

ANTIOXIDANT, ANTIMICROBIAL, ANTIINFLAMMATORY AND PCOD ACTIVITIES OF TRIBULUS TERRESTRIS FRUIT EXTRACT

Submitted in partial fulfillment of the requirements for the award of

Master of Technology degree in Medical Instrumentation

by

MOSHIKANALINI.K

(Reg. No. 41440009)



**DEPARTMENT OF BIOMEDICAL ENGINEERING
SCHOOL OF BIO AND CHEMICAL ENGINEERING**

SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

Accredited with Grade "A" by NAAC

JEPPIAAR NAGAR, RAJIV GANDHI SALAI, CHENNAI - 600 119

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BONAFIDE CERTIFICATE

This is to certify that this Project report is the bonafide work of **MOSHIKANALINI.K** (Reg. No. **41440009**) who carried out the project entitled "**ANTIOXIDANT, ANTIMICROBIAL, ANTIINFLAMMATORY AND PCOD ACTIVITIES OF TRIBULUS TERRESTRIS FRUIT EXTRACT**" under our supervision from **November 2022 to April 2023**.

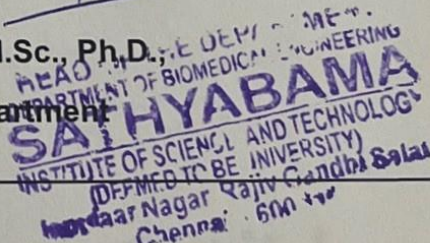
Dr. S. KRISHNAKUMAR, M.Sc., Ph. D., Dr. D. SARAVANAN M.Sc., M.Phil., Ph. D.,

Internal Guide

External Guide

Dr. T. SUDHAKAR, M.Sc., Ph.D.,

Head of the Department



Submitted for Viva-voce Examination held on **05-05-2023**

Internal Examiner

External Examiner

DECLARATION

I, **MOSHIKANALINI.K** (Reg. No. **41440009**) hereby declare that the Project report entitled “Antioxidant, Antimicrobial, Anti-inflammatory and PCOD activities of *Tribulus terrestris* fruit extract” done by me under the guidance of **Dr. S. Krishnakumar**, M.Sc., Ph.D., Department of Biomedical Engineering is submitted in partial fulfillment of the requirements for the award of **Master of Technology** degree in **Medical Instrumentation**.

DATE:05.05.2023

PLACE: CHENNAI

K.Moshikanalini
SIGNATURE OF THE CANDIDATE

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ABSTRACT

Tribulus terrestris is an annual plant in the caltrop family (Zygophyllaceae), commonly known as PCOD, has been used for a long time in both the Indian and Chinese systems of medicine for treatment of various kinds of diseases. Its various parts contain a variety of chemical constituents which are medicinally important, such as flavonoids, flavonol glycosides, steroidal saponins, and alkaloids. It has diuretic, aphrodisiac, antiurolithic, immunomodulatory, antidiabetic, absorption enhancing, hypolipidemic, cardiotonic, central nervous system, hepatoprotective, anti-inflammatory, analgesic, antispasmodic, anticancer, antibacterial, anthelmintic, larvicidal, and anticariogenic activities. For the last few decades or so, extensive research work has been done to prove its biological activities and the pharmacology of its extracts. The aim of the present study of PCOD activities of fruit extract of *Tribulus terrestris*, the different antioxidant assays such as DPPH ,radical scavenging assay, superoxide radical scavenging as well as Fe³⁺reducing power assay to be carried out for evaluating the antioxidant capacity of the fruit extract . Antibacterial activity of fruit extract of *Tribulus terrestris* was carried out by agar well diffusion method. Anti-inflammatory activity is carried by haemolysis inhibition assay method . PCOD inhibition activity is to be evaluated by using T-HESCs cell lines.

Keywords: *Tribulus terrestris*, DPPH, PCOD.

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LIST OF ABBREVIATIONS

PCOS	-	Poly Cystic Ovarian Syndrome
DM	-	Diabetes Mellitus
OSA	-	Obstructive Sleep Apnea
NAFLD	-	Non Alcoholic Fatty Liver Disease
NASH	-	Non Alcoholic Steato Hepatitis
FOH	-	Functional Ovarian Hyperandrogenism
GnRH	-	Gonadotropin - releasing hormone
FSH	-	Follicle Stimulating Hormone
LH	-	Luteinizing Hormone
FTIR	-	Fourier Transform Infrared
UV	-	Ultra Violet
EOC	-	Epithelial Ovarian Cells
WHO	-	World Health Organization
HGSOC	-	High Grade Serous Ovarian Cancer
CNV	-	Copy Number Variation
DMSO	-	Dimethyl Sulfoxide
DMEM	-	Dulbecco's Modified Eagle Medium
CNS	-	Central Neural System

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CHAPTER - 1

INTRODUCTION

The most prevalent endocrine disorder in females of reproductive age is polycystic ovarian syndrome (PCOS). Depending on the used diagnostic criteria, the prevalence ranges between 5% and 15%. According to specialist society recommendations, the presence of at least two of the following three conditions chronic anovulation, clinical or biological hyperandrogenism, and polycystic ovaries is required for the diagnosis of PCOS. (Morgan et al., 2020) It is an exclusionary diagnosis, thus conditions that resemble the clinical characteristics of PCOS must be ruled out. These include non-classical congenital adrenal hyperplasia, hyperprolactinemia, and thyroid illness. (Devi et al., 2011) If clinical signs point to alternative causes, certain individuals may require a more thorough workup.

Despite its high incidence, PCOS is underdiagnosed and frequently requires many visits or diagnoses from different doctors, which typically take longer than a year. For the patient, it is an extremely frustrating process. Delay in diagnosis can cause comorbidities to increase, making it more challenging to undertake lifestyle interventions, which are essential for improving the characteristics of PCOS and quality of life.

Infertility, metabolic syndrome, obesity, impaired glucose tolerance, type 2 diabetes mellitus (DM-2), cardiovascular risk, depression, obstructive sleep apnea (OSA), endometrial cancer, and nonalcoholic fatty liver disease/nonalcoholic steatohepatitis (NAFLD/NASH) are just a few of the numerous morbidities linked to PCOS. For each of these illnesses, there are distinct screening guidelines, but the clinician needs to have a low threshold. However, if any manifestation is seen in PCOS patients, the clinician must have a low threshold for workup for any of these disorders.

ETIOLOGY

A multifactorial illness, PCOS. The pathogenesis of the illness has been linked to a number of vulnerable genes. These genes play distinct roles in androgenic pathways and degrees of steroidogenesis. According to twin studies, heritability is

approximately 70%. The expression of these genes as well as the onset and course of the disease are both significantly influenced by the environment.

According to two well-known theories, PCOS characteristics are expressed in people who have a genetic predisposition when they are subjected to specific environmental conditions. The most prevalent environmental factors are insulin resistance and obesity. Fetal androgen exposure is another theory that has been put out.

EPIDEMIOLOGY

According to the diagnostic criteria, PCOS affects between 5% and 15% of reproductive-aged females globally, making it the most prevalent endocrine disorder. Infertility, metabolic syndrome, obesity, impaired glucose tolerance, DM-2, cardiovascular risk, depression, OSA, endometrial cancer, and NAFLD/NASH are just a few of the disorders that have been linked to PCOS. PCOS, prepubertal obesity, congenital virilizing diseases, above-average or low birth weight for gestational age, premature adrenarche, and usage of valproic acid as an antiepileptic medicine have all been linked to higher prevalence in first-degree relatives. There may be a higher incidence among Mexican Americans than among non-Hispanic whites and African Americans, according to studies.

PATHOPHYSIOLOGY

There is no other condition that can account for the oligo-anovulation and hyperandrogenic state of PCOS. It is an exclusionary diagnosis. But the majority of hyperandrogenic appearances are explained by it.

FOH, also known as functional ovarian hyperandrogenism, is the primary cause of PCOS. Typical functional ovarian hyperandrogenism, which is defined by dysregulated androgen production and an excess of 17-hydroxyprogesterone (17-OHP) in response to gonadotropin stimulation, is present in two-thirds of PCOS presentations. Although the 17-OHP overreaction in the remaining PCOS with atypical FOH is not present, testosterone increase can identify it when adrenal androgen production is suppressed. A related isolated functional adrenal hyperandrogenism

affects about 3% of PCOS patients. The remaining PCOS cases are often moderate. Since the majority of these patients are obese, practitioners believe that this explains why there is a lack of evidence of steroid secretory abnormalities. their unorthodox PCOS. The clinical value of specific testing for the FOH subgroup is still poor.

Functional hyperandrogenism of the ovaries Hyperandrogenism, oligo anovulation, and polycystic ovaries are the three main symptoms of PCOS. A combination of heritable and environmental variables contributes to functional ovarian hyperandrogenism, which is multifactorial in nature. Excess insulin, which is known to sensitize the ovary to luteinizing hormone (LH) by impeding the process of homologous desensitization to LH in the normal ovulation cycle, as well as an inherent imbalance among intraovarian regulatory systems, are the two main contributors to this dysregulation. Most steroidogenic enzymes and proteins involved in androgen production are overexpressed in Theca cells in PCOS, which suggests a significant anomaly at the level and activity of steroidogenic enzymes, including P450c17, which has been extensively identified. Overproduction of androgen and insulin is the main cause of early luteinization in granulosa cells.

The early recruitment of primordial follicles into the growth pool is facilitated by androgen excess. At the same time, it starts an early luteinization process, which hinders the choice of the dominant follicle. This causes the gross anatomical alterations and characteristic PCOS histopathologic symptoms that make up PCOM. Even when LH is elevated, PCOS is not caused by it. Although LH excess is prevalent and required for the development of gonadal steroidogenic enzymes and the release of sex hormones, it is less likely to be the main factor for ovarian androgen excess due to theca cell desensitization.

Atypical levels of insulin-resistant hyperinsulinism, which operate on theca cells to increase steroidogenesis, prematurely luteinize granulosa cells, and induce fat formation, are present in around half of patients with functional ovarian hyperandrogenism. LH production is increased by hyperandrogenism. This then affects the cycle that maintains the theca and luteinized granulosa.

A relative increase in LH vs FSH production and secretion may result from ovarian hormonal dysregulation altering the pulsatile gonadotropin-releasing hormone

(GnRH) release. The relative decline in FSH inhibits appropriate stimulation of aromatase activity within the granulosa cells, limiting androgen conversion to the powerful estrogen estradiol. LH promotes ovarian androgen synthesis. This develops into a noncyclic hormonal pattern that is self-sustaining.

In the peripheral, increased serum androgens are converted to estrogens, primarily estrone. Estrogen production will be increased in obese PCOS individuals because conversion takes place largely in the stromal cells of adipose tissue. As a result of this conversion, the hypothalamus and pituitary gland experience chronic feedback as opposed to the normal variations in feedback that are seen in the presence of a developing follicle and rapidly fluctuating estradiol levels. Endometrial hyperplasia may result from the endometrium being stimulated by estrogen without resistance.

An annual member of the Zygophyllaceae family, *TT* is also referred as tribulus, hard thorns, and goat head in China. It is primarily grown in subtropical and Mediterranean areas like India, China, South America, Mexico, Spain, Bulgaria, and Pakistan. It is a tiny, prostrate, hirsute or silky haired shrub, growing 10 to 60 cm tall. The leaves are elliptic or oblong lanceolate, opposite, paripinnate, pinnate from 5 to 8 pairs, and frequently uneven. The five mericarps' fruits have a diameter of 7-12 mm, are ax-shaped, 3-6 mm long, and oriented radially. They also have a rough feel. The root is light brown in colour, thin, fibrous, cylindrical, and frequently branching. It also has numerous little rootlets.

In China, the fruits and roots of *TT* have been utilised as folk medicine for thousands of years. It has just received certification for its medicinal activities that enhance cardiac health, improve sexual function, and have antioxidant, anti-urolithic, anti-diabetic, anti-inflammatory, antitumor, and antitumor properties.

The ethnobotanical use of *TT* as well as its phytochemical and pharmacological activities are presented and examined in the current review. These current research findings will be useful in comprehending the traits and benefits of this traditional Chinese medicine and will be applied in the future in the creation of new goods and herbal remedies.

1.1 TAXONOMICAL CLASSIFICATION

- Kingdom: Plantae
- Division: Phanerogams
- Subdivision: Angiospermae
- Class: Dicotyledonae
- Subclass: Polypetalae
- Series: Disciflorae
- Order: Giraniales
- Family: Zygophyllaceae
- Genus: *Tribulus*
- Species: *terrestris* Linn



Fig. 1.1: Whole plant of *Tribulus terrestris*



Fig. 1.2: Dry fruit of *Tribulus terrestris*

1.2 PLANT PROFILE

Gokshur (Sanskrit), puncture vine, land (or little) caltrops (English), Gokharu (Hindi), Bethagokharu or Nanagokharu (Gujarathi), Nerinjil (Tamil), and Kharekhusak khurd are some of the more frequent names for *TT* (Urdu). It is dispersed over a vast geographic area. All of India, including Kashmir, Ceylon, and all warm climates in both hemispheres have it up to a height of 11,000 feet. It is a typical weed of waste areas,

road sides, and grazing fields, especially in hot, arid, and sandy areas like Gujarat and West Rajasthan in India.

1.3 EXTRACTION

The first and most important step in the examination of medicinal plants is extraction since it is vital to separate and characterize the desired chemical components from the plant materials. The fundamental procedure included actions like pre-washing, freeze drying or drying of plant materials, grinding to get a homogeneous sample, and often boosting the kinetics of analytical extraction as well as increasing the contact of sample surface with solvent system. In order to prevent possible active ingredients from being lost, altered, or destroyed during the extraction of plant samples, the appropriate steps must be done. It is necessary to prepare the extract in accordance with the directions provided by the manufacturer if the plant was chosen based on its traditional applications (Fabricant and Farnsworth, 2001). traditional healer in an effort to as closely resemble the conventional 'herbal' drug.

The particular characteristics of the bioactive chemical being targeted determine the solvent system that should be used in major part. It is possible to extract the bioactive ingredient from natural goods using a variety of solvent systems. Polar solvents like methanol, ethanol, or ethyl acetate are used to extract hydrophilic substances.

Dichloromethane or a 1:1 combination of dichloromethane and methanol is used to extract more lipophilic substances. Chlorophyll can sometimes be eliminated using hexane extraction (Cos et al., 2006).

The applicability of the extraction techniques must be taken into account because the target compounds may range from non-polar to polar and be thermally labile. For the extraction of plant materials, a number of techniques are frequently utilized, including sonification, heating under reflux, soxhlet extraction, and others (United States Pharmacopeia and National Formulary, 2002; Pharmacopoeia of the People's Republic of China, 2000; The Japanese Pharmacopeia, 2001). Additionally, fresh green plants or dried powdered plant material are macerated or percolated in water and/or organic solvent systems to create plant extracts.

1.4 ANTIBACTERIAL ACTIVITY

With certain modifications, Zhang Y et al.,(2012) techniques were applied. Agar-well diffusion experiment was utilized to assess the antibacterial properties of the ag extract, and Sabouraud Dextrose agar (Difco) was employed for the culture of bacteria and fungus. One milliliter of the bacteria suspension (3×10^8 CFU/ml) was aseptically combined with 20 milliliters of the prescribed molten (45°C) agar before being put into 100 15 mm sterile Petri plates. A sterile cork borer was used to create 6 mm wells after the agar had set. 0.1 ml of the essential oil, which was produced using methanol (75%) as diluents and had different concentrations of 500, 1000, and 1500 l/ml, was independently poured into each well. The dishes were cultured for the bacteria for 24 hours at 37°C and the mould for 24 to 72 hours at room temperature. The positive controls for the bacterium species are ampicillin (10 g) and the positive controls for the Candida species are ketoconazole (10 g) (Pfizer). The diameter (mm) of the clearly defined zone of growth inhibition was used to measure the antibacterial activity. Every experiment used methanol (75%) as a negative control.

1.5 CHARACTERIZATION

1.5.1 ULTRAVIOLET -VISIBLE SPECTROSCOPY

UV-visible spectroscopy is the term used to describe the analytical evaluation of various types of solvents and chemicals. UV-visible spectroscopy has been widely employed over the past 37 years. Spectroscopy is frequently used because the equipment is less expensive and needs less upkeep, especially by small-scale businesses.

1.5.2 SPECTROSCOPY

The examination of electromagnetic radiation (EMR) absorbed and emitted by molecules, atoms, or ions as they change from one energy state to another in a sample is referred to as spectroscopy.

UV-visible spectroscopy uses light in the visible, UV, and near infrared ranges and is based on the Beer-Lambert equation, which stipulates that a solution's absorbance is directly proportional to its wavelength of ultraviolet and visible light. Since the energy levels of matter are quantized, only light with a precise amount of energy

that may cause transitions from one Level to another will be absorbed. using UV-visible spectroscopy UV-visible spectroscopy has many applications, including the structural elucidation of organic compounds, quantitative and qualitative drug evaluation, dissociation of acid and base constants, molecular weight determination, and Beer-Lambert law derivations as an HPLC detector. Due to its availability, usability, adaptability, and wide range of applications in a varied the UV-visible analytical technique has greatly increased in importance and recognition throughout the international scientific communities of domains, including analytical chemistry and biochemistry. The quantity of samples and reagents must currently be decreased in order to develop analytical procedures. In order to analyze materials, particularly uncommon samples or dangerous liquids, a UV-visible micro volume spectrometric device has been developed .

1.5.3 FTIR(*FOURIER TRANSFORM INFRARED SPECTROSCOPY*)

Over the past ten years, the science of Fourier transform infrared (FTIR) spectroscopy has advanced significantly, promising simpler, quicker, and more accurate diagnoses. The interactions between matter and electromagnetic radiation, which take the shape of a spectrum, are investigated using FTIR spectroscopy. Each molecule has a unique spectral fingerprint that enables separation from other molecules. Monitoring cellular changes with FTIR spectroscopy is another efficient and non-destructive technique. The characterization of various organ disorders and the quantification of various biomolecules, including proteins, nucleic acids, and lipids, have both been made possible by FTIR spectral analysis. Numerous studies emphasizing the value of spectroscopic methods in cancer detection have been disseminated in the literature. Focusing on specific bands or groups, FTIR seeks to differentiate and characterize cells and tissues by precisely identifying the molecular conformations, bonding types, functional groups, and intermolecular interactions that make up the material.

1.6 CELL LINE

Due to the lack of symptoms and delayed manifestation, epithelial ovarian cancer is frequently referred to as the silent killer. In more than 70% of patients, this

combined with the lack of specific diagnostic indicators and screening tools results in a diagnosis at a late stage of the disease. In spite of extensive research into ovarian cancer treatment, the overall mortality rate has not improved significantly over the past 20 years, with a 5-year overall survival rate of 30-39%. Ovarian cancer is the primary cause of gynecological cancer mortality globally. Ovarian carcinoma is still clinically treated with a combination of debulking surgery and platinum-based chemotherapy despite the fact that physicians have long recognized that it is a collection of diverse diseases. A heterogeneous in vitro model for the study of ovarian cancer is long overdue, according to the reported variance in the clinical behavior of the disease and the rising body of literature documenting molecular heterogeneity. An invaluable resource for researching biological processes at the molecular and cellular level is established cell lines. The advantages of strong proliferative ability, clonogenicity, and prolonged life span in culture are present in existing human ovarian cancer cell lines. Ovarian cancer cell lines, on the other hand, have infrequently come from patients who had never had chemotherapy; instead, many of them were created after viral transformation, such as using the SV40 Large antigen or xenograft passaged in immunocompromised mice. Due to the scarcity of cell lines derived from mixed histology tumors and the rarity of this type of tumor, dependable models are required for the investigation of mixed histology tumors. Furthermore, data suggests that numerous cell lines have considerable duplication, loss of integrity, and misidentification, making it desirable to develop new, thoroughly characterized models.

1.7 PHYTOCHEMICAL ANALYSIS

We understand the importance of plants. The plant kingdom contains a veritable pharmacy of possible drugs, and in recent years, interest in the utility of medicinal plants has grown. Numerous chemically active substances found in these plants, which have a certain physiological impact on the human body, have therapeutic potential. The most important of these bioactive plant components include alkaloids, tannin, flavonoids, and phenolic compounds. The plants that have been chosen for medicinal usage throughout thousands of years are the most obvious choice for looking at the contemporary quest for therapeutically effective innovative medications, such as anticancer drugs, antibacterial drugs, and antihepatotoxic chemicals. Worldwide

Organization for Health - The World Health Organization (WHO) claims that medicinal plants are the best source of a variety of nutrients.

Only a few of the chemical elements present in medicinal plants that have clear physiological impacts on people are tannins, alkaloids, sugars, terpenoids, steroids, and flavonoids. Numerous phytochemicals from different chemical classes have been shown to have inhibitory effects on all types of bacteria in vitro. Plant-based ingredients have been employed in phytomedicines since the dawn of humanity. This can be made from a variety of materials, including fruits, seeds, flowers, roots, and barks. Understanding the chemical components of plants will help one be able to synthesize complex chemical substances.

1.8 CANCER CELL

The majority of gynecological-related cancer deaths in Europe and North America are caused by ovarian cancer. Eighty percent of all ovarian tumors are epithelial ovarian cancers (EOC), which are now thought to be a heterogeneous disease made up of five basic histological subgroups with distinctive clinical and molecular characteristics. The majority of instances, or around 75%, are high-grade serous ovarian cancer (HGSOC), with the remaining 25% being low-grade serous (LGS), endometrioid, clear cell, and mucinous carcinoma. Mucinous carcinoma and endometrioid carcinoma are further divided into well-differentiated, moderately-differentiated, and poorly-differentiated tumors (grades 1 to 3, respectively). The gold standard for diagnosing each subtype of EOC is immunohistochemical analysis along with histological inspection. The distinct genomes of each EOC subtype may now be examined more closely thanks to the expansion of next-generation sequencing. A near-universal TP53 mutation and genome-wide copy-number variation (CNV) are features of HGSOC, while only 20% of cases have germline or somatic BRCA1/2 mutations. LGS exhibits TP53 mutation less commonly than mutations in the MAPK signaling pathway, such as KRAS, NRAS, and BRAF. Endometriosis and ARID-1A mutations are frequently linked to clear cell carcinomas and well-differentiated (i.e., grade 1) endometrioid carcinomas. Finally, ERBB2 amplifications and KRAS mutations are linked to mucinous ovarian cancer.

CHAPTER - 2

LITERATURE SURVEY

Aggarwal et.al., (2012) discussed about CaOx crystal adhesion to renal cells is a crucial process in kidney stones linked to severe hyperoxaluria. Phytotherapeutic medicines may be helpful as an adjuvant therapy because surgical techniques are not able to change the propensity of stone recurrence and lasting side effects. The goal of the current study is to evaluate the antilithiatic activity of the protein biomolecules of *Tribulus terrestris*, a plant that is frequently used in commercially available herbal urolithiasis remedies. Purification and characterization of the purified protein were carried out using several biochemical techniques with mass spectrometry. The protein's capacity to protect against oxalate-induced damage to renal epithelial cell lines (NRK 52E) was examined. Purification was done on an antilithiatic protein with a molecular weight of around 60 kDa.

Amin, et. al., (2006) Arabic traditional medicine uses *Tribulus terrestris* (TT) to cure a variety of illnesses. The investigation of TT's preventive benefits in diabetic mellitus (DM) was the main focus of this paper. It is well known that diabetes raises the level of reactive oxygen species (ROS), which in turn leads to the development of diabetes. Six groups of rats received either saline, glibenclamide (Glib), or TT treatment for 30 days.

Ayyanna, et. al., (2012) The dosage of metformin hydrochloride in tablet form was decreased using this extract. varied batches of tablets (F0 to F11) were made using the wet granulation method, with varied concentrations of gokhru extract and metformin hydrochloride, in order to optimise the dose of metformin hydrochloride. A number of factors, including weight fluctuation, hardness, friability, disintegration, dissolution, permeation, and stability, were assessed for tablets. Results showed that batch F7 and F8 had a similar level of permeation to the control batch F0. After examining all evaluation criteria, F7 was discovered to be the batch that was optimised because the tablet hardness was suitable for this batch and it did not absorb moisture

from the environment, in contrast to F8, whose hardness was easily absorbed by the environment and whose hardness was relatively lower than F7.

Chhatre et. al.,(2014) Its many portions include a range of chemical components that are crucial for medicine, including alkaloids, steroidal saponins, flavonoids, and flavonol glycosides. It has a variety of beneficial effects on the body, including diuretic, aphrodisiac, antiurolithic, immunomodulatory, antidiabetic, absorption-enhancing, hypolipidemic, cardiogenic, central nervous system, hepatoprotective, anti-inflammatory, analgesic, antispasmodic, anticancer, antibacterial, anthelmintic, larvicidal, and anticariogenic properties. Extensive study has been conducted in the last few decades to demonstrate its biological activities and the pharmacology of its extracts. The purpose of this review is to provide a database for future research on the plant's revealed phytochemical and pharmacological qualities. This will contribute to the validation of both its traditional use and its value-added use, ultimately resulting in increased plant revenues.

Lamba et. al.,(2011) In the current investigation, glibenclamide was used as the standard hypoglycemic treatment to compare the effects of various doses of *Tribulus terrestris* (T.T.) fruit extract on rat blood glucose levels in acute and chronic phases. Method: Sixty wistar male rats weighing 200-250 grammes were chosen and confined in identical conditions. A 50mg/kg intraperitoneal (ip) injection of streptozocin was used to produce diabetes. Rats with fasting blood sugar (FBS) levels over 200 mg/dl were chosen after 15 days and placed into 10 groups of six rats each. The T.T. fruit extract was chosen for intravenously injection in doses of 15, 30, 60, 150, 250, 500, 750, and 1000 mg/kg for the acute phase and 500, 750, 1000 mg/kg for the chronic phase. During the two months of the trial, FBG and 2 hour post-meal blood sugar (2hrPPBS) were measured every 20 days, and fasting blood insulin was measured at the conclusion of the study.

Li et. al., (2001) On normal mice, the decoction of *Tribulus terrestris* might considerably suppress gluconeogenesis and have an impact on glycometabolism. Additionally, the decoction may lower the plasma's triglyceride and cholesterol levels.

Evaluation of *Tribulus terrestris* fruit aqueous extract's cytological and genetic effects on cultured human lymphocytes.

Lim , et.al.,(2002) Saponin from *Tribulus terrestris*, which was at a rate of 26.25% and 40.67% in normal mice and diabetic mice, respectively, could dramatically lower the level of serum glucose.

Sangeetha et. al.,(1994) In this study, we investigate the impact of *Tribulus terrestris* aqueous extract on oxalate metabolism in male rats fed sodium glycolate. In addition to causing hyperoxaluria, glycolate feeding boosted the liver's ability to produce oxalate, specifically glycolate oxidase (GAO), glycolate dehydrogenase (GAD), and lactate dehydrogenase (LDH), while decreasing kidney LDH activity. In comparison to sodium glycolate-fed animals, *T. terrestris* administration to rats resulted in a considerable decrease in urine oxalate excretion and a large rise in urinary glyoxylate excretion. The liver's GAO and GAD activities were also decreased when sodium glycolate was added to *T. terrestris*, while liver LDH activity was unaffected. The kidney LDH isoenzyme pattern demonstrated that *T.terrestris* normalised kidney LDH.8. Sangeetha et. al(1994). In this study, we investigate the impact of *Tribulus terrestris* aqueous extract on oxalate metabolism in male rats fed sodium glycolate. In addition to causing hyperoxaluria, glycolate feeding boosted the liver's ability to produce oxalate, specifically glycolate oxidase (GAO), glycolate dehydrogenase (GAD), and lactate dehydrogenase (LDH), while decreasing kidney LDH activity. In comparison to sodium glycolate-fed animals, *T. terrestris* administration to rats resulted in a considerable decrease in urine oxalate excretion and a large rise in urinary glyoxylate excretion. The liver's GAO and GAD activities were also decreased when sodium glycolate was added to *T. terrestris*, while liver LDH activity was unaffected.

Shirfule et.al.,(2011) One of the key enzymes in the route of oxalate production is glycolate oxidase (GOX). By means of oxidation, it transforms glycolate into glyoxylate, which is then transformed into oxalate. Thus, the therapeutic use of GOX in this context was discovered to have promise in the management of calcium oxalate urolithiasis. In the current inquiry, we perused GOX to look for prospective leads in

more conventional sources. Using Glide 5.5.211 (Schrodinger™ suite), the identified leads quercetin and kaempferol's molecular structures were modelled. Since spinach glycolate oxidase (sGOX) and pure human glycolate oxidase (hGOX) share numerous conserved active site residues and have a 57% identity and 76% similarity, in vitro tests were carried out on sGOX instead of hGOX. We sought to determine a potential mode of action for the *Tribulus terrestris* anti-GOX leads that may be connected to the creation of anti-urolithic drugs. Future GOX inhibitory lead development is considered to be promising by this investigation.

Tilwari et. al.,(2011) The usage of traditional herbal remedies, herbal health products, pharmaceuticals, food supplements, cosmetics, etc. is on the rise nowadays (or what we can refer to as a "return to nature") because more people are realising the safety and lack of negative side effects of natural products. The purpose of this study was to examine the effects on Wistar rats of five different medicinal herbs used in Indian traditional medicine. Antibody titre, phagocytic carbon clearance, and a delayed type hypersensitivity test were used to examine the activity. The other groups got varying amounts of all five plant extracts, including *Tribulus terrestris*, *Cassia tora*, *Achyranthes aspera*, *Mucuna pruriens*, and *Abrus precatorius*, intraperitoneally for seven days straight. The control group received 0.1% carboxyl methyl cellulose. All the five plants exhibited immunostimulatory activity and out of the five plants, *T. terrestris* showed significant dose dependent increase in the 'humoral antibody titre and DTH response' as indicated by increase in footpad thickness. It also showed significant increase in the 'phagocytic index' in rats. The investigation revealed that all the mentioned plants have immunomodulatory activities.

CHAPTER - 3

AIM AND SCOPE

3.1 AIM

To analyze the extraction of *Tribulus terrestris* and its cytotoxic activity in ovarian cell line.

- To evaluate the potential of the extract to scavenge free radicals and protect cells from oxidative damage.
- To investigate the ability of the extract to inhibit the growth of pathogenic bacteria and prevent infections.
- To assess the extract's potential to reduce inflammation and alleviate symptoms associated with chronic inflammatory diseases.
- To examine the extract's effect on hormone levels and its potential as a natural remedy for PCOD.
- To determine the safety and efficacy of the extract for use in various health applications.

3.2 SCOPE

- Identification of the active components in *Tribulus terrestris* extract that have cytotoxic effects on ovarian cancer cells.
- Development of more specific and effective formulations of *Tribulus terrestris* extract that can target ovarian cancer cells while minimizing side effects on healthy cells.
- Conducting preclinical studies to evaluate the efficacy and safety of *Tribulus terrestris* extract in ovarian cancer treatment.
- Investigating the mechanism of action of *Tribulus terrestris* extract on ovarian cancer cells and exploring potential synergies with other anticancer agents.
- Conducting clinical trials to establish the appropriate dosages, treatment regimens, and patient selection criteria for *Tribulus terrestris* extract in ovarian cancer treatment.

- Exploring the potential of *Tribulus terrestris* extract as a complementary therapy to standard chemotherapy and radiation therapy in ovarian cancer treatment.
- Developing new delivery systems or formulations to enhance the bioavailability and therapeutic effect of *Tribulus terrestris* extract for ovarian cancer treatment.
- Investigating the potential of *Tribulus terrestris* extract as a source of novel lead compounds for the development of new ovarian cancer drugs.

CHAPTER - 4

MATERIALS AND METHODS

4.1 FLOW DIAGRAM

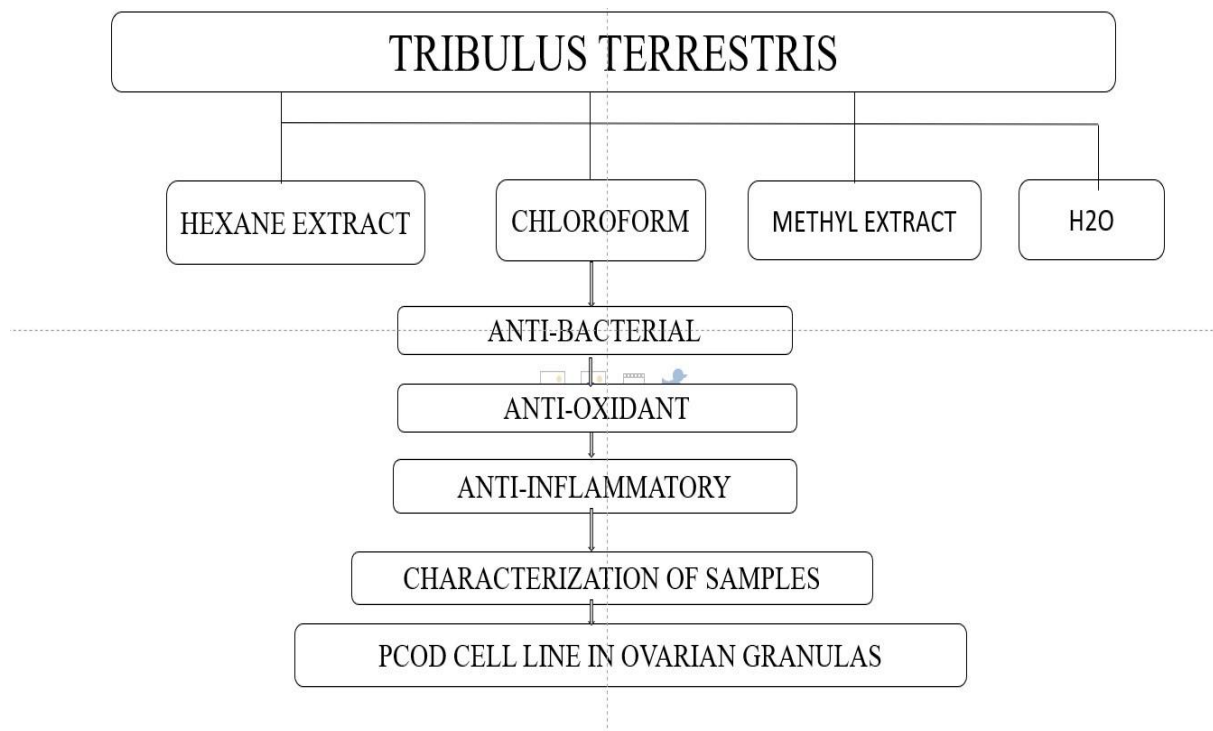


Fig. 4.1: Work flow of study

4.2 COLLECTION OF SAMPLE

The plant powdered 250g of *Tribulus terrestris* plant were collected from nearby hometown in the month of December 2022.

4.3 PREPARATION OF SAMPLES

The plant sample seeds were thoroughly cleaned with running tap water 2-3 times, then washed once more with distilled water, dried in the shade for seven days, and then dried in a low-temperature oven. After that, a mixer and grinder were used to grind up the dry seed components. Sequential extraction done with different solvents such as, hexane, chloroform, methanol and aqueous were used to extract 250g of plant powder, and the extracts were then evaporated. Extracts were put into pre-weighed sample containers and stored before being used for activities like antibacterial activity testing and phytochemical screening.

4.4 ANTIBACTERIAL ACTIVITY

The antibacterial activity of the extracts against *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus mutans*, and *candida albicans* was evaluated using the Agar well disc diffusion method. For the purpose of researching their antibacterial activities, essential Seed extracts were employed. Using the agar well disc diffusion method, a loopful of bacterial strains were inoculated into 5 ml of nutrient broth and cultured for 24 hours at 37°C to obtain an active strain. Mitchell Hinton 20ml of molten media was poured into sterilized Petri plates to create agar plates. After the media had solidified, the inoculum of the various strains was swabbed uniformly and given five minutes to dry. The dimethyl sulfoxide (DMSO) was used to dissolve the extracts. Using a micropipette, extract concentrations (500 g/disc) were placed onto sterile 5mm discs. The loaded disc was placed on the medium's surface, the compound was given five minutes to diffuse, and the plates were then retained for a 24-hour incubation period at 37°C. After the incubation period, millimeter measurements of the zones that had formed around the disc were taken. The diameter of the zone of inhibition was used to rank antibacterial susceptibility.

4.5 ANTIOXIDANT ACTIVITY

Tribulus terrestris seed extract's antioxidant capacity was evaluated using its ability to scavenge free radicals as determined by stable 1,1-diphenyl-2-picryl hydrazyl (DPPH) activity.

- 0.1ml of DPPH is added to 0.4ml of methanol extract.
- After correctly mixing, the reaction mixture was placed into well plates and incubated at 37C in the dark for 30 minutes.
- Using UV-VIS spectroscopy, absorbance was measured at 517 nm in comparison to a blank.
- An increase in radical scavenging activity was evidenced by a decrease in absorbance.
- The following formula was used to compute the percentage inhibition of DPPH:
$$\% \text{inhibition} = (\text{control} - \text{sample} / \text{control}) \times 100.$$

4.6 ANTI-INFLAMMATORY ACTIVITY

Egg Albumin Denaturation Assay

This procedure involved combining 2 mL of a test sample at a concentration of 100-400 g/mL with 0.5 mL of egg albumin and 1 mL of phosphate buffered saline (pH = 6.5).

- The reaction mixture was heated for 15 minutes at 70 °C after 20 minutes at 37 °C of incubation.
- Using a spectrophotometer, absorbance at 660 nm was measured after cooling to room temperature.
- Calculated the % inhibition according to the following equation: Denaturation-inhibiting activity (%) = $(1 - (AC/AS)) \times 100$
- where AS is the test sample's absorption and AC is the control sample's absorption.

The study describes how *Tribulus terrestris* seed extract inhibits denaturation of egg albumin and has good anti-inflammatory effects. The test sample performed well.

4.7 PHYTOCHEMICAL ANALYSIS

Tanins, anthraquinones, flavonoid, alkaloids, cardiac glycosides, saponins, sterols, terpenoids, and carotenoids were among the phytochemicals examined in the seed extract.

1. **Test for tannins:** mix a few drops of ferric chloride with the extract; the methanol will become yellow.
2. **Test for aquinones:** 10l of the extract, 2ml of Hcl, and 2ml of the ammonia solution were used. Distinct aquatic layer seen. Rose hue indicates presence.
3. **Test for flavonoid:** mix 10 liters of extract with a few drops of NAOH solution. Alkaloids give yellow to colorless solutions their color.
4. **Test for alkaloids:** Wagner's reagent and 1.5 ml of aqueous extract were combined. Alkaloids are present because of the reddish-brown coloration.
5. **Test for cardiac glycoside:** combine 1 milliliter of extract with 0.5 milliliters each of glacial acetic acid, ferric chloride, and conc H₂SO₄. Heart glycoside is shown by the color blue.

6. **Test for saponins:** Combine the extract with distilled water and thoroughly shake to check for saponins. Saponins are present when foam is present.
7. **Test for sterols:** mix the extract with a few drops of concentrated H₂SO₄ and thoroughly shake. The presence of sterols were indicated by reddish color.
8. **Test for terpenoids:** 2.0 ml of chloroform was added to 1.5 ml of aqueous plant extract, which was allowed to evaporate on the water route before being heated with 3 ml of concentrated H₂SO₄. Terpenoids were detected as a reddish-brown coating. 1 cc of chloroform and 85% sulphuric acid were added to the extract. A blue hue denotes the presence of carotenoids.

4.8 CHARACTERIZATION PROCESS

Identification of the bioactive molecule using FTIR and UV-Visible spectroscopy. To find the chemical having o-bonds, pair electrons, and aromatic rings, the uv-visible profile of seed extract was analyzed at 200 to 800 nm wavelength. The functional group present in the material is determined via FTIR analysis.

FTIR peak values of *Tribulus terrestris* methanolic extract. The FTIR peak values and functional groups are displayed in the above table. The methanolic extract included alkene, phenol, and halo compound, as shown by the FTIR spectrum.

4.9 ANTICANCER ACTIVITY

The National Centre for Cell Sciences (NCCS), Pune, India, provided PA-1 (Human ovarian teratocarcinoma epithelial cell line). In Dulbecco's modified eagle medium (DMEM), which is supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), 100 U/mL penicillin, and 100 g/mL streptomycin, cells were kept in the logarithmic phase of growth. They were kept in a 95% air humidified incubator at 37°C with 5% CO₂. With the help of the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay), the sample's anticancer activity was evaluated against the PA-1 cell line (Mossman, 1983). Incubated at 37°C for 48 hours in a 5% CO₂ incubator, the cells were seeded in 96-well microplates (1 x 10⁶ cells/well) and allowed to reach 70-80% confluence. After that, the medium was changed, and the

cells were exposed to various sample concentrations while being incubated for 24 hours. After 24 hours, the morphological alterations of the untreated (control) and treated cells were examined and documented using a digital inverted microscope (20X magnification). After using phosphate-buffer saline (PBS, pH 7.4) to wash the cells, 20 μ L of the (MTT) solution (5 mg/mL in PBS) was applied to each well. The plates were then let to stand in the dark at 37 °C for 2h. The absorbance was measured spectrophotometrically at 570 nm after the formazan crystals were dissolved in 100 μ L DMSO.

The formula Cell viability (%) = (Absorbance of sample/Absorbance of control) X 100 was used to determine the percentage of viable cells.

CHAPTER - 5

RESULTS AND DISCUSSION

5.1 RESULTS

Gokhru (Sanskrit) is a significant herb that is frequently utilized for a variety of purposes in folk medicine throughout the world. *T. terrestris*' fruits have been shown to have diuretic, anti-urolithiatic, antimicrobial, antifungal, CNS-stimulating, antioxidant, and antihypertensive properties in rats and other beneficial effects. The biologically rich chemicals found in *T. terrestris*, including steroids, saponins, flavonoids, alkaloids, and unsaturated acids, are responsible for stimulating a variety of physiological responses. The leaves promote menstrual flow and are gonorrhea-curing. The fruits can help with impotence, severe micturation, and urinary issues.

5.1.1 PLANT SEED SAMPLE



Fig. 5.1: Powdered sample

Fig.5.1 shows the powdered sample of *Tribulus terrestris* plant.

5.1.2 EXTRACTION PROCESS



Fig. 5.2: Solvent extraction of *Tribulus terrestris*

Samples are weighed. Hexane (200ml), methanol, chloroform, and water are then added. regularly shaking. 24 hours were spent at room temperature. followed by filtering out and setting aside for evaporation.

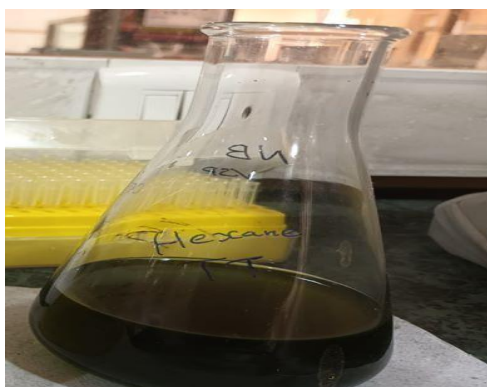


Fig.5.3:Hexane extraction of TT

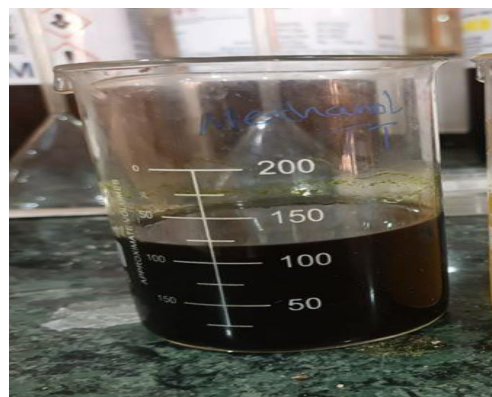


Fig.5.4:Methanol extraction of TT

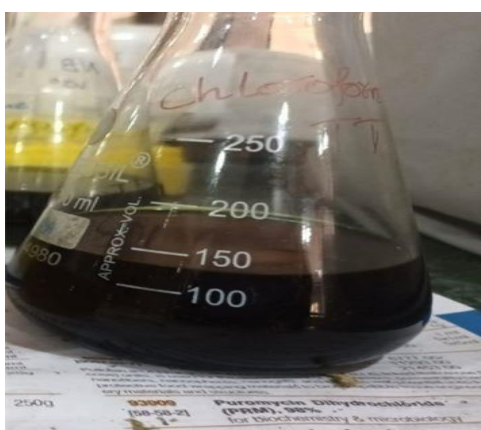


Fig. 5.5: Chloroform extraction of TT

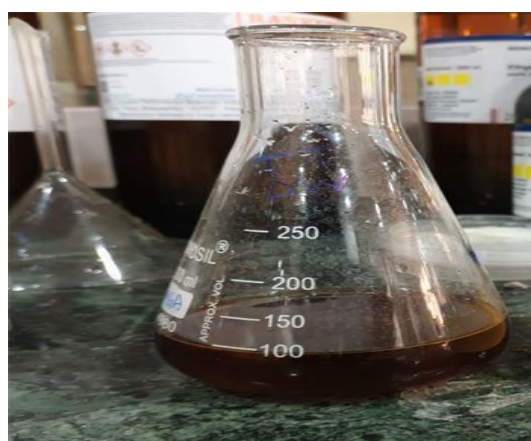
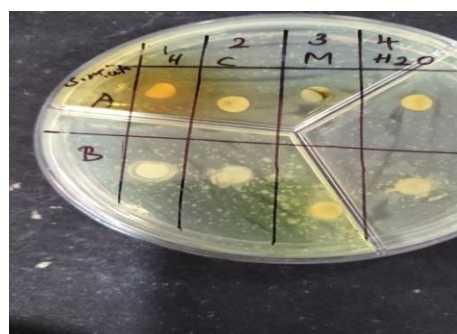


Fig.5.6: H2O extraction of TT

5.1.3 ANTI-BACTERIAL ACTIVITY



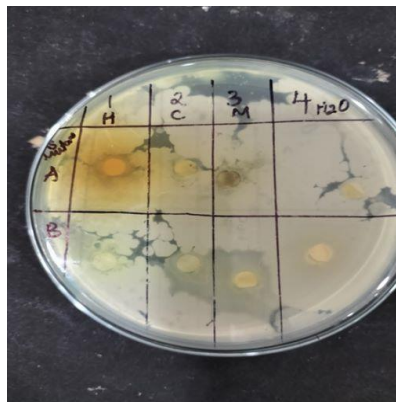
(a) Escherichia-coli



(b)Streptococcus mutans



(c) *Staphylococcus aureus*



(d) *Pseudomonas aeruginosa*



(e) ANTIFUNGAL ACTIVITY: *Candida albicans*

Fig. 5.7: Antibacterial activities of plant extract

Fig.5.7.(a,b,c,d,e) shows the antibacterial activity of plant extract showed a positive for few bacteria.Hexane extract showed 7mm of inhibition against *candida albicans*.Chloroform extract showed 8mm zone of inhibition against *E.coli* and *staphylococcus aureus* .Methanol extract showed inhibition of 6mm against *E.coli* and *staphylococcus aureus*,8mm against *candida albicans*.

Table 5.1: The zone of inhibition of plant extract against different bacterial strain

SOLVENT	Zone of inhibition in mm				
	<i>E.Coli</i>	<i>Streptococcus mutans</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Candida albicans</i>
HEXANE	-	-	-	-	7
CHLOROFORM	8	-	8	-	-
METHANOL	6	-	6	-	8
H2O	-	-	-	-	-

5.1.4 ANTIOXIDANT ACTIVITY

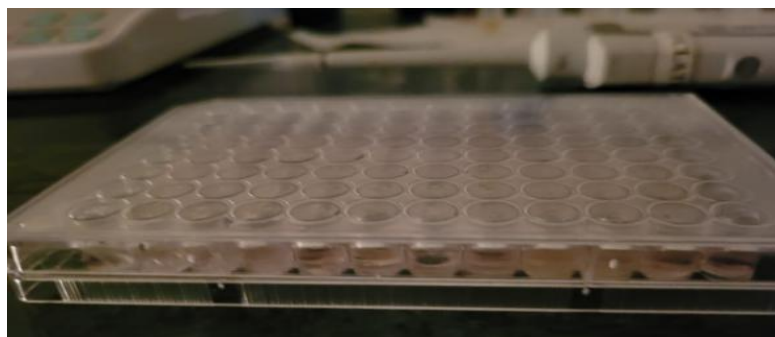


Fig. 5.8: 96 Well-plate

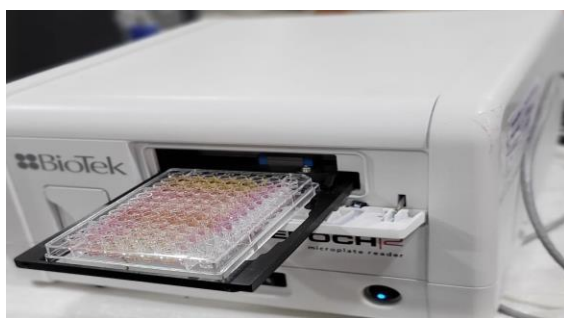


Fig. 5.9: Sample loaded in UV Spectroscopy

CALCULATION OF % RADICAL SCAVENGING AND IC50 FROM DPPH ASSAY

Methanol Extract

Table 5.2: DPPH activity of Methanol Extract

CONCENTRATION	CONTROL AVERAGE	SAMPLE 1	SAMPLE 2	SAMPLE AVERAGE	% RSA	IC 50
10	0.2555	0.07	0.079	0.1095	57.14285714	6.972287118
5	0.2555	0.068	0.071	0.1035	59.49119374	7.375090631
2.5	0.2555	0.067	0.073	0.1035	59.49119374	7.576492387
1.25	0.2555	0.069	0.117	0.1275	50.09784736	7.677193265
0.625	0.2555	0.112	0.147	0.1855	27.39726027	7.727543704
0.312	0.2555	0.153	0.238	0.272	-6.457925636	7.752759204
0.156	0.2555	0.197	0.173	0.2835	-10.95890411	7.76326674
0.078	0.2555	0.189	0.184	0.281	-9.980430528	7.771610408

Table 5.2 IC50 values of the extract evaluated in this study are shown

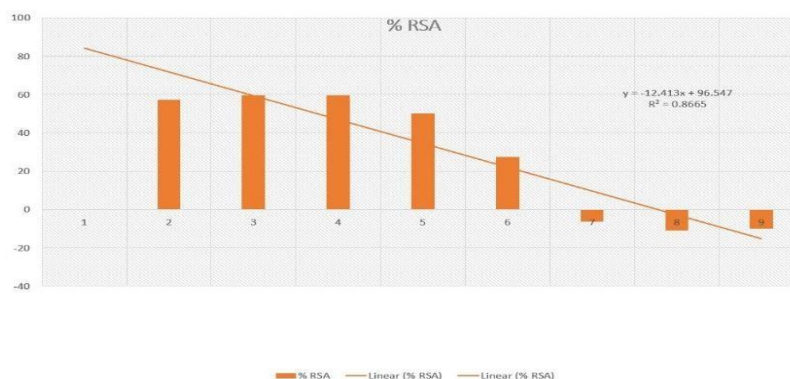


Fig. 5.10: DPPH assay activity of Methanol Extract

Further, the antioxidant activity of methanol extract of *Tribulus terrestris* was evaluate by DPPH assay. Different concentration of methanol extract of (0.078-10mg) its used in assay it was found that IC50 value of Methanol extract was found to be 7.727 at 0.625mg.

H₂O Extract

Table 5.3: DPPH activity of H₂O Extract

concentration	Control average	sample 1	sample 2	sample average	%RSA	IC50
10	0.2555	0.075	0.074	0.112	56.1643836	7.55560121
5	0.2555	0.061	0.059	0.0905	64.5792564	8.09620604
2.5	0.2555	0.06	0.055	0.0875	65.7534247	8.36650845
1.25	0.2555	0.12	0.082	0.161	36.9863014	8.50165966
0.156	0.2555	0.158	0.112	0.214	16.9863014	8.61994399
0.078	0.2555	0.129	0.141	0.1995	21.9178082	8.62837743

Table 5.3.IC50 values of the extract evaluated in this study are shown

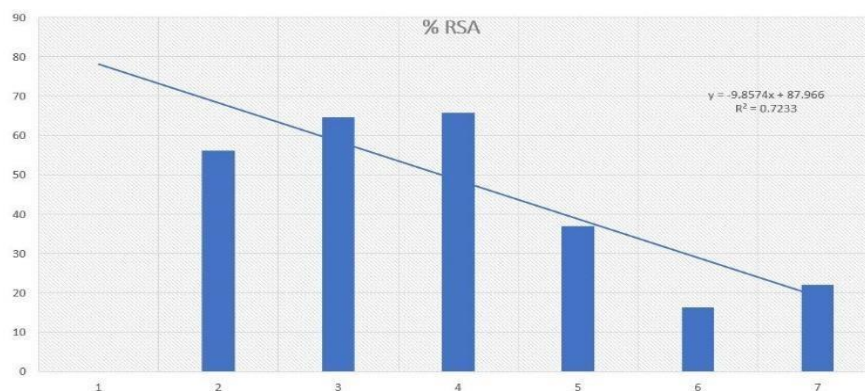


Fig. 5.11: DPPH assay activity of H₂O Extract

Further, the antioxidant activity of methanol extract of *Tribulus terrestris* was evaluate by DPPH assay. Different concentration of methanol extract of (0.078-10mg) its used in assay it was found that IC50 value of Methanol extract was found to be 8.61at 0.156mg.

5.1.5 ANTI-INFLAMMATORY ACTIVITY

Egg Albumin Denaturation Assay

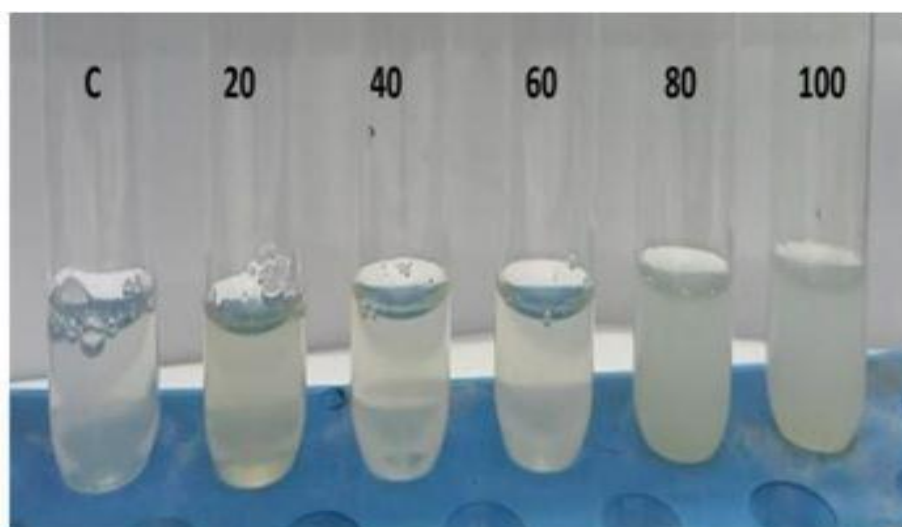


Fig. 5.12: Egg Albumin Denaturation Assay

Fig.5.12 shows the Anti-Inflammatory Activity of Denaturation Assay.

Table 5.4: Anti-Inflammatory Activity (egg albumin)

CONCENTRATION (µg/ml)	ABSORBANCE	INHIBITION (%)
Control	0.175	
20	0.222	11.72414
40	0.289	23.44828
60	0.401	36.55172
80	0.599	51.03448
100	0.743	68.96552

The study describes how *Tribulus terrestris* seed extract inhibits denaturation of egg albumin and has good anti-inflammatory effects. The test sample performed well.

5.1.6 UV-VISIBLE SPECTROSCOPY

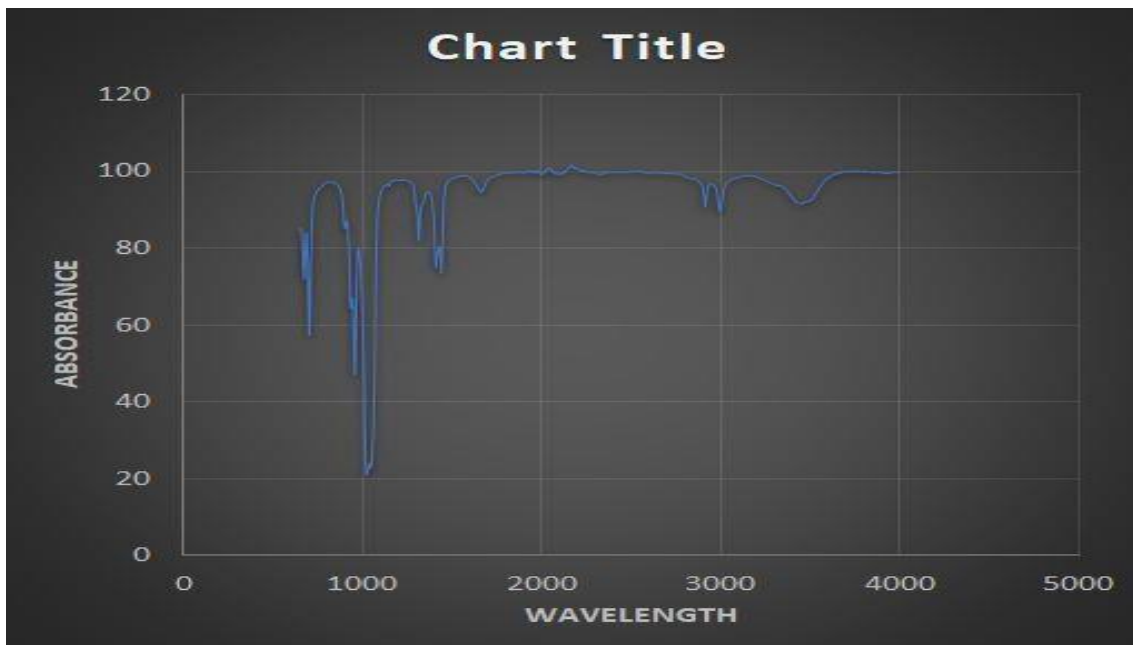


Fig. 5.13: UV-Visible spectrum of Methanolic Extract

Fig.5.13 shows the compound dissolved in methanol exhibited two absorption peaks (650 and 667 nm).

Table 5.5: UV-Visible peak values of methanolic extract of *Tribulus terrestris*

WAVELENGTH nm	INTENSITY
650.4205	85.29127
652.2841	84.84154
654.1478	84.50053
656.0115	83.70613
657.8751	82.07048
659.7388	80.00431
661.6025	77.80234
663.4661	75.00064
665.3298	73.08053
667.1935	72.29126

5.1.7 FOURIER TRANSFORM INFRARED SPECTROSCOPY

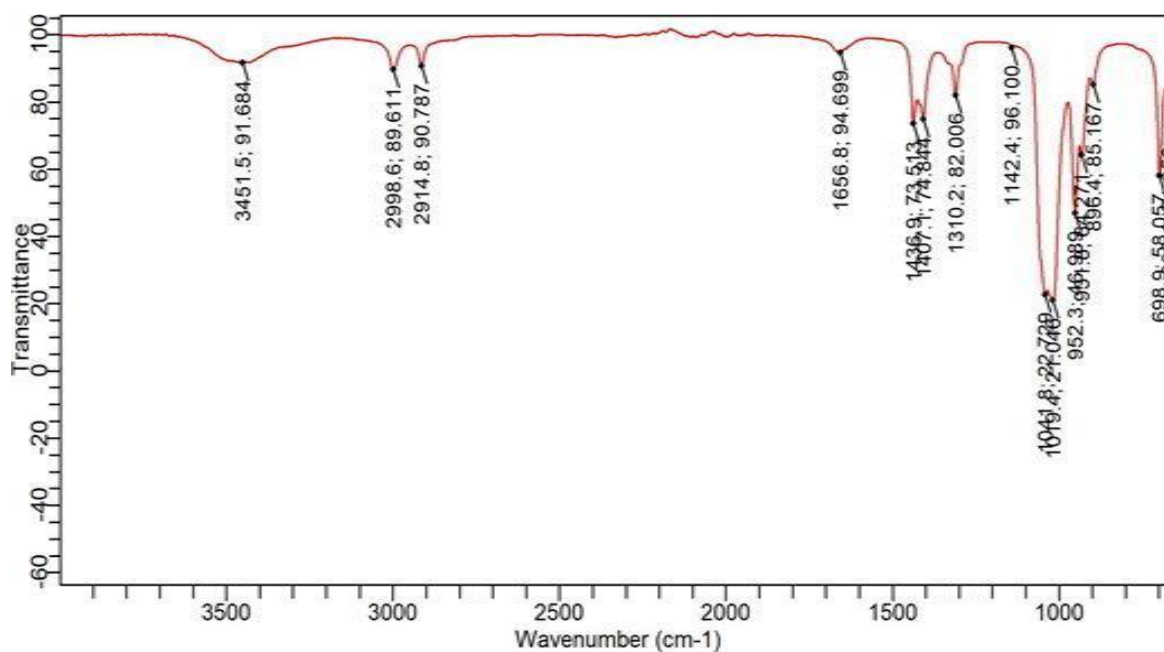


Fig. 5.14: FTIR analysis of methanolic extract

Table 5.6: FTIR peak values of Methanolic extract of Tribulus terrestris

PEAK VALUE	FUNCTIONAL GROUP	FUNTIONAL GROUP NAME	Appearance
669.05714	C-Br stretching	halo compound	Strong
698.87584	C=C Bending	alkene	Strong
896.42475	C-H Bending	1,2,4-trisubstituted	Strong
1041.79093	CO-O-CO stretching	Anhydride	strong, broad
1310.15925	O-H Bending	phenol	Medium
1407.07003	S=O stretching	sulfonyl chloride	Strong
1436.88874	O-H Bending	carboxylic acid	Medium

The FTIR structure characteristics of compound present or functional group present in the component methanol extract. Structural characteristic of component present in methanol concentration of *Tribulus terrestris* was investigated by UV visible & FTIR spectroscopy. It confirms that results presents of Halogen , alkene , phenol, sulfonyl chloride , carboxylic acid , anhydride , 1,2,4-trisubstituted. Halogen compound its confirmed is 669.05 , Alkene compound its confirmed is 698.87, phenol compound its confirmed is 1310.15.

5.1.8 ANTICANCER ACTIVITY

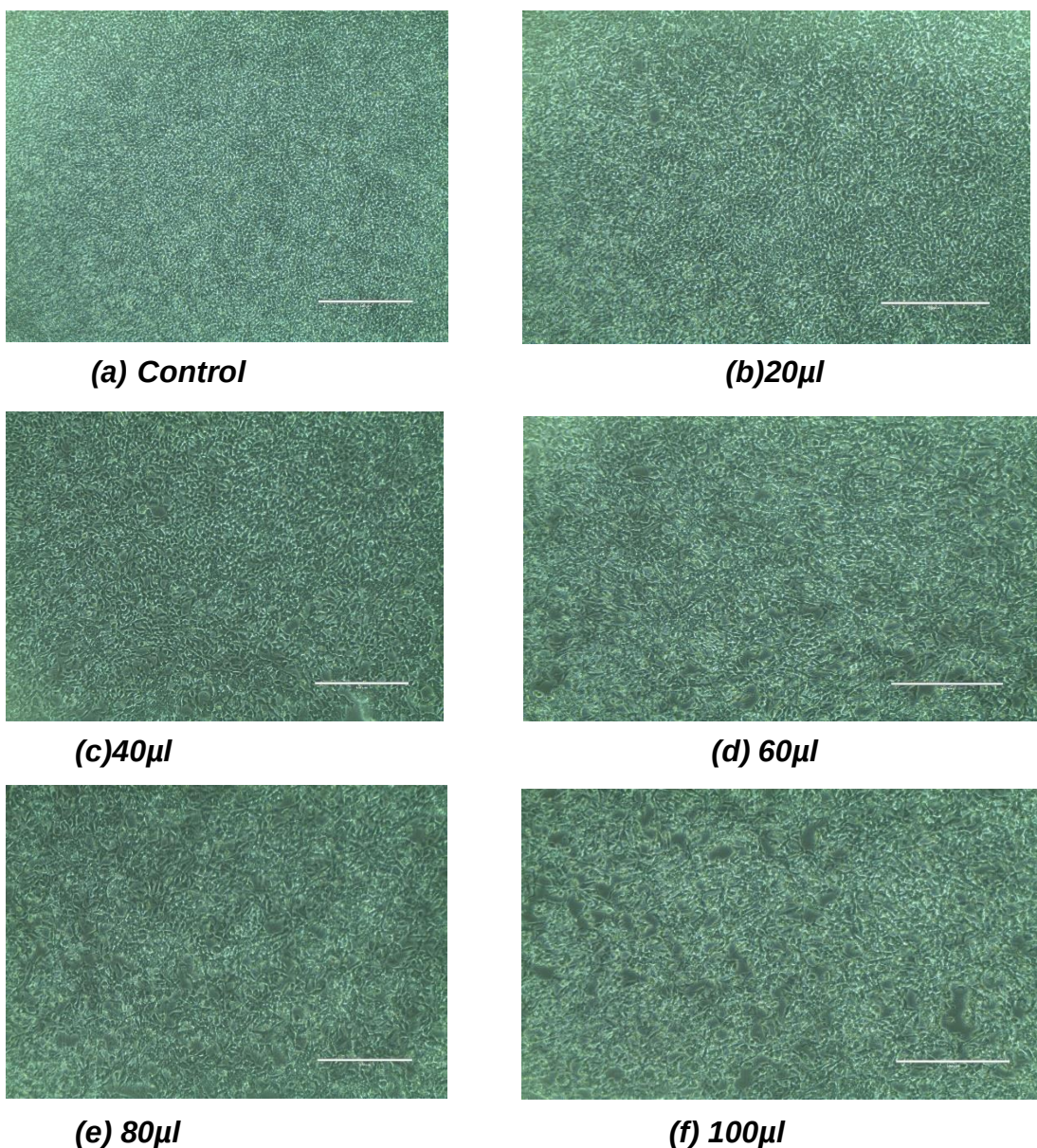


Fig.5.15:Anticancer activity of MTT assay against ovarian cell line

Fig.5.15 (a,b,c,d,e,f) shows the different concentration of methanol extract of MTT Assay against ovarian cell line.

Table 5.7: *Tribulus terrestris* methanolic extract of MTT assay in cell line

CONCENTRATION ($\mu\text{g/ml}$)	ABSORBANCE		AVERAGE	CELL AVAILABILITY (%)
	I	II		
Control	0.943	0.95	0.9465	100
20	0.821	0.836	0.8285	87.53301638
40	0.733	0.728	0.7305	77.17908082
60	0.565	0.549	0.557	58.8483888
80	0.401	0.413	0.407	43.00052826
100	0.262	0.254	0.258	27.25832013

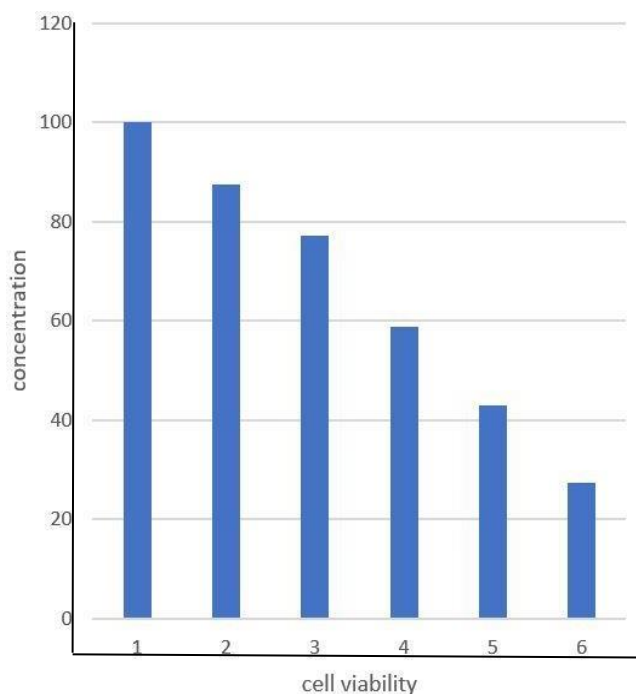


Fig. 5.16: The results of the *Tribulus terrestris* methanol extract

The results of the *Tribulus terrestris* methanol extract are depicted in the photographs, and it was discovered that when the concentration was increased, the percentage of cell viability fell. Based on the findings, it was evident that the PA-1 cell line may be susceptible to cytotoxicity from *Tribulus terrestris* methanolic extract.

5.2 DISCUSSION

Gokhru (Sanskrit) is a significant herb that is frequently utilized for a variety of purposes in folk medicine throughout the world. *T. terrestris*' fruits have been shown to have diuretic, anti-urolithiatic, antimicrobial, antifungal, CNS-stimulating, antioxidant, and antihypertensive properties in rats (Zhang et al., 2010); and other beneficial effects. The biologically rich chemicals found in *T. terrestris*, including steroids, saponins, flavonoids, alkaloids, and unsaturated acids, are responsible for stimulating a variety of physiological responses (Yan et al., 1996). Thimation. The leaves promote menstrual flow and are gonorrhea-curing. The fruits can help with impotence, severe micturation, and urinary issues. *Tribulus Terrestris* is a plant that has been used in traditional medicine for centuries due to its medicinal properties. Its fruit extract is known to possess several health benefits, including antioxidant, antibacterial, anti-inflammatory, and PCOD activities. The fruit extract of *Tribulus Terrestris* contains several antioxidant compounds such as flavonoids, alkaloids, and phenolic compounds. These compounds are responsible for scavenging free radicals in the body, which helps prevent oxidative damage to the cells. Antioxidants also help in reducing inflammation in the body, which can lead to various diseases. The fruit extract of *Tribulus Terrestris* has been found to possess antibacterial activity against several strains of bacteria, including *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. This activity is due to the presence of various bioactive compounds in the extract that interfere with the growth and replication of the bacteria. The fruit extract of *Tribulus Terrestris* has anti-inflammatory properties that can help reduce inflammation in the body. This activity is due to the presence of various bioactive compounds in the extract that inhibit the production of pro-inflammatory cytokines in the body. This activity can be beneficial in conditions such as arthritis, where inflammation plays a significant role in the progression of the disease. Polycystic ovary syndrome (PCOS) is a common hormonal disorder in women that can lead to several complications, including infertility. Studies have shown that the fruit extract of *Tribulus Terrestris* can help regulate hormonal imbalances in women with PCOS. This activity is due to the presence of various bioactive compounds in the extract that help regulate the levels of hormones such as estrogen and testosterone in the body.

CHAPTER - 6

SUMMARY & CONCLUSION

6.1 SUMMARY

Tribulus terrestris, a traditional medicinal plant, has been found to have numerous health benefits, including its ability to manage polycystic ovary syndrome (PCOS). PCOS is a common hormonal disorder that affects women, causing irregular periods, excessive hair growth, and infertility. Studies have shown that *Tribulus terrestris* can help regulate menstrual cycles, increase ovulation, and reduce insulin resistance in women with PCOS. It also has anti-inflammatory and antioxidant properties, which can help reduce the risk of developing complications associated with PCOS. With its potential to manage PCOS, *Tribulus terrestris* has become a popular natural supplement for women looking to manage their symptoms and support their overall health. However, it's important to note that while *Tribulus terrestris* may be helpful in managing PCOS, it should not be used as a sole treatment method. A comprehensive approach to PCOS management, including lifestyle changes and medical interventions, should be considered for optimal recovery.

6.2 CONCLUSION

The primary discrepancies in the therapeutic benefits recorded over the years in the literature are explained by different phytochemical profiles of the herbal medications from *TT*, underlined both in the concentration of the main active components and in the absence of some active compounds. Although the majority of the pharmacological study on *TT* has been concentrated on sexual issues, additional significant benefits, such as anti-hyperglycemic, anti-inflammatory, antioxidant, and antibacterial, have been shown in vitro and in vivo trials. Despite their limitations, toxicological investigations have revealed the possibility of nephrotoxicity after the administration of *TT* supplements. However, more research is required to identify some of the medicinal active chemicals reported in *Tribulus* extracts that have molecular mechanisms of action that are currently unknown. Despite the fact that *TT* has been thoroughly studied, more research is required to elucidate key issues like a more precise correlation between the phytochemical and pharmacological profiles, pharmacokinetic studies of

the most significant compounds, and the assessment of potential pharmacokinetic and pharmacodynamic interactions with other substances. Researchers must disclose all relevant details regarding the origin of the plant and the tested organ. To get reproducible findings, standardization techniques are required. As of now, it appears that the chemical components in *TT* are capable of activating a variety of pathways, which accounts for the variety of effects.

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