



Savitribai Phule Pune University

Project Report  
on

“EXTRACTION OF BETA SITOSTEROL FROM FICUS  
RELIGIOSA”

*Submitted By*  
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*Carried out at*  
(Pune)

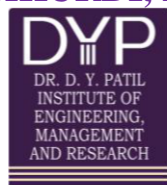
*Under The Guidance of*

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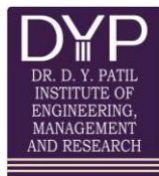
DEPARTMENT OF CHEMICAL ENGINEERING

**DR.D.Y.PATIL INSTITUTE OF ENGINEERING, MANAGEMENT AND  
RESEARCH, AKURDI, PUNE – 411044**



2021-2022

**DR. D. Y. PATIL INSTITUTE OF ENGINEERING, MANAGEMENT  
AND RESEARCH, AKURDI, PUNE – 411044**



**DEPARTMENT OF CHEMICAL ENGINEERING**

**CERTIFICATE**

*This is to certify that, **Chandrakant Ingale and Akshay Nannaware** of B.E. Chemical Engineering has successfully completed the Project Phase-I, “**EXTRACTION OF BETA SITOSTEROL FROM FICUS RELIGIOSA**” towards the partial fulfillment for the requirements of the Degree of Engineering course under the Savitribai Phule Pune University, Pune during the academic year 2021-22.*

*Prof. Mrs. Kirti Zare  
Project Guide*

*Dr. Mrs.  
A.V.Patil  
Principal*

*External Examiner*

## **PROJECT APPROVAL SHEET**

**THIS IS TO CERTIFY THAT PROJECT PHASE – I REPORT ENTITLED**

**“EXTRACTION OF BETA SITOSTEROL FROM FICUS RELIGIOSA”**

**SUBMITTED BY**

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are approved for evaluation during their examination of final year of Chemical Engineering under the **Savitribai Phule Pune University**.

**Examiner**

**Guide**

**DEPARTMENT OF CHEMICAL ENGINEERING  
DR. D.Y. PATIL INSTITUTE OF ENGINEERING,  
MANAGEMENT & RESEARCH AKURDI,  
PUNE – 44**

## **DECLARATION**

*We hereby declare that, entire project work entitled “**EXTRACTION OF BETA SITOSTEROL FROM FICUS RELIGIOSA**” is a project report of original work done by us and to the best of our knowledge and belief. No part of it has been submitted for any degree or diploma of any institution previously.*

*This Project work is submitted to **Savitribai Phule Pune University, Pune** in the **Dr. D.Y Patil Institute of Engineering, Management and Research**, Akurdi, Pune during the academic year **2021-22**.*

Place: Pune

Date:

2.

Signature of Students

1.

## **ACKNOWLEDGEMENT**

The successful completion of any work would be incomplete unless, we mention the names of people whose guidance and encouragement served as beacon light and crowned our effort with success.

We express sincere thanks to our internal guide (**Prof. Mrs. Kirti Zare**) who motivated, guided and encouraged us at each and every step and their guidance was of utmost importance.

We also take this opportunity of expressing our gratitude to **Dr. Shailesh Ghodke**, Head, Department of Chemical Engineering, for constant support throughout the progress of project work.

We also owe our thanks to **Dr. (Mrs) A. V. Patil, Principal** for having provided us the academic environment that nurtured our practical skills.

We would like to thank our beloved parents and other family members whose constant love, support and encouragement have been the main source for whoever we are and it will continue everlasting.

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Name of the Students

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## ABSTRACT

Herbs have always been the principal form of medicine in India. *Ficus religiosa* (L.), commonly known as peepal belonging to the family Moraceae, is used traditionally as antiulcer, antibacterial, antidiabetic, in the treatment of gonorrhea and skin diseases and heart disease. The plant was subjected to extraction and also fractionated with two various solvent like methanol, ethanol, n-hexane, diethyl ether and n-butanol. The Phytochemical test, UV spectrometer, TLC and HPTLC reports shows presence of compound only in methanol and n-butanol fraction. So by using the batch extraction and soxhlet apparatus we are done the extraction of Beta-sitosterol and also find optimized condition at we get maximum concentration of Beta-sitosterol. We done the analysis of that extracted product by using UV spectrophotometer. This fraction subjected to structural elucidation. The identified compound was beta-sitosterol.



## **Aim and Objective**

Our Aim is to detect chemical compound Beta-sitosterol from ficus religiosa (peepal tree) and to analyze different parameters for better extraction of Beta sitosterol in batch reactor and Soxhlet apparatus and to review post analysis of obtained extract from extraction process and to study and review upon optimized parameters. Our Objective is to follow Standard Methods for sample analysis and to detect Beta-sitosterol using ultra-violet spectrometer from extracted solution and obtain optimized conditions for better extraction.

# CHAPTER 1

## INTRODUCTION

### 1.1. Ficus Religiosa (Peepal Tree)

*Ficus religiosa* Linn is often called Peepal belongs to Moraceae. In India, since past it's got nice mythological, spiritual and medical importance. this can be thought of because the oldest tree in Indian art literature. There square measure several chemical compounds that are extracted out from completely different plants as they as they need important use in medicative field. These compounds play terribly|a really|a awfully} necessary role in as medicative usages is increasing worldwide very quickly, it's important to induce alternate sources of medicine with effective results and with no side-effects.

The genus *Ficus* consists of concerning 800 species and 2000 varieties that square measure cosmopolitan in Republic of India and throughout the globe particularly in tropical and sub-tropical regions. *F. religiosa* has got mythological, medical and spiritual importance since past. consistent with spiritual purpose of read, *F. religiosa* is important because it is believed that Gautam Buddha achieved enlightenment below this tree. attributable to this, it's additionally named as “Bodhi tree or Bo tree”.

In medicative field, *F. religiosa* is gaining nice attention as a result of it's several compounds that square measure useful in treatment of the many diseases like polygenic disorder, skin diseases, metabolism disorders, central system disorder, stomachic issues etc.

#### **Classification:**

Domain: Eukaryota

Kingdom: plant kingdom

Subkingdom: Viridiplantae

Phylum: Tracheophyta

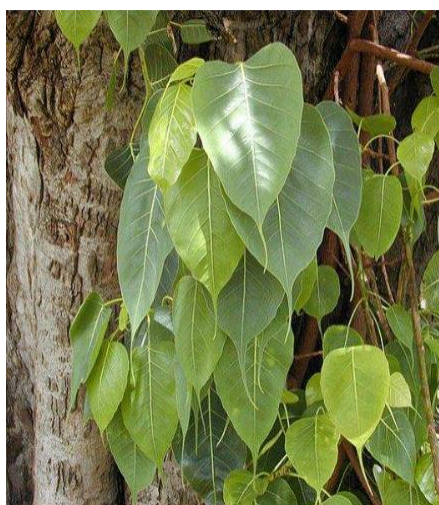
Order: order Urticales

Family: Moraceae

Genus: genus *Ficus* Species: *F. religiosa*

### 1.1.1. Vernacular Names:

Pippala (Sanskrit), Ahant (Assamese), Asvattha, Ashud, Ashvattha (Bengali), sacred fig (English), Piplo, Jari, Piparo, Pipalo (Gujrati), Pipala, pipul (Hindi), Arlo, Ranji, Basri, Ashvatthanara, Ashwatha, Aralimara, Aralegida, Ashvathamara, Basari, Ashvattha (Kannada), dangerous (Kashmiri), Arayal (Malayalam), Pipal, Pimpal, Pippal (Marathi), Aswatha (Oriya), Pipal, Pippal (Punjabi), Ashwarthan, Arasamaram, Arasan, Arasu, Arara (Tamil), Ravichettu (Telugu).



a)



b)



c)

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### **1.1.2. Botanical Description:**

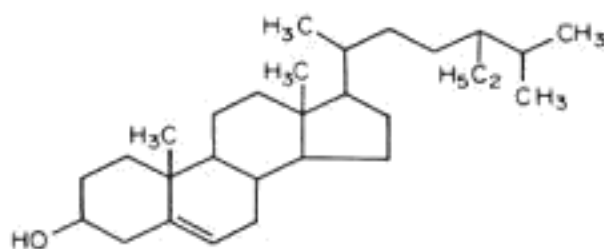
*F. religiosa* is an outsized deciduous tree with or no aerial roots that is often found in Republic of India. It's native from Republic of India to Southeast Asia that grows up to 5000ft with the trunk that reaches up to one meter (Fig one.1c). Copy is finished by layering, bungalow and seeds. The stem is yellow by that many roots square measure coalesced. Leaves square measure terribly shiny, skinny and bear 5-7 veins that square measure alternate, long, petiolate, and serrate or heart formed at the bottom or typically rounded (Fig.1.1).

The young leaves square measure pink in color, changes to copper and eventually to inexperienced at maturity. The bark is of white or brown in color. Fruits square measure circular in form referred to as as Figs that is coarctate in flowering. Once fruits square measure raw, they're inexperienced in color throughout summer however once ripening they flip black through time of year

### **1.1.3. Phytochemical Study:**

Phytochemistry is outlined as chemistry of plants or chemical composition of plants. In pharmacy, it's delineate because the chemistry of these natural merchandise which might be used as medication or plant components with the stress on organic chemistry. These compounds is therapeutically active or inactive. The inactive constituents square measure structural constituents of the plants like starch, sugars or proteins. they need sensible pharmaceutical uses. The active constituents square measure in the main secondary metabolites like alkaloids, glycosides, volatile oils, tannins etc. The secondary metabolites square measure fashioned from primary merchandise. they can not be utilized by plants and thus gets deposited within the cells. The reportable phytoconstituents of stem bark of *F. religiosa* Linn. square measure phenols, tannins, steroids, alkaloids, flavonoid,  $\beta$ -sitosterol-d-glucoside, vitamin K, noctacosanol, alkyl oleanolate, lanosterol, stigmasterol, lupen-3-one. the foremost lush flavonoid gift in *Ficus religiosa* is quercetin.

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**β-sitosterol**

**Figure 1.2: active compounds present in *f. religiosa*.**

#### **1.1.4. Medicative uses of *Ficus religiosa*:**

*F. religiosa* exhibits many biological activities and it's effectively used for the treatment of various diseases. virtually each a part of the tree is used as medication. *F. religiosa* is reportable to own varied therapeutic uses in people medication. This tree has lush medical uses. they're thought of as herbs. Six a part of the tree (leaves, seeds, fruits, bark, roots and latex) square measure valued for his or her medicative qualities (Table one.1).

**Table 1.1: chemical compounds contained by different parts of *f. religiosa*.**

	Plant part	Compounds present
1.	Bark	Phenols, tannins, steroids, alkaloids and flavonoids, β-sitosteryl-d-glucoside, vitamin K, noctacosanol,
		methyl oleanolate, lanosterol, stigmasterol, lupen-3-one. Tannin, wax, saponin, Leucoanthocyanins. delphinindin-3-0-α-Lrhamnoside (11), Pelargonidin-3-0-α-Lrhamnoside, Leucocyanidin-3-0- β-D-galactosyl-cellobioside (111), Leucoanthocyanidin, 20- tetratriaconten-2-one , pentatriacontan- 5-one , 6 heptatria content-10-one, mesoanisotal
2.	Root	β-sitosteryl-dglucoside
3.	Fruit	Protein (4.9%), Essential amino acids (isoleucine, and phenylalanine) , Flavonols ( kaempeferol, quercetin, and myricetin), Also contains good amount of total phenolic contents, total flavonoid, and percent inhibition of linoleic acid 11, Asgaragine, tyrosine, undecane, tridecane, tetradecane,

		(e)- $\beta$ -ocimene, $\alpha$ - thujene, $\alpha$ -pinene, $\beta$ -pinene, $\alpha$ -terpinene, limonene, dendrolasine, dendrolasine $\alpha$ -ylangene, $\alpha$ -copaene, $\beta$ -bourbonene, $\beta$ -caryophyllene, $\alpha$ -trans bergamotene.
4.	Seed	Phytosterolin $\beta$ -sitosterol, and its glycoside, albuminoids, carbohydrate, fatty matter, Coloring matter, Caoutchoue 0.7-5.1%
5.	Leaves	campesterol, stigmasterol, isofucosterol, $\alpha$ -amyrin, lupeol, tannic acid, arginine, serine, aspartic acid, glycine, threonine, alanine, proline, tryptophan, tryosine, methionine, valine, isoleucine, leucine, n-nonacosane, n-hentricontanen, hexa-cosanol and n-octacosan
6.	Aqueous extract of dried bark	phytosterols, flavonoids, tannins, furanocoumarin derivatives namely bergapten and begaptol

## 1.2. Medical specialty activities gift in genus *Ficus religiosa*:

*F. religiosa* possess a broad range of pharmacological activities as shown in figure 1.3. Fresh plant materials, crude extracts and extracted components of *F. religiosa* showed a wide spectrum of in vitro and in vivo pharmacological activities like, antidiabetic, anti-inflammatory, wound healing, anticonvulsant, anti-inflammatory, analgesic, antimicrobial, antioxidant, antiasthmatic, antitumor, antiulcer, antianxiety, antihelmintic, and proteolytic activity.



**Figure 1.3: different types of pharmacological activities present in *f. religiosa***

### **1.2.1. Medicament activity:**

It has been rumored that binary compound extract of *F. religiosa* in doses of fifty and a hundred mg/kg showed pronounced reduction in glucose levels. The impact was compared with glybenclamide, a widely known hypoglycemic drug. thenceforth binary compound extract of *F. religiosa* showed important increase in liquid body substance hypoglycemic agent, weight, polysaccharide content in liver and musculus of experimental diabetic rats, conjointly reduced the liquid body substance lipid and total sterol level. The results instructed potential ancient use of *F. religiosa*. conjointly the bark root showed most fall of the blood glucose level. binary compound extract of *F. religiosa* orally decreases the fast glucose. *F. religiosa* modulates the enzymes of inhibitor weapons system to combat aerobic stress.

As a result glutathione was rebuilt and smothered the formation of malondialdehyde, proving its anti-diabetic activity together with inhibitor potential.

### **1.2.2. Antimicrobial activity:**

Aqueous extract of *F. religiosa* shows high antimicrobial activity against chosen infective organisms. High activity has been found in *B. subtilis* with concerning 24mm inhibition zone. And conjointly the expansion of *P. Aeruginosa* (multi drug resistant) has been remarkably smothered by the plant extract nineteen. The preliminary screening of bactericide activity of *F. religiosa* by agar-well diffusion assay was investigated. bactericide activity of *F. religiosa* against *B Cereus* twenty and *escherichia* twenty one has conjointly been rumored earlier; equally the chloroform extracts of *F. Religiosa* showed a robust repressing activity against growth infectious typhoid bacillus, enteric bacteria typhimurium and *Proteus vulgaris* at a MIC of thirty-nine, five and twenty µg/ml severally.

### **1.2.3. Anti-inflammatory drug activity:**

The methanolic extract of stem bark of *F. religiosa* has shown important anti-inflammatory drug activities orally. A big anti-inflammatory drug impact has been determined in acute and chronic models of inflammation; the extract conjointly protected mast cells from degranulation elicited by varied degranulators. A paste of the powdery bark could be a sensible absorbent for inflammatory swellings and might be accustomed treat burns. Earlier study on constant wood alcohol extract incontestible that the extract smothered professional induction of gas and pro inflammatory cytokines in LPS-stimulated neuroglia via the MAPK.

### **1.2.4. Analgesic activity:**

The analgesic activity of the *F. religiosa* stem bark methanolic extract victimization the carboxylic acid acid-induced moving (extension of hind paw) model in mice. pain pill were used as standards medication. It exhibited reduction within the variety of moving. this implies that extract showed the analgesic impact in all probability by inhibiting synthesis or action of prostaglandins.

### **1.2.5. Inhibitor activity:**

The binary compound and alcoholic extract of roots possess outstanding inhibitor activity showing inflated levels of peroxidase (GPX), glutathione S-transferase (GST), glutathione enzyme (GRD), enzyme (SOD) and enzyme (CAT) and decreased level of lipide peroxidation (LPO). *F. religiosa* root extracts showed important inhibitor activity against carbon tetrachloride-induced liver injury in rats twenty eight. it's sensible superoxide scavenging potential admire that of water-soluble vitamin and most subtractive potential admire that of acid and phenol. Recent study has conjointly disclosed that the wood alcohol extract of *F. religiosa* containing high total phenoplast and total flavonoids contents, exhibits high inhibitor activity. Studies have shown that plants of fig tree will grow in adverse habitats having fifty fifth or higher H<sub>2</sub>O<sub>2</sub> production with concerning half-hour increase in oxidase activity. Guaiacol, ascorbate and o-dianisidine square measure the 3 most most well-liked substrates of *F. religiosa* tested for oxidase activity.



### **1.2.6. Antiepileptic activity:**

The wood alcohol extract of figs of *F. religiosa* exhibits dose-dependent antiepileptic activity against most electroshock- and picrotoxin-induced convulsions through serotonergic pathways modulation. The antiepileptic activity of the extract is studied in strychnine-, pentylenetetrazole-, picrotoxin- and isoniazid-induced seizures in mice.

### **1.2.7. Wound healing activity:**

The wound healing activity was investigated by excision and incision wound models victimization *F. religiosa* leaf extracts. High rate of wound contraction, decrease within the amount for epithelialisation, high skin breaking strength were determined in animals treated with 100% leaf extract ointment when put next to the management cluster of animals treated with normal drug Povidine iodine. it's been rumored that tannins possess ability to extend the albuminoid content, that is one among the issue for promotion of wound healing what is more, the alcohol bark extract of *F. religiosa* was rumored to possess wound healing thirty five. A patient with cutaneous sensation or skin condition is suggested to drink a stewing of peepal bark.

### **1.2.8. Anti-amnesic activity:**

The anti-amnesic activity was investigated victimization *F. religiosa* wood alcohol extract of figs on scopolamine-induced anterograde and blackout in mice. Figs were noted to contain a high serotonergic content, and modulation of serotonergic neurotransmission plays a vital role within the pathological process of memory loss.

### **1.2.9. Acetylcholinestrerase activity:**

Methanolic extract of the stem bark of *F. religiosa* is found to inhibit the acetylcholinestrerase catalyst, thereby prolonging the half-life of neurotransmitter. it's been rumored that the majority accepted ways in Alzheimer's diseases treatment is that the use of enzyme inhibitors.

### **1.2.10. Chemical action activity:**

A comparison of the chemical action activity of the latex of forty six species of Ficus has been done by cataphoretic and natural process properties of the macromolecule elements and *F. religiosa* has showed a big chemical action activity.

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### **1.3. Significance of peepul within the field of drugs**

The roots, bark-skin, fruits, and leaves of *F. religiosa* have nice medicative worth. The bark is cooling and astringent and is understood to heal wounds. it's helpful in inflammations and organ swellings of neck. The bark is also wont to treat jaundiced patients by reducing the number of micturition.

According to Unani system of drugs, root, bark is aphrodisiac and additionally smart for backache. Bark is employed in healing ulcers, varied skin diseases and itch and in treatment of polygenic disease. Roots are aforesaid to be smart for those folks that tormented by urarthritis during which acid builds up within the joints, inflicting a lot of pain. this kind of inflammatory disease is cured by the utilization of root as a result of it decreases the degree of acid. The roots also are chewed to stop gum unwellness.

Root bark is nice for rubor, clean ulcers and it's astringent in leucorrhoea and promotes granulations. The fruit is laxative, promotes digestion, aphrodisiac and checks projection. Ripe fruits are alexipharmic (an counterpoison or defensive remedy against poison, venom or infection). The fine fruit is taken for bronchial asthma. The fruit powder is additionally given to reinforce fertility and employed in infectious disease, female internal reproductive organ troubles, ulcers, biliousness, bitter tonic, in blood diseases. contemporary fruit is additionally wont to treat dehydration and forestall heart condition. fine dry fruit destroys sorrows of someone.

The seeds are cooling and refrigerant. Seeds are helpful in urinary troubles. Seeds are laxative and if taken 3 days throughout period, sterilize girls for very long time. The leaves are the foremost helpful elements of *F. religiosa*. The leaves alone are wont to treat constipation. The leaves and young shoots along are purgative (strong laxative). The leaves are wont to alleviate fevers, injury wounds, constipation, dysentery, bruises, boils and infectious disease. The

leaves will either be consumed or poured on the wound, boil or mump. Leaf juice has been used for the treatment of bronchial asthma, cough, sexual disorders, diarrhea, hematuria, ache and odontalgia, migrane, eye troubles, stomachic issues and itch.

Leaf simmering has been used as AN analgesic for odontalgia. AN infusion or simmering of the bark is employed with some honey for the treatment of clap, ulcers, skin diseases and itch. Its power bark has been wont to heal the injuries for years. Charaka and Sushruta prescribed a simmering of the bark of Ashvatha in haemorrhages; leaves for covering wounds; the paste of tender roots or the bark for skin infections. Sushruta administered a simmering in urinary disorders and duct discharges. Milk cocked with the fruit, leaf bud, bark and therefore the root extra with sugar and honey, was prescribed as AN aphrodisiac. Powder of the dried bark was dusted over burns. A paste of the bark and leaves was prescribed in rubor. Ash water of the dried bark was given for checking projection. simmering of the basis bark with salt and induce anti-diuretic property.

#### **1.4. Extraction Of Beta-Sitosterol From Peepal Leaves Powder**

For extraction of beta-sitosterol by solvent method weight concerning 20g of dried leaves were grounded to a moderately coarse powder in domestic electrical grinder extra into the batch reactor. in an exceedingly batch reactor around two hundred mil of wood spirit is extra and therefore the mixtuere is heated at temperature vary of 30-55°C, simulteneously the stirrer is about with AN rate of vary 100-300 rate following with the time in minutes.

#### **1.5. TLC Confirmation**

Thin layer natural action is wont to monitor the progress of a reaction, establish compounds gift in an exceedingly given mixture, and confirm the purity of a substance<sup>7</sup>. Silicagel G used as adsorbent and benzene:chloroform within the quantitative relation of 7:3 used as a mobile part. Plate was ready by gushing colloid on glass plate and activated by heating at 110°C for thirty min. Then the sample was noticed on the plate and therefore the plate was keptin the solvent system till it reaches to 3/4 th of the plate. The spots ar detected beneath long actinic radiation at 365 nm and Rf values ar calculated.

## **1.6. UV-Spectrophotometer**

Different concentrations of beta-sitosterol were ready with wood spirit solvents and scanned within the actinic radiation region. the wave length 204nm was hand-picked for beta-sitosterol wherever it shows most absorbance. lower concentrations don't show any absorbance. absorbance was afrethought versus several concentrations.

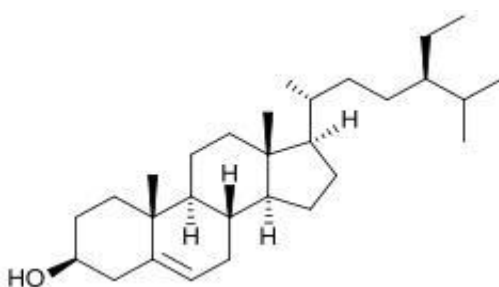
## **1.7. High performance liquid natural action (HPLC)**

HPTLC is characterised by economical separation used either for identification or quantitation of chemical substances. metallic element plates ar commonly used. colloid is that the most generally used adsorbent. HPTLC plates ar created from four.5 five colloid with AN inert binder to make to make layer. Plates exposed to high humidness ar unbroken resolute build them activated by inserting in AN kitchen appliance at 110-120°C for thirty min before sample spotting<sup>8</sup>. Benzene:chloroform within the quantitative relation of 7:3. Sample ar solubilized with nominal solvent of the extract. The sample volume commonly applied on to the plate is around zero.2 µl. Streaking the sample on the plate leads to higher separation than recognizing. In Ascending development the optimum separation distance is 20-25 millimetre with separation time of concerning four min. Detection of colored substances or colourless substances riveting in longer wave actinic radiation region(365 nm) or substances with intrinsic light is simply detected. quantisation was performed with photometrical measuring of absorbed light-weight or emitted light. In absorption mensuration, the spots in HPTLC plates ar scanned by a beam of monochromatic light-weight.

## 1.8. Beta-Sitosterol

### 1.8.1. Introduction

A term “Phytochemicals” (plant primarily based chemicals), was introduced to the globe in 1994 and promptly became a trend and frontier for researchers and scientists, of that phytosterols square measure a subgroup of the steroids as a vital category of bioorganic molecules. Phytosterols square measure widespread in plants and animals moreover as fungi, and have structural similarity to steroid alcohol. Phytosterols play essential roles within the physiology of organism organisms. for example, steroid alcohol is that the main a part of the cellular membrane in animals, poignant the cell membrane's liquidity and serving as secondary courier in biological process signal. the foremost necessary profit for these natural metabolites is their entering amongst the health promoting constituents of natural foods that contains them. the ecu Foods Safety Authority (EFSA) recommends overwhelming concerning one.5 - 2.4 g/day of phytosterols and/or stanols so as to scale back blood steroid alcohol. what is more, government agency has approved the role of foods containing phytosterol esters within an occasional saturated fat and steroid alcohol diet in reducing the danger of heart condition, particularly consumption of a minimum of one.3 g/day sterols, doubly on a daily basis.



**Figure 1.4: Structure of Beta-Sitosterol**

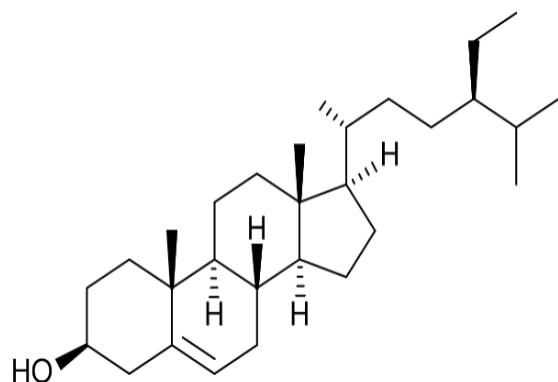
IUPAC Name	17-(5-Ethyl-6-methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol
Chemical formula	C <sub>29</sub> H <sub>50</sub> O
Molar mass	414.72 g·mol <sup>-1</sup>
Appearance	White crystalline solid
Density	0.97 g/cm <sup>3</sup>
Melting point	130°C -145 °C
Boiling point	502°C at 1 atm
Solubility in water	Insoluble
Solubility in methanol	Soluble

**Table 1.2: Physical properties of Beta-sitosterol**

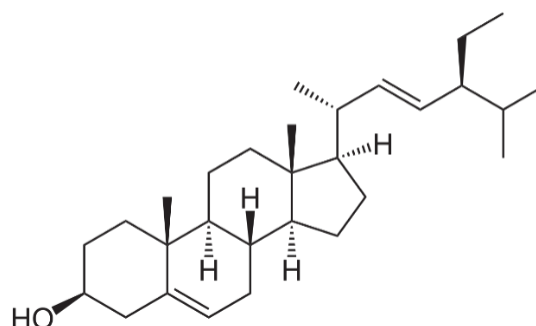
The natural foods and high phytosterol-containing dietary has been incessantly marketed for many years in numerous countries. Vegetable oils and merchandise made up of them, nuts, cereal merchandise, vegetables, fruit and berries are classified as wealthiest or considerably rich sources of phytosterols. 3 phytosterols together with together with, campesterol and stigmasterol square measure predominant sterols within the human flavouring nutrition forming sixty fifth, half-hour and three of diet contents, severally. Phytosterols, with a protracted history of consumption as food or pharmaceutical merchandise, have typically recognized as safe (GRAS), and no undesirable aspect effects are reportable. associate exception is associate unwellness named “phytosterolaemia”, a genetically illness, associated with some mutations within the ABCG5/G8 supermolecules that play the role of protein pump to enter the sterols into enterocytes and hepatocytes.

### 1.8.2. Synthesis of Beta-Sitosterol

Although though has not been utterly synthesized to date, it's been made from pure stigmasterol via 2 ways that. within the 1st rout, the aspect chain  $\Delta^{22-23}$  aliphatic compound is by selection change to supply to supply with numerous levels of stigmasterol and totally saturated stigmasterol, whereas this selective chemical action among protection of  $\Delta^5$ – half-dozen aliphatic compound to cyclopropylcarbiny ether is purposed within the second approach. This method ought to follow by chemical action of the  $\Delta^{22-23}$  covalent bond and additionally solvolysation of the inhalation anaesthetic so as to supply the C3-alcohol and  $\Delta^5-6$  aliphatic compound once more. The latter methodology looks terribly helpful thanks to action of  $\beta$ -sitosterol in high purity. As a fact, semi-synthesis of  $\beta$ -sitosterol continues to be a challenge as a result of manufacturing the alkyl radical ether by merchandise, whose removal is troublesome.



Beta-Sitosterol



Stigmasterol

### 1.8.3. Synthesis Of B-Sitosterol

Biosynthesis of the phytosterols is control throughout membrane biogenesis. The literature showed that  $\beta$ -sitosterol is biologically synthesized from each mevalonate and deoxyxylulose pathways. been studied and though varies found in keeping with the organism used, cycloartenol has been known as associate initial substrate. Actually, one molecule of isopentenyl-diphosphate (IPP) joins to 2 molecules of dimethylallyldiphosphate (DMAPP) to supply farnesyldiphosphate (FPP). 2 of the later molecule (FPP) square measure then combined tail-to-tail to lead to formation of squalene, as a triterpene and at last cycloartenol.

#### 1.8.4. Isolation And Identification Of B-Sitosterol From flavouring Extracts

$\beta$ -sitosterol is that the dominant phytosterol, which can endure aerobic

method similar to

steroid alcohol, leading to leading to. This makes isolation of pure  $\beta$ -sitosterol a challenge thanks to presence of sitosterol oxides. The common isolation procedure is making ready a chloroform extract from a plant, then playacting numerous natural action separations on colloid column and watching the fractions on aid. Sometimes, the fraction containing  $\beta$ -sitosterol is dissolved during a mixture of chloroform: fermentation alcohol (2:3) followed by heating on a water bathtub. Needle crystals could be appeared by exploit the answer undisturbed during a white goods. HPLC with reverse part stationary part (RP-18) is one in every of the foremost applied natural action techniques for this purpose. Capillary gas chromatography-mass spectroscopic analysis (GC/MS) technique is additionally utilized to see either sitosterol oxides in vegetable oils or alcohol esters. Moreover, plant sterols can be analyzed victimization high performance liquid chromatography-atmospheric pressure chemical ionization spectrographic analysis (HPLC-APCI-MS). ne'er the less  $\beta$ -sitosterol-D-glycoside is a lot of polar than  $\beta$ -sitosterol itself, it's been reportable to break away crude oil ether fraction of *Ocimum sanctum*, as a yellow amorphous solid, soluble in crude oil ether, ester, chloroform and methylene chloride. once associate flavouring extract contains each each and stigmasterol, isolation of those similar analogs isn't straightforward. However,  $\beta$ -sitosterol and stigmasterol square measure oft isolated and sublime from crude oil ether fraction of crude wood spirit extract via activity ways. Phytosterols, found profusely in non-polar fractions of plants and marines, square measure consumed (200-400 mg daily) in human diets. a number of these compounds square measure structurally resembled steroid alcohol (such as  $\beta$ -sitosterol, stigmasterol and their analogues) and be ready to inhibit the absorption of steroid alcohol, cancer-cell growth, growing, invasion and metastasis. Moreover, numerous biological activities square measure ascertained victimization these natural compounds or the extracts, within which concerned, e.g. trypanocidal, two-winged insects larvicidal, and as neutralizing agent

on ophidian and elapid snake venom. Among the higher than mentioned sterols,  $\beta$ -sitosterol is well-known natural alcohol in composition of notable flavouring medication for treatment of benign endocrine gland dysplasia and prostatic adenocarcinoma. Besides, the compound elevated protein and protein inhibitor in cells creating it effective anti-diabetic, neuroprotective and chemoprotective agent moreover. High potential of this compound and its analogues in treatment of varied diseases, classifies this compound because the noteworthy drug of the longer term, though its role in treatment of benign prostatic hyperplasia is currently approved via clinical test confirmations.

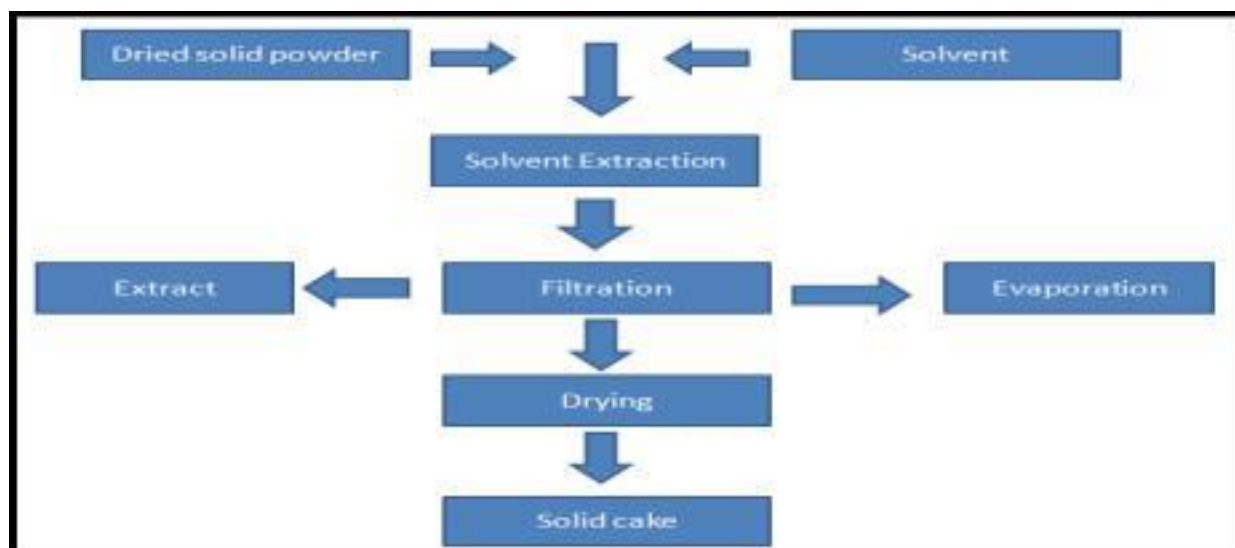
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#### **1.8.4. Uses**



$\beta$ -sitosterol is typically used for cardiovascular disease, hypercholesteremia, modulating the system, bar of cancer, similarly as for autoimmune disorder, infectious disease, cervical cancer, hair loss and benign endocrine gland dysplasia. moreover, various biological activities whereby natural compounds or the extracts were thought of as well as trypanocidal and two-winged insects larvicidal, even neutralization of snake and elapid venom characteristics was recorded. a number of the on top of indications square measure proof based mostly, however others square measure still doubtful and want a lot of investigations to verify its effectivity and safety.

**Figure 1.5: Chemical Structures of Some Phytosterols**



**Figure 3.1: General Unit Operation for Solvent Extraction**

## **CHAPTER 2**

## LITERATURE REVIEW

SarithaVedulla, S. Angala Parameswari , C. Gopinath had explained in his analysis paper of “Isolation of Active Compound in FicusReligiosaLinn” within which they delineated regarding Herbs have continuously been the principal style of medication in Asian country. Ficusreligiosa(L.), ordinarily referred to as peepal tree to the mulberry family, is employed historically as antiulcer, medicine, antidiabetic drug, within the treatment of Venus's curse and skin diseases. The plant was subjected to extraction and conjointly fractionated with 2 numerous solvent like ethoxyethane and nbutanol. The Phytochemical take a look at, TLC and HPTLC reports shows presence of compound solely in n- butyl alcohol fraction. So this fraction subjected to structural elucidation. The known compound was stigmasterol. Structural elucidation may be a major part of analytical work to verify pharmaceutical medicine. during this study deals with isolation of active molecule in pipul leaf. The extraction was done by victimisation wood alcohol as a result of wood alcohol was solubilized all the active constituents within the plant or drug. The extract targeted to isolate by half technique victimisation ethoxyethane, n-butanol. Then the fraction was concentrated , the residue was tested by TLC and nbutanol: glacial carboxylic acid acid: water within the magnitude relation of 4:0.5:5. The compound was screened through chemical tests. It shows the presence of steroids. the share yield of the composition screened by HPTLC. It shows high share of composition and conjointly single peak. The structure was confirmed through IR, magnetic resonance and mass spectroscopy[1].

SanskritiGautam, Anju Meshram , Sameer S. Bhagyawant and Nidhi Srivastava had explained in his Paper of “Ficus Religiosa– Potential Role In Pharmaceuticals” within which they delineated , In ancient medication, healthful plants are used for the treatment of assorted diseases. Ficusreligiosais renowned to be a sacred plant in Asian country. Since terribly ancient time, it's nice healthful and non secular significance. In Ayurveda, Unani and medical care, this plant is necessary supply of drugs. the assorted elements of the plants like stem bark, fruits, buds, latex ar employed in treatment of various diseases like infectious disease, mumps, jaundice, heart diseases, constipation, skin diseases, etc. in keeping with Ayurvedic system of drugs, F. Religiosa(Peepal tree) is renowned to be helpful in polygenic disease. Since last few years it's conjointly been investigated for the presence of assorted phytoconstituents (phenolics, sterols, flavonoids etc). F. religiosashowed various vary of

medical specialty activities like, antiepileptic drug, antidiabetic drug, medication, antimicrobial, analgesic, wound-healing, inhibitor, acetylcholinesterase, chemical action, and anti-amnesic. the current review is to compile up-to-date info of this plant that covers its natural phytochemical, organic chemistry, ethnobotanical and medical specialty significance. *F. religiosa* may be a wide branched deciduous tree with tough, heart formed, long tipped leaves, employed in the Indian system of drugs since terribly earlier period. The multiple advantages of *F. religiosa* (Peepal) created it a real miracle of nature. it's one in every of the foremost versatile plant having a good sort of healthful activities thus employed in treatment of many sorts of diseases for eg. Diarrhoea, diabetes, urinary disorder, burns, haemorrhoids, gastrohelcosis, skin diseases, convulsion, TB, fever, paralysis, aerobic stress, microorganism infection etc. this can be the distinctive supply of assorted sorts of compounds having various chemical structure. varied studies are conducted on totally different elements of *F. religiosa*, however still this plant has not nevertheless developed as a drug by pharmaceutical corporations. an intensive and systematic study is needed for identification, cataloguing and documentation of plants, which give a big manner for the promotion of the standard data of the flavourer healthful plants. currently it's important to try and do a lot of analysis work on this plant so a drug with multiple and negligible side-effects are out there within the future market

Ramhari J. Bagade, Sachin V. Bangale had explained in his journal of “Pharmacological Studies in genus *Ficus religiosa*” within which they delineated regarding, ancient medication, healthful plants are used for the treatment of assorted diseases. pipul is thought to be a sacred plant in Asian country. Since terribly ancient time, it's nice healthful and non secular significance. In Ayurveda, Unani and medical care, this plant is necessary supply of drugs. the assorted elements of the plants like stem bark, fruits, buds, latex are employed in treatment of various diseases like infectious disease, mumps, jaundice, heart diseases, constipation, skin diseases, etc. in keeping with Ayurvedic system of drugs, *F. Religiosa* (Peepal tree) is renowned to be helpful in polygenic disease. Since last few years it's conjointly been investigated for the presence of assorted phytoconstituents (phenolics, sterols, flavonoids etc). *F. religiosa* showed various vary of medical specialty activities like, antiepileptic drug, antidiabetic drug, medication, antimicrobial, analgesic, wound-healing, inhibitor, acetylcholinesterase, chemical action, and anti-amnesic. the current review is to compile up-to-date info of this plant that covers its natural phytochemical, organic chemistry, ethnobotanical and medical specialty significance. *Ficus religiosa* is a wide branched tree with

tough, heart formed, long folklore medication additionally claims its use in symptom, diabetes, urinary disorder, burns, haemorrhoids, gastrohelcosis, skin diseases, convulsion, infectious disease, fever, paralysis, aerophilic stress, microorganism infection etc. analysis administrated victimization completely different in-vitro and in-vivo techniques of biological analysis support most of those claims. Presently there's associate increasing interest worldwide in flavourer medicines amid redoubled laboratory investigation into the medicine properties of the bioactive ingredients and their ability to treat numerous diseases. various medicine have entered the international through exploration of ethnopharmacology and ancient medication. though scientific studies are administrated on an outsized range of Indian botanicals, a significantly smaller range of marketable medicine or phytochemical entities have entered the evidence-based medical specialty. Efforts area unit thus required to determine and validate proof concerning safety and practices of Ayurvedic medicines

## **CHAPTER 3**

### **METHODOLOGY**

#### **3.1 Solid- Liquid Extraction**

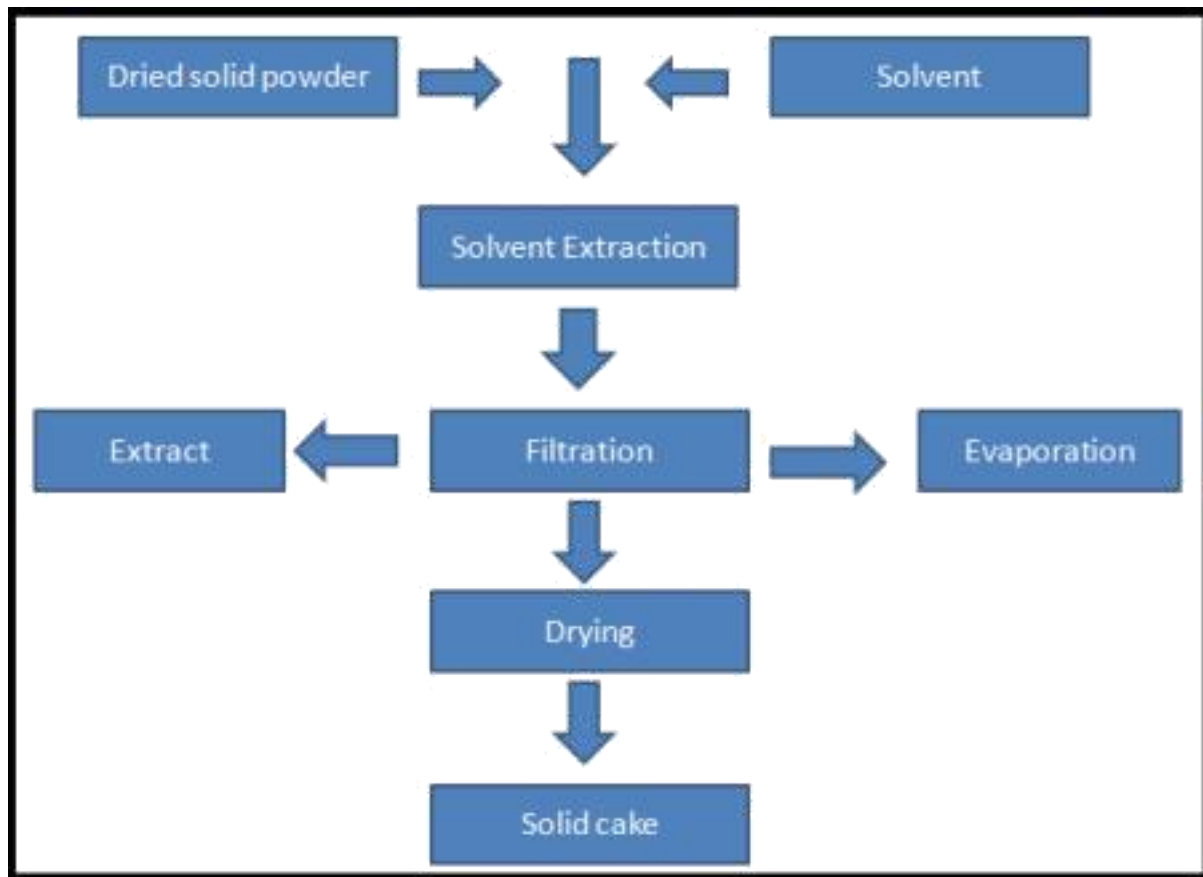
Leaching may be a separation technique within which the solid mixture is contacted with a liquid solvent for removal of 1 or additional soluble constituents of the solid mixture. the answer obtained is named as leach solution and therefore the wet solid is named as leached solid. the method of natural process consists of 2 steps:

- i. Contacting the solid with the selective solvent so as to dissolve the soluble matter within the solvent.
- ii. Separation of insoluble phases i.e. separation of the liquid part physically from the solid part physically subsidence, filtration etc.

The various solvents utilized in the method of natural process are- dissolvent, iso-octane, toluene, chloroform, chloride, tetrahydrofuran, diethyl ether, ester, acetone, acetonitrile, isopropanol, methanol, water, etc.

#### **Factors moving rate of natural process are:**

1. Particle size: - Smaller the particle size, bigger the surface space between the solid and liquid and bigger the speed of natural process.
2. Solvent: - The solvent used ought to be selective and will have low consistency for free of charge circulation.
3. Temperature: - The solubility of soluble material will increase with increase in temperature, therefore warmth favors the natural process operation.
4. Agitation: - Agitation will increase the eddy diffusion and therefore will increase the speed of natural process. Agitation conjointly prevents the subsidence of solids, keeps them in suspension.



**Figure 3.1: General Unit Operation for Solvent Extraction**

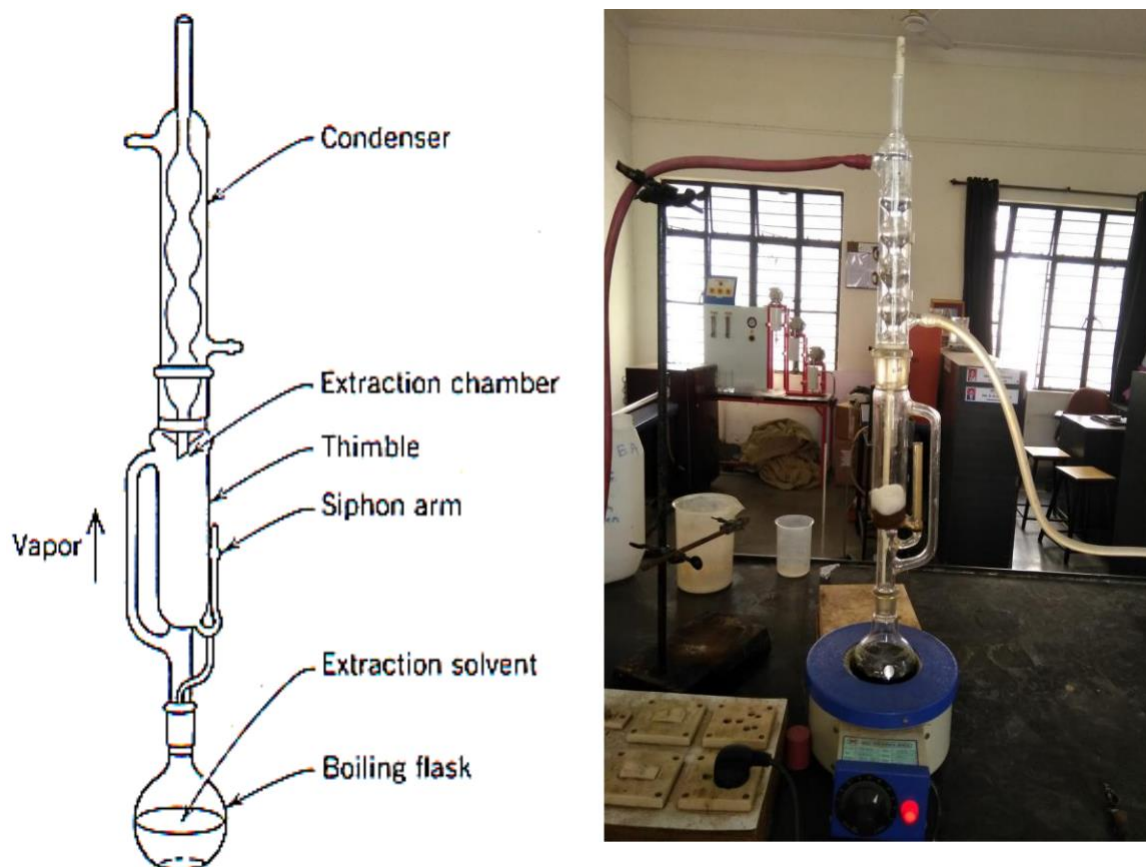
### **3.2. Conventional Method:**

#### **3.2.1. Soxhlet Extraction:**

In a typical Soxhlet system, material is placed in an exceedingly thimble-holder, and stuffed with condensed contemporary solvent from a distillation flask. once the liquid reaches the overflow level, a siphon aspirates the answer of the thimble-holder and unloads it back to the distillation flask, carrying extracted solutes into the majority liquid. within the solvent flask, matter is separated from the solvent victimisation distillation. matter island within the flask and contemporary solvent passes back to the plant solid bed. The operation is perennial till complete extraction is achieved.

### a) Experimental set-up and procedure:

The equipment for Soxhlet extraction consists of a solvent reservoir, extractor body, an electrical heat supply and a cool condenser.



**Figure 3.2: Schematic of Soxhlet extraction**

In a typical Soxhlet system as shown in figure three.2, material is placed in an exceedingly thimble-case, and stuffed with condensed contemporary solvent from a distillation flask. once the liquid reaches the overflow level, a siphon aspirates the answer of the thimble-holder and unloads it back to the distillation flask, carrying extracted solutes into the majority liquid. within the solvent flask, matter is separated from the solvent victimisation distillation. matter is left within the flask and contemporary solvent passes back to the plant solid bed. The operation is perennial till complete extraction is achieved. throughout Soxhlet extraction, the solvent is sometimes recovered by evaporation. The extraction and evaporation temperatures have a major result on the standard of ultimate merchandise.

### **b) Benefits of Soxhlet extraction**

1. Displacement of transfer equilibrium by repeatedly brings contemporary solvent into contact with the solid matrix.
2. Maintaining a comparatively high extraction temperature with heat from the distillation flask.
3. No filtration demand once natural process.
4. The Soxhlet methodology is extremely easy and low cost.

### **c) Disadvantages of Soxhlet extraction**

1. The extraction time is long.
2. an outsized quantity of solvent is needed.
3. Agitation can't be provided within the Soxhlet device to accelerate the method.
4. the massive quantity of solvent used needs AN evaporation/concentration procedure.
5. the chance of thermal decomposition of the target compounds can't be unnoticed as the extraction typically happens at the boiling purpose of the solvent for an extended time.

### **d) Applications of Soxhlet extraction:**

Soxhlet extraction, that has been used for an extended time, is customary technique and therefore the main reference for evaluating the performance of different solid–liquid extraction ways. The aromatic material is packed in an exceedingly still and a comfortable amount of water is more and dropped at a boil; instead, steam is injected into the plant charge. thanks to the influence of quandary and steam, the oil is free of the oil glands within the plant part. The vapor mixture of water and oil is condensed by indirect cooling with water. From the condenser, liquid flows into a setup, wherever oil separates mechanically from the liquid water.



### **3.2.2. Hydrotropic Extraction:**

#### **a) Introduction:**

Hydrotropy refers to the power of extremely soluble however gently active amphiphilic organic salts known as hydrotropes to extend the solubility of meagrely soluble or water insoluble organic compounds in binary compound solutions. Hydrotropy may be a collective molecular development like micellar solubilization however with a way higher capability. it's a consequence of the tendency of amphiphilic hydro figure of speech molecules to combination among themselves and possibly with different hydrophobic molecules. These aggregates square measure purportedly abundant smaller than wetting agent micelles and much less co-operative. Another identifying feature of hydro tropes, in contrast to surfactants, is their ability to completely differentiate among different organic constituents of a combination, even closely connected substances. it's this ability of molecular recognition that ought to be helpful for the discriminatory extraction of a compound from present raw materials. The high solubilisation capability of hydro figure of speech solutions ought to cause high extraction capacities for otherwise insoluble organic-active parts. we tend to demonstrate here the power of hydrotropic solutions, in a similar manner, to disrupt plant cell structures and aid within the extraction of hydrophobic constituents from the complicated bio matrix. The hydrotropic result is critical higher than a minimum hydro figure of speech concentration (MHC) that's a characteristic of a given hydro figure of speech, analogous to the crucial particle concentration (CMC) of a wetting agent. However, as a result of hydro tropes have comparatively short organic compound chains or hydrophobic teams, their MHCs square measure typically within the molar vary. The solubility of AN compound in an exceedingly hydro figure of speech answer rises nearly exponentially in real time higher than the MHC, however at higher concentrations of the hydro figure of speech, it would level to a upland counting on the character of the matter.

b) **Experiment:**

We explore the extraction of piperin from *Piper Nigrum* using hydrotropic solubilisation. Whole pepper fruits were initially fine to a rough powder and so separated into batches of various sizes using mechanical sieves. Particles with a mean size of 50  $\mu\text{m}$  were used for the extraction studies unless declared otherwise. The extraction experiments were carried out during a totally baffled cylindrical glass vessel (9-cm height, 7.0-cm i.d.) equipped with a six-bladed rotary engine blade (i.d. 2 cm). This whole assembly was unbroken during a constant-temperature run throughout experimentation. A 10-g sample of ground pepper was added to 0.1 dm<sup>3</sup> of hydro figure of speech answer of a better-known concentration within the range 0.05–3.4 mol/dm<sup>3</sup> within the glass vessel. The suspension was agitated smartly at 1100 rpm for a amount of two h at 30° C. The answer was then allowed to accept another hour and was afterwards filtered below vacuum inside ten min. A transparent brown-colored liquid was obtained because the filtrate. The solid residue, that consisted largely of the starch content of pepper, was soft and nonwoody however failed to hinder the filtration method. The cake was more washed with hydro figure of speech answer (0.01 dm<sup>3</sup>) of an equivalent concentration as within the extraction stage to get rid of extract residue adhering thereto, if any. The wash answer was added to the ultimate extract. The filtrate was then diluted with water at thirty °C to bring the hydro figure of speech concentration below its MHC. Piperin precipitated from the answer as fine crystals over a amount of one h. The suspension was then centrifuged at 2822g for fifteen min to separate the solid product from the remaining answer.

The precipitate was dried and analyzed for purity using HPLC with a 5- $\mu\text{m}$  Novopak C-18 column. The column was at the start rinsed with wood spirit and DCM and so equilibrated with the eluting solvent (DCM/MeOH 100:4).<sup>15</sup> The column was mounted on a Tosho HPLC chromatograph equipped with a 20- $\mu\text{L}$  loop. The mobile phase flow was 0.6 mL/min, and therefore the detection wavelength was 343 nm. The analysis was isocratic and was carried out for fifteen min.

### **c) Advantages:**

- i. simple recovery of merchandise and extremely high property.
- ii. Most of the hydro tropes square measure stable and low-cost.
- iii. Hydrotropic extraction will offer a competitive various to critical fluid extraction.
- iv. Compounds having relatively free aquophilic cluster was extracted additional within the liquid hydrotropic answer.

### **Types of Hydro-distillation:**

#### **i) Water Distillation**

In this technique, the fabric is totally immersed in water, that is cooked by applying heat by firing, steam jacket, closed steam jacket, closed steam coil or open steam coil. the most characteristic of this method is that there's direct contact between boiling water and stuff.

Disadvantages of Water Distillation:

Oil elements like esters square measure sensitive to reaction whereas others like acyclic monoterpene hydrocarbons and aldehydes square measure prone to chemical process. aerated elements like phenols have a bent to dissolve within the still water, therefore their complete removal by distillation isn't potential.

#### **ii) Steam Distillation**

Steam distillation is that the method of distilling stuff with steam generated outside the still during a satellite steam generator usually observed as a boiler. As in water and steam distillation, the stuff is supported on a perforated grid higher than the steam recess. a true advantage of satellite steam generation is that the quantity of steam may be without delay controlled. as a result of steam is generated during a satellite boiler, the stuff is heated no over 100° C and, consequently, it mustn't bear thermal degradation. Steam distillation is that the most generally accepted method for the assembly of essential oils on massive scale. Throughout the flavour and fragrance offer business, it's a typical apply. In some things, like the large-scale production of inexpensive oils, the planet market costs of the oils square measure barely high enough to justify their production by steam distillation while not amortizing the cost needed to create the ability over a amount of ten years or additional.

### **Disadvantage of Steam Distillation**

- A lot of higher cost required to ascertain this activity than for the opposite 2 processes

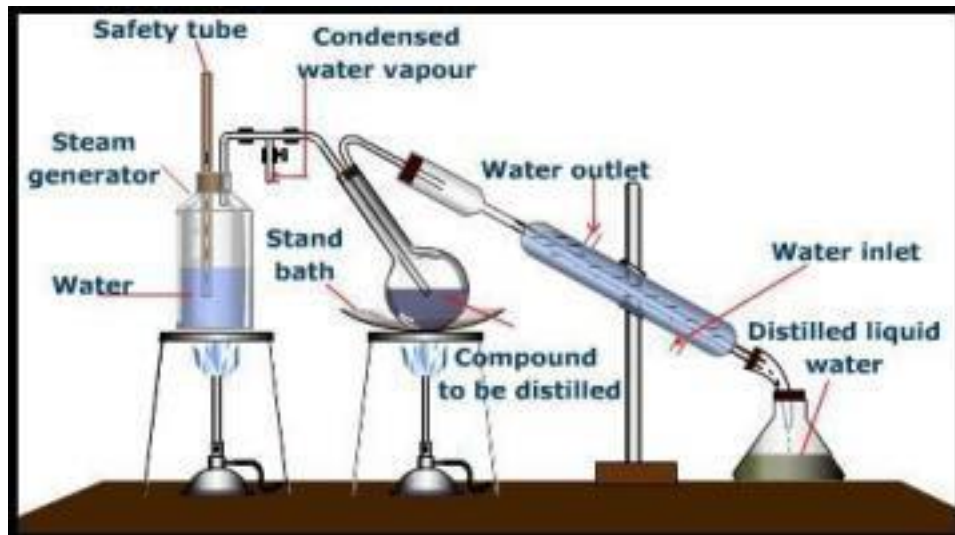


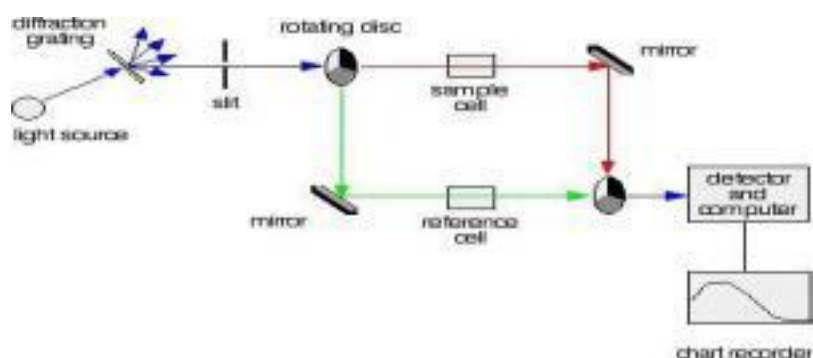
Figure 3.3: Schematic diagram of steam distillation

### **3.3. Analysis Technique:**

#### **3.3.1. UV Spectrophotometer**

##### ***Working:***

**The light sources:** you would like a lightweight source which supplies the whole color spectrum and the close to ultra-violet so you're covering the vary from regarding two hundred nm to regarding 800 nm. you cannot get this vary of wavelengths from one lamp, and then a mix of 2 is employed - a isotope lamp for the UV a part of the spectrum, and a metallic element / grouping lamp for the visible half.



**Figure 3.4: Schematic of UV Spectrophotometer**

##### **The diffraction grating and the slit:**

The blue arrows show the method the varied wavelengths of the sunshine square measure sent off in numerous directions. The slit solely permits light-weight of a awfully slim vary of wavelengths through into the remainder of the mass spectrometer.

### ***The rotating discs:***

The light coming back from the optical device and slit can hit the rotating disc and one in all 3 things will happen. If it hits the clear section, it'll go straight through and submit to the cell containing the sample. it's then bounced by a mirror onto a second rotating disc. This disc is rotating such once the sunshine arrives from the primary disc, it meets the reflected section of the second disc. That bounces it onto the detector.

1. If the initial beam of sunshine from the slit hits the reflected section of the primary rotating disc, it's bounced down on the inexperienced path. once the mirror, it passes through a reference cell (more this later). Finally the light gets to the second disc which is rotating in such a way that it meets the transparent section. It goes straight through to the detector.
2. If the light meets the first disc at the black section, it is blocked - and for a very short while no light passes through the spectrometer. This just allows the computer to make allowance for any current generated by the detector in the absence of any light. These are small rectangular glass or quartz containers. They are often designed so that the light beam travels a distance of 1 cm through the contents. The reference cell just contains the pure solvent.

### ***The detector and computer:***

The detector converts the incoming light into a current. Higher the current, greater intensity of light.

## **CHAPTER 4**

### **EXPERIMENTATION**

#### **4.1 Extraction**

##### **a) Material used:**

The Peepal leaves were collected from the Alandi premises. Then they were washed well with H<sub>2</sub>O and dried underneath sun rays (at 28°C for 5-6 days)/ receptacle drier and grinded it then the grinded powder was taken for more experimentation.

##### **b) Apparatus:**

Glass reactor of capability five hundred metric capacity unit, paper, beaker, round shape flask, stirring rod, UV-spectrophotometer unit, micropipette, meter flasks, funnel, advisement pan, sample bottles, water bath, batch setup, soxhlet setup.

##### **c) Batch extraction:**

20 metric weight unit of shed dry and crushed leaves (particle size three hundred - seventy five  $\mu\text{m}$ ) was subjected to totally baffled 500ml stirred salt glass vessel beside fuel (200 ml) as solvent. A four blade rotary engine kind trouble maker was used for stirring at totally different rev to confirm all particles stay in suspended condition.

Extraction of Beta-Sitosterol was allotted at totally different temperature for various hours. The schematic experimental setup is as shown in Figure. The samples were collected in AN interval of ten minutes. The collected samples were filtered through a standard paper primarily then filtered through whatman paper and analyzed in actinic ray photometer for determination of Beta-Sitosterol contents in mixtures.

##### **d) Soxhlet extraction:**

It was run so as to seek out out grievous bodily harm concentration that may be extracted get into the various time intervals. For this purpose, the we have a tendency to had place 200ml solvent in round shape flask and 20gm Peepal leaves powder within the thimble and endure 2hrs,4 and 6hrs. Conc. of was checked in actinic ray prism spectroscop.

### **4.1.1 Procedure:**

#### **a. Pre-Processing:**

- Fresh Peepal leaves wherever collected from the Alandi premises. From this the little twigs, massive particle, dangerous leaves and little braches of leaves wherever removed.
- They were washed well so as to get rid of the mud particles.
- Then unbroken in receptacle drier for ten min so as to get rid of wet content then unbroken underneath sun for 5-6 days.
- Then the leaves square measure grinded/ small-grained in mixer well.

#### **b. Process:**

- Water tub is switched ON.
- -20gms of small-grained Peepal leaves (raw material) wherever measured victimisation balance.
- -Weighed powder beside 200ml of fuel (Solvent) was poured into the extractor.
- -Then the baffle was inserted in to the extractor and it had been properly tightened.
- The trouble maker was switched ON and therefore the rev was set (as one hundred, 200 & 300).
- -Samples square measure collected from extractor at AN interval of ten minutes up-till three hrs.
- -Then the setup was shifted utterly.
- -Each of the sample bottles square measure labelled and picked up during a beaker containing water is either affected for refrigeration (in order forestall|to stop|to forestall} contamination or prevent microbic activity) or directly taken for actinic ray analysis in analytical workplace.
- -Before analysis the samples square measure properly filtered by primarily traditional paper then with whatman paper.
- -The remaining sample in extractor is send to Soxhlet for solvent recovery and to urge neem tree oil one by one.
- -The filtrate, residue and remaining extract square measure weighed properly for mass balance.



## **4.2. Analysis of extract victimisation UV- photometer**

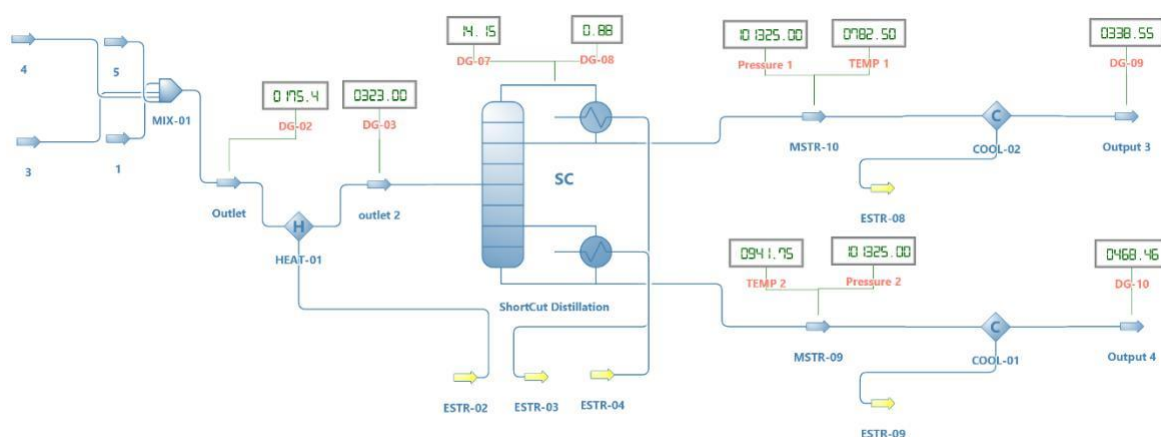
The UV- photometer we've used is of model analysis 201. All samples were measured victimisation UV- qualitative analysis. The system consists of UV- photometer set-up. fuel was taken as reference sample in each cuvettes. hand-picked the bottom line for measure. After that, we have a tendency to took sample in second cuvette to live the absorbance of Beta-Sitosterol in extracted sample at wavelength ( $\lambda=204\text{nm}$ ). The results square measure obtained in terms of peaks on the pc screen. These peaks square measure compared with the height of normal Beta-Sitosterol sample, so concentration of Beta-Sitosterol is simply determined.

## CHAPTER 5

### PROCESS AND DESIGN

#### 5.1 Development of Process Flow Diagram:

The development of a process for the production of Extraction of Beta-sitosterol has been developed and simulated in this work using DWSIM. This was achieved by picking the pieces of process equipment of the plant from the appropriate section of the DWSIM environment and connecting them together through appropriate streams. In addition, the components involved in the process were selected from the DWSIM *data bank*. Peng-Robinson Stryjek-Vera (*PRSV*) is used as the fluid package of the developed process for property estimation during the simulation.



#### Process of Extraction of Beta-Sitosterol

#### 5.2 Developing the base of Simulation in DWSIM:

##### 5.2.1 Steps of Simulation

1. Calling proper thermodynamic packages.
2. Collection of compounds required from the simulation.
3. Choosing suitable/convenient unit system for the calculations.
4. Finally, simulation the process according to flow diagram.

### 5.3 Simulation in DWSIM

For the simulation given in the above picture we have used the components present in the Ficus Religiosa aka Peepal tree Leaves and have added the mixture in the following inlets . The components are Beta-sitosterol , Beta Cartotene , Lenoleic , Palmitoleic Acid. The four components are mixed at the mixture and directed towards outlet 1. The mixture is heated at heater and is passed on to outlet 2 . Then the mixture is feed to Shortcut Distillation where the separation of the components is taking place .After the separation the material stream is cooled and we acquire the following compound

## CHAPTER 6

### RESULT AND DISCUSSION

In the experimental work we studied different temperature (250C, 350C, 450C & 550C), different rpm's (100rpm, 200rpm & 300rpm), different time (1hr, 2hr, 3hr, 4hr) & different particle size. Sample was pipette out after every 10 min for UV analysis when the batch extraction started. Results are obtained by UV that shows the concentration of beta-sitosterol in the extract phase at that particular time. By using the optimized condition that we have obtained that gives different values at different time. All values are noted down in fig. 5.1 below:

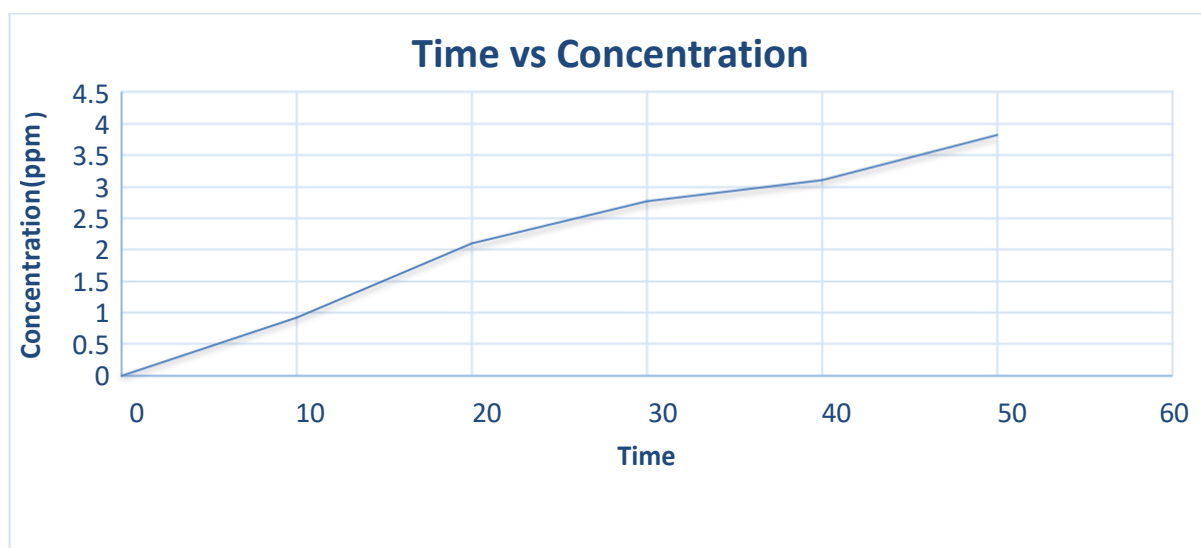


Figure 5.1: concentration of Beta-Sitosterol vs Time

#### **6.1. Soxhlet Extraction**

We have taken 3 runs in Soxhlet extraction at boiling point of solvent (640C) i.e. for 2hrs, 4hrs & 6hrs. Result shows concentration of beta-sitosterol in the extract phase is increased with the increase in extraction time. For 2hrs run the concentration is less as compared to 4hrs & 6hrs. After completion of particular time we checked the concentration of beta-sitosterol in the extract phase by UV spectrometer and whenever the extraction time increases, the concentration of beta-sitosterol increases in the extracted product.

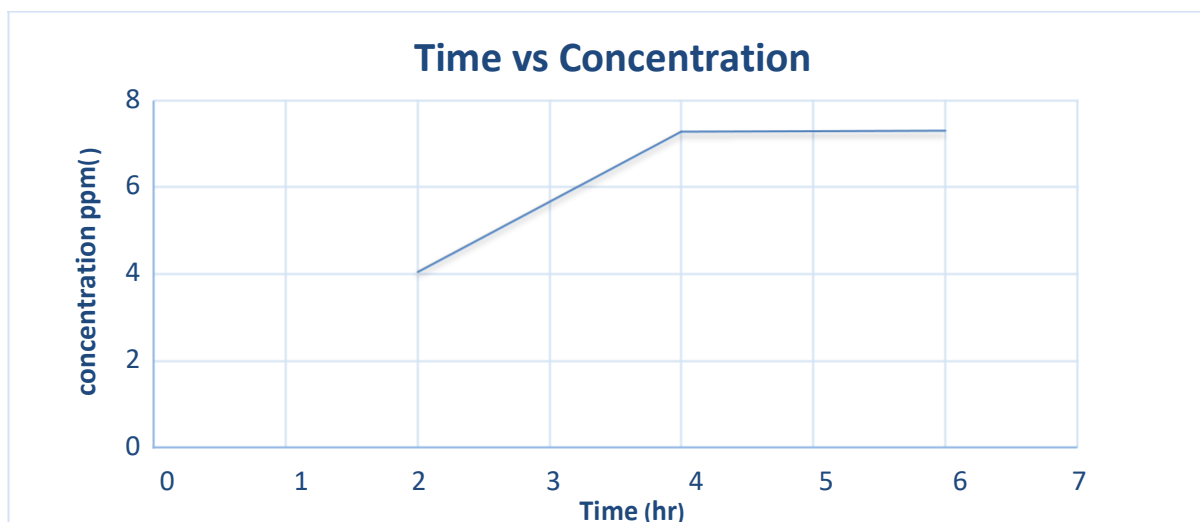


Fig. 5.2: Concentration of Beta-Sitosterol obtained from Soxhlet Extraction

## **6.2 Speed of agitation:**

### **6.2.1 For 200 rpm & 30<sup>0</sup>C:**

About 20 gms of powder of Neem Leaves was weighted and it was fed to Batch Reactor with 200ml of ethanol. Process was carried out at temperature of 300C different intervals for 2.30 hours. At this 200 rpm the effect of % Extraction was analyzed on UV unit.

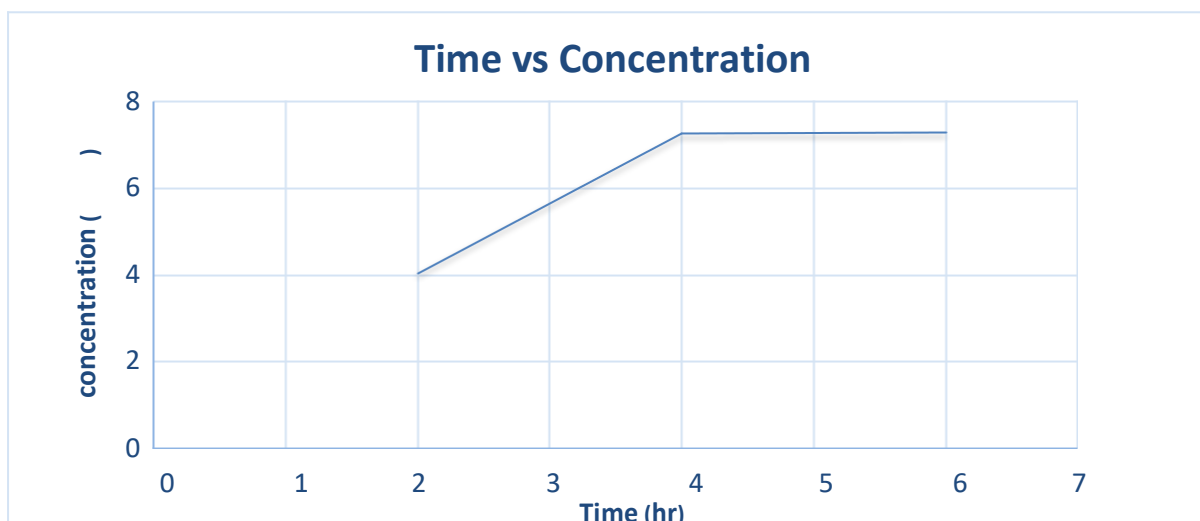


Fig. 6.2: Concentration of oleic acid obtained from Batch at 300C at 200RPM

### 6.2.2 For 400 rpm & 30<sup>0</sup>C

About 20 gms of powder of Neem Leaves was weighted and it was fed to Batch Reactor with 200ml of ethanol. Process was carried out at different intervals for 2.30 hours. At this 400 rpm the effect of % Extraction was analyzed on UV unit.

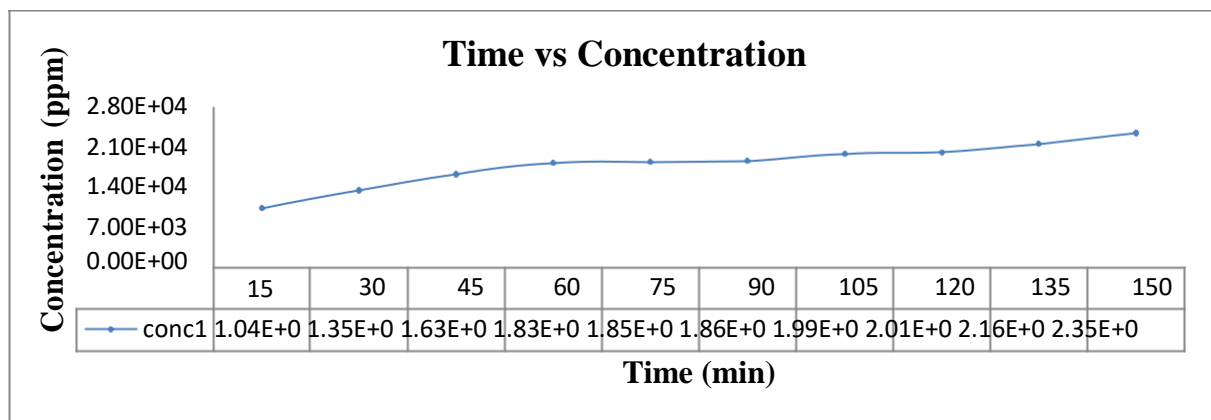


Fig. 5.3: Concentration of oleic acid obtained from Batch at 300C at 400 RPM

### 6.2.3 For 200 rpm & 40<sup>0</sup>C:

About 20 gms of powder of Neem Leaves was weighted and it was fed to Batch Reactor with 200ml of ethanol. Process was carried out at different intervals for 2.30 hours. At this 200 rpm the effect of % Extraction was analyzed on UV unit.

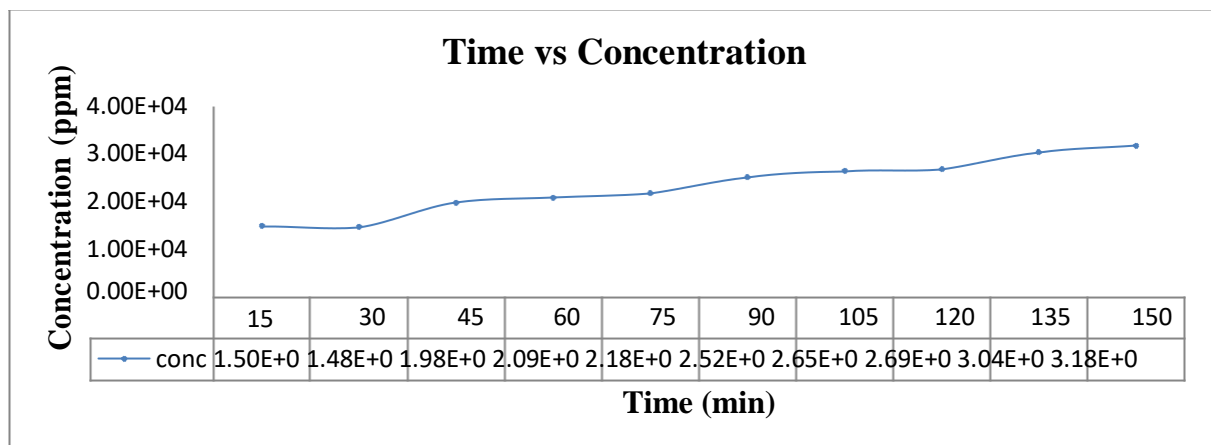


Fig. 5.6: Concentration of oleic acid obtained from Batch at 400C 200 RPM

### 6.3. Effect of Temperature:

With increase in temperature, the solubility of beta-sitosterol in the methanol increases and it was expected that the extraction would also increase. Therefore, extraction is carried out at different bath temperatures i.e. 25°C, 35°C, 45°C & 55°C & results are shown in fig.5.3 below. Solubility of beta-sitosterol in methanol increases with increase in temperature, so the extraction also increases.

Extraction temperature is the most important parameter because chemical constituent of the plants is mostly heat sensitive. The solubility of solute and the diffusion co-efficient increases with increase working temperature which favors extraction. But, at certain temperature compound stability may be affected due to chemical degradation or losses by thermal decomposition. Time and temperature are important parameter to be optimized in order to reduce energy cost of the process. The extraction temperature could markedly influence the recovery of bioactive ingredient during liquid-solid extraction.

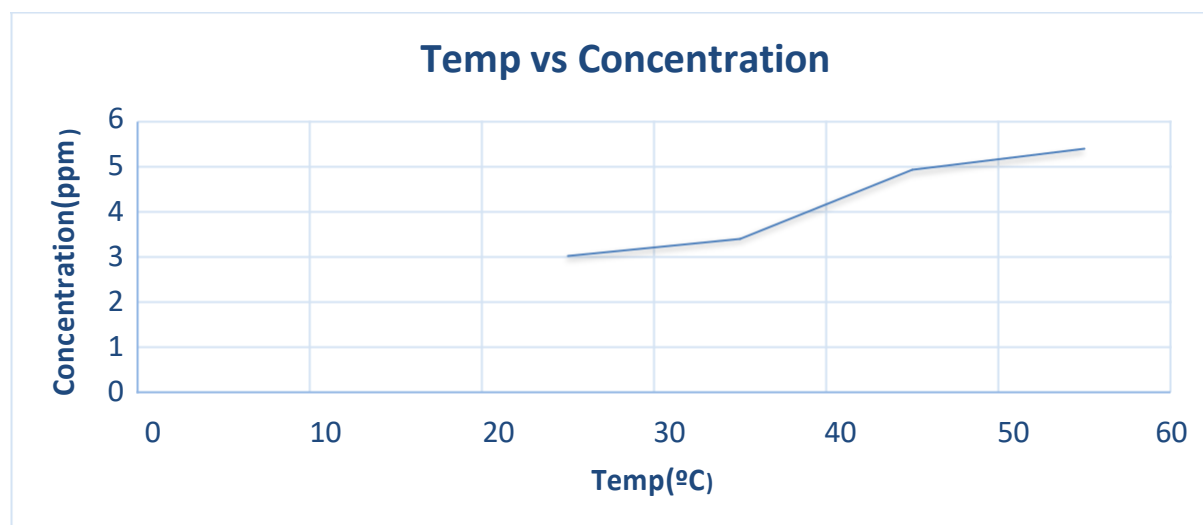


Figure 6.3: Effect of different temperature on Concentration of beta-sitosterol

#### 6.4. Effect of RPM:

At high speed of agitation more turbulence is generated which result in high mass transfer rate in batch reactor. It is clear from result show in graph that with increase in the speed of agitation from 100 rpm to 300 rpm, there is slightly decrease in concentration because of vertex formation the more turbulence is not done and the concentration of Beta-Sitosterol is decrease.

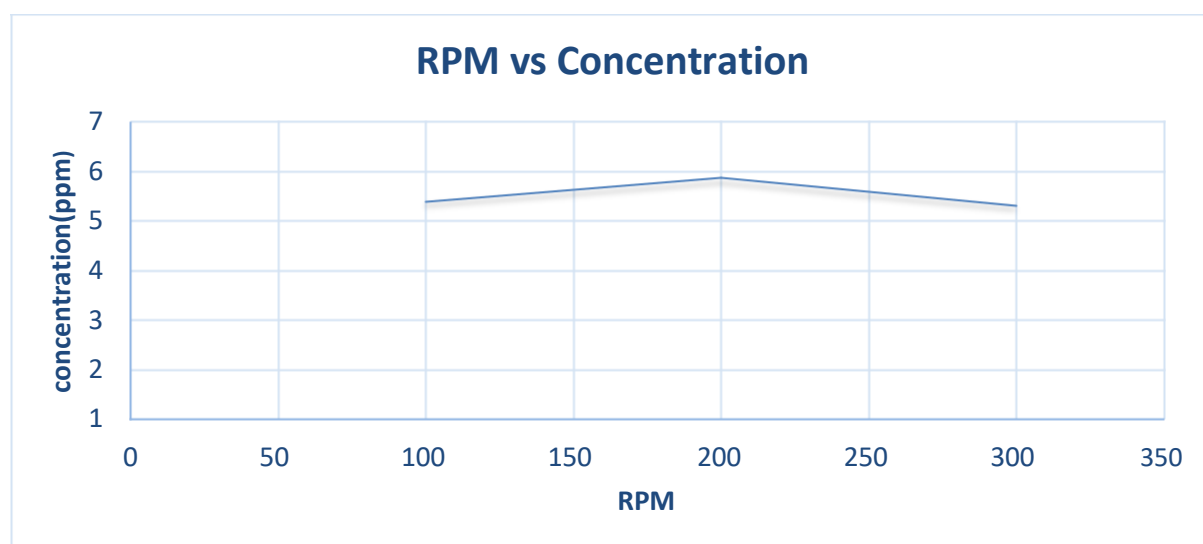


Figure 5.4: Effect of different RPM at 550C on Concentration of beta-Sitosterol

Further increase in the speed of agitation has no significant effect on yield of extraction which clearly shows that external mass transfer resistance is negligible at 200 rpm. Thus further experiment has been carried out using 200 rpm.

#### 6.5 Effect of time:

As increase in time, the concentration of beta-sitosterol in the methanol decrease. It is clear from result show in graph that with increase in time from 1hr to 4hr there is decrease in concentration. Further increase in the time has no significant effect on yield of extraction. Thus further experiment has been carried out using 3hr.

#### 6.6 Effect of particle size of dry leave power:



As increase in particle size, the concentration of beta-sitosterol in the methanol decreases. It is clear from result show in graph that with decrease in particle size from 90 micron(sieve), 75 micron(sieve) to 53 micron(sieve) there is increase in concentration., Thus further experiment has been carried out using 53 micron(sieve).

## **6.7 Selection of Solvents**

The effect of solvent on extraction of BA from Vitex negundo is studied to select the best solvent. Different solvent like methanol, ethanol, isopropyl alcohol, and water were used to find out the best solvent for BA at room temperature. A probable reason for selecting methanol is, it could be low molecular weight which helps to penetrate better inside the cellulose matrix of Vitex Negundo. Other alcohols like ethanol or IPA is bulky thus they do not penetrate properly, hence gradually lowers the extraction. BA is shows any solubility in water.

## **7. FUTURE SCOPE**

There are several herbs which provide oleic acid as their extract namely olive leaves and palm leaves from which we can isolate and use for the same purpose. But neem leaves were taken for consideration for its easy availability. So in future we would like to do more works on other raw materials which we had not gone through till now. Also there are several other methods like ultra-sonic, micro-wave and super critical fluid extraction (SFE) by which we can obtain the pre-mentioned extract which can be obtained. We have to optimize the working model according to that. The particle size is a dependent factor which can be varied on the basis of which we can get more extract should be varied. More test like iodine test and carbohydrates can be performed. Also the analysis of many other components inside neem oil can be done using GC and HPLC by which we can get the complete composition of our product thus obtained. Different aspects are self-addressed during this chapter. The new integrated approach consisting of a screening of extraction conditions AND AN in vitro mensuration of purposeful activities along side an complete chemical characterization can offer US with a replacement tool to get new bioactive compounds and to assist the any style of processes to get such merchandise within the greenest, property, and economical means, obliging with the foundations of inexperienced chemistry and inexperienced engineering. during this chapter we have a tendency to discuss and gift some elect applications of the extraction of target compounds with, among others, inhibitor, antimicrobial, and anti-proliferative activities from totally different property marine sources. We have shown several prospects at laboratory scale, that square measure the idea of the data, which may be used because the opening move for a much bigger production scale. beyond question, the replacement of environmentally heavy solvents like acetonitrile, methanol, chloride, and dissolving agent, long utilized in such extraction processes, for additional inexperienced solvents like dioxide, ethanol, and water is one in all the goals and one in all the proposals that we have a tendency to square measure providing to the reader during this chapter.

## **CONCLUSION**

It was concluded from the present investigation that the selected species of *Ficus religiosa* contains various active phytosterols, flavonoids, tannins. It also contains the core ingredient of the project that is, Betasitosterol which is a very expensive and highly medicinal chemical found in *ficus religiosa* which is used in the treatment of various diseases. Diseases like tuberculosis, heart problems, skin problems and many more.

As the compound is very useful but found rare as well as the compound is very expensive therefore in our project we are analyzing the exact method for detection as well as high concentration extraction of betasitosterol. All parameters unit operation is studied in project work and betasirosterol is detected by using ultra violet spectrophotometer. Hence we conclude that we have detected our compound that is betasitosterol from our extract which contains methanol and powdered peepal leaves as well as optimized conditions were analyzed.

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