RESEARCH PLAN PROPOSAL

COMPARATIVE PHYTOCHEMICAL STUDIES OF TWO VARIETIES OF CYAMOPSIS TETRAGONOLOBA AND THEIR BIOEFFICACY

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# Table of contents

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Content</th>
<th>Pg. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Introduction</td>
<td>3-8</td>
</tr>
<tr>
<td>2.</td>
<td>Review of literature</td>
<td>9-16</td>
</tr>
<tr>
<td>3.</td>
<td>Objectives of study</td>
<td>17</td>
</tr>
<tr>
<td>4.</td>
<td>Significance of study</td>
<td>18</td>
</tr>
<tr>
<td>5.</td>
<td>Methodology</td>
<td>19-21</td>
</tr>
<tr>
<td>6.</td>
<td>Plan of work</td>
<td>22</td>
</tr>
<tr>
<td>7.</td>
<td>References</td>
<td>23-24</td>
</tr>
</tbody>
</table>
INTRODUCTION

The use of local plants in folk medical practices has a long history. The resource base of the traditional medical practices prevalent in rural and tribal villages of India and abroad is mainly the plants. (Lakshman et al 2012) The plants which have medicinal properties are used to maintain and promote healthy life, prevent disease and cure ailments. Record show that even today, 80% of the world population depends on herbal traditional medicine for their primary health care. (Qureshi et al 2008).

Plants are rich in a wide variety of secondary metabolites like tannins, terpenoids, alkaloids and flavanoids, which have been found antimicrobial properties. Laboratories are all over the world have found, literally, thousands of phytochemicals which have inhibitory effects on all types of micro-organisms (Cown 1999).

The meaning of phytochemistry is the study of phytochemicals. These are chemicals derived from plants. Phytochemicals are non- nutritive plant chemicals that have protective or disease preventive properties. They are nonessential nutrients, means they are not required by the human body for sustaining life. We known that plant produces these chemicals to protect them-selves but recent research demonstrate that they can also protect humans against diseases. (Lakshman et al 2012).
In the field of phytochemistry, the techniques which are commonly used are extraction, isolation and structural elucidation (MS, 1D and 2D NMR) of natural products, as well as various chromatography techniques (HPLC, GC-MS). Phytochemistry is mostly used in the field of indigenous medicine especially in the field of herbal medicine.

For thousands of years, medicine depended exclusively on leaves, flowers and barks of plants because they consist of a wide variety of chemical compounds that offer a promising source of new antimicrobial agents. The use and search for drugs and food supplements obtained from natural sources like plant extracts have increased in recent years. Plant are rich source of secondary metabolites are which exhibit significant pharmacological effects. Recently many new synthetic drugs have come into use and in many instances these are carbon copies of chemicals identified in plants (Conway 1973). Hence phytochemical methods are important to screen and analyze bioactive components, not only for the quality control of crude drugs but also for the elucidation of their therapeutic mechanism.

The Fabaceae or Leguminosae commonly known as the legume, pea, or bean family, are a large and economically important family of flowering plants. It includes trees, shrubs and herbaceous plants perennials or annuals, which are easily recognized by their fruit (legume) and their compound, stipulated leaves. Records demonstrate that this group is widely distributed and is the third-largest land plant family in terms of number of species, behind only the Orchidaceae and Asteraceae, with 730 genera and over 19,400 species. The largest genera are Astragalus (over 2,400 species), Acacia (over 950 species), Indigofera (around 700 species), Crotalaria (around 700 species),
and Mimosa (around 500 species), which contain around 9.4% of all flowering plant species. Tropical rainforests and dry forests in America and Africa are where the Fabaceae family is most commonly found.

Many members of Fabaceae family show a special character. Their roots are colonized by a bacteria known as Rhizobium that have the ability to take nitrogen gas (N\(_2\)) out of the air and convert it in the form of usable nitrogen in the form of NO\(_3^-\) or NH\(_3\). This process is called nitrogen fixation. In this relationship, the legume, acting as a host, and rhizobia, acting as a provider of usable nitrate, form a symbiotic relationship. In all Fabaceae family comprises of 470 genera and 14,000 species.

*Cyamopsis tetragonoloba* which is commonly known as Guar has been grown in India since ancient time for vegetables and fodder purposes. At present it is grown more for mucilaginous gum production. The presence of a number of wild relatives of guar in Africa suggests that it was most probably originated in Africa. In India, it is grown in Rajasthan, Gujarat, Haryana, Punjab, Uttar Pradesh, Madhya Pradesh and Orissa. Guar plant produces a cluster of flowers, therefore, it is also known as cluster bean. Its taproot system is well developed. Therefore, it is also popular as drought hardy plant and can survive better under comparatively dry conditions.
Classification (as per Bentham and Hooker system):

Kingdom: Plantae
Division: Dicotyledonae
Order: Rosales
Family: Fabaceae
Sub family: Papillionatae
Genus: *Cyamopsis*
Species: *tetragonoloba*

Guar is a coarse, upright, bushy, drought-resistant, shrub ranging from 2 to 9 feet in height. It has pointed, saw-toothed, trifoliate leaves up to 10 cm long and small purplish flowers borne along the axis of a spikelet. The pods are straight, hairy, pale green, up to 12 cm long and contain 5 to 12 hard seeds (beans) each. There are both dwarf and tall cultivars. Guar flowers are self-pollinating. A mature unopened bud starts out white and then changes to a light pink as petals begin to open. Finally, the flower is deep blue. However, the plant morphology is highly variable. Guar has a deep tap root system and with the help of this they can find moisture deep below the soil surface. (Lakshman *et al* 2012)

*C. tetragonoloba* L. is a well-known traditional plant used in folklore medicine. It acts as an appetizer, cooling agent, digestive aid, laxative, and is useful in dyspepsia and anorexia and has anti-ulcer, anti-secretory, cytoprotective,
hypoglycemic, hypolipidemic and anticancer effects. (Mukhtar et al 2004) The pods contains condensed tannins together with p-coumaric, caffeic, gallic and gentisic acids, quercetin, kaemfeol and its 3-arabinoside, p-hydroxycinamyl and coniferyl and coniferyl alcohol.

Because of its nutritional qualities, beans can successfully replace meat. Its rich containment of proteins (approximately 70% of the necessary amino acids which can be found in meat), carbohydrates and the lack of toxins, it is best recommended for its beneficial effects on health. Green beans contain about twice as much iron than spinach. Besides its protein content, this bean also contains organic and mineral substances (sodium, potassium, calcium, magnesium, silica, nickel, copper, and cobalt), vitamins (A1, B1, B2, B5, B6, C, E, K and P1). Green bean carotenoids include lutein, beta-carotene, violaxanthin, and neoxanthin and flavonoids include quercetin, kaemferol, catechins, epicatechins, and procyanidins. Bean also contains fibers (soluble and insoluble) with an important role in the body: it reduces the level of cholesterol, prevents constipation and colon cancer. Beans also benefit the immune system through its anti-infectious effect which facilitates the growing of the number of leucocytes, destroying injurious microorganisms in the body. This effect is present because of chromium - a substance present in beans with the role of stimulating lymphatic ganglions. (Chaurasia et al 2012).

The Present studies deals with the identification of phytochemicals and there quantitative estimation, antimicrobial activity of Cyamopsis tetragonoloba and its important medicinal qualities.
Fig: showing production of *Cyamopsis tetragonoloba* in India

Fig: A twig of *Cyamopsis tetragonoloba* (Guar)  
Fig: Seeds of *Cyamopsis tetragonoloba*
REVIEW OF LITERATURE

Khan *et al* (1981) estimated the effect of guar gum in capsule form on serum total cholesterol, triglycerides, and lipoprotein cholesterol (very low-density lipoproteins, low-density lipoproteins, and high-density lipoproteins) in healthy volunteers in a double-blind study.

Sarathy *et al* (1983) shows that tender cluster bean pod (*Cyamopsis tetragonoloba*) is a vegetable commonly consumed in South India and contains 20% of guar gum on dry weight basis. Effects of incorporating this vegetable into a hypercholesterolemic diet of rats were studied. All test materials--cluster bean 0.5 and 2.5% levels, commercial guar gum (0.5%), cluster beans partially free from guar gum (1.6%), neutral detergent fiber of cluster bean (0.6%)--exerted a hypocholesterolemic effect and also lowered liver cholesterol concentration, but did not alter liver weights. Neutral detergent fiber of cluster bean and guar gum lowered cholesterol levels.

Khatta *et al* (1988) examined four cultivars of *C. tetragonoloba* L. and found 24.5-32.9% crude proteins, 2.4 - 3.3% crude fats, 3.2 - 4.0% ash and 9.0 - 10.2% crude fibers. Hafedh *et al* (1998) reported that the *C. tetragonoloba* L. beans had 32.81% crude proteins, 3.18% crude fats, 4.19% ash and 10.87% crude fibers.

Miettinen *et al* (1989) examined short-term viscous dietary fibres, Plantago ovata and guar gum preparations, decreased serum cholesterol, mainly LDL cholesterol, as compared to low fibre or nonviscous high fibre periods, through enhancing cholesterol elimination as fecal bile acids. These changes were associated with significant increases in serum levels of cholesterol precursors, both in methylsterols and demethylated precursor sterols, while that of cholestanol (saturated cholesterol derivative) was decreased.
According to Todd et al (1990) guar gum has pharmacological properties and can be used as a dietary adjunct in hypercholesterolaemia. Clinical trials indicate that, when used alone, guar gum may reduce serum total cholesterol by 10 to 15%. Guar gum as monotherapy may be considered at most modestly effective in reducing serum cholesterol levels.

Oral feeding of the aqueous extract of beans of *Cyamopsis tetragonoloba* was investigated on fasting blood glucose levels in glucose loaded, normal and alloxan-induced diabetic rats and compared with gliclazide, a reference drug. The aqueous extract of beans at 250 mg/kg body wt significantly lowered blood glucose levels in alloxan-induced diabetic rats within 3 hr of administration. Continued administration of the extract at the same dose daily for 10 days produced statistically significant reduction in the blood glucose levels of alloxan induced diabetic rats while marginal activity was seen in normal and glucose-loaded rats. (Mukhtar et al 2004)

Brennan (2005) explain the use of dietary fibres, especially soluble dietary fibres (such as guar gum, locust bean gum, and *Psyllium* fibres), resistant starch, and slowly digestible carbohydrates. These have been shown to alter food structure, texture, and viscosity, and hence the rate of starch degradation during digestion. And shows association between the rate of carbohydrate degradation during digestion, and the regulation of postprandial blood sugar and insulin levels. Kaushal et al (2006) outlined that guar beans contained flavonoids in the range of 0.13 to 0.23%. And also reported that gallic acid as well as its derivatives, kaempferol, caffeic acid and ellagic acid were derived from *C.tetragonoloba* L. beans.
Mukhtar et al (2006) shows that *C. tetragonoloba* L. is a well-known traditional plant used in folklore medicine. It acts as an appetizer, cooling agent, digestive aid, laxative, and is useful in dyspepsia and anorexia, anti-ulcer, anti-secretory, cytoprotective, hypoglycemic, hypolipidemic and anti-hyperglycemic effects.

Kays et al (2006) found that the protein, fat, carbohydrate and ash contents of *C.tetragonoloba* L. beans were ranged between 22.9 - 30.6%, 2.88 - 3.45%, 50.2 - 59.9% and 3.04 - 3.53%, respectively.

Prieto et al (2006) shows Short-term and long-term effects of guar on postprandial plasma glucose, insulin and glucagon-like peptide 1 concentration in healthy rats. And examined guar gum decreases postprandial glycemia and insulinemia and improves sensitivity to insulin in diabetic patients and several animal models of diabetes.

According to Ozaslan et al (2007) there is a growing trend in the use of medicinal plants because of their medical effectiveness and low toxicity. As a results many natural anticancer agents have been derived from these.

Guar beans are potentially high sources of additional phytochemicals. (Wang et al 2007) He stated that *C. tetragonoloba* L. beans are a good source of quercetin and kaempferol.

*Cyamopsis tetragonoloba* (L) Taub is a moderate sized annual herb found throughout India as a cultivar for its pods used as vegetable. Plant is popular in indigenous system of medicines like Ayurveda, Siddha and discussed in various traditional literatures. In traditional medicines various parts such as leaves, seeds and pods are used in diabetes, asthma, inflammation, as laxative, antibilious agents, appetite depressor (weight loss not observed) and hypolipidemic agent. (Sharma et al 2007)
Therapeutic and medicinal properties lies in the soluble dietary fiber content of guar gum to improve the serum biochemical profile of human and non-human primates, reducing total serum cholesterol, triglycerides, increasing the high density lipoprotein cholesterol level, and the management of glycemic indices and obesity and it is effective in reducing the cardiovascular disease risk, diabetes and weight loss programs. (Butt et al 2007)

According to Goyali et al (2007) fleshy green tender pods of cluster bean, are a typical tropical warm climate vegetable crop, grown in subtropical area during summer and used as vegetable. The rough shined hairy types are used as fodder for the cattle and as green manuring crops. It is rich source of protein (3.2%), fibre (3.2%), carbohydrate (10.8%), calcium (130 mg%), phosphorus (57 mg%), and iron (0.6 mg%). Galactomanan, the essential ingredient of guar gum is highly mucilagnous and used in various industries. Guar gum of cluster bean being rich in soluble fibre, i.e. non starchy polysaccharides is useful for patients suffering from obesity, diabetes and hyperlipidemia.

Hypocholesterolemic effects associated with soluble fiber consumption are clear from animal model and human clinical investigations. Guar gum use as soluble fibers in the regulation of cholesterol metabolism. (Rideout et al 2008)

Hassan et al (2009) reported haemolytic and antimicrobial activities of saponin-rich extracts from guar meal. Results indicated that only 100% MeOH fraction and its 16 min peak sub-fraction exhibited both haemolytic and anti-bacterial activities against Staphylococcus aureus, Salmonella typhimurium and Escherichia coli, but 20% and 60% MeOH fractions stimulated Lactobacillus spp. growth.

According to Sharma et al (2010) Cyamopsis tetragonoloba is a weed of Rajasthan and has ethno-botanical importance. When land is cultivated to raise crops, weeds
spring up naturally along with the crop plants. Weeds are defined as “a plant out of place or an unwanted plant or a plant with a negative effect or plants that compete with man for the soil” (Kasera et al 1998).

Plants that are specifically employed for the treatment of diabetes are Acacia nilotica, Acacia senegal, Aegle marmelos, Calotropis procera, Capparis deciduas, Cassia auriculata, Cassia sophera, Cayratia trifolia, Cyamopsis tetragonoloba, Dalbergia sisso, Gymnema sylvestre, Momordica charantia, Syzygium cumini, Withania somnifera. (Batra et al 2011)

Thirty one genotypes of clusterbean [Cyamopsis tetragonoloba (L.)] were evaluated to estimate variability, broad sense heritability and genetic advance over mean for vegetable pod yield and related attributes, during Kharif 2009. Maximum range of variability was observed for number of branches, plant height, clusters per plant, pod length and pod yield per plant. High heritability coupled with high genetic gain in percentage was observed for pod yield per plant, number of pods per plant, days to 50% flowering, number of branches and plant height. High heritability with low genetic advance was shown for pods per cluster, number of seeds per pod and pod width. The results of present investigation suggests that selection based on plant height, number of branches, days to 50% flowering, clusters per plant and pod yield per plant might bring simultaneous improvement in vegetable pod yield of cluster bean. (Shabharshrai et al 2011).

Mohamed et al (2011) observed biochemical studies on Plantago major L. and Cyamopsis tetragonoloba L. Guar beans had high contents of proteins, fats and total hydrolysable carbohydrates. Plantago leaves had high percentage of linolenic acid (56.19%), While its seeds and Guar beans had high percentages of linoleic acid (25.41 and 48.99%, respectively). Essential and non essential amino acids
were present in all samples and Guar beans had high amounts of glutamic, arginine, aspartic and leucine.

_Cyamopsis tetragonoloba_ fruit and _Cyperus rotundus_ rhizome were analysed by GC-MS analysis to assess their phytochemical constituents. The ethanolic extract of _C. tetragonoloba_ fruit showed the presence of thirty four phytochemical constituents. The major phytochemical compounds were inositol, ethyl alpha-d-glucopyranosid end stigmasterol. The ethanolic extract of _C. rotundus_ showed the presence of twenty two phytochemical constituents. The major phytochemical compounds were 7-isopropenyl-1,4a-5,6,7,8-hexahydro-3H-naphthalen-2-one, zierone and (+)-cis-longipinan. (Surendran et al 2012)

Quality assessment of guar gum (Endosperm) of guar (C. tetragonoloba) was done by Murwan et al (2012) Six guar genotypes were used for this study The results revealed chemical compositions of endosperm of the guar seed as : 4.8-8.7 % moisture, 3.5-5.5 % protein, 0.5-0.9 % fat, 0.5- 1.3 % ash, 1.4- 2.0 % fiber and 83.3-87.5 % carbohydrates.

Pande et al (2012) shows that Cluster beans (C. tetragonoloba) are rich source of soluble fiber content and are known for their cholesterol lowering effect. The beneficial anti-hyper cholesterolaeamic effect of whole dietary cluster beans as a source of dietary fiber was evaluated in high cholesterol diet induced hyper cholesterolaeamia in experimental rats.

Chaurasia et al (2012) shows the antibacterial activity of four different varieties of green beans. Fresh extracts of pods of _Phaseolus vulgaris_ (french beans), _Vicia faba_ (broad beans; saem), _Cyamopsis tetragonoloba_ (cluster beans; guar) and _Vigna unguiculata_ (cowpea; lobia) were tested against two human pathogenic bacteria: _Escherichia coli_ (gram negative) and _Bacillus subtilis_ (gram positive).
using disc diffusion assay. The streptomycin was used as the control. The results showed that all the extracts possess antibacterial activity (bacteriostatic activity). The range varied from 9 mm to 15 mm against *E.coli* and 10 to 12 mm against *B. subtilis*.

Sharma *et al* (2012) analysed genetic and Phytochemical constitute of Cluster bean (*Cyamopsis tetragonaloba* L.) by RAPD and HPLC in which five varieties of guar RGC-936, 1002, 1003, 1031 and 1017 were taken. Phenolic acids such as sinapic acid, cholorogenic acid, caffeic acid and gallic acids were detected among all cultivars. Whereas flavanoids i.e. kaempferol and myricetin were showing variations among all cultivars. The phytochemical analysis of guar may expand its nutraceutical and pharmaceutical utilization and information from this study will be useful to breeding programmes for improving guar seed quality.

Lakshman *et al* (2012) done the phytochemical screening, quantitative estimation total phenolics and total flavonoids, anti microbial evaluation of *Cyamopsis tetragonoloba*. According to them Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. They are nonessential nutrients, meaning that they are not required by the human body for sustaining life. It is well-known that plant produces these chemicals to protect themselves but recent research demonstrate that they can also protect humans against diseases. *Cyamopsis tetragonoloba* is also traditionally known used as a laxative. It is also used for treating diarrhea, irritable bowel syndrome (IBS), obesity, and diabetes, for reducing cholesterol. The medicinal properties of the plant are due to the anti oxidants present in the plant. Present studies deals with the identification of phytochemicals and there quantitative estimation, antimicrobial activity of *Cyamopsis tetragonoloba* and its important medicinal qualities provided
one does not play around with poisonous plants they are totally free of harmful side effects - unlike the modern drug industry.

Singh et al (2013) Show that ethanol and aqueous extracts from leaf, stem and fruits of *Cyamopsis tetragonoloba* (L.) Taub. were investigated for their anthelmintic activity by using four concentrations (12.5, 25, 50, 100 mg/ml) of each extract against *Pheretima posthuma* as test worms.

According to Srinivasan et al (2013) Soluble fiber-rich tender cluster bean pod (*Cyamopsis tetragonoloba*) and a combination of CB and capsaicin, a thermogenic spice compound, were evaluated for weight-reducing effect in high-fat-fed Wistar rats.

According to Pandey et al (2013) Gastro intestinal protective effect of soluble, dietary fiber-rich tender cluster beans, with respect to the activities of antioxidant enzymes and the concentration of antioxidant molecules, was examined in Wistar rats. Dietary intervention with tender cluster beans significantly enhanced the activities of antioxidant enzymes and the concentrations of antioxidant molecules in both gastric and intestinal mucosa. The gastroprotective effect of the cluster bean was also reflected in its positive effect on gastric mucosal glycoproteins, resulting in a lowering of mucosal injury. Incidentally, the serum and liver also showed an elevated antioxidant status, thus suggesting desirable lowered oxidative stress results when tender cluster beans are consumed.
OBJECTIVES

Considering the above facts, this research work entitled, “Comparative phytochemical studies of two varieties of *Cyamopsis tetragonoloba* and their bioefficacy” has been taken up with the following objectives:

1. To collect healthy plant seeds and confirm their variety.
2. To analyze physicochemical constituents of the plant.
3. To purify and identify the isolated component by chromatographic techniques.
4. To study the biological activity of the crude extracts as well as the components. (antibacteria or antihelminthic activity)
5. To elucidate the structure of the isolated components by spectroscopy.
6. Identify the hypolipidemic and hypocholesterolemic effect of plant extract as well as the component on particular sample.
SIGNIFICANCE

It is widely accepted that research on medicinal plants should be focussed primarily on species whose pharmaceutical activities have already been demonstrated. The present study is therefore designed to provide scientific evidence for the selected plants to be used as a traditional folk remedy by investigating the phytochemical constitution of the plant as well as the antibacterial activity. Essential oils would be evaluated for antimicrobial activity against gram-positive and gram-negative bacteria strains obtained from standard agencies. Developing countries have the potential of plant resources that exhibit a wide range of biological activities which may help in the development of cheaper and more potent anti-microbial agents. Many plants have been used because of their antimicrobial traits, which are chiefly synthesized during secondary metabolism of the plant. Nearly, all culture and civilizations from ancient times to the present day have depended fully or partially on herbal medicine because of their effectiveness, affordability, availability, low toxicity and acceptability. Therefore, newer plants should be investigated to better understand their properties, safety and efficacy.

The abuse of drugs for ailments is increasing with time which leads to side effects & sometimes death. It is, therefore, very important to search for effective but low cost and reliable traditional therapeutic agents. This work is therefore aimed at studying of photochemical properties of two varieties of *Cyamopsis tetragonoloba* and their biological activities.
METHODOLOGY

1. Identification and authentication of plant seeds of *Cyamopsis tetragonoloba*.

2. Samples which are collected are shade dried and transported to the laboratory in accordance with standard procedures.

3. Seeds will be selected as a sample from *Cyamopsis tetragonoloba*.

4. Powdering of shade dried Plant material for extraction.

5. Hot soxhlet extraction for various phytochemicals using solvent series (n-hexane, chloroform, ethyl acetate, methanol and water) followed by Physico-chemical analysis test according to Harborne’s standard guide.

6. Qualitative estimation

   - Tests for carbohydrates
     
     (By Molisch’s test, Fehling’s test, Benedict’s test)

   - Tests for glycosides
     
     (By Legal’s test, Borntrager’s test)

   - Tests for alkaloids
     
     (By Mayer’s test, Dragendorff’s test, Hager’s test, Wagner’s test)

   - Tests for Phyto steroids
     
     (By Salkowski test, Liebermann- Burchard test)

   - Tests for flavanoids
(By Shinoda test, Ferric chloride test, Alkaline reagent test)

- Tests for saponins
  (By foam test)

- Tests for Proteins and amino acids
  (By Biuret test, Million’s test, Xanthoprotein test, Ninhydrin test)

- Tests for Tannins and Phenolic compounds
  (By 5% Fecl₃ sol., Lead acetate sol., Acetic acid sol., Dilute iodine sol., Dilute HNO₃)

- Tests for fixed oils and fats
  (By Spot test, Saponification test)

7. Quantitative estimation -

  (Estimated content is calculated as % of the starting materials)

8. Extracted crude samples will be redissolve in respective solvents & preserved as mother sample for further analysis of biological activity.

9. Isolation -

Samples collected will be processed further for Isolation, Purification and Identification of unknown compounds using Chromatography technique. (Thin layer chromatography or Column Chromatography)

Characterization each of the component individually by some standard techniques by GC-MS & NMR.
10. Purified extract will be treated against selected bacterial strains (by Disc Diffusion method / MIC / Agar well Diffusion method or Kirby-Bour method 1961) for further assessment of antimicrobial activity & evolution of the various biochemical parameters.
**PLAN OF WORK**

**PHASE I** - Sample collection from some of the selected areas

**PHASE II** - Crude extract preparation by the help of soxhlet extractor

**PHASE III** - Storage and preservation of samples for some Physico-chemical Analysis via qualitative and quantitative estimation & analysis of their biological activity.

**PHASE IV** - Isolation & Purification of bioactive compounds by TLC and column chromatography technique.

**PHASE V** - Characterize each of the component individually by GC-MS and further characterization by NMR

**PHASE VI** - Assess the antimicrobial activity of the individual compound against selected bacterial strain (By Disc Diffusion method / MIC / Agar

**PHASE VII** - Screening the effects of these isolated compounds for further possible uses

**PHASE VIII** - Documentation and Thesis Writing
REFERENCES


