Synopsis of the proposed research plan

Title of research proposal

“PHARMACOLOGICAL EVALUATION OF Aegle marmelos L., Helicteres isora L. AND Artocarpus heterophyllus Lam.”

Submitted for the degree of

Doctor of Philosophy in

BOTANY

Under

Faculty of science & technology

Submitted by

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(M.Sc. Botany, B.Ed., PET)

Under the guidance of

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N.E.S. Science college, Nanded.

Re-accredited with “A” Grade by NAAC (CGPA 3.38)

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INTRODUCTION

Diabetes mellitus commonly known as diabetes is a chronic metabolic disease in which there is an increase in blood glucose level in the body. This increase in blood glucose levels is known as hyperglycemia. It occurs due to the insulin deficiency, insulin resistance or both. If the disease is not treated properly it may result into the prolonged complications like kidney failure, heart disease, foot ulcers, damage to the eyes, weight gain hypoglycemia, gastrointestinal disturbances liver toxicity. Diabetes can be characterized by abnormal metabolism of carbohydrates, lipids, lipoproteins. It is one of the most common disorders emerging from worldwide. (Credo et al., 2018)

Jaradat et al. (2021) worked on Orobanche aegyptiaca. They reported that diabetes and obesity are global health care problems that have posed a serious threat to both human and environmental ecosystems The goals of the present investigations are to investigate the phytoconstituents.

Medicinal plants constitute effective source of both traditional and modern medicines. Medicinal herbs have remained popular as an alternative medicine due to their low cost, effectiveness and historical, cultural and religious preferences. Herbal medicine has been shown to have genuine utility. About 80% of rural population depends on it as a primary health care. Medicinal plants are the richest bio-resource. Natural bioactive compounds found in different parts of the plant like fruit, flower, stem, leaf, root etc.

Credo et al. (2010) studied that, the currently available modern medicines used for diabetes management include insulin injections and oral hypoglycemic agents have not been able to cure the disease. Use of allopathic medicines causes adverse side effects and complications to the patients after prolonged use. Due to this antidiabetic drug discovery has shifted to medicinal plants as an alternative source of new anti-diabetes. Based on traditional medicine information, diabetes can be treated with herbs and medicinal plants for a long time.

Singh et al. (2022) did their work om characterization of bioactive compounds. India is recognized as the worlds botanical garden because it produces most of the medicinal plants. Among the list of 21,000 medicinal of diabetes by the medicinal plants involves the use of those plants which show hypoglycemic activity. Among 450 verified medicinal plants are with antidiabetic potential. The discovery of novel medicinal plants extensively relies on traditional knowledge and the historical literature. Because of efficacy in human clinical trials and the low side effects of drugs derived from medicinal herbs, medicinal plants have emerged as a major
priority in the search of novel antidiabetic medicine. Because of the increasing prevalence, there is a growing need to establish an integrated approach for diabetes management and prevention by exploring the efficacy of traditional herbal remedies. There are several reports highlighting variety of medicinal plants and plant-based formulations that exhibit antidiabetic properties. This activity mainly belongs to the different families like Leguminosae, Lamiaceae, Liliaceae, Cucurbitaceae, Asteraceae, Moraceae, Rosaceae, Araliaceae etc.

The few scientific evaluated medicinal plants reported to show antidiabetic potential were shown to have useful active phytochemical compounds which includes terpenoids, alkaloids, phenolics, flavonoids, saponins, glycosides.

Among ancient civilizations, India has been known to be rich repository of medicinal plants. The forest in India is the principal repository of medicinal plants. These medicinal plants are largely collected as raw materials for manufacture of drugs and perfumery products. Treatment with medicinal plants is considered very safe as there is no side effects or minimal side effects. Most important fact is that use of herbal treatments is independent of any age groups and sexes. Medicinal plants are considered as a rich source of ingredients which can be used in drug development. Apart from that these plants play a critical role in the development of human cultures around the whole world. Some medicinal plants are considered as important source of nutrition.

**REVIEW OF LITERATURE:**

Das et. al. (2018) did the phytochemical screening of various secondary metabolites, antioxidant and anthelmintic activity of *Coscinium fenestratum* fruit pulp. Preliminary phytochemical screening of CF fruit extracts revealed the presence of alkaloids, phenols, flavonoids, tannins, steroids, resins etc which are responsible for biological properties. The combined aqueous and methanol extract resulted in significant anthelmintic and antioxidant order to find possible isolated compounds as a bio source for future novel antioxidants in food and pharmaceutical formulations. The findings in this report indicate for the first time that the CF fruit pulp has therapeutic value with prominent antioxidant and anthelmintic properties.

Credo et. al. (2021) evaluated the antidiabetic activity of extracts from five selected medicinal plants used traditionally to manage diabetes in Tanzanian. These five medicinal plants are *Afzelia quanzensis, Bridelia dувigneaudii, Cyphomandra crassifolia,*
Dioscora praehensilis, Ficus fischeri. The study was conducted from March to August 2017. Phytochemical screening indicated the presence of terpenoids, phenolics, saponins and glycosides. The root extract exhibited the most significant antidiabetic activity.

Marcia et. al. (2015) reviewed pharmacological activities of Myrcia. The non-volatile compounds isolated from Myrcia are usually flavonoids, tannins, acetophenone derivatives and triterpenes. Anti-inflammatory, antioxidant, antimicrobial activities have been described to Myrcia essential oils, while hypoglycemic, anti-hemorrhagic and antioxidant activities were attributed to the extracts. Flavonoid glucosides and acetophenone derivatives showed aldose reductase and $\alpha$-glucosidase inhibition, and could explain the traditional use of Myrcia species to treat diabetes.

Psidium guajava Linn. (Guava) is used not only as a food but also as a folk medicine in subtropical areas around the world because of its pharmacologic activities. In particular, the leaf extract of guava has traditionally been used for the treatment of diabetes in East Asia and other countries. Many pharmacological studies have demonstrated the ability of this plant exhibit antioxidant, hepatoprotective, anti-allergic, antimicrobial, antigenotoxic, antiplasmodial, cytotoxic, cardioactive, anticough, antidiabetic, anti-inflammatory activities, supporting its traditional uses (Neeta Chaudhary and Shalini Tripathi 2013)

Gupta et. al. (2017) studied antihyperglycemic action of different extract of medicinal plants including Trigonella foenum-graecum, Bauhinia purpurea, Syzygium cumuni, Cymbopogon citratus, Triticum aestivum and Momordica charantia in a diabetic rat model.

Fatemeh et. al. 2013) studied the antidiabetic action of garlic juice (Allium sativum). The study includes change in biochemical and histopathological factors in STZ induced diabetic rats. On treatment of garlic juice the serum cholesterol and triglycerides were decreased. The results of test revealed that the fresh garlic juice might be helpful to avoid various complication of diabetes.

Kulkarni et. al. (2011) studied the different pharmacognostic evaluation parameters to ascertain the presence of various phytochemical constituents in Chonemorpha grandiflora belonging to Apocynaceae family. In vivo and In vitro methods were adopted for investigation. The presence of total alkaloids was found high percentage in stem and bark as compared to leaves. The phytochemical investigation showed the presence of steroids, alkaloids and tannins in stem and leaves extract.
Bhat et. al. (2008) studied about the inhibitory activity of α-amylase and α-glucosidase with different extract. The chloroform, methanol, and aqueous extracts of Murraya koenigii, Azadirachta indica, Oscimum tenuflorum, Linum usitatissimum, Syzygium cumini and Bougainvillea spectabilis were prepared from leaves and seeds. These extracts were tested for glucosidase inhibition activity. The results revealed that the chloroform extract of Oscimum tenuflorum, Bougainvillea spectabilis, Murraya koenigii Syzygium cumini have potent significant α-amylase activity. Glucosidase inhibitory activity was found in plants extract against murine pancreatic, liver and small intestinal crude enzyme.

Panchal et. al. (2019) did the phytochemical analysis of medicinal herb Oscimum sanctum. The main chemical constituents of Tulsi are Oleanolic acid, Ursolic acid, Rosmarinic acid, Eugenol, Carvacrol, Linalool etc. Plant extract continues the numerous searches for more effective drugs of plant origin which are less toxic and available for low socio-economic population in the treatment of diseases caused by pathogenic bacteria. Monoterpenes are obtained from the volatile oils such as camphene, myrcene, sabinene, in which some monoterpenes produced oxygen such as linalool, borneol. Phytochemical analysis of this medicinal herb can identify the nature of compounds present in extract of Oscimum sanctum. It is also for identifying the bioactive compound and their effect. They are commonly helpful as model for the synthesis of new medicine.

**OBJECTIVES OF RESEARCH:**

1. Collection and identification of plant material.
2. Phytochemical analysis (HPTLC) of selected medicinal plants extracts.
3. Determination of antimicrobial activity of selected medicinal plant extracts.
4. Determination of antioxidant activities of selected medicinal plant extracts.
5. Determination of antidiabetic activity of selected plant extracts.
6. To perform LC-MS analysis of selected plants. LC-MS is used to elucidate the structural and chemical properties of isolated compounds. It provides structural information.
METHODOLOGY TO BE ADOPTED:

The current research work will be completed using standard and established protocols

Phase 1: Literature survey collection and identification of plant materials:

The following medicinal plants will be selected,

1. *Aegle marmelos* L. (Rutaceae)
2. *Helicteres isora* L. (Malvaceae).

In the first phase of research work extensive literature survey will be done in order to know latest scenario of research in undertaken and allied area. The collection of plant material will be done by giving personal visit to field and also by interacting with some local tribal healers. The collected plant materials will be identified by using standard floras (Naik V. N. 1998).

Phase 2: Plant extraction and phytochemical analysis:

Samples will be collected from the fields. And extracted for phytochemical analysis by using prescribed methods. (Harborne 1973; Sadasivam and Manikam 2005; Thimmaiah 1999; Evans 1997)

Phase 3: Antimicrobial studies:

By using standard protocols, in the third phase the antimicrobial activity of selected medicinal plants will be tested against different fungal and bacterial human pathogens.

Phase 4: HPTLC analysis:

In this phase emphasis will be given on HPTLC analysis of selected medicinal plant extract in central instrumentation facility available at N E S Science College, Nanded using standard protocols. (Sethi 1996; Mona et. al. 2012)
Phase 5: Antioxidant and antidiabetic activities:

In this phase the focus will be given on study of antioxidant and antidiabetic activity by using standard protocols recommended by Gulnazer et al. (2014); Abdullah and Kashim (2017); Oktay, et. al. (2003); Kamleshiya, et al (2012).

Phase 6: LC-MS analysis:

In this phase the focus will be given to perform and study the LC-MS analysis. LC-MS is a powerful analytical technique used for separation, identification and quantification of both unknown and known compounds as well as to elucidate the structure and chemical properties of different molecules. (K. L. Lynch 2017)

Phase 7: Thesis writing and submission:

Scientific writing of thesis will be done for final submission to university.

IMPORTANCE OF STUDY/SOCIETY APPLICATION:

International status:

*Syzygium polyanthum* (Wight) Walp is one of the important medicinal plants showing antidiabetic properties. In the literature search in Science Direct, Scopus and Google Scholar databases, it was found that *Syzygium polyanthum* has valuable therapeutic potentials including antidiabetic, antihypertensive, antimicrobial, antioxidant, anticancer, antitumor, antiinflammatory, acetylcholine esterase inhibitory properties. However, few research areas, especially its toxicological profiles, mechanism of action, as well as identification of its bioactive compound, require more in-depth studies. (Azlini et al. 2016)

*Anchusa officinalis* is a wild plant native to Europe. This plant has wide application in folk medicine, both exhibiting antimicrobial, antitumour, antiviral, anti-inflammatory, antidiabetic and many other activities. *Melilotus officinalis* is a widespread plant in Europe and Asia, but it also grows throughout the United States and Canada and has been used traditionally for anti-inflammatory and antioedematous activities, antiaging skin, diuretic and sedative. It contains high presence of coumarins and because of that *Melilotus officinalis* can be used for the treatment of diabetic foot ulcers. (Gabriela et al. 2020).
Naseer et. al. (2018) did their work on *Psidium guajava* in Pakistan. The plant can be applied for the treatment of diabetes, hypertension, dysentery and carries pain relief. The leaf extract of *Psidium guajava* is used as a medicine in cough, diarrhoea, oral ulcers. Its fruit is rich in vitamin A, C, iron, phosphorus and minerals. It contains high content of secondary metabolites like antioxidants, polyphenols, antiviral compounds, anti-inflammatory compounds.

Roots of *Uvaria chamae* were obtained from a farm in Uromi, Edo state, Nigeria during rainy season. *Uvaria chamae* has antidiabetic and hypolipidemic activities. The extract also caused weight loss, making it beneficial for diabetic patients that are overweight. The anti-fungal, anti-malarial and anti-inflammatory activities have also been reported. (Emordi et. al. 2016)

**NATIONAL STATUS:**

*Annona squamosa* Linn, commonly called as custard apple is cultivated throughout India. The pharmacological active ingredients are present in seeds, leaves and aerial parts of plants. The plant possesses both hypoglycemic and antidiabetic activity. The extract obtained from leaves of this plant is useful in maintaining healthy blood sugar and cholesterol level. *Bougainvillea spectabilis* Linn. is a very familiar ornamental plant commonly grown in Indian gardens. The traditional plant has antidiabetic potential. The ethanolic extract of the leaves has antihyperglycemic activity. (Malviya et. al. 2010)

India is the largest sources of medicinal plants in the whole world. *Oscimum sanctum* is employed in the treatment of various diseases such as antimicrobial infection, antifungal, anticancer, arthritis, chronic fever, antifertility, eye disease, analgesic etc. This medicinal herb has been shown to reduce blood glucose levels, making it an effective treatment of diabetes. (Panchal et. al. 2019)

*Trigonella foenum-graecum* is one of the medicinal plants which are important in the management of diabetes. It belongs to family Fabaceae. The study investigated the effect of fenugreek seed powder solution on the lipid profile of newly diagnosed type II diabetic patients. The plant is rich reservoir of different phytoconstituents attributed to their diverse pharmacological effects. (Geberemeskel et. al. 2019)

*Curcuma longa* an important dietary plant which possess several pharmacological activities including antioxidant, antimicrobial, anti-inflammatory, anti-
cancer and antidiabetic activities. In the HPLC chromatogram of *Curcuma longa* rhizome extract 15 phenolic compounds were identified. The ethanolic extract concluded that *Curcuma longa* is a rich source of curcumin and contains several important phenolics. (Sabir et. al. 2020)

**SIGNIFICANCE OF THE STUDY:**

Plants have been used for medicinal purposes long before prehistoric period. Treatment with medicinal plants is considered as very safe as there is no or minimal side effects. The treatment with medicinal plants is independent of any age groups and sexes. Human beings are depending on medicinal plants directly or indirectly for the treatment of various ailments since ancient time. According to the World Health Organization, reports nearly 80% psychological and physical health mainly because of the two reasons once is they cannot afford the products of Western pharmaceutical industries and the second reason is due to side effects of the synthetic drugs followed by lack of healthcare facilities. India has a rich culture of medicinal herbs and spices, with high potential abilities for Ayurvedic, Unani, Siddha traditional medicines but only very few have been investigated chemically and pharmacologically for their potential medicinal value. Rural masses of many developing countries still rely on traditional medicine for their primary health care needs and have found a place in day-to-day life. These medicines are relatively safer and cheaper than synthetic or modern medicine.
PROPOSED WORK PLAN / FORMULATION AND STRUCTURE OF STUDY / YEAR WISE PLAN OF WORK AND TARGET TO BE ACHIEVED:

The current research work planned for three years as follows,

First year:

1. Literature survey/ Review of literature
2. Collection and identification of plants
3. Extraction of plant materials.

Second year:

1. Phytochemical analysis of selected plants
2. HPTLC analysis of plant extracts
3. Antimicrobial activity of selected plants.

Third year:

1. Anti-diabetic activity of selected plant extracts
2. LC-MS analysis of selected plant extracts
3. Data analysis and thesis writing for the submission to the university.

Presentation of the research work by attending conference workshops will be done alongside all the activities as per mentioned above.

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