Formulation and Characterization of *Solanum lycopersicum* Extracts for Anti-depressant Activity

project report submitted in partial fulfillment of the requirements for the degree of

BACHELOR OF PHARMACY

Submitted by

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Under the Supervision of

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May 2023

GALGOTIAS UNIVERSITY

CERTIFICATE

This is to certify that project work entitled **"Formulation and Characterization of *Solanum lycopersicum* Extracts for Anti-depressant Activity"** done by **Mr. Kapil Rana,** is a bonafide research work done under the supervision and guidance of **Ms. Awaneet Kaur**, Assistant Professor, School of Medical and Allied Sciences, Greater Noida. The work is completed and ready for evaluation in partial fulfillment for the award of Bachelor of Pharmacy during the academic year 2022-2023. The project report has not formed the basis for the award of any Degree/Diploma/Fellowship or other similar title to any candidate of any University.

Date:

Prof. (Dr.) Pramod Kumar Sharma
Dean
School of Medical and Allied Sciences
Galgotias University
Greater Noida (U.P.)

BONAFIDE CERTIFICATE

This to certify that the project work entitled **"Formulation and Characterization of *Solanum lycopersicum* Extracts for Anti-depressant Activity"** is the bonafide research work done by **Mr. Kapil Rana**, who carried out the research work under my supervision and guidance for the award of Bachelor of Pharmacy under Galgotias University, Greater Noida during the academic year 2022-2023. To the best of my knowledge the work reported herein is not submitted for award of any other degree or diploma of any other Institute or University.

Ms. Awaneet Kaur
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DECLARATION

I hereby declare that the work embodied in this project report entitled **"Formulation and Characterization of *Solanum lycopersicum* Extracts for Anti-depressant Activity"** in Partial fulfillment of the requirements for the award of Bachelor of Pharmacy, is a record of original and independent research work done by me during the academic year 2022-23 under the supervision and guidance of **Ms. Awaneet Kaur,** Assistant Professor, School of Medical and Allied Sciences, Depatment of Pharmacy Galgotias University, Greater Noida. I have not submitted this project for award of any other degree or diploma of any other Institute or University.

Date:

Place: Greater Noida **Name and Signature of Candidate**

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I would like to thanks all my teachers, laboratory technician and other staff for helping me in completion of this project.

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ABSTRACT

Research Problem

Depression is a multifaceted mental health disorder characterized by persistent feelings of sadness, loss of interest or pleasure in activities, changes in appetite or sleep patterns, low energy, and difficulties in concentration or decision-making. It is a significant global health concern, affecting people of all ages and backgrounds. This abstract explores the problem of depression by examining its prevalence, risk factors, impact on individuals and society, and available treatment options. Depression affects a substantial portion of the global population, with estimates suggesting that more than 264 million people worldwide experience depression. It is a complex condition influenced by a combination of genetic, biological, environmental, and psychological factors. Common risk factors include a personal or family history of depression, chronic medical conditions, substance abuse, significant life changes or stressors, and social isolation. Most of the antidepressant agents like moclobemide, amitriptyline etc are used for depression . However, most drugs still induce adverse effects. These disadvantages motivate to find a herbal treatment for depression.

Objective

To Develop and optimize the *Solanum lycopersicum* extracts for the evaluation of the antidepressant activity based nasal drug delivery.

Methodology

The powdered of *Solanum lycopersicum* (tomato) were extracted by maceration process for seven days, using water as a solvent. The sample: solvent ratio was 50:100. The crude extract obtained was collected and concentrated on a water bath. Cubosome formulation developmemt using top-down and bottom up apporach with homogenizer utilizing of PLG, GMO, PLGA, SOBITOL, DISTILLED WATER.

Evaluation Parameters

- Physical appearance

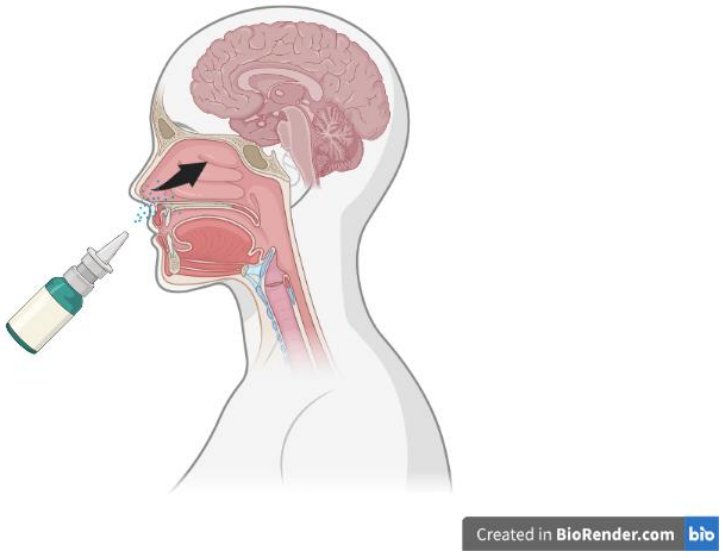
- FTIR spectroscopy
- Percentage yield and Entrapment efficiency
- In-Vitro release study

Conclusion

Extract of *Solanum lycopersicum* (tomato) exhibited antioxidant and antidepressant capacity. The chemical constituents were mainly responsible for these *in vitro* biological activities and ultimately improved the depression of the treatment groups. The present study suggests that plant can provide the therapeutic potential for management of depression.

Future Aspects

Results obtained from Evaluation Parameters revealed that *Solanum lycopersicum* extract showed effective use of the plant in depression. Exploration of phytoconstituents from natural resources; identification and isolation of phytoconstituents are simultaneously presenting enormous scope for their better therapeutic application for combating various diseases. Thus, in the near future S*olanum lycopersicum* may play a very important role as a potential in depression.



Nasal administration of cubosomal anti-depressant formulation

CHAPTER 1

INTRODUCTION

INTRODUCTION

According to the World Health Organization (WHO), depression is a mood disorder that is characterized by a number of distinct symptoms, such as sadness, loss of interest, anhedonia (loss of pleasure), lack of appetite, guilt-related feelings, low self-esteem or self-worth, disturbed sleep, fatigued feelings, and difficulty concentrating [1]. People who are depressed often experience various levels of helplessness and hopelessness, as well as difficulty concentrating, insomnia, appetite loss, a lack of interest in previously enjoyable activities, feelings of extreme sadness and guilt, and sometimes even thoughts of suicide. Depression causes a significant reduction in capacity to perform and may be persistent or long-lasting. The signs of depression must last for at least two weeks before a diagnosis may be made.[2]

There are several forms of depression, some of which are brought on by certain events. Major depression is characterised by depressive symptoms that have persisted for at least two weeks and are generally disruptive to one's ability to work, sleep, study, and eat.

Dysthymia, or persistent depressive illness, is characterised by less severe depressed symptoms that persist for at least two years on average.

Perinatal depression is when a woman develops a serious depressive disorder while pregnant or after giving birth (postpartum depression).

Seasonal affective disorder is a condition that comes and goes with the changing of the seasons, usually beginning in the late autumn or early winter and ending in the spring or summer. [3-5]

A severe kind of depression known as depression with psychotic symptoms occurs when a person also exhibits psychotic symptoms like hallucinations or delusions, which are unsettling erroneous fixed beliefs.

People who have bipolar disorder (previously known as manic depression or manic-depressive illness) also go through depressive episodes during which they feel down and out and have very little energy. However, a person with bipolar disorder can also have manic episodes, which are periods of unusually elevated moods during which the person may feel extremely happy, agitated, or "up," with a noticeable increase in activity.[6]

Symptoms

Although you might only experience depression once in your lifetime, most people experience multiple episodes. During these episodes, symptoms might involve and last for the most of the day, almost every day.

Sadness, tears, emptiness, or a sense of futility irrational behaviour, irritation, or annoyance, even over trivial issues, the inability to enjoy most or all of the common activities, such as sex, hobbies, or sports sleep disorders, such as insomnia or excessive sleeping, fatigue and lack of energy, which makes even little chores more difficult, increased food cravings and weight gain, or decreased appetite and weight reduction, an agitated or anxious state, sluggish speech, posture, or other physical motions, thoughts of suicide, death, or other suicidal behaviour on a regular basis or repeatedly [5]

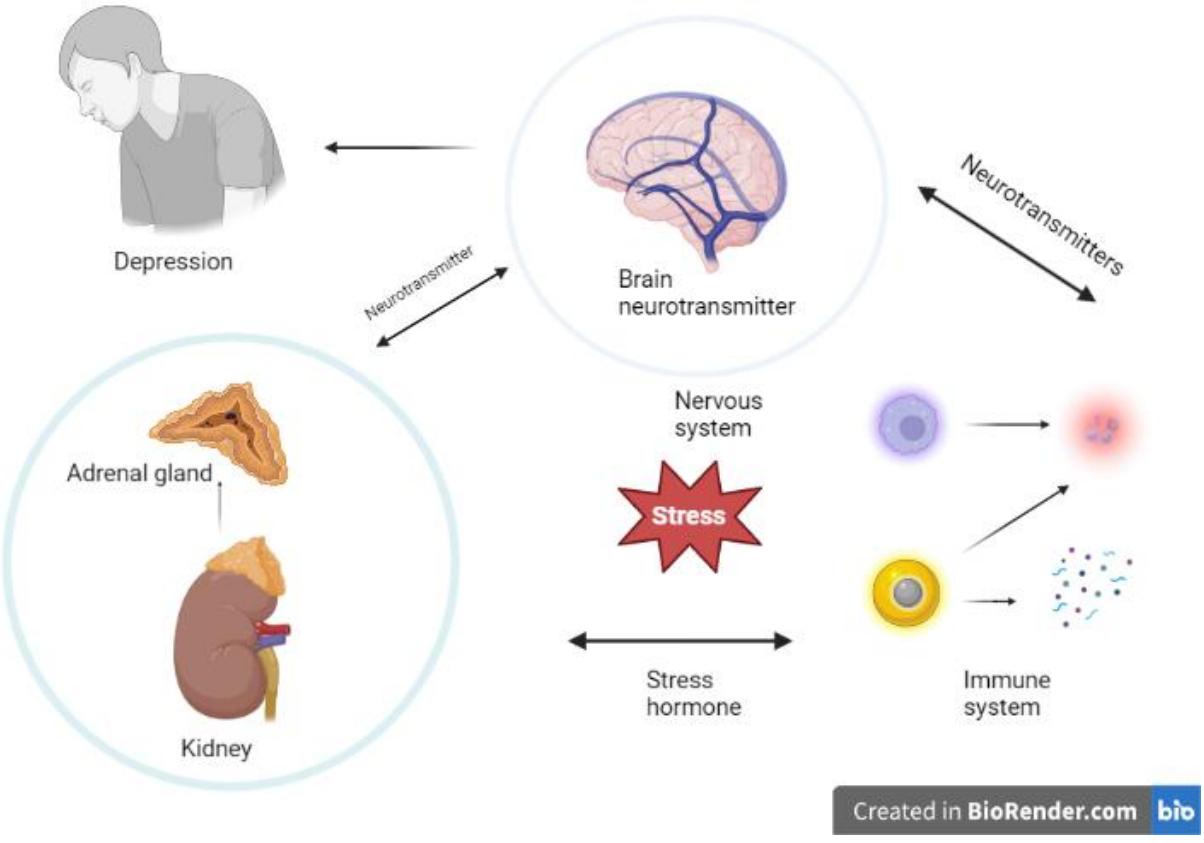


Figure-1: Signs of depression in adolescents and children

Although there may be some differences, the typical signs and symptoms of depression in children and teenagers are similar to those in adults. Depression in young children might manifest as melancholy, impatience, clinginess, concern, aches and pains, refusal to attend school, or underweight.

Teens may experience symptoms such as sadness, irritability, feeling down and unworthy, anger, poor performance or poor attendance at school, feeling misunderstood and overly sensitive, using alcohol or drugs recreationally, eating excessively, engaging in self-harm, losing interest in regular activities, and avoiding social interaction. [4]

Depression symptoms in older adults

Depression is never to be taken lightly because it is not a typical aspect of ageing. Unfortunately, older adults with depression frequently go undiagnosed and untreated, and they may be hesitant to get help. Older persons may experience different or less noticeable signs of depression, such as: Memory issues or behavioural changes, Physical pains or discomfort, Suicidal thoughts or sentiments, especially in older males. Fatigue, lack of appetite, sleep issues, or loss of interest in sex—not due to a medical condition or medicine. Wanting to remain home rather than going out to socialise or try new things. [6]

Common Causes of Depression

According to researchers, depression may have a variety of reasons and may not always be avoidable. Depression may result from a number of factors, including:

- Genetics
- Brain chemistry
- Certain medical conditions
- Substance use
- Stress
- Poor nutrition

There is no one cause of depression. The likelihood that a person may acquire the disease is influenced by a variety of variables. 10.5% of women experience depression compared to 6.2% of men,2 which experts speculate may be attributable to hormonal causes.

Pathophysiology of Depression

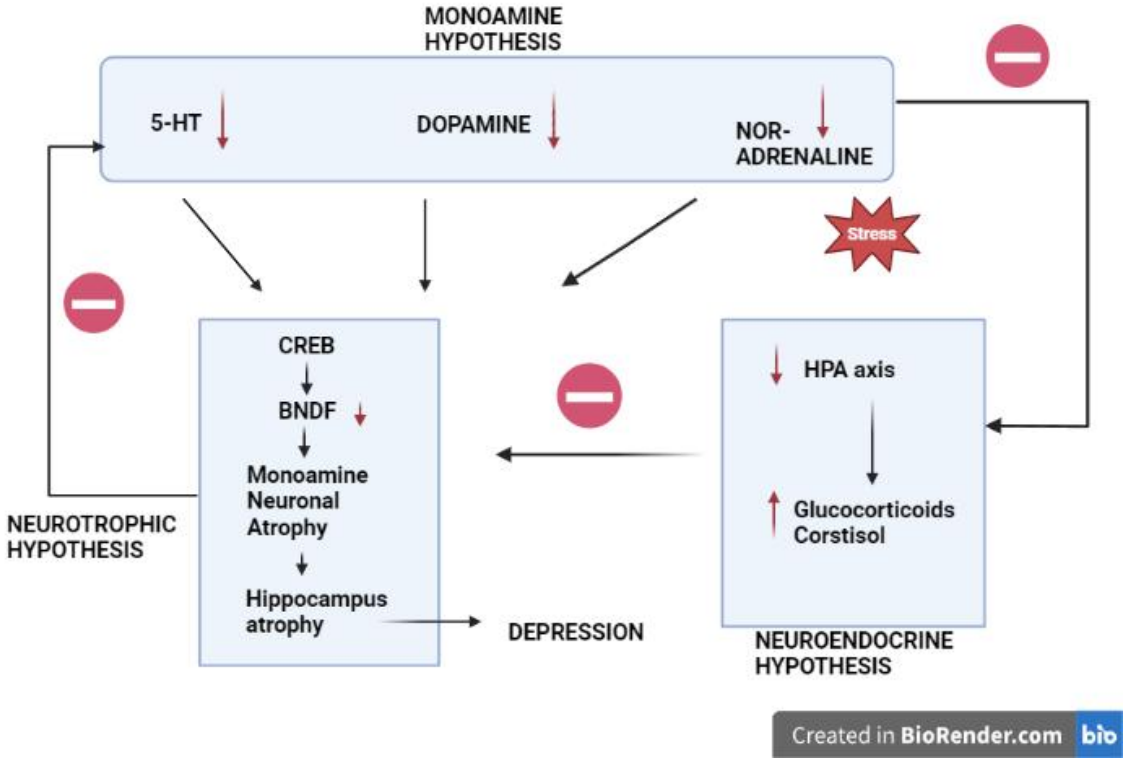


Figure-2: Pathophysiology Of Depression

The basic G-E interaction model, which is also used to explain the aetiology of other complex diseases including cancer, hypertension, and diabetes, best captures the present understanding of the causes of depression. Three key monoamine systems—serotonin (5-hydroxytryptamine, 5HT), norepinephrine (NE), and dopamine (DA)—have been the focus of the model. Functional brain imaging and the newly developed molecular neurobiology technologies have added to the evidence for the contribution of these three systems. [7]

Serotonin Alterations in Depression

A significant involvement for the central nervous system's (CNS) 5HT systems in depression is supported by evidence from an astonishing array of investigations. Reduced serotonergic neuron activity has been seen in depressed individuals in postmortem, cerebrospinal fluid (CSF), and neuroendocrine investigations.New data

from postmortem And positron emission tomography (PET) imaging studies demonstrate a reduction in the number of serotonin transporter (SERT) binding sites (the site of action of selective Serotonin reuptake inhibitors, SSRIs) in the midbrain and amygdala of drug-free Depressed patients, as well as a reduction in both presynaptic (in the midbrain) and Postsynaptic (in the mesiotemporal cortex) 5HT receptor subtypes of depressed Patients.

5–6 Previous research showed an increase in the density of 5HT2 receptors, possibly as a result of a relative decline in the availability of 5HT. The observation that depressed patients in remission following treatment with SSRIs, when confronted with an experimental manoeuvre that decreases CNS 5HT availability (i.e., tryptophan depletion), exhibit a rapid return of depressive symptoms, in many cases within a few hours, supports the involvement of 5HT circuits in depression. Additional evidence is provided by the ground-breaking discovery that people with the s allele of the SERT gene's promoter region (SLC 6A4) are unusually susceptible to the depressogenic effects of early life stress, such as child maltreatment or neglect. The SERT protein plays a crucial role in controlling the amount of serotonin in the central nervous system. The s allele of the SERT gene is associated with reduced functional capacity of the SERT. [8]

Norepinephrine Alterations in Depression

Another theory for the involvement of NE-containing circuits in mood disorders Nortriptyline and reboxetine are examples of NE reuptake inhibitors that are efficient antidepressants, much like medications that increase the availability of 5HT. A role for NE Dysfunction in depression is also supported by neurochemical and neuroendocrine investigations in depressed individuals and by postmortem results.Patients with treatment-resistant depression may also be more likely to see changes in noradrenergic circuitry. The effectiveness of antidepressants that are thought to operate on both 5HT and NE neurons in comparison to those that just target 5HT or NE neurons is still debatable, although new meta studies imply that, if an advantage exists, it is only marginal. [9]

Dopamine Alterations in Depression

Although DA-containing neuronal pathways have been heavily linked to the pathophysiology of schizophrenia, there is growing evidence that CNS DA circuits play a significant role in depression. In fact, several researchers11 contend that the relative lack of action of SSRIs and selective serotonin-norepinephrine reuptake inhibitors (SNRIs) on brain DA circuits may contribute to the unsatisfactory therapeutic responses to these drugs. A potential involvement for CNS DA circuits in depression has been proposed, in part as a result of the poor remission rates in clinical studies with SSRIs and SNRI.

This development Given that anhedonia, the inability to experience pleasure, is frequently cited as the most significant pathognomonic symptom of depression and that pleasure, whether connected to eating, socialising, or sexual behaviour, is primarily mediated by activation of DA neurons, the existence of a DA hypothesis of depression is not surprising. [8][10]

Drug treatment

For the first antidepressant, 50–65% of patients show improvement. No antidepressant has a higher level of effectiveness or faster time to action than any other. The ability to match a patient's symptoms to a side effect profile, the existence of medical and mental health co-morbidity, and previous responses can all influence a decision. Also to be taken into account are relative costs (such as generics). Citalopram (Celexa ®) and generic Fluoxetine are the preferred treatments at UMHS. Antidepressant-treated patients should be continuously monitored for potential worsening of depression or suicidality, particularly at the start of medication or when the dosage is changed.

By inhibiting the enzyme monoamine oxidase, monoamine oxidase inhibitors (MAOIs) prevent the breakdown of the monoamine neurotransmitters serotonin, norepinephrine, and dopamine. This results in higher levels of these neurotransmitters in the brain and an increase in neurotransmission. [9][11]

Tricyclic antidepressants (TCAs):

serotonin, norepinephrine, and to a much lesser extent dopamine are among the neurotransmitters that are prevented from being reuptaken. The most widely used antidepressants today are selective serotonin reuptake inhibitors (SSRIs), which prevent serotonin from being reabsorbed and raise the amount of active serotonin in

brain synapses. Different nerve cell receptors or norepinephrine reuptake are affected by other novel antidepressants.

Although SSRIs, MAOIs, and TCAs raise serotonin levels, other medications stop serotonin from binding to 5-HT2A receptors, arguing that it is oversimplified to refer to serotonin as the "happy hormone." In fact, it's typical for patients to feel worse during the first few weeks of treatment since the prior antidepressants accumulate in the circulation and the serotonin level rises.

Another theory is that antidepressants' encouragement of neurogenesis in the hippocampus, an effect shown in rodents, may have some longer-term benefits. According to other animal studies, antidepressants may have an impact on "clock genes," which regulate how genes are expressed in brain cells. [12]

By combining data from the outside world with signals produced by the internal environment, complex operations are carried out. The BBB, which serves as a barrier between the brain and capillary endothelium, divides the two. The human brain is encased in a protective shell made up of the skull, meninges, blood-CSF barrier, and blood-brain barrier (BBB). Due to the blood-brain barrier's (BBB) very lipophilic nature, which makes it challenging for medications to access the brain's cells, meningitis, Parkinson's disease (PD), brain abscess, MS (multiple sclerosis), epilepsy, and late-stage neurological trypanosomiasis are all challenging to treat.

The BBB was altered to allow the release of medicines into the brain by osmotic, chemical, and carrier/transport system activation.

The most frequent and preferred route for providing medications with systemic effects, oral therapy, has a number of drawbacks, including sluggish action and limited bioavailability (40 to 45%), nausea and partial pain relief, and headache recurrence. Oral formulations shouldn't be used for hepatic first-pass metabolism to hepatic and renal system clean-up agents or metabolism pass since they have a half-life of 1-2 hours. [13]

Functions of The Brain

You can see and feel everything around you thanks to your brain. Three different types of neurons are present in this main organ: sensory neurons, motor neurons, and interneurons. It affects a wide range of biological processes, such as the senses of

touch, hearing, movement, and sight. Language and mechanics are both governed by motor neuron activity. The function of the brain is to control and manage the brain's emotional responses to sensations like pain and pleasure. It controls practically all bodily processes. Information is sent from neuron to neuron in the brain.

Blood-Brain Barrier

Toxins, viruses, and other potentially harmful substances cannot enter the circulatory system past the BBB's protective role and reach the brain. "Selectively permeable barrier structures," or BCECs, are endothelial cell monolayers that make up this layer of endothelial cells. Large molecules (> 400 Da) are impermeable to around 98% of them through the close connections of individual BCEC cells. The BBB, which is a more complex and useful notion than the endothelial cells that line our intraluminal brain capillaries, includes peridothelial accessory framework.

Pericytes, astrocytes, extracellular matrix (ECM), and neurons are also present in the BBB. Usually, capillaries are spaced apart by 40 microns. Each brain cell has a 20 micrometre gap between it and the vascular capillary, which may be filled up by diffusion in under a second. Endothelial cells in the BBB's capillaries experience less transcytosis as a result.[14]

Tight junctions (TJs) and adherent junctions (AJs) are the main junctional complexes of the BBB. The majority of AJs are made up of caderin proteins, which maintain the spinal cord by covering its cross-section and attaching to cantenin proteins in the cytoplasm of the cell. Although endothelial cell adhesion molecules are unknown, they are thought to be crucial for preserving cell polarity, providing cohesion to endothelial cells, and responding to stimuli through interactions with Catherine and actin proteins. According to the study, AJs also affect TJs.

The BBB is a protein complex molecule that forms rows of massive, overlapping occlusions between endothelial microvascular cells in the central nervous system, severely restricting paracellular movement of polar and macromolecules. Occludin, claudins, and JAM are all involved in how the TJ works. The form, structure, and complexity of BBB endothelial TJs differ from those of the epithelium and peripheral endothelium.

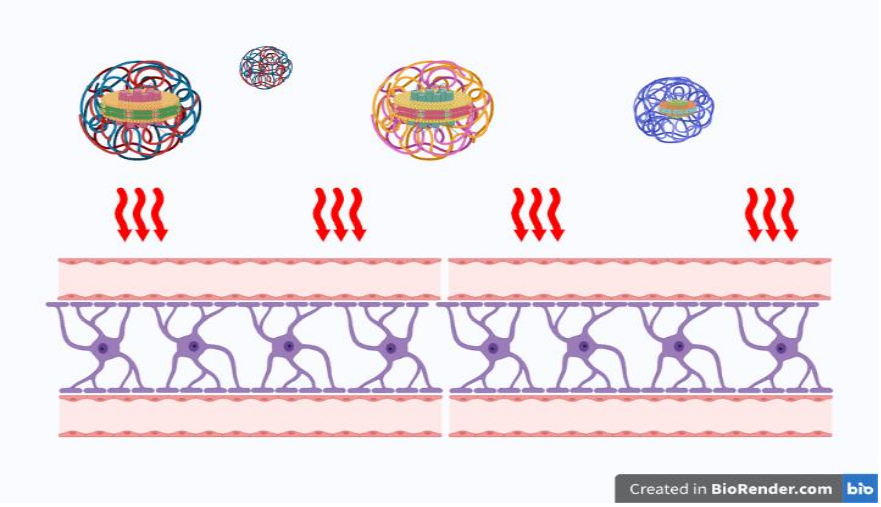


Figure-3: Cubosomal Permeation Through Blood Brain Barrier

Through intraparenchymal endothelial cells, the impedance of these unfenestrated capillaries consolidated in with intercellular tight junctions may reach up to 2000 cm2. The BBB serves as a significant barrier to the CNS in the presence of drugs and other substances and shields the brain from blood-borne compounds.[15]

Because the BBB barrier, which is essential for controlling brain transport, cannot function properly, therapeutic active substances cannot reach the brain. A small number of molecular drugs may be able to cure conditions including epilepsy, chronic pain, depression, and anxiety. The BBB makes it challenging to treat a number of CNS-related illnesses and afflictions. To comprehend how the BBB regulates transport, which is essential for drug detection and CNS disease therapy, it is necessary to analyse drug and other molecule transport through the BBB. [15][16]

Transport Mechanism across the BBB

The BBB barrier, which is essential for regulating brain transport, is dysfunctional, preventing therapeutic active chemicals from reaching the brain. A small number of molecular medicines may be able to treat a variety of conditions, including epilepsy, chronic pain, depression, and anxiety. The BBB makes a number of CNS-related illnesses and afflictions challenging to treat. Understanding how the BBB regulates transport is essential for drug detection and the treatment of CNS disorders. To do this, it is necessary to analyse how drugs and other molecules are transported through the BBB.

Factors involved in brain targeting

Physiological Factor

Passive diffusion

With this vehicle system, uncharged particles (500 g/ml), tiny atomic size, lipophilicity, and decreased hydrogen holding capacity are the main factors influencing drug diffusion through the BBB. This might be enhanced therapeutically by decreasing the atomic size and increasing the ionization-dependent lipophilicity.

Transport through vesicular

Two types of transit are in use: The two forms of endocytosis are fluid stage and adsorption stage. This mechanism also links the wonder-restraining capacity of the plasma cell layer to the underlying stage. By the selectivity of its ligands and the controllability of the process, adsorbtive endocytosis may be separated. Many macromolecules, including endocytosis interfering receptors, are transported from the plasma to the brain via endocytosis. This includes insulin as well as the aim transfer's development.[17]

Active mediated transport

It is common to refer to this type of transportation as “transporter-involved transport.” LAT1 and GLUT1 are examples of carriers intercepted by transporters. They have a greater range and are more durable. To improve pharmaceutical transcellular access across the BBB, new strategies are being employed. Drug-related endogenous mixtures may be directed away from the BBB using this method [13].

Other factors are:

Drug lipophilicity, Efflux protein affinity, Drug clearance rate, Drug concentration gradient, Drug molecular weight, Cell sequestration, Enzymatic cellular stability, Pathological status, Enzymatic systemic stability, and another tissue metabolism.

Future aspect of brain targeting

Typically used to treat neurodegenerative diseases, sustained-release medications have two characteristics: biocompatibility and biodegradability.

The processes by which genes and cells are targeted.Systems for nanomedicine can deliver large quantities of medications to specific areas in a controlled and sustainable manner.

By recognizing changes in the physical or magnetic properties of the device used to bind ligands to paramagnetic nanoparticles, it may be used to calculate the quantity of ligands.

Improved disease indicators in regard to specificity and sensitivity.Nanoparticles for tissue engineering are created to stimulate regeneration and differentiation, control cytokines, and support cellular growth.[18]

Herbs and their Constituents

The use of herbs as an alternative treatment for several neurological illnesses is possible. Various bioactive substances that have been isolated from herbs are being used effectively to treat neurological disorders. Herbal therapies are becoming more and more well-liked as a result of the negative consequences of chemical medications. Numerous scientific studies have shown that different herbal extracts and their active ingredients can treat nerve disorders and elevate mood.

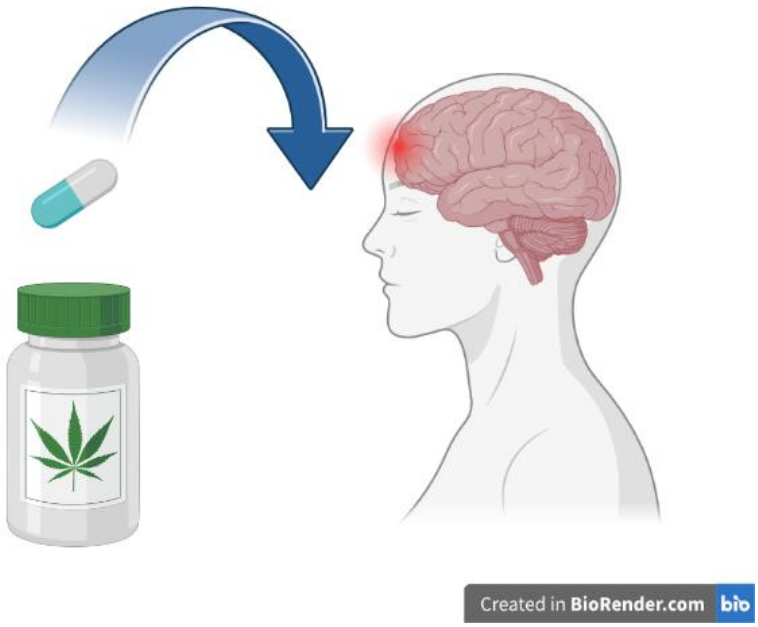


Figure-4: Herbal drug for treating neurological disorder

PLANT PROFILE

Solanum Lycopersicum L.

Common name –Tomato

Table-1: Taxonomical classification of *Solanum lycopersicum*

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Solanales
Family	Solanaceae
Genus	Solanum L.
Species	*Solanum lycopersicum* L.

One of the most significant vegetable plants in the world is the tomato (Solanum lycopersicum). It is believed that domestication took place in Central America, while its origins are in western South America.

Tomato has been developed to increase production, fruit quality, and resistance to biotic and abiotic stressors because of its significance as a food crop. The tomato has been extensively utilised both as food and as a research tool. The tomato plant has numerous intriguing characteristics that other model plants (such as rice and Arabidopsis) lack, such as meaty fruit, a sympodial Shoot, and compound leaves.[19-21]

The majority of these features are crucial for farming, hence they can't be investigated in other model plant systems. There are 13 recognised species of wild tomatoes that exhibit a wide range of phenotypes and can cross with domesticated tomatoes. These untamed tomatoes are crucial for evolutionary research, breeding, and as sources of desirable features.

The tomato genome sequencing project is currently making progress, which has produced significant data to aid in the research of tomatoes. The tomato is also closely related to other

economically significant plants, including the potato, aubergine, peppers, tobacco and petunias, and it is a member of the enormously huge Solanaceae family. Because these plants are easily applicable to the knowledge gained from studies on tomatoes, these plants are crucial research subjects. These characteristics make the tomato a model organism for the Solanaceae family. also particularly for plants that produce succulent fruits.[21]

Chemical constituents

Narigenin, Lycopene, antioxidants, ascorbic acid and carotenoids, Bioflavonoids, Manganese, Magnesium, Iron Etc. [22]

Traditional uses

Anti-inflammatory, Cardiovascular Diseases, Antioxidant, Different types of cancer Asthma and Diabetes etc.[23]

Antioxidant And Neuroprotective Effect of Naringenin

When linked to chronic illnesses including cardiovascular, diabetes, cancer, and neurodegenerative disorders, naringenin's beneficial benefits on oxidative stress disorders have been well shown.7 By scavenging reactive species and reducing oxidative damage, the flavonoid can function directly as an antioxidant. It can also change the activities of antioxidant enzymes and regulate the expression of genes that improve antioxidant defence.35 Naringenin supplementation's anti-oxidant effects on the animal model Naringenin have been employed as a nutraceutical substance on a variety of neurological problem models, including Alzheimer's disease and Parkinson's disease, and has consistently provided health advantages by suppressing or reducing the oxidative environment as a result of these models.[24-27]

Approaches to herbal formulation

Plants have long been used for both nutrition and medicine. Since the dawn of time, humans have relied on them to combat numerous illnesses and diseases. The most common type of treatment, phytomedicine, or plant-based medicine, is still used extensively around the world. The contemporary pharmaceutical industry had its start in the early nineteenth century, and ever since, its main driver has been the search for new plant-based pharmacological substances. A drug created from plants, either in raw or prepared form, is referred to as a herbal medicine or phytopharmaceutical preparation. According to a WHO

study, between 70% and 90% of people in many developing nations rely mostly on traditional remedies for therapy.

There are several secondary metabolites in plants. The food, agricultural, and pharmaceutical sectors all employ secondary metabolites. It has been discovered that other water-soluble phytoconstituents, such flavonoids, tannins, and terpenoids, have therapeutic benefits. Approximately 10,000 species of higher plants have been identified as having medicinal potential out of the nearly 270,000 terrestrial plants that have been taxonomically defined. [28][29]

Out of 210 therapeutic small molecules, 17 are derived from plants, according to the WHO's Model List of Essential Medicines. These phytoconstituents have a lot of potential despite their high molecular size and low lipid solubility. These medications are poorly absorbed and only reach their target regions of action at low concentrations due to their low bioavailability. The current formulations of these phytomedicines have a variety of additional problems in addition to not being target-specific.

Recently, there has been a lot of interest in the creation of innovative drug delivery systems based on herbal substances such phytoconstituents or extract. Many scientists are using nanotechnology as a delivery system to spread pharmaceuticals made from plants. As pharmaceutical medication composition has improved, herbal dosage forms have evolved from straightforward tablets, mixes, and solutions to highly complex drug-delivery systems.

Stability has enhanced as a result of the delivery of herbal products using cutting-edge technologies. The toxicity and pre-systemic metabolism of herbal medications have also been decreased due to the buildup of chemicals in non-targeted locations. Using a special DDS to target pharmacologically active substances to certain bodily areas is one way to overcome the substantial issues with herbal drugs. Carriers can be utilised to control how herbal and synthetic medications are distributed throughout the body, allowing them to work faster and more efficiently.

Pharmaceutics, molecular biology, polymer science, bioconjugate chemistry, as well as special physicochemical properties like controllable size, high reaction rates, large surface area to mass ratios, and functional ability, can be combined to create a cutting-edge drug delivery system. By using nano-formulations, which have superior pharmacokinetic and pharmacodynamic qualities as well as increased chemical and physical stability, it is possible to get beyond the limitations of conventional herbal formulations, such as solubility and bioavailability.

These methods address the underlying flaws in the herbal formulations that are currently used [83]. For site-specific drug delivery, the medication's increased surface area results in quicker absorption and action at the intended location. Hydrophilic herbal extracts may traverse the Blood-Brain Barrier (BBB) more effectively than with conventional methods when administered with nanocarrier drugs. [30]

It has been demonstrated that new DDS, like as nanoparticles, nanocapsules, liposomes, and phytosomes, are better suited than previous techniques for delivering phytomedicine components. In addition to providing physical and chemical stability that lengthens the drug's half-life, these delivery vehicles have increased pharmacological efficacy, bioavailability, and delivery time.

One strategy for increasing the potency of herbal formulations while reducing the danger of side effects is novel drug delivery. In recent years, there has been a lot of interest in the creation of innovative methods for delivering herbal medicines. Many scientists are working to develop innovative delivery methods for natural medicines. Herbal dosage forms have changed from simple mixes and tablets to incredibly complicated technology-based medication delivery systems as pharmaceutical medicine research advances in science and technology. The stability of plant-based medicines has increased thanks to the application of modern and contemporary technology. Pre-systemic metabolism and the negative effects of herbal medicines are decreased by drug accumulation in non-targeted locations.The various health advantages that herbal medicines offer have sparked a growing interest in finding novel ways to use them. Herbal medications can be delivered in nano-formulations using polymeric nanoparticles, liposomes, Cubosomes, ethosomes, nano emulsions, transferosomes, and phytosomes. [31]

Cubosomes

The name "Cubosomes" derives from its structure, which is "phases" followed by "some" and their cubic crystal lattice. Cubosomes, also known as "viscous isotropic phases," are bicontinuous cubic phase liquid crystals with non-intersecting hydrophilic areas and non-intersecting lipid bilayers that are twisted into periodic minimum surfaces with zero curvature. According to Larsson, who used X-ray diffraction, NMR, and other techniques to study the structure of aqueous monoglyceride cubic phases, the cubosomes have continuous regions that are both hydrophobic and hydrophilic in nature. This discovery led Larsson to the conclusion that the cubic phase structures can be explained by the concept of differential

geometry and periodic minimal surfaces. Cubosomes are nanoparticles or more precisely, nanostructured particles that self-assemble into liquid crystals with the rheology of a solid. [32-37]

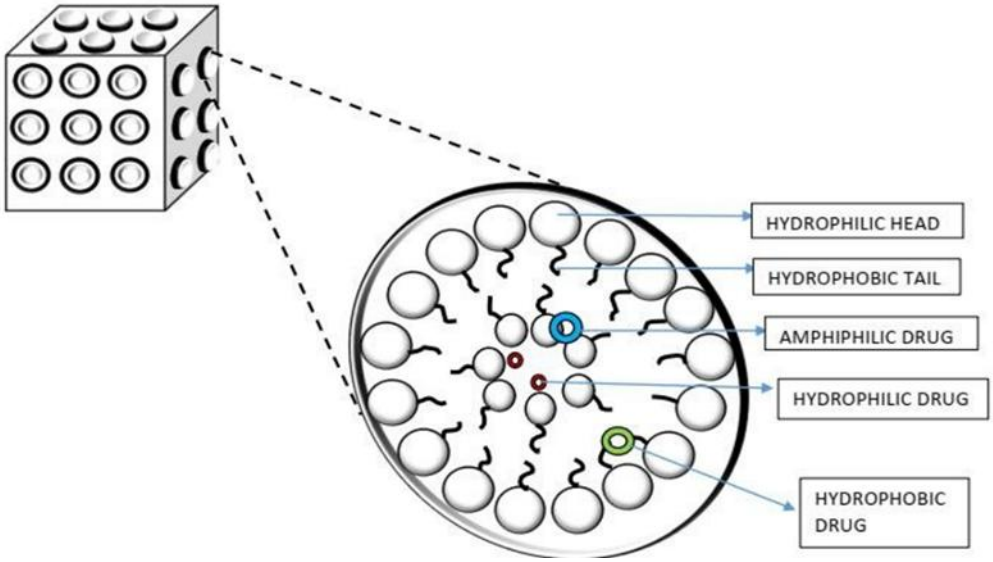


Figure-5: 3D Representation of cubosome

Cubosomes were provided, created, and tested for medication administration because they adhere to the geometric concept. Cubosomes are the name given to the particles that are originally created by the mechanical fragmentation of the lipid-water cubic phase in a three-cubic phase area that contains a liposome dispersion and differs from liposomes. The cubosome structure preserves the effectiveness and stability of substances like proteins and vitamins. Cubosomes occur in an aqueous surfactant solution with a reasonably high concentration of amphiphile and have a sufficient average degree of molecular orientation to be recognised by structural symmetry. [38]

Top-down Approach

The bulk cubic phase is first created and then dispersed by high energy processing into Cubosomes nanoparticles, making it the most often employed method in research. While cubic phases resemble a liquid crystalline structure, bulk cubic phases resemble a clear, rigid gel made of water-swollen, crossed-linked polymer chains.4 The cubic phases have a yield stress that rises when the amount of oils and surfactants that form bilayers is increased. [37]

Structure-forming lipids and stabilisers are combined to create the extreme viscous bulk phase, which is subsequently dispersed into aqueous solution using high energy (such as high-pressure homogenization [HPH], sonication, or shearing) to produce LLC nanoparticles. Currently, HPH is the method most frequently used to create LLC nanoparticles (cubosomes).

Bottom-up approach

Cubosomes are permitted to develop or crystallise in this process from their progenitors. According to Almgren et al., Cubosomes are formed by spreading L2 or inverse micellar phase droplets in water at 80 °C, allowing the droplets to gently cool, and then crystallising the droplets into Cubosomes. [36] This is more durable when Cubosomes are produced on a wide scale. By combining aqueous poloxamer 407 solution with monoolein-ethanol solution, Spicer et al. created cubosomes at room temperature. By emulsifying, the cubosomes spontaneously develop. Hydrotrope, which can breakdown water-insoluble lipids to produce liquid precursors and stop the production of liquid crystals at high concentrations, is a fundamental component of the bottom-up strategy. This dilution-based strategy, as opposed to the top-down strategy, may create Cubosomes without time-consuming fragmentation. [39]

Mechanism of drug release from cubosomes

Several investigations have indicated that the integrated drug's physiochemical characteristics characterise the drug's release from cubosomes. As seen in, One of two principles—the partition coefficient and drug diffusion inside the lipid bilayer or drug diffusion into an aqueous environment—controls the release of drugs from cubosomes. if the integrated substance is hydrophilic in nature. For hydrophobic medications, the diffusion mechanism is all that is needed to release the pharmaceuticals from cubosomes. A concentration gradient was discovered to be critical for the release of drugs from cubosomes in several similar investigations. The Higuchi or Fick Diffusion equation may be the greatest fit for describing the release of drugs from cubosomes, based on all of the drug release principles discovered. [39-40]

Preparation methods for cubosomes

Many medical applications need homogenous particle size distribution in nanostructured aqueous dispersion. It could be useful to create a range from two perspectives: from the top down or the bottom up. A colloidal stabilizer, such as P407, is needed in both methods to avoid cubosome dispersion aggregation, notwithstanding the variations. Many pharmaceutical applications depend on the ability to make nanostructured aqueous

dispersions with homogenous particle sizes. For therapeutic delivery, cubosomes have been prepared using a variety of techniques. For parenteral dosage forms, the usage of cubosomes as pharmaceutical dosage forms is on the rise because of their improved compatibility and easy manufacturing method, The following are some of the ways that have been describe

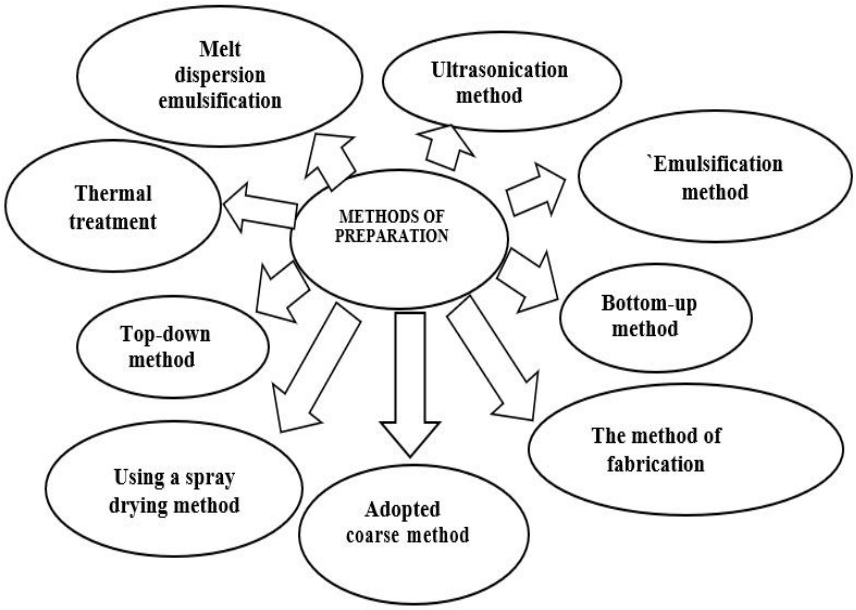


Figure-6: Different methods of preparation

Intranasal drug delivery system-

When it comes to treating central nervous system (CNS) illnesses, Small cubosomes of odorranalectin have been developed for the administration of mucosal medicines to the brain through the intranasal route. [31][41]

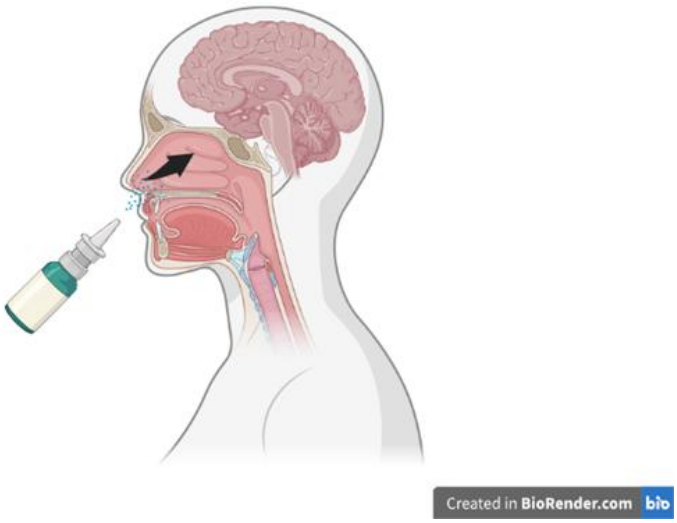


Figure-7: Nasal administration of cubosomal anti-depressant formulation

CHAPTER-2

LITERATURE REVIEW

LITERATURE REVIEW

Kumar et al., (2018) reviewed features 65 phytochemically studied species of Solanum between 1990 and 2018, fetched from SciFinder, Pubmed, ScienceDirect, Wikipedia and Baidu, using “Solanum” and the species’ names as search terms (“all felds”). Previous phytochemical investigations on Solanum species led to the identifcation of steroidal saponins, steroidal alkaloids, terpenes, favonoids, lignans, sterols, phenolic comopunds, coumarins, amongst other compounds. Many species belonging to this genus present huge range of pharmacological activities such as cytotoxicity to diferent tumors as breast cancer (4T1 and EMT), colorectal cancer (HCT116, HT29, and SW480), and prostate cancer (DU145) cell lines. The biological activities have been attributed to a number of steroidal saponins, steroidal alkaloids and phenols.

Elizalde-Romero et al., (2021) review provided the information about antioxidant compounds, their bio-accessibility and bioavailability, and their health-promoting effects. Bio-accessibility and bioavailability studies must be considered when evaluating the bioactive properties of health-promoting molecules like those from the Solanum genus. Various reports have shown that flavonoids, phenolic acids, alkaloids, saponins, and other molecules can be found in these plants. These molecules are associated with various health-promoting properties against many non-communicable diseases, the main causes of death globally. Nonetheless, the transformations of the structure of antioxidants caused by cooking methods and gastrointestinal digestion impact their potential benefits and must be considered.

Salehi et al., (2019) Naringenin is a flavonoid belonging to flavanones subclass. the data reported have been obtained from *in vitro* or *in vivo* studies. Although some clinical studies have also been performed, the main focus is on naringenin bioavailability and cardioprotective action. In addition, these studies were done in compromised patients (i.e., hypercholesterolemic and overweight), with a dosage ranging between 600 and 800 μM/day, whereas the effect on healthy volunteers is still debatable. In fact, naringenin ability to improve endothelial function has been well-established. Indeed, the currently available data are very promising, but further research on pharmacokinetic and pharmacodynamic aspects is encouraged to improve both available production and delivery methods and to achieve feasible naringenin-based clinical formulations.

Tutunchi et al., (2020) reviewed, the effects and possible mechanisms of action of naringenin, a citrus-derived flavonoid, against COVID-19 were discussed. They searched PubMed/Medline, Science direct, Scopus, and Google Scholar databases up to March 2020 using the definitive keywords. The evidence reviewed here indicates that naringenin might exert therapeutic effects against COVID-19 through the inhibition of COVID-19 main protease, 3-chymotrypsin-like protease (3CLpro), and reduction of angiotensin converting enzyme receptors activity. One of the other mechanisms by which naringenin might exert therapeutic effects against COVID-19 is, at least partly, by attenuating inflammatory responses. The antiviral activity of the flavanone naringenin against some viruses has also been reported.

Elzoghbya et al., (2016) The nanoparticles are discussed with respect to their formulation aspects, advantages, limitations, as well as the major outcomes of the in vitro and in vivo investigations. Modification of the nanoparticle surface with specific brain targeting ligands or by coating with certain surfactants for enhanced brain delivery is also reviewed. In addition, the mechanisms of the nanoparticle-mediated drug transport across the BBB are also discussed.

Peng et al., (2015) the cubosomes, which are based on Glycerol Monooleate, were created as a focused and sustained manner of transdermal delivery of capsaicin. In addition to shape and particle size distribution, the cubosomes were also studied using transmission electron microscopy (TEM) and photon correlation spectroscopy. As a result of tiny angle scattered X-rays, their crystallographic space group Im3m has been verified. A study of capsaicin release from cubosomes in vitro demonstrated that they offered a sustained release mechanism, according to an in vitro diffusion investigation employing Franz diffusion cells. The cubosome formulations were shown to be stable for up to 10 days when exposed to intense light and high temperatures. Cubosomes and cream containing capsaicin irritated mouse skin very slightly, causing little adverse effects. Cubosomes may be a viable skin-targeted and sustained delivery method for capsaicin transdermal treatment, according to the study.

Aleandri et al., (2015) As an alternative method for developing dose delivery systems capable of actively targeting specific cancerous cells surfaces which are (over)expressed, biotin-functionalized cubosomes have been disclosed. Using SAXS and dynamic laser light scattering(DLS), we detail the design, syntheses, assembly, and characterization of these new cubosome nanoparticles and demonstrate their applicability to the human adenocarcinoma

cell line Hela.As a result of the use of a biotin-based block copolymer, these cubosomes may transport paclitaxel, an effective anticancer medication, plus a hydrophobic fluorescent dye to cancer cells. As a result, biotinylated cubosomes might be used for cancer cell targeting, medication delivery, and monitoring of treatment response.

Azmi et al., (2014) the nonlamellar liquid crystalline states are interesting substrates for medicament dissolution and tailored delivery, according to the report. Amphiphilic, hydrophobic, and hydrophilic medicines can be solubilized and released with this formulation principle due to its nano structural flexibility, compatibility, digestibility, and bioadhesive characteristics, as well as its capacity to solubilize and maintain medicine release. In the progression in the mechanism for delivering drugs, nonlamellar liquid crystalline states provide2 different and hopeful approaches. Cubosomes and hexosomes are examples of ISAsomes (internally self-assembled'somes' or particles), as are parenteral dosage forms with adjustable nanostructures that are formed in situ at the point of delivery. Review of the unique properties of cubosomes and hexosomes as potential medication delivery devices.

Nanjwade et al., (2014) the determination and learning of cubosomes is an identical history that reaches decades. When amphiphilic lipid systems are placed in an aqueous natural habitat, they self-assemble into nanostructured dispersed particles known as "Cubosomes," which range in size from 10 to 500 nanometers in diameter. This is because cubosomal drug delivery systems have significant promise for use in melanoma treatment. It has become more popular to study cubosomes because to its thermodynamically stable cubic shape, biodegradability, and the ability to encapsulate hydrophobic and hydrophilic molecules. A cuboidal drug delivery device will be reviewed in terms of production processes, characterization and applications.

Zhou et al., (2014) Cubosome nanoparticles were analyzed quantitatively using HPLC. By theuse of an isocratic system, the chromatographic technique was conducted. Methanol-PBS (pH6.8)-triethylamine (50:50:0.1%) made up the mobile phase, which was pumped at 1 ml/minat a wavelength of 322 nm. The acceptable size or diameter (the regression equation is A=10835C+1058; R=1.0) and complete regaining are required to separate Sinomenine from its metabolites (102.2 %). In cubosome nanoparticles, the chromatography procedures were suitable for quality control.

Pan et al., (2013) "cubic phase" is formed when certain amphiphilic lipids automatically

self- assemble in case of moisture. Different cubic stages can encapsulate hydrophilic, amphiphilic,and hydrophobic medicines in order to deliver. By fragmenting cubic phase gels or using lyotropic techniques, nanostructured cubosomes preserve the cubic phase's internal structure, but have a considerably greater surface area and reduced viscosity. This makes cubosomes effective drug delivery vehicles for oral, mucosal, transdermal, and parenteral drug administration, due to their unique characteristics. An overview of cubosome research, including its production, features, and pharmaceutic uses, was provided in this article.

Ravindran et al., (2013) Transporting numerous medicines over biological membranes is difficult. Hydrophilicity also hinders the development of several medicines. Such medicines have a poor bioavailability, which is determined by their capacity to penetrate the membrane, and considerable intra- and intrasubject variability. At the moment, formulation scientists are working on a number of initiatives to distribute active chemicals via transdermal, nasal, and other routes. It is wise to study alternate delivery methods. As a systemic and topical delivery vehicle, cubosomes allow active substances to be transported and customized. Bicontinuous cubic liquid crystal phase cubosomes are self-assembling nanoparticles of lipid and surfactant systems that are distributed or self-assembled. The most often used components in cubosomes formulation are monoolein, poloxamer 407, and polyvinyl alcohol (PVA). Cubosomes' internal and structural alterations can be controlled by changing their lipid content. Pn3m, la3d, and Im3m are three architectures of cubosomes based on nodal surfaces. Formulating a very viscous bulk material and an aggregate from a precursor using top down or bottom-up methods is common. Cubosomes have been studied and applied for a long time. This page offers a bird's eye view of cubosome engineering, characterization, and assessment.

CHAPTER-3
AIMS
&
OBJECTIVES

AIMS AND OBJECTIVES

AIM

To Develop and optimize the *Solanum lycopersicum* extracts for the evaluation of the antidepressant activity based nasal drug delivery.

OBJECTIVE

The objectives of the proposed research work were:

- Selection of medicinal plants based on experimental evidences and traditional use
- Preparation of extracts
- Formulation of Cubosomes of extracts for better bioavailability
- Characterization of Cubosomes such as particle size determination, drug contents,
- Entrapment efficacy, spectroscopic evaluation etc.
- To evaluate optimized formulations.

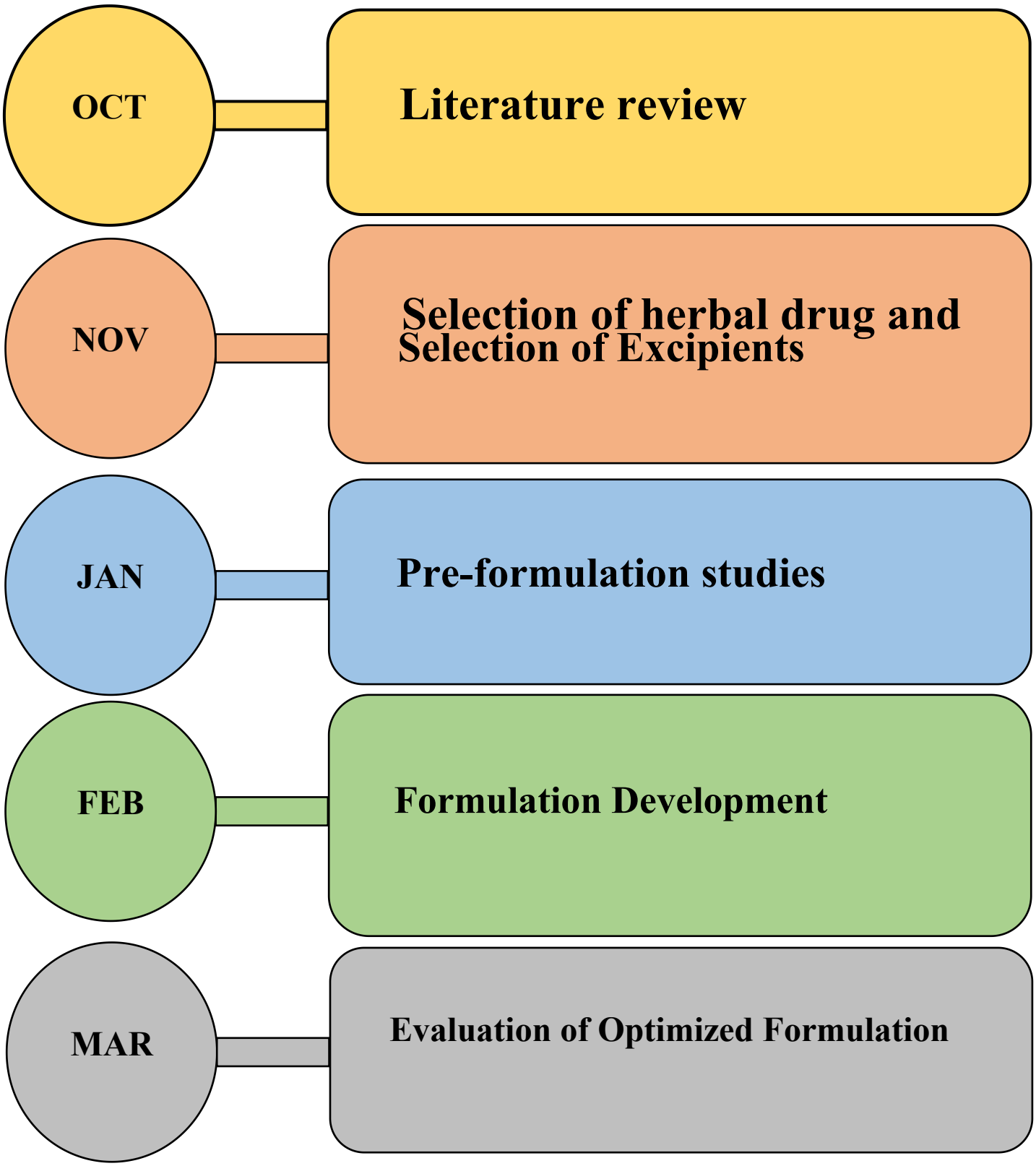
EVALUATION PARAMETERS

- Physical appearance
- FTIR spectroscopy
- Percentage yield and Entrapment efficiency
- In-Vitro release study

CHAPTER 4

PLAN OF WORK

PLAN OF WORK


OCT
Literature review
NOV
Selection of herbal drug and Selection of Excipients
JAN
Pre-formulation studies
FEB
Formulation Development
MAR
Evaluation of Optimized Formulation

CHAPTER 5

PLANT PROFILE

PLANT PROFILE

Figure-8: *Solanum lycopersicum* and microscopy (peel)

Phytochemical investigations include various steps like:

- Collection, identification and authentication of plant material.
- Preparation of extract and various fractions.
- Phytochemical screening for identification of chemical components.

Collection of Plant Material

The plant of *Solanum Lycopersicum* were gathered from the neighborhood Greater Noida, Swarn Nagri market, India.

Drying of Plant Material

- The plant material was shed dried.
- Course powder of Plant Material.

The *Solanum Lycopersicum* were squashed and afterward powdered with the assistance of manual homogenizer and blender.

Extraction

Extraction is a separation process, consisting of separating a substance from a matrix.

Preparation of Crude Extract

The powdered plant material was extracted by Successive extraction measure, utilizing petrol ether, chloroform, methanol, ethanol and water,The quantity of the solvent used was 500 ml. The crude extract obtained was collected and concentrated in a oven at 70°C.

Percentage Yield of Extract

The percentage yield of the crude extract of seeds was calculated by using the formula given below-

Percentage Yield = Weight of Extract (gm)/ Weight of Dry Powder (gm) ×100

Table-2: Successive solvent extraction using a different solvent with time duration and temperature.

S. No.	Solvent	Quantity (ml)	Temp. (°C)	Duration
1.	Petroleum Ether	500	60	3 hrs.
2.	Chloroform	500	61.2	3 hrs.
3.	Methanol	500	64.5	6 hrs.
4.	Ethanol	500	78	8 hrs.
5.	Water	500	100	8 hrs.

Preliminary Phytochemical Studies

The extract of the *Solanum Lycopersicum* was subjected to different chemical tests separately, for the identification of various active phytoconstituents. The tests were as follows-

Tests for Alkaloids

Dragendroff's Test:

Dragendroff's reagent (potassium bismuth iodide solution) was combined with the test solution. Alkaloids were present because a reddish-brown precipitate formed.

Wagner's Test:

Iodine potassium iodide solution and the test solution were combined. Alkaloids were present, as evidenced by the precipitate's reddish-brown color.

Mayer's Test:

Test solution (1mL) was mixed with one drop of Mayer's reagent (mixture of mercuric chloride and potassium iodide). Formation of a cream-colored precipitate confirmed the presence of alkaloids.

Hager's Test:

Test solution was mixed with Hager's reagent (saturated solution of picric acid). Formation of a yellow precipitate confirmed the presence of alkaloids.

Tests for Steroids

Libermann Burchard Test:

Test solution was treated with a few drops of acetic anhydride, boiled and cooled. Then, concentrated sulphuric acid was added from the side of the test tube. A brown ring was formed at the junction of two layers and the upper layer turned green which showed the presence of steroids and formation of a deep red color indicated the presence of triterpenoids.

Salkowski Test:

Test solution was treated with 1 mL of chloroform. Then, 1 mL of concentrated sulphuric acid was added carefully and shaken gently. A reddish-brown color in the lower layer indicated the presence of a steroidal ring, i.e., glycone portion of the glycoside.

Tests for Flavonoids:

Test solution was mixed with 20% sodium hydroxide solution, a yellow colour was formed which disappeared on addition of dilute HCl.

Tests for Saponins

Foam Test:

Test solution was mixed with 5 mL of distilled water in a test tube and then it was shaken vigorously. The formation of a stable foam was taken as the indication for the presence of saponins.

Tests for Glycosides

Legal Test:

Test solution was treated with pyridine and alkaline sodium nitroprusside solution was added. Appearance of blood red color indicated the presence of glycosides.

Baljet Test:

Test solution was treated with picric acid. Formation of an orange color indicated the presence of glycosides.

Tests for Amino Acids

Ninhydrin Test:

Test solution when boiled with 0.2% solution of ninhydrin, formed a violet color which indicated the presence of amino acids.

Tests for Proteins

Millon's Test:

Test solution, when mixed with 2 mL of Millon's reagent (solution of mercuric nitrate and nitrous acid), gave a white precipitate which turned red upon gentle heating, confirming the presence of proteins.

Tests for Phenolic Compounds

Test solution was treated with ferric chloride solution. The formation of a blue or a green colour indicated the presence of phenolic compounds.

Tests for Fixed Oils

Spot Test:

A small quantity of test solution was pressed between filter papers. Oil stains on the paper indicated the presence of fixed oils.

Tests for Carbohydrates

Molisch's Test:

Test solution was mixed with few drops of Molisch's reagent (alcoholic α-naphthol) and the mixture was shaken properly. Few drops of concentrated sulphuric acid were added carefully, along the side of the test tube. Appearance of a violet ring at the inter-phase indicated the presence of carbohydrates.

Fehling's Test:

Equal volume of Fehling A and Fehling B reagents were mixed together and 2ml of test solution was added to it and boiled gently. A brick red precipitate appeared at the bottom of the test tube indicating the presence of reducing sugars.

Benedict's Test:

Test solution, when mixed with 2 mL of Benedict's reagent and boiled, gave a reddish-brown precipitate indicating the presence of carbohydrates.

CHAPTER 6

PREFORMULATION STUDIES

Preformulation Studies

Preformulation studies are a series of tests and experiments conducted to understand the physicochemical properties of a drug substance and to establish the feasibility of developing a stable and effective drug product. These studies are conducted during the early stages of drug development and are an important part of the drug development process.

The primary objectives of preformulation studies are to:

1. Characterize the physicochemical properties of the drug substance, such as solubility, melting point, stability, particle size, and polymorphism.

2. Determine the compatibility of the drug substance with excipients and the effect of the manufacturing process on the drug substance.

3. Identify potential degradation pathways and degradation products.

4. Establish the appropriate formulation and manufacturing process for the drug product.

Melting Point

Melting point is the temperature at which a solid substance changes its state from solid to liquid at a specific atmospheric pressure. At the melting point, the solid and liquid phases of the substance are in equilibrium with each other.

The melting point of a substance depends on various factors, including the strength of the intermolecular forces between its particles, the size and shape of its molecules, and the presence of impurities or other substances.

The melting point is a physical property that is often used to identify and characterize substances, particularly in chemistry and materials science. It is typically measured using a melting point apparatus, which heats a sample of the substance gradually while monitoring its temperature until it melts completely. The melting point of a substance is usually reported in degrees Celsius or Fahrenheit.

FTIR

FTIR stands for Fourier Transform Infrared Spectroscopy. It is a type of analytical technique used in chemistry to identify and quantify the chemical bonds present in a sample.

In FTIR spectroscopy, infrared light is passed through a sample and the resulting spectrum is analyzed. The spectrum contains information about the molecular vibrations within the sample, which can be used to identify the functional groups and chemical bonds present.

The Fourier transform is used to convert the raw data obtained from the spectrum into a usable format. This allows for the identification of specific peaks in the spectrum, which correspond to specific molecular vibrations.

FTIR spectroscopy is commonly used in the analysis of organic compounds, as well as in the characterization of polymers, pharmaceuticals, and other materials. It is a powerful analytical tool that can provide information about the chemical composition of a sample, as well as its molecular structure and bonding.

CHAPTER 7

FORMULATION AND DEVELOPMENT

Formulation and Development

Materials and Method

Materials

Cubosomes were formulated by using Top and Bottom method by using homogenizer and utilizing

Materials

- PLG
- GMO
- PLGA
- SOBITOL
- DISTILLED WATER

Top-down Approach

The bulk cubic phase is first created and then dispersed by high energy processing into Cubosomes nanoparticles, making it the most often employed method in research. While cubic phases resemble a liquid crystalline structure, bulk cubic phases resemble a clear, rigid gel made of water-swollen, crossed-linked polymer chains.The cubic phases have a yield stress that rises when the amount of oils and surfactants that form bilayers is increased.

Structure-forming lipids and stabilisers are combined to create the extreme viscous bulk phase, which is subsequently dispersed into aqueous solution using high energy (such as high-pressure homogenization [HPH], sonication, or shearing) to produce LLC nanoparticles. Currently, HPH is the method most frequently used to create LLC nanoparticles (cubosomes).

Bottom-up approach

Cubosomes are permitted to develop or crystallise in this process from their progenitors. According to Almgren et al., Cubosomes are formed by spreading L2 or inverse micellar phase droplets in water at 80 °C, allowing the droplets to gently cool, and then crystallising the droplets into Cubosomes. This is more durable when Cubosomes are produced on a wide scale. By combining aqueous poloxamer 407 solution with monoolein-ethanol solution, Spicer et al. created cubosomes at room temperature. By emulsifying, the cubosomes spontaneously develop. Hydrotrope, which can breakdown water-insoluble lipids to produce liquid precursors and stop the production of liquid crystals at high concentrations, is a

fundamental component of the bottom-up strategy. This dilution-based strategy, as opposed to the top-down strategy, may create Cubosomes without time-consuming fragmentation.

Table-3: Factorial design of formulation

FACTORIAL DESIGN	PLG {Peptide} [mg]	GMO {Carrier lipid} [mg]	PLGA {Polymer} [mg]	DW [ml}	SORBITOL {Carrier} [ml]
F1	15	100	10	50	500
F2	20	100	10	50	500
F3	25	100	10	50	500
F4	15	100	15	50	500
F5	20	100	15	50	500
F6	25	100	15	50	500
F7	15	100	20	50	500
F8	20	100	20	50	500
F9	25	100	20	50	500
F10	20	100	20	50	500

Characterization of Formulation

Encapsulation efficiency

The drug entrapment of prepared formulation of the 5-fluorouracil loaded cubosomes with different polymer was done by centrifugation method. 1ml of the formulation was taken and mixed with methanol and sonicated if required for 10-15 min to obtain the clear homogenous mixture. The mixture was centrifuged at high speed for around 30-45 min at 37°C. Supernatant was carefully separated and diluted further if needed. The amount of the drug was calculated spectrophotometrically at 283nm as λmax.

$$Entrapment\ efficiency\ (\%) = (Calculated\ drug\ content/\ Theoretical\ drugcontent) \times 100$$

Drug Release

Drug release refers to the process by which a drug is released from a dosage form or drug delivery system (such as a tablet, capsule, patch, or implant) and becomes available for absorption by the body. The rate and extent of drug release can have a significant impact on the drug's therapeutic efficacy, safety, and pharmacokinetics.

There are several factors that can affect drug release, including the chemical and physical properties of the drug and the dosage form, the formulation and manufacturing process, and the physiological environment of the site of administration.

Various techniques can be used to control drug release, such as modifying the drug's chemical structure, altering the formulation of the dosage form, or using specialized drug delivery systems that can release the drug at a specific rate or location in the body.

The goal of drug release control is often to optimize the therapeutic effect of the drug, minimize side effects, and improve patient compliance by reducing the frequency of dosing.

CHAPTER 8

RESULT AND DISCUSSION

RESULT AND DISCUSSION

RESULTS OF PHYTOCHEMICAL INVESTIGATION OF *Solanum lycopersicum*

Percentage Yield of the Aqueous Extract of *Solanum lycopersicum*

Table-4: Percentage Yield of *Solanum lycopersicum*

S. No.	Solvent	Quantity (ml)	Temp. (°C)	Duration	%Yield
1.	Petroleum Ether	500	60	3 hrs.	10.5%
2.	Chloroform	500	61.2	3 hrs.	12%
3.	Methanol	500	64.5	6 hrs.	45%
4.	Ethanol	500	78	8 hrs.	68%
5.	Water	500	100	8 hrs.	21%

Preliminary Phytochemical Screening

Table-5: Phytochemical testing of *Solanum lycopersicum*

Test of alkaloids

S.NO	test	present	absent
1.	Dragendroff's/ Kraut's test	✓	-
2.	Hager's test	-	×
3.	Mayer's/ Bertrand's/ Valser's test	-	×
4.	Wagner's test	-	×
5.	Picric acid test	-	×
6.	Iodine Test	-	×

Test of flavonoids

S.NO	test	present	absent
1.	Alkaline reagent test	✓	-
2.	Lead acetate test	✓	-
3.	Shinoda's test/ Mg-hydrochloride		

	reduction test	✓	-
4.	Ferric chloride test	✓	-
5.	Conc. H2SO4 test	✓	
6.	Ammonia test	✓	

Test of glycoside

s.no	test	present	absent
1.	Killer killani test	✓	-
2.	Legal test	✓	-
3.	Liebermann test	✓	-
4.	coumarins	-	×

Test of phenolic acid

s.no	Test	present	absent
1.	Iodine test	✓	-
2.	Ferric chloride test	✓	-
3.	Gelatin test	✓	-
4.	Lead acetate test	✓	-
5.	Test for Cartenoids	✓	-
6.	Potassium dichromate test	-	×
7.	Ellagic Acid Test	-	×

Test of tannins

s.no	Test	present	absent
1.	Gelatin test	✓	-
2.	Braymer's test	✓	-
3.	Bromine water test	✓	-
4.	10% NaOH test	✓	-

Test of anthocyanin

1.	Hcl test	✓	-

(✓) indicates presence of particular constituents

Results of Pre-formulation and Formulations

Table-6: Melting point of Polymers are

S.No.	Compound	Theoretical Value	Practical Value
1.	PLGA	45-50°C	49°C
2.	GMO	45-60°C	55°C
3.	SOBITOL	95-98°C	98°C

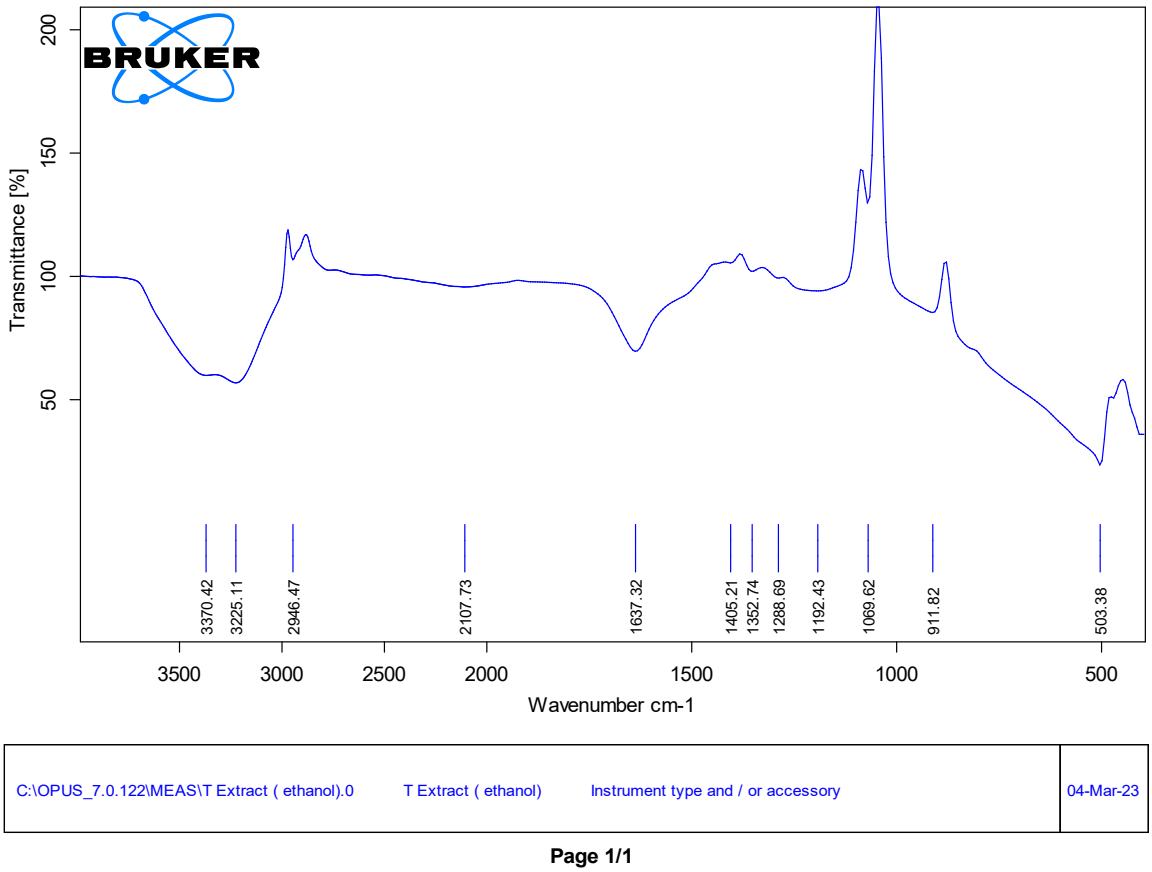


Figure-9: FTIR of Ethanolic Extract

Formulation Result

In- Vitro Release

Table shows the in vitro drug release profile of the optimized batch of cubosomes containing ethanolic plant leaves. The results demonstrated that the cubosomal

formulation's in vitro drug release was greatly improved for up to 2 hours. % release of drug absorbance shown in table and represented graphically.

Table-7: Cubosomal formulation's in vitro drug release

Time points (mins)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
5	5.637	6.524	8.951	7.438	5.602	6.201	8.304	7.501	8.394
10	13.807	11.630	12.102	14.426	16.589	15.207	11.964	13.520	12.695
15	18.361	17.555	18.300	19.644	21.433	20.690	18.354	17.329	21.213
30	27.652	28.420	29.200	24.066	26.231	28.366	29.590	27.496	24.280
45	34.527	31.512	33.504	35.688	36.450	34.548	33.546	31.958	40.357
90	41.258	45.291	39.563	43.860	41.357	42.298	45.316	39.679	43.619
120	48.666	51.203	53.428	55.368	57.264	51.258	54.963	52.659	57.843

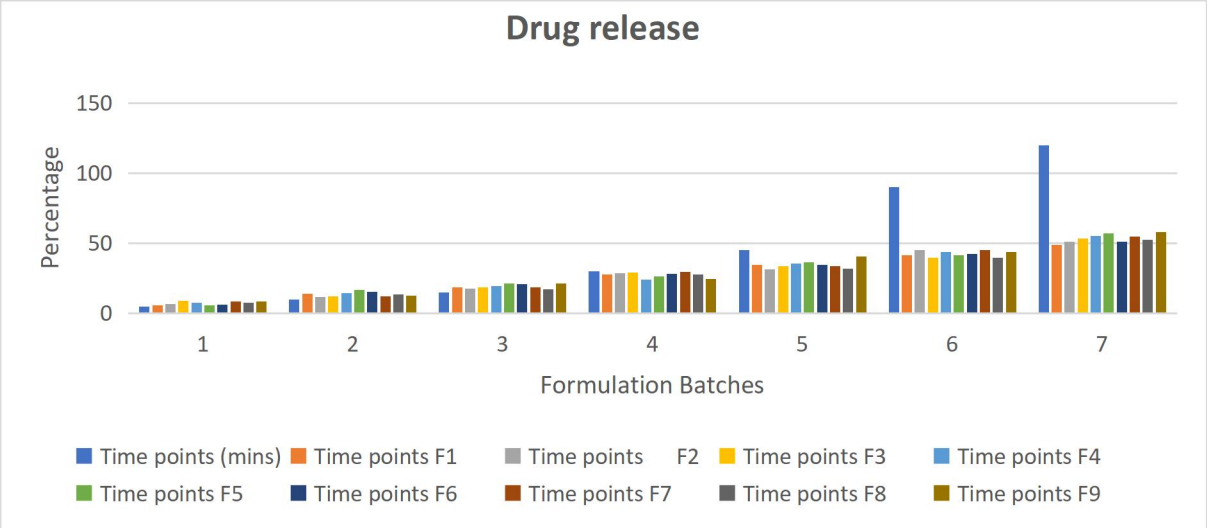


Figure-10: Graphical representation of drug release

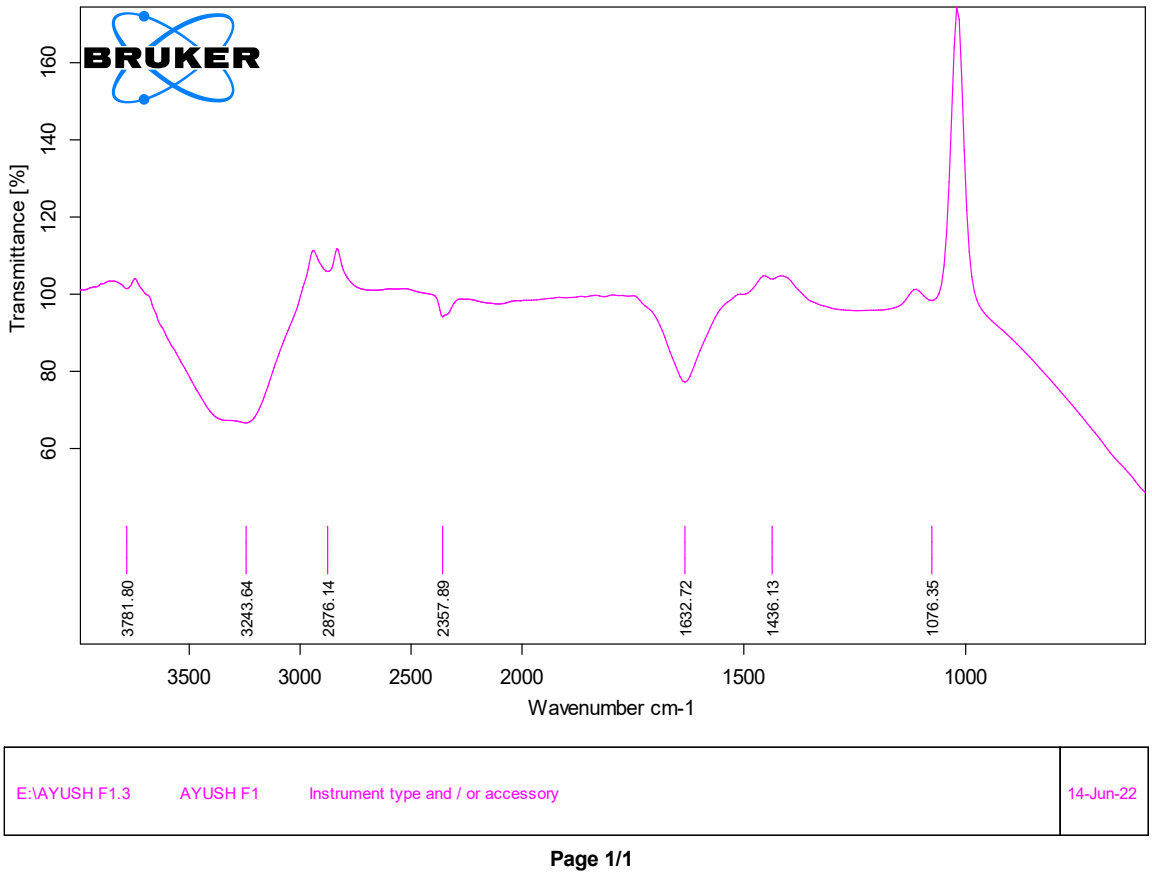


Figure-11: FTIR of cubosomal Formulation

Morphology of optimized formulation

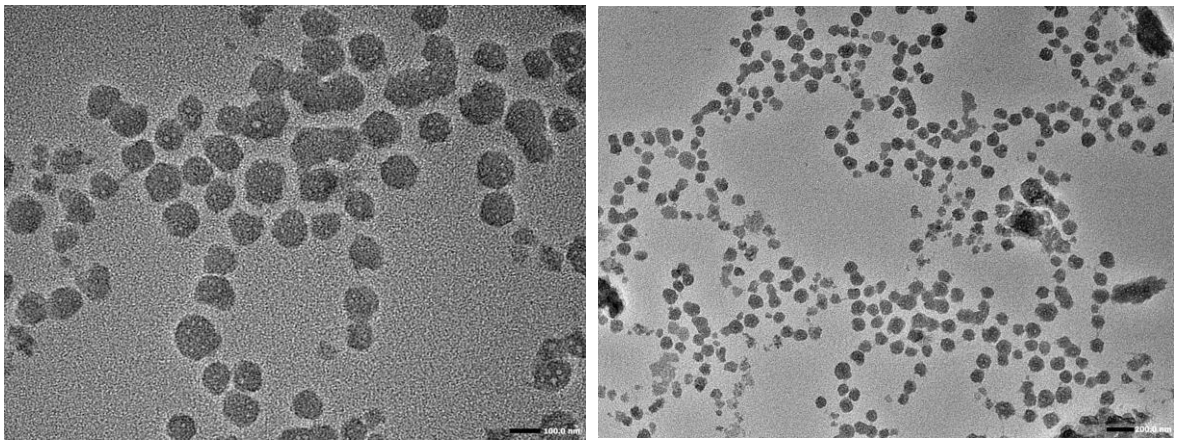


Figure-12: TEM image of cubosomal Formulation

CHAPTER 9

REFERENCE

REFERENCES

1. Trivedi MH, Rush AJ, Wisniewski SR, Nierenberg AA, Warden D, Ritz L, Norquist G, Howland RH, Lebowitz B, McGrath PJ, Shores-Wilson K. Evaluation of outcomes with citalopram for depression using measurement-based care in STAR* D: implications for clinical practice. American journal of Psychiatry. 2006 Jan 1;163(1):28-40.
2. Sullivan PF, Neale MC, Kendler KS. Genetic epidemiology of major depression: review and meta-analysis. American journal of psychiatry. 2000 Oct 1;157(10):1552-62.
3. Mann JJ, Malone KM, Sweeney JA, Brown RP, Linnoila M, Stanley B, Stanley M. Attempted suicide characteristics and cerebrospinal fluid amine metabolites in depressed inpatients. Neuropsychopharmacology. 1996 Dec;15(6):576-86.
4. Meltzer CC, Price JC, Mathis CA, Butters MA, Ziolko SK, Moses-Kolko E, Mazumdar S, Mulsant BH, Houck PR, Lopresti BJ, Weissfeld LA. Serotonin 1A receptor binding and treatment response in late-life depression. Neuropsychopharmacology. 2004 Dec;29(12):2258-65.
5. Charney DS. Monoamine dysfunction and the pathophysiology and treatment of depression. Journal of Clinical Psychiatry. 1998 Jan 1;59(14):11-4.
6. Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, McClay J, Mill J, Martin J, Braithwaite A, Poulton R. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science. 2003 Jul 18;301(5631):386-9.
7. Ressler KJ, Nemeroff CB. Role of norepinephrine in the pathophysiology of neuropsychiatric disorders. CNS spectrums. 2001 Aug;6(8):663-70.
8. Nemeroff CB, Entsuah R, Benattia I, Demitrack M, Sloan DM, Thase ME. Comprehensive analysis of remission (COMPARE) with venlafaxine versus SSRIs. Biological psychiatry. 2008 Feb 15;63(4):424-34.
9. Dunlop BW, Nemeroff CB. The role of dopamine in the pathophysiology of depression. Archives of general psychiatry. 2007 Mar 1;64(3):327-37.
10. Meyer JH, Krüger S, Wilson AA, Christensen BK, Goulding VS, Schaffer A, Minifie C, Houle S, Hussey D, Kennedy SH. Lower dopamine transporter binding potential in striatum during depression. Neuroreport. 2001 Dec 21;12(18):4121-5.
11. Nestler EJ, Carlezon Jr WA. The mesolimbic dopamine reward circuit in depression. Biological psychiatry. 2006 Jun 15;59(12):1151-9.
12. MacMillan HL, Patterson CJ, Wathen CN. Screening for Depression in Primary Care: Updated Recommendations From the Canadian Task Force on Preventive Health Care. London, Ontario, Canada: Canadian Task Force on Preventive Health Care. 2004 Jul.
13. Arthur A, Jagger C, Lindesay J, Graham C, Clarke M. Using an annual over-75 health check to screen for depression: validation of the short Geriatric Depression Scale (GDS15) within general practice. International journal of geriatric psychiatry. 1999 Jun;14(6):431-9.
14. Banerjee S, Shamash K, Macdonald AJ, Mann AH. The use of the SelfCARE (D) as a screening tool for depression in the clients of local authority home care services—a preliminary study. International journal of geriatric psychiatry. 1998 Oct;13(10):695-9.

15. Bashir K., Blizard R., Jenkins R. and Mann A., Validation of the 12-item general health questionnaire in British general practice, Primary Care Psychiatry, 2, 4-7 (2006)
16. Geerlings SW, Beekman AT, Deeg DJ, Tilburg WV, Smit JH. The Center for Epidemiologic Studies Depression scale (CES-D) in a mixed-mode repeated measurements design: sex and age effects in older adults. International Journal of Methods in Psychiatric Research. 1999 Jun;8(2):102-9.
17. Bird AS, Macdonald AJ, Mann AH, Philpot MP. Preliminary experience with the SELFCARE (D): A self-rating depression questionnaire for use in elderly, non-institutionalized subjects. International Journal of Geriatric Psychiatry. 1987 Jan;2(1):31-8.
18. Broadhead WE, Leon AC, Weissman MM, Barrett JE, Blacklow RS, Gilbert TT, Keller MB, Olfson M, Higgins ES. Development and validation of the SDDS-PC screen for multiple mental disorders in primary care.
19. Burnam MA, Wells KB, Leake B, Landsverk J. Development of a brief screening instrument for detecting depressive disorders. Medical care. 1988 Aug 1:775-89.
20. D'ATH PE, Katona P, Mullan E, Evans S, Katona C. Screening, detection and management of depression in elderly primary care attenders. I: The acceptability and performance of the 15 item Geriatric Depression Scale (GDS15) and the development of short versions. Family practice. 1994 Sep 1;11(3):260-6.
21. Fechner-Bates S, Coyne JC, Schwenk TL. The relationship of self-reported distress to depressive disorders and other psychopathology. Journal of consulting and clinical psychology. 1994 Jun;62(3):550.
22. Geisser ME, Roth RS, Robinson ME. Assessing depression among persons with chronic pain using the Center for Epidemiological Studies-Depression Scale and the Beck Depression Inventory: a comparative analysis. The Clinical journal of pain. 1997 Jun 1;13(2):163-70.
23. Gerety MB, Williams Jr JW, Mulrow CD, Cornell JE, Kadri AA, Rosenberg J, Chiodo LK, Long M. Performance of case-finding tools for depression in the nursing home: influence of clinical and functional characteristics and selection of optimal threshold scores. Journal of the American Geriatrics Society. 1994 Oct;42(10):1103-9.
24. Serio F, oSMAn AyAlA AB. AnD Pietro SAntAMAriA2. World.;83:115-950.
25. Raiola A, Rigano MM, Calafiore R, Frusciante L, Barone A. Enhancing the health-promoting effects of tomato fruit for biofortified food. Mediators Inflamm. 2014;2014:139873. doi: 10.1155/2014/139873. Epub 2014 Mar 12. PMID: 24744504; PMCID: PMC3972926.
26. Chidambaram K, Alqahtani T, Alghazwani Y, Aldahish A, Annadurai S, Venkatesan K, Dhandapani K, Thilagam E, Venkatesan K, Paulsamy P, Vasudevan R, Kandasamy G. Medicinal Plants of *Solanum* Species: The Promising Sources of Phyto-Insecticidal Compounds. J Trop Med. 2022 Sep 21;2022:4952221. doi: 10.1155/2022/4952221. PMID: 36187457; PMCID: PMC9519333.
27. Elizalde-Romero CA, Montoya-Inzunza LA, Contreras-Angulo LA, Heredia JB, Gutiérrez-Grijalva EP. Solanum fruits: phytochemicals, bioaccessibility and

bioavailability, and their relationship with their health-promoting effects. Frontiers in Nutrition. 2021 Nov 25;8:790582.
28. Kaunda JS, Zhang YJ. The genus solanum: an ethnopharmacological, phytochemical and biological properties review. Natural products and bioprospecting. 2019 Apr;9:77-137.
29. Kumar MS, Pal AK, Singh AK. Studies on heterosis and inbreeding depression for quality traits and yield in tomato (Solanum lycopersicum L.). Int. J. Curr. Microbiol. App. Sci. 2018;7(6):3682-6.
30. Vogel JT, Tieman DM, Sims CA, Odabasi AZ, Clark DG, Klee HJ. Carotenoid content impacts flavor acceptability in tomato (Solanum lycopersicum). Journal of the Science of Food and Agriculture. 2010 Oct;90(13):2233-40.
31. Esposito, E., et al., Cubosome dispersions as delivery systems for percutaneous administration of indomethacin. Pharmaceutical research, 2005. 22(12): p. 2163-2173 https://doi.org/10.1007/s11095-005-8176-x .
32. Thadanki M, Kumari PS, Prabha KS. Overview of cubosomes: a nano particle. Int J Res Pharm Chem. 2011;1(3):535-41.
33. Chaudhary K, Sharma D. Cubosomes: a potential drug delivery system. Asian journal of pharmaceutical research and development. 2021 Oct 15;9(5):93-101.
34. Spicer P. Cubosome processing: industrial nanoparticle technology development. Chemical Engineering Research and Design. 2005 Nov 1;83(11):1283-6.
35. Chaudhary K, Sharma D. Cubosomes: a potential drug delivery system. Asian journal of pharmaceutical research and development. 2021 Oct 15;9(5):93-101.
36. Nithya, R.; Jerold, P.; Siram, K. Cubosomes of dapsone enhanced permeation across the skin. J. Drug Deliv. Sci. Technol., 2018, 48, 75-81.
37. Ahirrao, M.; Shrotriya, S. In vitro and in vivo evaluation of cubosomal in situ nasal gel
38. containing resveratrol for brain targeting. Drug Dev. Ind. Pharm., 2017, 43(10), 1686-1693.http://dx.doi.org/10.1080/03639045.2017.1338721 PMID: 28574732.
39. A. Cytryniak, E. Nazaruk, R. Bilewicz, E. G orzy nska, K. Zelechowska Matysiak, R. Walczak, et al., Lipidic cubic-phase nanoparticles (cubosomes) loaded with doxorubicin and labeled with 177Lu as a potential tool for combined chemo and internal radiotherapy for cancers, Nanomaterials 10 (11) (2020 Nov 16) 2272. Available from: https://doi.org/10.3390/nano10112272.
40. M. Nasr, M.K. Ghorab, A. Abdelazem, In vitro and in vivo evaluation of cubosomes containing 5-fluorouracil for liver targeting, Acta Pharm. Sin. B 5 (1) (2015 Jan) 79–88 Available from: https://doi.org/10.1016/j.apsb.2014.12.001.
41. C. Chang, T.G. Meikle, C.J. Drummond, Y. Yang, C.E. Conn, Comparison of cubosomes and liposomes for the encapsulation and delivery of curcumin, Soft Matter 17 (12) (2021), 3306–3313. Available from: https://doi.org/10.1039/d0sm01655a.

CHAPTER 10
ANNEXURE

Azadi Ka Amrit Mahotsav

सीएसआईआर - राष्ट्रीय विज्ञान संचार एवं नीति अनुसंधान संस्थान
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वैज्ञानिक तथा औद्योगिक अनुसंधान परिषद् Council of Scientific & Industrial Research
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NISCPR सीएसआईआर-निस्पर

Raw Material Herbarium And Museum Delhi

Authentication No-RHMD/2023/56 12/2/2023

CERTIFICATE FOR CRUDE DRUG SAMPLE AUTHENTICATION

This is to certify that fruit sample of *Solanum lycopersicum*, Tomato, recieved from Ms. Awneet Kaur vide letter No Nil Dated 12th February 2023 has been found correct as fruit of *Solanum lycopersicum,* commonly known as Tomato. The identification has been done on the basis of macroscopic studies of the sample followed by detailed scrutiny of literature and matching the sample with authentic sample deposited in the Raw Material Herbarium and Museum Delhi (RHMD)

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Formulation and Characterization of *Solanum lycopersicum* Extracts for Anti-depressant Activity

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Dear Dr. Kapil Rana,

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The manuscript has been submitted to the journal by Awaneet Kaur who is listed as the main author and who will be authorized to track the status of the paper after login.

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