone (Ashok *et al*, 2013). Pure curcumin can be isolated from crude curcumin(turmeric rhizome powder was extracted with ethyl acetate in a soxhlet assembly until all the colouring matter is extracted. The obtained crude extract was concentrated to semisolid brown coloured mass)by recrystallization process. Different organic solvents and combinations were tried for recrystallization. The melting point of the recrystallized powder was determined by capillary method. The identity of the purified curcumin was further confirmed by Thin Layer Chromatography and FTIR spectroscopy. Curcuminoids vary in chemical structures, physico-chemical characteristics as well as the functional properties. Curcumin exhibits poor solubility in water.As a liposoluble compound, curcumin can be extracted from turmeric rhizomes with organic solvents. Acetone as solvent was slightly superior to alcohol and ethyl acetate, the curcuminoids content also is on the high side suggesting selective extraction. During isolation and purification of curcumin from oleoresin, the volatile oil present in turmeric solubilizes curcumin creating problem in recrystallization process.In this work, oleoresins were separated from turmeric rhizome powder using ethyl acetate. Crude curcuminoids were separated from ethyl acetate extract using hexane by simple stirring method. During stirring, volatile oil and other resinous interfering substances were eliminated from ethyl acetate extract.

The separated curcuminoids were further purified by recrystallization method. Various organic solvents and their combinations were tried for selective recrystallization of curcuminoids. The crude curcuminoid powder obtained was further purified by recrystallizing it in the mixture of hot isopropyl alcohol:hexane (1:1.5) was found to be the

best recrystallization solvent for purification of curcuminoids. The total curcumin content of crude curcuminoid powder was found to be 76.82% w/w whereas in recrystallized powder the purity was increased to 99.45% w/w. The pure curcumin powder obtained after recrystallization was orange-yellow coloured crystalline powder with melting point of 183^0c.Curcuminoids complex, found in the rhizome of turmeric(2.5-6%) comprises curcumin (curcumin I), demethoxycurcumin (curcumin II) and bisdemethoxycurcumin(curcumin III).These three curcuminoids were well separated by Thin Layer Chromatography (Harshal *et al*, 2018).

Plant phenolics are important constituents that contribute to functional quality, colour and flavour and have significant roles both as singlet oxygen quenchers and free radical scavengers, helping to minimize molecular damage. The health benefits of phenolics are primarily derived from their antioxidant potentials because the radicals produced after hydrogen or electron donation are resonance stabilized and thus relatively stable. Ethanol is an organic polar solvent suitable for the extraction of phenolic compounds and is safe for human consumption (Tanvir *et al*, 2017).

Flavonoids are the plant pigments responsible for plant colours and exert their health promoting activities through their high pharmacological potentials as radical scavengers. The TFC of turmeric varieties ranged between 0.29% and 0.67% in aqueous extract but was higher(between 4.28% and 9.66%) in ethanolic extracts (Tanvir *et al*, 2017).

Flavanoids can be extracted using coventional soxhlet extraction using 70% ethanol for 6 h. Different solvents with different polarities were used to determine which one gives the highest recoveries of bioactive flavonoid compounds.Four solvents were used(1) methanol, (2) pure ethanol, (3) ethanol(70%) and (4) petroleum ether. Based on the obtained results, the highest extraction yield(267.3 mg/g)was found with methanol extraction and then with a little difference followed by ethanol 70% extraction(257.6 mg/g)(Mandana *et al*, 2011).

There are various methods to increase the bioavailability of curcumin. Besides these natural compounds have been also used to increase the bioavailability of curcumin. One of them is piperine, a major component of black pepper, known as inhibitor of hepatic and intestinal glucuronidation and is also shown to increase the bioavailability of curcumin. This effect of piperine on the pharmacokinetics of curcumin has been shown to be much greater inhumans than in rats. In humans, curcumin bioavailability was increased by two thousant percentage at 45 minutes after co- administering curcumin with piperine. The study shows that in the dosage used, piperine enhances the serum concentration, extent of absorption and bioavailability of curcumin in humans with no adverse effects (Sahdeo *et al*, 2014).

Black pepper is one of the most commonly consumed spices and its pungency is due to the presence of an alkaloid known as piperine, volatile chemical constituents and essential oils. Piperine is found in black pepper (*Piper nigrum* L.), white pepper and long pepper (*Piper longum* L.) belonging to the family Piperaceae. Piperine represents diverse biological activities such as anti-inflammatory, anticancer, antiviral, anti-larvicidal, anti-Alzheimer's, anti-depressant and most importantly piperineis known as the bioavailability enhancer. Piperine is used in traditional therapies of Chinese as well as in Indian medicine. Piperine can be used widely in pain management, chills, rheumatism, arthritis, influenza and fever. Piperine is reported to be used for the enhancement of blood circulation, salivation and stimulation of appetite. Piperine has a multifaceted biological profile that includes pain management, hypotension, vascular cell modulation and anti-cancer activity. Piperine has shown various biological activities such as anti-infective, antimicrobial, insecticidal, antiamoebic and antiulcer activities. Different studies on piperine suggest that piperine acts as an excellent bioenhancer to improve the bioavailability of drugs with poor ADMET properties. Some studies also indicate that piperine shows synergistic effects when taken in combination with various classes of drugs (Anshuly Tiwari *et al.*, 2020).

Piperine can be extracted from black pepper seeds using soxhlet extraction

using 95% ethanol for 2-3h. The yield of piperine was found to be 3.2%. Piperine can be used to improve the efficacy and reduce the dosing frequency of various xenobiotic agents. By combining piperine with the medication, side effects and toxicity of drugs can be reduced due to a lower dose and increased availability at the site of action (Sreevidya *et al*).

Human adipose tissues are made up of triglycerides. These triglycerides consist of fatty acids and glycerol. Seven fatty acids, myristic(14:0), palmitic(16:0), palmitoleic(16:1), stearic(18:0), oleic(18:1), linoleic(18:2) and linolenic(18:3) acid constituted >98% of the fatty acids in every tissue (Mariluz *et al*, 1999).

Fat burning is a complex physiological process. When the body loses fat, the fat cell does not go anywhere or move into the muscle cell to be burned. The fat cell itself stays right where it was under the skin in thighs, hips, arms etc. and on the top of the muscles (Porter *et al*, 2009). Fat is stored inside the fat cell in the form of triglycerol. The fat is not burned right there in the fat cell;it must be liberated from the fat cell through somewhat complex hormonal/enzymatic pathways. When stimulated to do so, the fat cell simply releases triglycerol into the bloodstream as free fatty acids (FFA's), and they are transported through the blood to the tissues where the energy is needed (Manore *et al*,2011). By lipolysis, each molecule of triglycerol splits into glycerol and fatty acids.The reaction is catalyzed by hormone-sensitive lipase (HSL). Fat can be synthesised artificially through Fischer esterification reaction (Turcotte, 2000).

MATERIALS AND METHODS

PLANT MATERIALS

In this work we used two plant materials such as turmeric and black pepper.

1. TURMERIC

SYSTEMATIC POSITION

KINGDOM : Plantae

DIVISION : Phanerogamae

CLASS : Monocotyledonae

SERIES : Epigynae

FAMILY : Scitamineae

SUBFAMILY :Zingiberaceae

GENUS : Curcuma

SPECIES : *Curcuma Longa* L.

Curcuma longa L. is a flowering plant of the ginger family, Zingiberaceae, the rhizomes of which are used in medicines and for culinary purposes. Turmeric have been studied in numerous clinical trials for various human diseases and conditions, with no high-quality evidence of any anti-disease effect or health benefit. There is no scientific evidence that turmeric reduces inflammation, as of 2020.

BOTANICAL DESCRIPTION

Turmeric is a perennial herbaceous plant that reaches upto 1 m tall. It has highly branched, yellow to orange, cylindrical, aromatic rhizomes. The leaves are alternate and

arranged in two rows. They are divided into leaf sheath, petiole and leaf blade. From the leaf sheaths, a false stem is formed. At the top of the inflorescence, stem bracts are present on which no flowers occur; these are white to green and sometimes tinged reddish-purple, and the upper ends are tapered. The hermaphrodite flowers are zygomorphic and threefold. Sepals 3,long, fused and white and have fluffy hairs, the three calyx teeth are unequal. Petals 3, bright yellow coloured and fused into a corolla tube. While the average corolla lobe is larger than the twolateral, only the median stamen of the inner circle is fertile. All other stamens are converted to staminodes. The labellum is yellowish and obovate.Carpels 3, under a constant, trilobed ovary adherent, which is sparsely hairy. Fruit is a capsule and it opens with three compartments.

1. BLACK PEPPER

SYSTEMATIC POSITION

KINGDOM : Plantae

DIVISION : Phanerogamae

CLASS : Dicotyledonae

SUBCLASS : Monochlamydeae

SERIES : Microembryeae

FAMILY : Piperaceae

GENUS : Piper

SPECIES : Piper Nigrum L.

Black pepper is native to the Malabar Coast of India, and the Malabar pepper is extensively cultivated there and in other tropical regions. Ground, dried and cooked peppercorns have been used since antiquity, both for flavour and as a traditional medicine. Its spiciness and medicinal value is due to the chemical compound piperine, which is a different kind of spicy from the capsaicin characteristic of chilli peppers. Piperine is under study for its potential to increase absorption of selenium, vitamin B12, beta-carotene and curcumin, as well as other compounds.

BOTANICAL DESCRIPTION

Black pepper, *Piper nigrum* L., is a climbing perennial plant in the family piperaceae which is grown for its fruits. The fruits are used to produce black, white and green peppercorns which are commonly used as a spice in cooking. Black pepper may be vining or have bushy, wooden stems. The plant has simple, alternating leaves which are oval in shape and produces clusters, or spikes, of 50 to 150 flowers. The fruits develop on the flower spike and are small spherical fruits which are green and ripen to red. Each stem can produce 20-30 spikes. Fruits are drupes and they become yellowish red at maturity and bear a single seed.

EXTRACTION OF PHYTOCHEMICALS

1) EXTRACTION OF PHENOL FROM TURMERIC

a) Collection of plant material: Fresh and good quality rhizomes of turmeric were collected from Chennithala (Mavelikara).

b) Processing of plant material: The rhizomes were washed in boiling water, dried and powdered. The powder was filtered through a sieve to obtain uniform powder.

c) Conventional extraction using soxhlet: 20 g ground turmeric powder was weighed and wrapped in a blotting paper and packed in the soxhlet apparatus which was gradually filled with 300 ml of 95% ethanol as the extraction solvent. The extraction experiment was carried out at 78.37^{o}C for 8h. Upon completion of the extraction, the ethanol was separated from the extract using hot air oven at 80^{0}C. The residue (oleoresin) was dried, taken in a pre- weighed petriplate and weighed. Weight of the extract was calculated by using the equation,

Weight of extract = Final weight of the petriplate – Initial weight of the petriplate

2) EXTRACTION OF FLAVANOID FROM TURMERIC

a) Collection of plant material: Fresh and good quality turmeric rhizomes were collected from Chennithala(Mavelikara).

b) Preparing plant material for extraction: The rhizomes were washed in hot water, air dried and powdered.The powder was filtered through a sieve to obtain uniform powder.

c) Extraction using soxhlet: 6 g of dried and ground turmeric rhizomes were placed in a soxhlet apparatus. Extraction was performed with 300 ml of 70% ethanol for 6h.After extraction, the extract along with the solvent was taken in a pre-weighed petriplate and was allowed to evaporate in the hot air oven to remove the solvent.Take the weight of the petriplate.

The weight of the extract was calculated using the formula given below

Weight of the extract = Final weight of the petriplate – Initial weight of the petriplate

3) EXTRACTION OF ALKALOID (PIPERINE FROM BLACK PEPPER)

a) Collection of plant material: Good quality pepper seeds were collected from Charummood, washed, air dried and powdered.

b) The powder was filtered through a sieve to obtain uniform powder.

c) Extraction using soxhlet: 20g of powdered black pepper was weighed and wrapped in a blotting paper and put in the soxhlet apparatus. Extraction was performed with 300 ml of 95% ethanol for 2-3 h. After extraction, the extract

along with the solvent was taken in a pre-weighed petriplate and was allowed to evaporate in the hot air oven at 80^0c to remove the solvent.Weigh the petriplate after drying.

Weight of the extract was calculated using the formula given below

Weight of the extract = Final weight of the petriplate – Initial weight of thepetriplate

IN VITRO SYNTHESIS OF FAT/TRIGLYCERIDE

Esterification is the process of combining an organic acid(RCOOH) with an alcohol(ROH) to form an ester(RCOOR) and water. The two most common fatty acids stored in human adipose tissue are oleate($C_{18}H_{34}O_2$) and palmitate($C_{16}H_{32}O_2$) which esterify to form $C_{55}H_{104}O_6$(1-Stearoyl-2- palmitoyl-3-oleoyl-glycerol). The standard method to synthesize triglyceride is the Fischer esterification process. Through esterification, fatty acids and glycerol combine in the presence of an acid catalyst to form fatty acids. There are seven fatty acids which are present in abundance in human adipose tissue. Among them oleic acid and palmitic acid are the dominant one. These fatty acids and glycerol were purchased from Kelvin Labs, Kayamkulam.

FISCHER ESTERIFICATION

Before starting the experiment ensure that all glasswares were clean and dry.If it was wet, dry it in an oven before proceeding. Add 10 ml of glycerol followed by 15 ml of oleic acid and 15 g of palmitic acid to a 50 mL round bottom flask. Place a magnetic stirrer into the flask. While stirring, add 1.0 mL of concentrated sulphuric acid dropwise under a fume hood. Reflux using a water-cooled condenser. Check the apparatus before proceeding. Adjust the heat until the reaction boils gently. Continue heating under reflux for 60 minutes. When the reflux period is completed, disconnect the heating source and

let the mixture cool. When the reaction is completed and had cooled to room temperature, disassemble the apparatus and transfer the reaction mixture to a separatory funnel. Add 15 mL of ice cold water and mix the phases by careful shaking and venting. Allow the phases to separate, and then discard the aqueous layer. Wash the organic layer by adding 5 mL of 5% aqueous sodium bicarbonate, then shake and vent. Again, discard the aqueous layer. Wash one final time with 5 mL of saturated aqueous sodium chloride and discard the final aqueous layer. Transfer the product to a clean beaker and add a scoop of anhydrous sodium sulphate to the organic layer containing the crude ester.Cap the mixture and let it stand for about 10-15 minutes. Filter to remove the sodium sulphate.

PREPARING DIFFERENT COMBINATIONS OF THE EXTRACT

At the end of extraction, we got three extracts.

1. Phenol from turmeric
2. Flavanoid from turmeric
3. Alkaloid from black pepper

The extracted phytochemicals have to be tested for analyzing their fat burning ability. For this, different combinations of the extract has to be used. The ratios representing each phytochemical in different combinations used are as follows:

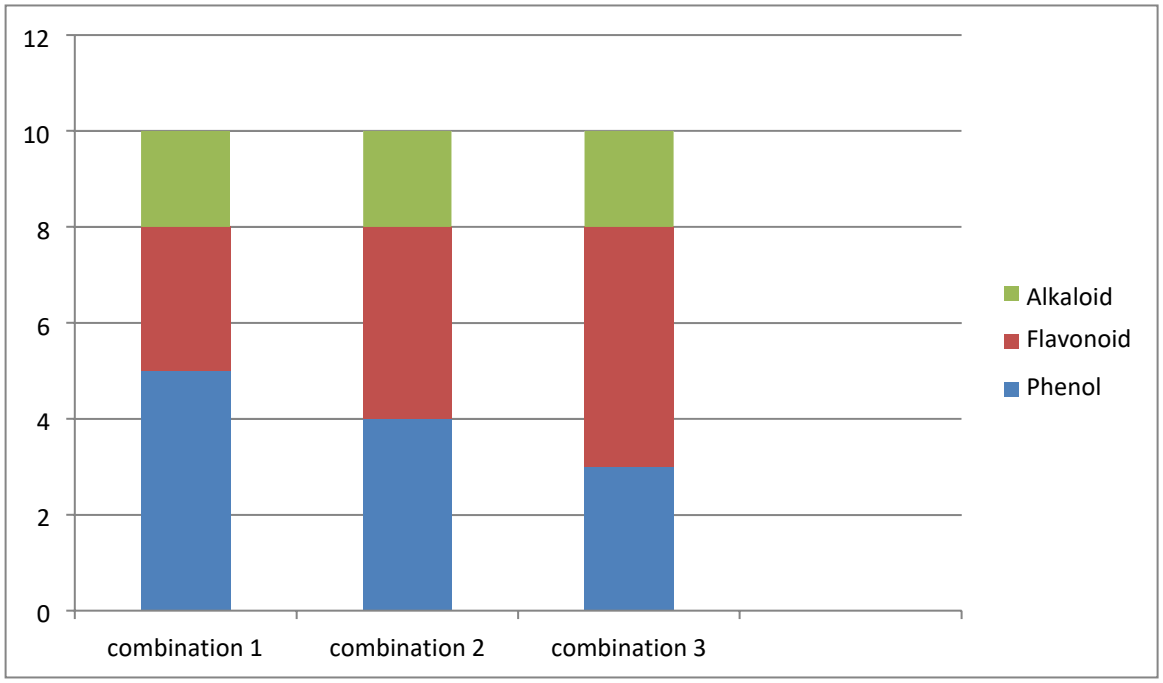
	PHENOL (g)	:	FLAVANOID (g)	:	ALKALOID (g)
COMBINATION I -	5	:	3	:	2
COMBINATION II -	4	:	4	:	2

COMBINATION III - 3 : 5 : 2

These combinations were mixed in prescribed amount (according to the ratio)and were directly applied to the fat synthesized in the laboratory. For our work, to find out the fat burning rate of different combinations of phytochemicals such as phenol and flavonoid from turmeric and alkaloid from black pepper, three different combinations were used.For this, 2 ml of fat were taken in three petriplates.In petriplate 1, 0.05 g of phenol, 0.03 g of flavonoid and 0.02 g of alkaloid were added and mixed with the fat.In petriplate 2, equal amount of phenol and flavonoid were taken.ie, 0.04 g each and 0.02 g alkaloid were added and mixed with the fat. In petriplate 3, 0.03 g of phenol, 0.05 g of flavonoid and 0.02 g of alkaloid were taken and mixed with the fat. The three petriplates were covered with three larger petriplates and were carefully observed.

RESULT

1) RESULT OF THE FAT BURNING EXPERIMENT



In this work, to find out the fat burning rate of different combinations of phytochemicals such as phenol and flavonoid from turmeric and alkaloid from black pepper, three different combinations were used. For this, 2 ml of fat were taken in three petriplates. In petriplate 1, 0.05 g of phenol, 0.03 g of flavonoid and 0.02 g of alkaloid were added and mixed with the fat. In petriplate 2, equal amount of phenol and flavonoid were taken.ie, 0.04 g each and 0.02 g alkaloid were added and mixed with the fat. In petriplate 3, 0.03 g of phenol, 0.05 g of flavonoid and 0.02 g of alkaloid were taken and mixed with the fat.

COMBINATIONS	PHENOL (g)	FLAVONOID (g)	ALKALOID (g)	AMOUNT OF FAT (ml)	% OF DISSOCIATION OF FAT
1	0.05 g	0.03 g	0.02 g	2 ml	30%
2	0.04 g	0.04 g	0.02 g	2 ml	50%
3	0.03 g	0.05 g	0.02 g	2 ml	25%

Out of the three combinations, the combination containing equal amounts of phenol and flavonoid shows 50% fat burning action. The other two combinations also showed acertain extent of antioxidant activity, but lower than the above mentioned combination. The percentage dissolution of fat by the first combination is found to be 30% and is found to be 25 % by the third combination.

2) CONVENTIONAL SOXHLET EXTRACTION YIELDS

Name of the extract	Amount of plant material taken (g)	Solvent used	Amount ofsolvent used(ml)	Time for extraction (hr)	Amount of extract obtained (g)
PHENOL	20	95% ethanol	300	8	1.19
FLAVONOID	6	70% ethanol	300	6	0.96
ALKALOID	20	95% ethanol	300	3	1.02

Conventional Soxhlet extraction method was used for the extraction of phenol, flavanoid and alkaloid which yeilds 1.19 g of phenol, 0.96 g of flavonoid and 1.02 g of alkaloid. The solvent in the extracts were removed by evaporating using a hot air oven at 80^0C.

1. Extraction of phenol

Amount of phenol produced = Weight of the petriplate along with the extract-Initial weight of the petriplate / 47.24 - 46.05 = 1.19 g

2. Extraction of flavonoid

Amount of flavonoid obtained = Weight of petriplate along with the extract – Initialweight of the petriplate / 47.24 – 46.28 = 0.96 g

3. Extraction of alkaloid

Amount of alkaloid produced = Weight of petriplate along with the extract – Initialweight of the petriplate / 104.32 – 103.30 = 1.02 g

3) RESULT OF ESTERIFICATION

The fat produced through condensation had a liquid nature(About 20 ml). It was then purified to remove the acid catalyst(Concentrated sulphuric acid) by adding 5% aqueous sodium bicarbonate,saturated aqueous sodium chloride and anhydrous sodium sulphate one after the other.Finally, a brown coloured semisolid substance with a distinct odour was obtained.

Amount of fat produced = 31.13 g or 10 ml.

DISCUSSION

Accumulation of fatty tissue between the skin layer and the muscle is the major factor that contributes to the formation of lipomas. These are mainly originated at the mesenchymal region. Lipoma tumor frequently develops, where fatty tissues are predominant. Lipomas are usually detected in middle ages. Some people have more than one lipoma. The cause of lipoma isn't fully understood. They tend to run in families, so genetic factors likely play a role in their development. Fat-containing tumors are the most common soft-tissue tumors encountered clinically. The vast majority of fat-containing soft-tissue masses are benign. Lipomas are the most common benign fat-containing masses and demonstrate a characteristic appearance at magnetic resonance(MR)imaging. The root cause for the development of lipomas are the excessive accumulation of fat under the skin. Fats are formed by the reaction between fattyacids and glycerol. The process of formation of fat is known as esterification. There are three differen types of fat cells in the human body. They are white, brown and beige. Fat cells can be stored in three ways: essential, subcutaneous or visceral fat. Subcutaneous fat makes up most of the bodily fat and is stored under the skin. This type of fat is responsible for lipomas. In a study, the fatty acid composition of human adipose tissue was found to contain seven types of fatty acids. They are myristic acid(14:0), palmitic acid(16:0), palmitoleic acid(16:1), stearic acid(18:0), oleic acid(18:1), linoleic acid(18:2) and linolenic acid(18:3). Among them oleic acid and palmitic acid comprises about 70% of the human body fat.

Burning of the subcutaneous fat deposited in the lump is the cure for lipomas. Fat burning compounds in plants are usually termed as anti-oxidants. The process of burning of fat cells is quite a complex physiological process. When the body loses fat, the fat were liberated from the fat cells through somewhat complex hormonal/enzymatic pathways. The fat cells simply releases triglycerol into the

bloodstream as free fatty acids and they are transported through the blood to the tissues where the energy is needed. By lipolysis, each molecule of triaglycerol splits into glycerol and three fatty acids.

In this experiment, three combinations of plant extracts including phenol, flavonoid and alkaloid are evaluated for the fat burning activity. For this, phenol and flavonoid were extracted from *Curcuma longa* L. and alkaloid were extracted from *Piper nigrum* L. using conventional soxhlet extraction method. The phytochemicals thus extracted were combined in three ratios for testing their fat burning ability. Out of the three combinations, the combination containing equal amounts of phenol and flavonoid shows 50% fat burning action. The other two combinations also showed a certain extent of antioxidant activity, but lower than the above mentioned combination. The percentage dissolution of fat by the first combination is found to be 30% and found to be 25 % by the third combination. While comparing other methods for treating lipomas, the utilization of natural phytochemical extracts are beneficial since they possess the least side effects when compared to other methods and are thus safe to use. Thus the use of phytotherapy is proven to be significant with the least side effects. The in vitro results revealed that the combination of plant extracts shows fat burning ability in the ratio 4:4:2 (Phenol: Flavonoid: Alkaloid) followed by 5:3:2.

SUMMARY AND CONCLUSION

For our investigation, phytochemicals such as phenol and flavonoid from *Curcuma longa* L. (Turmeric) and alkaloid from *Piper nigrum* L. (Black pepper) were extracted using the conventional soxhlet extraction method. For the extraction of phenol from 20 g of turmeric, 300ml of 95% ethanol is used as the solvent and 1.19 g of phenol were obtained. 6g of dried and ground turmeric rhizome were extracted with 300 ml of 70% ethanol for extracting 0.96g of flavonoid from turmeric. The alkaloid present in black pepper is known as piperine. It is well known for its anti- inflammatory property and is used to increase the bioavailability of turmeric in human body. 20g of blackpepper was extracted using 300 ml of 95% ethanol to yield 1.02 g alkaloid.

The *in vitro* lipolytic potential of the extracts were evaluated through the estimation of dissolution of fat into fatty acids and glycerol. For estimating the ability of extracts to burn fat, an *in vitro* set up is required to test whether they burn fat or not. For this, fat was synthesized artificially in the laboratory which have resesmblance with human fat through Fischer esterification method. It results in a brown coloured semi-solid substance with a characteristic odour. The amount of fat produced is 31.13g (10 ml).

For testing the lipolytic ability of extracts, three combinations of the extracts wereused (phenol: flavanoid: alkaloid). In the first combination, phenol is taken in large amount than flavonoid (5:3:2). In the second combination, equal amount of phenol and flavonoid (4:4:2). And the third combination contains more flavonoid than phenol (3:5:2), the composition in which they are present in turmeric. A small amount (0.02 g) of alkaloid were added to all the three combinations to increase the bioavailability of these phytochemicals. From the present study it has been concluded that the second

combination shows more effectiveness in burning fat. The combination containing equal amount of phenol and flavonoid can be used to treat diseases like lipoma. This combination dissolves fat into fatty acid and glycerol. That is why the fat containing the combination became colourless after 6 hours of application. Surgery is known to be the major cure for lipomas. Through this study, an alternative natural remedy using the combination of phenol, flavonoid and alkaloid (4:4:2) could be used to cure lipomas.

This study has given the primary evidence for using the combined action of the phenol and flavonoid from *Curcuma longa* L. and alkaloid from *Piper nigrum* L. for lipolysis. As reported earlier, both flavonoid and phenol possess antioxidant activity. To develop a medicine for lipoma, the *in vitro* results should be confirmed by the *in vivo* analysis, as the combination tested were advantageous in burning fat.

IN VITRO LIPOLYTIC ASSAY

Plate 1: Extraction process using conventional soxhlet apparatus

Results of extraction

Plate 2: Petriplate containing phenol extract

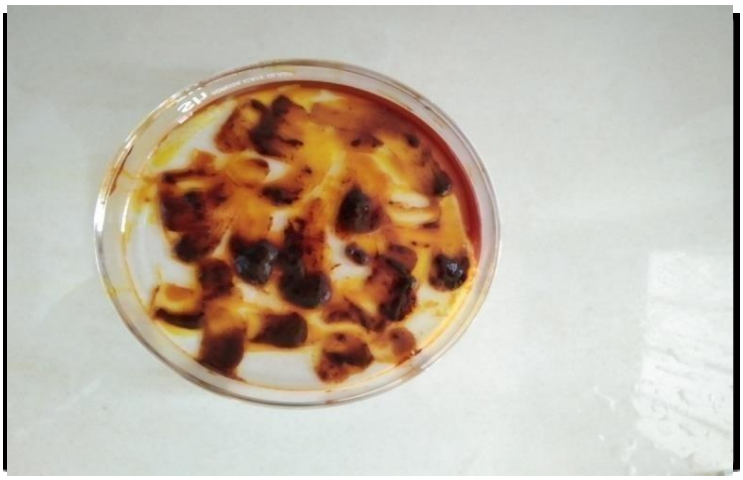


Plate 3: Petriplate containing flavonoid extract

Plate 4: Petriplate containing alkaloid extract

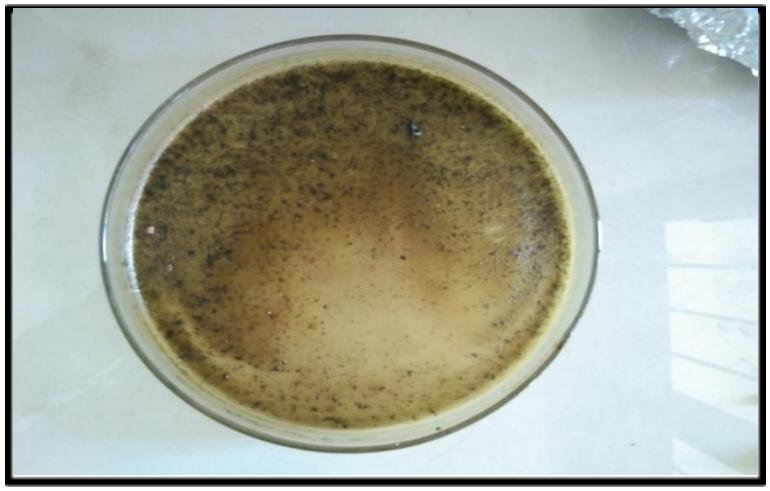


Plate 5: Beaker containing fat produced through esterification(Before purification)

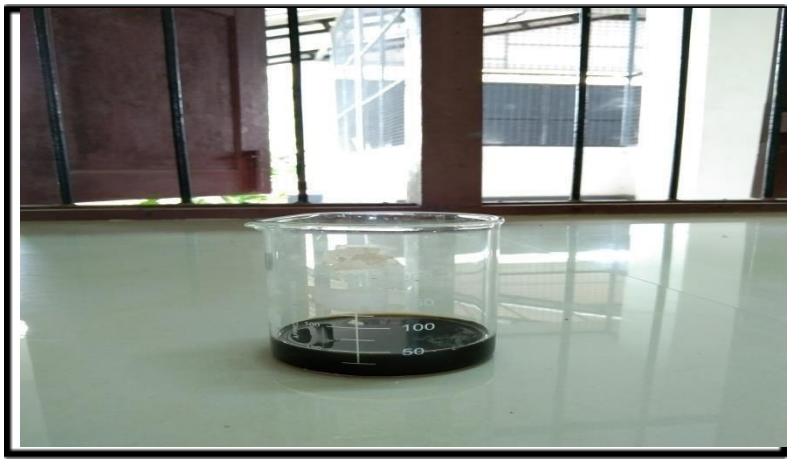
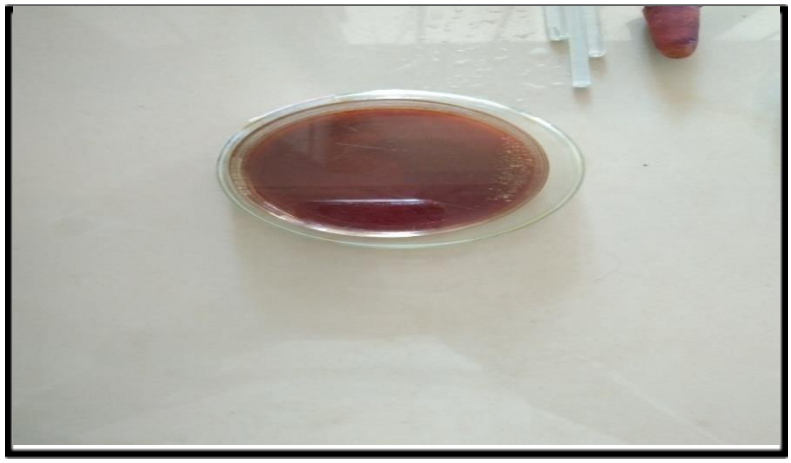


Plate 6:Petriplate containing fat(After purification)



<u>In vitro lipolytic experiment</u>

Plate 7: Preparing three different

combinations

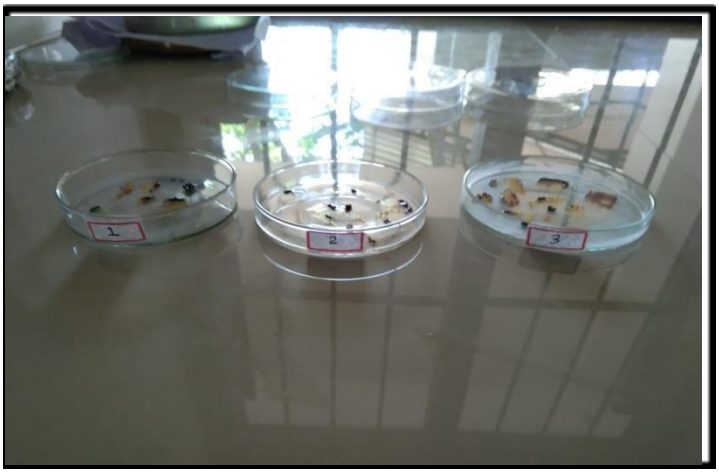
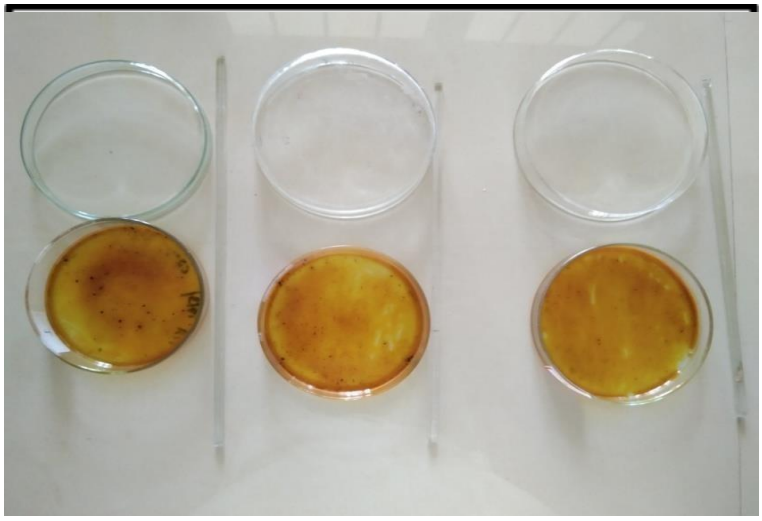


Plate 8: Addition of fat to test the fat burning ability



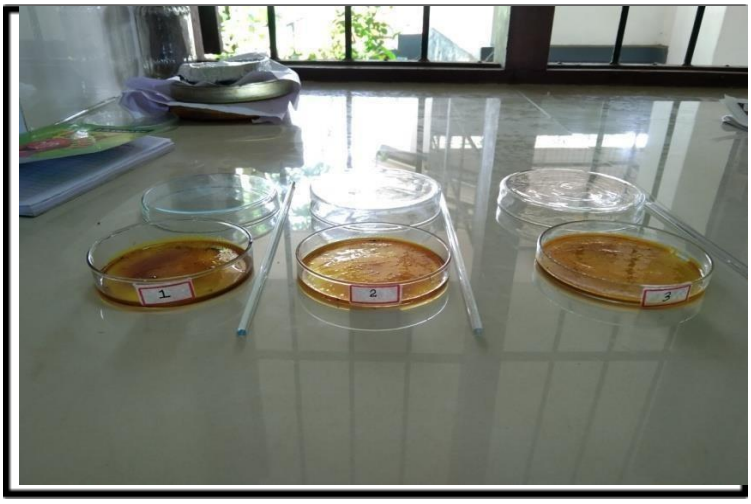


Plate 9: Petriplates after 3 hours

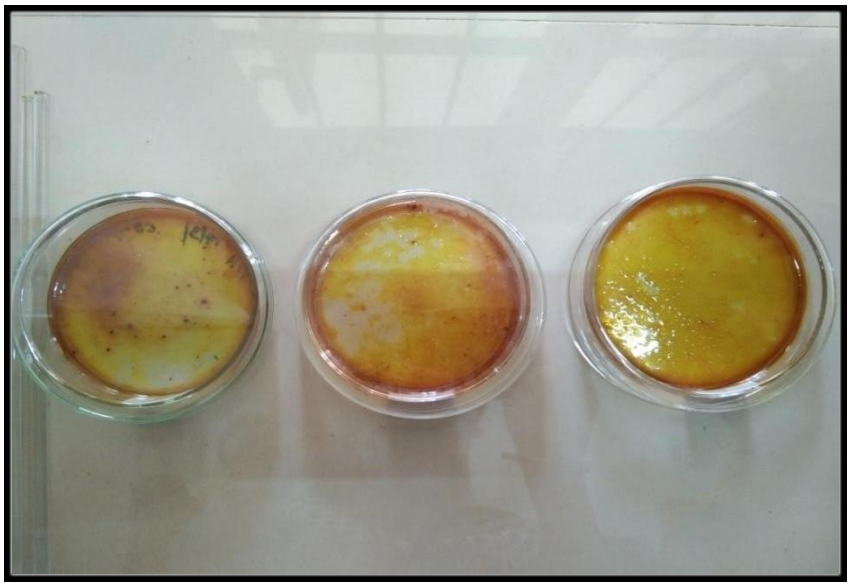
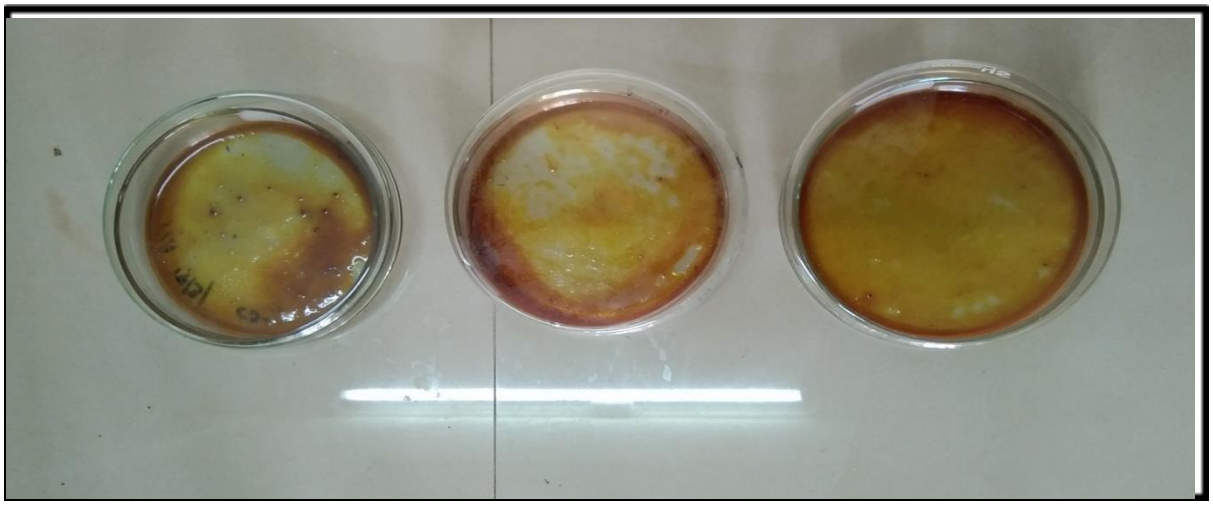
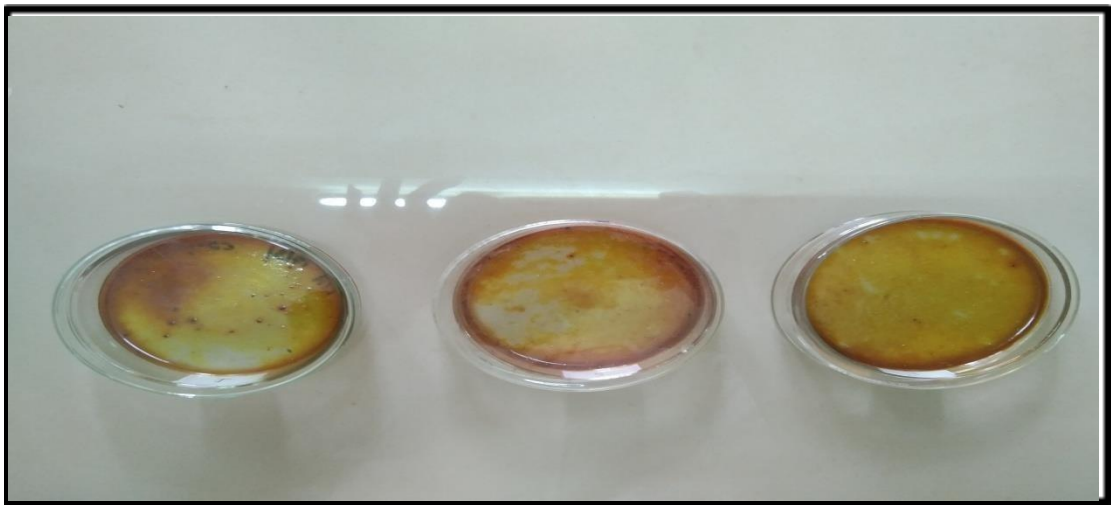


Plate 10: Colour change of fat in the petriplates after 6 hours





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