

SCREENING OF PHARMACOLOGICAL COMPOUND FOR THE PREVENTION OF POLYCYSTIC OVARIAN DISEASE(PCOD)

Submitted in partial fulfilment of the requirements for the award of
Bachelor of Technology Degree in Biotechnology

By

DIVYASRI.R (37230014)

ARTHI.R (37230007)



**DEPARTMENT OF BIOTECHNOLOGY
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.**

APRIL – 2021.



SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)

Accredited with "A" grade by NAAC
Jeppiaar Nagar, Rajiv Gandhi Salai,
Chennai – 600 119



www.sathyabama.ac.in

DEPARTMENT OF BIOTECHNOLOGY

BONAFIDE CERTIFICATE

This is to certify that this Project Report is the Bonafide work of Arthi. R (37230007) and Divyasri. R (37230014) who carried out the project entitled "**SCREENING OF PHARMACOLOGICAL COMPOUND FOR THE PREVENTION OF POLYCYSTIC OVARIAN DISEASE(PCOD)**" under our supervision from December 2020 to March 2021.

Internal Guide

Dr. M. MASILAMANI SELVAM
(ASSOCIATE PROFESSOR)

External Guide

Dr. C. SIVARAJ
(ARMATS BIOTEK)

Head of the Department

(DR. RAMESH KUMAR)

Submitted for Viva-voce Examination held on 15.04.2021

Internal Examiner

External Examiner



ARMATS BIOTEK

SERVE HUMANITY THROUGH SCIENCE

TRAINING AND RESEARCH INSITUTE (ABTRI)

ISO

14001:2015

CERTIFICATE

This is to certify that the dissertation entitled "*SCREENING OF PHARMACOLOGICAL COMPOUND FOR THE PREVENTION OF PCOD*" submitted by Ms. DIVYASRI. R (37230014), of Sathyabama Institute of Science and Technology for the degree of B. Tech Biotechnology is a record of project work carried out by her at Armats Biotek Training and Research Institute, Guindy, Chennai during the period December 2020- March 2021 under my guidance and that the dissertation has not formed the basis for the award of any degree, diploma, associate, fellowship or titles in this University or any other University or Institution of higher learning.

Dr. C. SIVARAJ
SUPERVISOR



Dr. P. ARUMUGAM
DIRECTOR

PLACE: Chennai

DATE : 09.03.2021

14/18c, Mettu Street, Link Road, Maduvankarai, Guindy, Chennai -600 032

Telephone: 044-43502783; Mobile: 0-9444845204 & 0-9789010039

E-mail: armatsbiotek@gmail.com & armatsbiotekstaffs@gmail.com, www.armatsbioteklab.in



ARMATS BIOTEK

SERVE HUMANITY THROUGH SCIENCE

TRAINING AND RESEARCH INSTITUTE (ABTRI)



14001:2015

CERTIFICATE

This is to certify that the dissertation entitled "**SCREENING OF PHARMACOLOGICAL COMPOUND FOR THE PREVENTION OF PCOD**" submitted by **Ms. ARTHI. R (37230007)**, of **Sathyabama Institute of Science and Technology** for the degree of **B. Tech Biotechnology** is a record of project work carried out by her at **Armats Biotech Training and Research Institute, Guindy, Chennai** during the period **December 2020- March 2021** under my guidance and that the dissertation has not formed the basis for the award of any degree, diploma, associate, fellowship or titles in this University or any other University or Institution of higher learning.

Dr. C. SIVARAJ
SUPERVISOR



Dr. P. ARUMUGAM
DIRECTOR

PLACE: Chennai

DATE : 09.03.2021

14/18c, Mettu Street, Link Road, Maduvankarai, Guindy, Chennai -600 032

Telephone: 044-43502783; Mobile: 0-9444845204 & 0-9789010039

E-mail: armatsbiotech@gmail.com & armatsbiotechstaffs@gmail.com, www.armatsbiotechlab.in

Scanned By Scanner Go

DECLARATION

We **Arthi. R (37230007)** and **Divyasri. R (37230014)** hereby declare that the Project Report entitled **SCREENING OF PHARMACOLOGICAL COMPOUND FOR THE PREVENTION OF POLYCYSTIC OVARIAN DISEASE(PCOD)** done by us under the guidance of Dr.M. Masilamani Selvam (Internal) and Dr.C. Sivaraj (External) at Armats Biotek Training Institute are submitted in partial fulfillment of the requirements for the award of Bachelor of Technology degree in Biotechnology.

Handwritten signatures of the candidates, Arthi. R and Divyasri. R, in black ink.

DATE: 11.04.2021

PLACE: CHENNAI

SIGNATURE OF THE CANDIDATE

ACKNOWLEDGEMENT

I am pleased to acknowledge my sincere thanks to the Board of Management of SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY for their kind encouragement in doing this project and for completing it successfully. I am grateful to them.

I convey my thanks to Dr. Mariazeena Johnson, Chancellor; Dr. Marie Johnson, President; Dr. Aruni Wilson, Pro Vice-Chancellor School of Bio and Chemical Engineering, Dr.V. Ramesh Kumar, Ph.D., Head of the Department, Dept. of Biotechnology and Dr. Renuga Devi. K, Year Coordinator for providing me necessary support and details at the right time during the progressive reviews.

I would like to express my sincere and deep sense of gratitude to my Project Guide Dr. Masilamani Selvam.M, Ph.D., Associate Professor, Dept. of Biotechnology and Dr. Sivaraj.C, Scientist, Armats Biotek Training and Research Institute for their valuable guidance, suggestions, and constant encouragement paved way for the successful completion of my project work.

I wish to express my thanks to all Teaching and Non-teaching staff members of the Department of Biotechnology who were helpful in many ways for the completion of the project. I would like to express our gratitude to my loving parents for being the strength in my life and support me during this entire project.

ABSTRACT

Medicinal plants have been used as a source of medicine to alleviate diseases. The use of medicinal plants as a source for medicine to relieve illness, which comes out by the early civilization in India and documented. This herb nicker bean (kalarchikkai) depends on the Leguminosae or Fabaceae family. The native of this plant is India, Burma, and Sri Lanka. The nicker bean is rich in proteins, minerals, and vitamins, and also, this herb has a lot of medicinal properties, such as antimalarial, antibacterial, anti-diabetic, and anticancer properties. This medicinal plant helps to cure many health problems, such as diabetes, women hormones, liver problems, acne, malaria, intermittent fever. The study aimed to evaluate the radical scavenging activities of aqueous and chloroform seed extract of nicker bean and to evaluate its potential to cure Polycystic Ovarian Syndrome (PCOD).

TABLE OF CONTENTS

CHAPTER NO.	TITLE	PAGE NO
	ABSTRACT	i
	LIST OF FIGURES	v
	LIST OF TABLES	vi
	ABBREVIATIONS	vii
1	INTRODUCTION	1
	1.1 HISTORY OF TRADITIONAL MEDICINE	1
	1.1.1 INTEREST IN MEDICINAL PLANTS	3
	1.1.2 GEOGRAPHICAL DISTRIBUTION	4
	1.1.3 TAXONOMICAL DISTRIBUTION	5
	1.1.4 MORPHOLOGY OF PLANT	7
	1.1.5 THERAPEUTIC USES OF PLANT	7
	1.2 INTRODUCTION – PCOD	8
	1.3 DEFINITION OF PCOD	8
	1.4 ETIOLOGY OF PCOD	9
	1.4.1 FACTORS INFLUENCING PCOD	9
	1.4.2 COMMON SYMPTOMS OF PCOD	10
	1.5 EPIDEMIOLOGY OF PCOD	12
	1.6 MENSTRUAL CYCLE IRREGULARITY	12
2	REVIEW OF LITERATURE	14

3	AIM AND SCOPE	26
	3.1 AIM	26
	3.2 SCOPE	26
4	MATERIALS AND METHOD	27
	4.1 EQUIPMENT AND GLASSWARE	27
	4.2 CHEMICALS	27
	4.3 REAGENTS	27
	4.4 PREPARATION OF EXTRACT	28
	4.5 IN VITRO ANTIOXIDANT ASSAYS	29
	4.5.1 DPPH [•] RADICAL SCAVENGING ACTIVITY	29
	4.5.2 PHOSPHOMOLYBDENUM ASSAY	29
	4.5.3 FERRIC(Fe ³⁺) REDUCING POWER ASSAY	30
	4.5.4 ANTI HEMOLYTIC ASSAY	30
	4.5.5 COLUMN CHROMATOGRAPHY	31
	4.5.6 GC-MS	32
5	RESULTS AND DISCUSSION	34
	5.1 DPPH [•] RADICAL SCAVENGING ACTIVITY	34
	5.2 PHOSPHOMOLYBDENUM ACTIVITY	36
	5.3 FERRIC(Fe ³⁺) REDUCING POWER ACTIVITY	38
	5.4 ANTI HEMOLYTIC ACTIVITY	40
	5.5 COLUMN CHROMATOGRAPHY	42
	5.6. IDENTIFICATION OF COMPONENTS	44
	5.7 MTT ASSAY	48
	5.7.1 CHEMICALS AND REAGENTS	48

	5.7.2 CELL CULTURE	48
	5.7.3 CELL GROWTH INHIBITION STUDIES	48
	5.7.4 RESEARCH AND DEVELOPMENT	49
6	SUMMARY AND CONCLUSION	52
	SUMMARY	52
	CONCLUSION	52
	REFERENCES	53

LIST OF FIGURES

FIGURE NO.	TITLE	PAGE NO.
1.1	Seeds of <i>Caesalpinia bonduc</i>	4
1.2	Geographical distribution map	5
1.1.3	<i>Caesalpinia Bonduc L</i> Plant	6
1.3	Comparison of ovary	9
4.4	Soxhlet extraction	28
4.5.5	Column chromatography	32
4.5.6	GC-MS	33
5.1	DPPH [•] radical scavenging activity	35
5.2	Phosphomolybdenum reduction assay	37
5.3	Ferric (Fe ³⁺) reducing power activity	39
5.4	Anti-Hemolytic assay	41
5.4	RBC Suspension	42
5.5	Column chromatography	44
5.7.4	Microscopic studies of HESCs	49

LIST OF TABLES

TABLE NO.	TITLE	PAGE NO.
5.1	DPPH' Radical Scavenging Activity	34
5.2	Phosphomolybdenum Reduction Activity	36
5.3	Ferric (Fe ³⁺) Reducing Power Activity	38
5.4	Anti-Hemolytic Activity	40
5.5	Column Chromatography	43
5.1	Identification of compounds through GC-MS	46
5.7.4	Percentage of Live HESCs Table	50

LIST OF ABBREVIATIONS

1. **FSH** Follicle-stimulating hormone
2. **DPPH[•]** 2,2-diphenyl-1-picrylhydrazyl
3. **PCOS** Polycystic ovarian disease
4. **GST** Glutathione S-Transferase
5. **CBSCE** Caesalpinia bonduc seed coat extract
6. **CF** Cystic fibrosis
7. **CL** Corpus luteum
8. **LDL** Low-density cholesterol
9. **HDL** High-density cholesterol
10. **HAE** Haemagglutinating antibody
11. **Na₂HPO₄** Disodium hydrogen phosphate
12. **NaH₂PO₄** Sodium dihydrogen phosphate
13. **K₃Fe (CN)₆** Potassium ferricyanide
14. **FeCl₃** Ferric chloride
15. **RBC** Red blood cells
16. **GC-MS** Gas chromatography - mass spectroscopy
17. **Ic₅₀** Inhibitory concentration at 50 %
18. **Rc₅₀** Reduction concentration at 50%
19. **CHCl₃** Chloroform
20. **HESCs** Human Embryonic Stem Cell

CHAPTER -1

INTRODUCTION

Medicinal plants, as a hidden source of medicinal aids, have played an important role in health care for both humans and animals all over the world, not only in diseased conditions but also as a possible material for preserving good health. However, it is necessary to understand which parts of the medicinal herb are responsible for its curative properties (Ali et al., 2008). In recent years, there has been a greater emphasis on plant science around the world, and a wide body of evidence has been gathered to demonstrate the enormous potential of medicinal plants used in various conventional systems. There is a renewed interest in traditional medicine today, as well as growing demand for more medicines derived from plants (Archana et al., 2005). This surge in interest in plant-based medicines is largely due to the general perception that "green medicine" is safer and more dependable than the more expensive synthetic drugs with negative side effects. Herbal medicines and medicinal plants, as well as their extracts and isolated compounds, have shown a wide range of biological activities. Such has been used and continued to be used as medicine in tradition or food supplement for various disorders.

Today, there is a lot of interest in the use of herbal remedies among the general public. Herbal medicines or medicinal plants, as well as their extracts and isolated compound(s), have shown a wide range of biological activities. Furthermore, plant extracts were the source of many western medicines (Chakrabarti et al., 2000). There are many herbs, which are predominantly used to treat cardiovascular problems, liver disorders, central nervous system, digestive and metabolic disorders. Given their potential to produce a significant therapeutic effect, they can be useful as a drug or supplement in the treatment management of various diseases (Mazumde et al., 2005).

1.1 HISTORY AND DEVELOPMENT OF TRADITIONAL MEDICINE

The history of traditional medicine can be traced back to thousands of years in both Western and Eastern traditions. Traditional medicine can be classified into phytotherapy, over-the-counter herbal, and traditional herbalism. The role of folklore

medicine started to decline after the 1960s as vast quantities of resources and money were used to promote synthetic medication. Aside from that, advancements in the human genome increased understanding of the structure and function of proteins, and the perception that synthetic drugs are safer and have less side effects have all contributed to synthetic drug popularity. However, there are a number of significant limitations to these developments (Saeed and Sabir, 2001). The number of known compounds that may theoretically act as drug candidates has already outgrown the number of possible new drug targets, and chemistry has a limit when it comes to synthesizing new drug structures. Herbal medicine has progressed in the last decade, with societal acceptance of their therapeutic effects increasing at a faster rate. New lead drug developments as well as safe and effective plant-based medicines are being developed in this sector (Gupta et al., 2004). As a result, there are more commercialized medicinal herb sales and, more significantly, an increasing number of pharmaceutical companies involved in plant research and development as a source of modern medicine... What chemists have been desperately seeking, Mother Nature has already plenty of stock (*Fatma, 2008*). The realization that herbal medicines are effective in different therapies. Many proofs are indicating the importance of herbal plant use in the different conventional systems. The realization that herbal medicines are more reliable has increased the interest in these medicines. It is observed that many medicinal plants are used to cure diseases like digestive problems, cardiovascular disorders, metabolic problems, liver disorders, and the disorders of nervous systems. The plant extract is predominantly used as a source of many western drugs. The quality of medicine, its protection, and its efficacy must all be guaranteed before using conventional herbal plants safely. Phytochemicals are important for the defense of both plants and humans against a variety of diseases (Shukla et al., 2006). This edition tries to expound on the importance of plant-based products in modern drug development by examining their roles in modern drug development.

India has one of the world's most extensive plant medical traditions. It's a tradition that has a lot of contemporary significance when it comes to maintaining health protection. In India, there are an estimated 25,000 effective plant-based formulations used in traditional medicine and known to rural communities (*Yadav and Arora, 2007*). There are over 1.5 million practitioners of the traditional medicinal system using medicinal

plants in preventive, promotional, and curative applications. It is estimated that there are over 7800 medicinal drug manufacturing units in India, which consume about 2000 tons of herbs annually.

Just about 5% of the world's 300,000 plant species have been scientifically investigated for medicinal purposes. Researchers have discovered that developing countries rely on herbal plants to treat diseases, especially in areas where hospitals are scarce (Bagul and Ragani, 2005). Ethnopharmacological studies on herbs/medicinally important plants continue to interest investigators throughout the world (Jabbar *et al.*, 2007). However, one of the impediments in the acceptance of the Ayurvedha or Siddha formulation is the lack of standard quality control profiles (Jayakrishnan *et al.*, 2014). Medicinal plants are plants that have properties or compounds that can be used for therapeutic purposes, or plants that synthesize metabolites to create effective drugs, according to the World Health Organization (WHO).

1.1.1 Interest in Medicinal plants

Caesalpinia bonducella is an Indian herb reported in Ayurveda, the ancient Hindi medicine system of India. *Caesalpinia bonducella* is classified under the family of Caesalpiniaceae. It is also known as *C. bonducella* Flem and *C. crista* Linn. Commonly it is called Fever Nut, Bonduc Nut, and Nicker Nut also. The name "Bonducella" comes from the Arabic word "Bonduce," which means "a little ball," and refers to the seed's globular form (Wadkar, 2009). In the conventional system of Indian medicine, Ayurveda, *Caesalpinia bonducella* is largely used for its antiperiodic, antipyretic, anti-inflammatory, anthelmintic, antimalarial, and also for different diseases like skin diseases, hydrocele, leprosy, convulsions, orchitis, paralysis, and analogical nervous complaints. Antioxidant, antibacterial, antitumor, and anti-diabetic properties have also been recorded. In Chinese traditional medicine, it is useful to prepare the lotion for the treatment of pain type cellulitis (Kapoor, 2000). Despite the widespread use of *Caesalpinia bonducella* in conventional medicine, few systematic pharmacological studies evaluating its therapeutic properties have been published to date. (Bleyere, 2014).

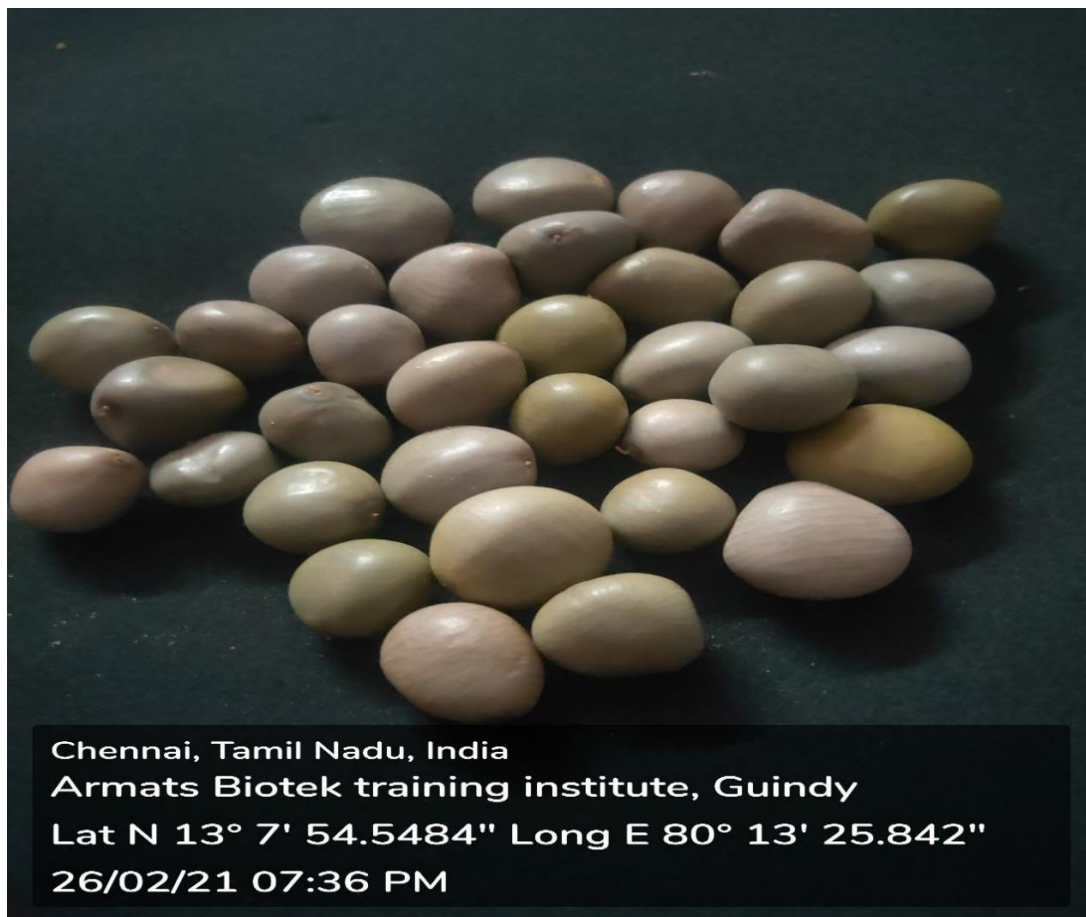


FIG 1: Seeds of *Caesalpinia bonduc*

1.1.2 Geographical Distribution

The plant is found in the tropical and subtropical parts of Asia. It is distributed in Srilanka, Bangladesh, Myanmar, China, and Vietnam. It is also found in Andaman, Nicobar, Islands, and India especially in the tropical region (*Khatun, 2013*). *Caesalpinia bonduc* Linn. is a large scandent prickly shrub, found throughout the hotter parts of India, Burma, and Ceylon, particularly along the sea coast, and up to 2,500 feet on the hills. It is common in Bengal, Burma, and south India. It is a climber up to 15 m long, throughout India, near the sea coast, especially all over Bengal, Bombay, and the whole of Southern India (*Sagar, 2009*).

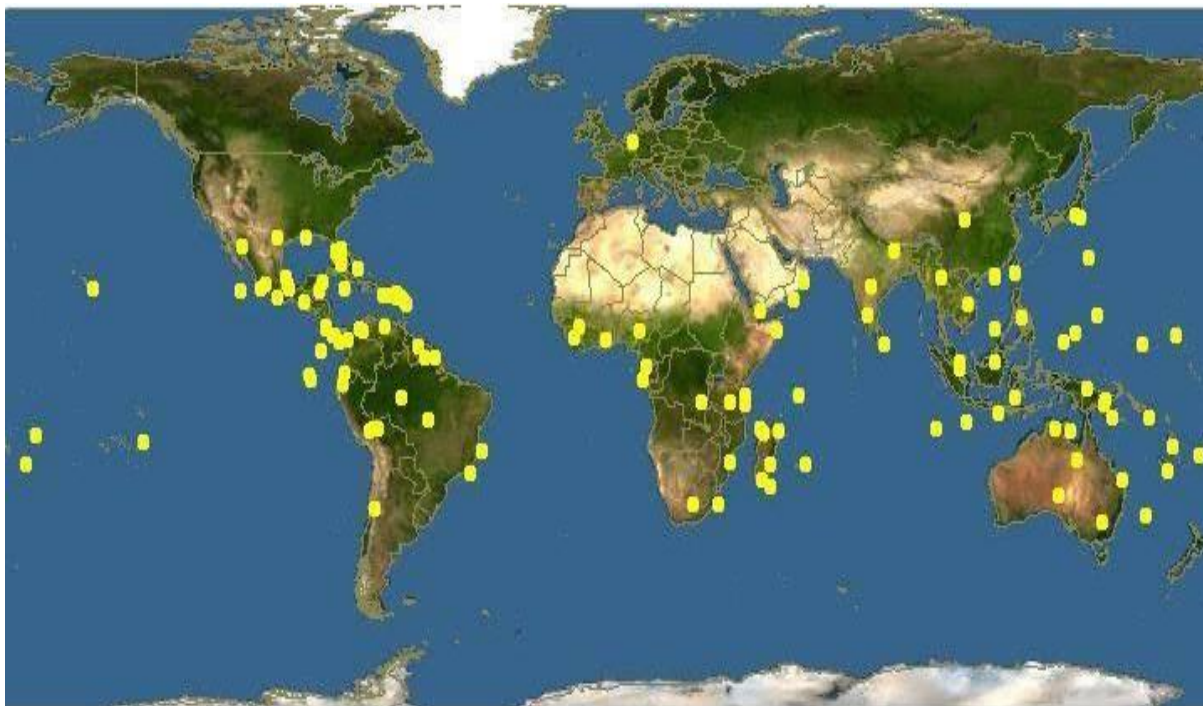


FIG 2: Geographical distribution map

1.1.3 Taxonomical distribution

Kingdom Plantae – Plants

Subkingdom Tracheobionta – Vascular plants

Superdivision Spermatophyta – Seed plants

Division Magnoliophyta – Flowering plants

Class Magnoliopsida – Dicotyledons

Subclass- Rosidae

Order -Fabales

Family Fabaceae/Leguminosae – Pea family

Genus -Caesalpinia L. – nicker

Species- Caesalpinia bonduc (L.) Roxb. – yellow nicker.

(Vedavyasa Sagar and Ahamad,2015)

Taxonomic tree

Domain: Eukaryota
Kingdom: Plantae
Phylum: Spermatophyta
Subphylum: Angiospermae
Class: Dicotyledonae
Order: Fabales
Family: Fabaceae
Subfamily: Caesalpinioideae
Genus: *Caesalpinia*
Species: *Caesalpinia bonduc*



FIG 3: CAESALPINEA BONDOC L Plant

1.1.4 Morphology of plant

The morphology of seeds is almost globular in shape, grey in color, hard with a smooth shiny surface. The shell, which is thick and brittle, encloses a yellowish-white, bitter, fatty kernel. Leaves are bipinnate, large, stipules foliaceous, pinnae 7 pairs, leaflet 3-8 pairs with 1-2 small recurved prickles between them on the underside. Flowers are yellow, in dense long peduncled supra-auxiliary racemes at the top. Fruits are inflated pods, covered with wiry prickles. Pods are oblong, densely armed with sharp wiry prickles, dehiscent. Branches are armed with recurved prickles (*Sharma et al., 2000*).

1.1.5 Therapeutic uses of the plant

The seeds of the plant contain Bonducin, Protein, Saponin, Starch, Sucrose, two Phytosterols namely Sitosterol and Heptacosane, a new Homoisoflavone Bonducelline and Citrulline, Fatty acids such as Palmitic, Stearic, Lignoceric, Oleic, Linolenic acids. The seed kernels of the plant contain α -, β -, γ - and δ -Caesalpins, CaesalpinF and Amino acids (*Vaidya, 2005*). Seeds are bitter, astringent, acrid, anti-inflammatory, anthelmintic, antiperiodic, digestive, stomachic, liver tonic, expectorant, antipyretic, aphrodisiac and tonic. They are also useful in cough, asthma, leprosy, skin diseases, dyspepsia, dysentery, hepatopathy, plexopathy, diabetes, and intermittent fevers. They are used externally for dispersing Inflammatory, Swelling (*Gyanendra Pandey and Dravyaguna Vijnana, 2001*). Oil from seeds is an Emollient, used to remove freckles from the face. Kernels are used as a Tonic in fever. The Ethanolic extracts of the defatted seed kernels of plant drug show antimalarial activity. A decoction of the roasted seed is used in asthma. The fatty oil obtained from the nucleus of the seeds is useful in Convulsion and Paralysis (*Sivakumar, 2005*).

1.2 INTRODUCTION- PCOD

If we come across the survey of diseases over the world, the hormonal problem is the most hearable one. One of them is PCOD - Polycystic ovarian disease/syndrome, which affects man women. PCOD is a problem with hormones that affects women

during their childbearing years (ages 15 to 44). Between 2.2 and 26.7 percent of women in this age group have PCOD. Many women have PCOD but don't know it. In one study, up to 70 percent of women with PCOS hadn't been diagnosed (*Anderson et al., 2009*).

PCOD is a condition that affects a woman's ovaries, which are the reproductive organs that contain estrogen and progesterone, which control the menstrual cycle. A small number of male hormones called androgens are also produced by the ovaries (*Grebe et al., 2010*). While it was historically thought to be an adult female condition, new research indicates that PCOD is a lifelong syndrome that begins before birth.

1.3 DEFINITION OF PCOD (POLYCYSTIC OVARIAN DISEASE)

PCOD is a syndrome characterized by a hormonal imbalance that causes enlarged ovaries with a large number of cysts which is filled by a fluid. If it is untreated it may result in infertility, which is troublesome. The menstrual cycle depends on hormone regulation. During this cycle, a hormone is produced from the pituitary gland of the brain, i.e., FSH (Follicle-stimulating hormone). This hormone is in charge of creating the water sac that covers the egg (*Spritzer, 2014*). The pituitary gland secretes LH after the egg is formed (Luteinizing Hormone). The follicle is induced to release an egg, which settles in the fallopian tube for ovulation. The follicle gradually disappears after forming an egg. The ovaries produce estrogen and progesterone after ovulation, which reinforces the uterus in preparation for pregnancy. At this time ovary secretes a small amount of androgen which slowly converts into estrogen. But for the patients with PCOD, this follicle stops its maturity and the ovulation does not occur. Thus, the follicle does not disappear and forms a cyst that settles in the ovary. B ultrasound diagnosis can be identified by the presence of more than 12 follicles in an ovary (*Bhagyashri, 2013*). This cyst could trigger a hormonal imbalance, with an elevated level of testosterone, which could lead to excessive facial and body hair development. It's called a syndrome because it exhibits male characteristics. Polycystic ovary disorder is also known as Stein Leventhal syndrome or hyper androgen anovulation syndrome. In this case of PCOS, there are more than 12 tin cysts in the ovary.

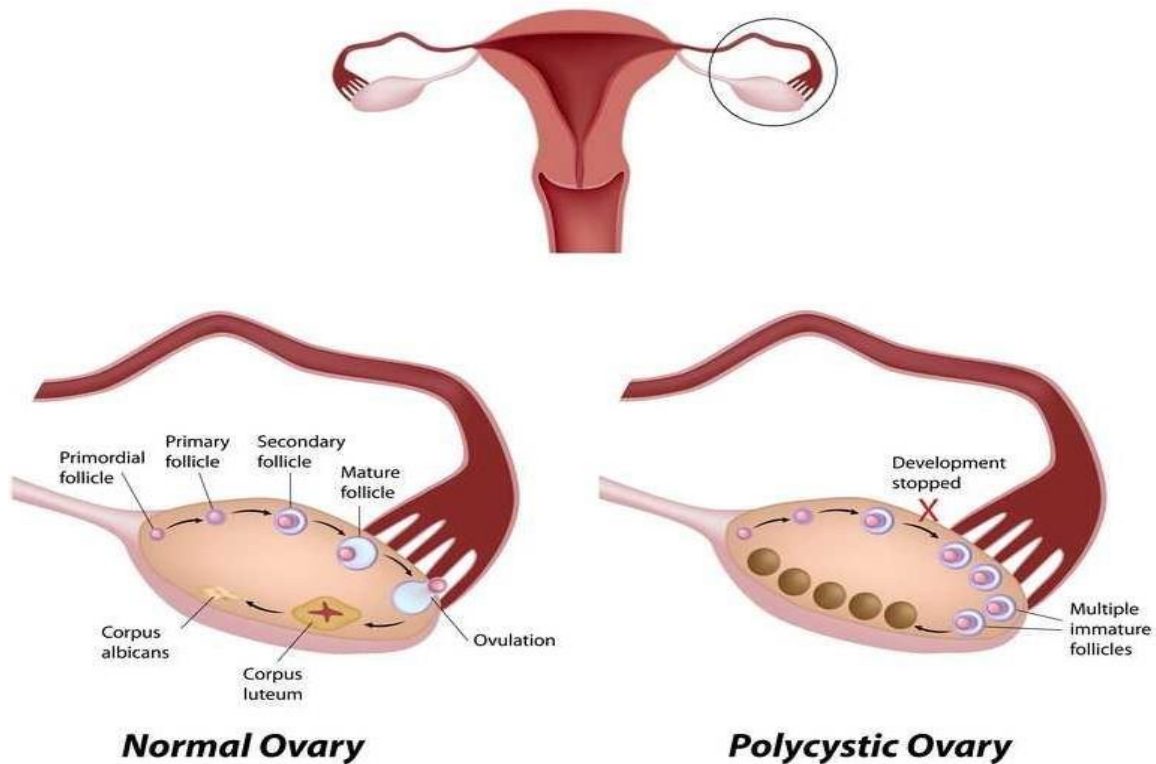


FIG 4: Comparison of the ovary

1.4 ETIOLOGY OF PCOD

Women are exposed to a variety of food preferences and cultures on a regular basis, which can lead to hormonal imbalances that they are unaware of. PCOS is a “syndrome,” or set of symptoms affecting the ovaries and ovulation (Wang, 2001). It has three major characteristics:

- cysts in the ovaries
- high levels of male hormones
- irregular or skipped periods

1.4.1 Factors that cause PCOD

-Genetic problems

-Health issues

•Increased weight gain

•Excess insulin

- Improper diet maintenance
- Low-grade inflammation
- Increased androgen level
- Cholesterol and blood fat
- High-stress level
- Obesity
- Sleeping disorder

1.4.2 Common symptoms of PCOD

- Pregnancy problems
- Abnormal menstrual cycle
- Unwanted hair growth
- Acne formation
- Excess weight gain around hip and stomach
- Decreases insulin resistance so that more chance for diabetes or gestational diabetes.
- Improper cholesterol level
- Imbalance in lipids
- Infertility
- Triglyceride problems
- Increase in androgen level
- Hair loss
- Weight gain
- Skin dryness

(Akshata et al.,2007)

•**Increase in androgen level:** It may cause unwanted hair growth on the face, thighs, back, tum and nipples.

•**Weight gain:** Women with overweight are more suspected of PCOD than others. However, few women with a healthy weight range may also have PCOD. In many cases, overweight may lead to anovulation.

•**Menstrual problems:** Due to the disappearance of follicles results in cysts which lead to an increase in androgen level. This causes to irregular menstrual cycle which increases weight.

•**Hair loss from scalp:** Excessive hair loss from the scalp is called alopecia. This may occur due to increased testosterone.

•**Acne and oil skin:** Increased androgen level may also cause acne which may appear on the face, back and other parts of the body. It can be treated with oral contraceptive pills, topical creams, and oral antibiotics.

•**Insulin resistance:** The women with PCOD are mostly affected with insulin resistance i.e. the normal action of insulin can be affected. Insulin is the hormone that regulates blood sugar levels. When a high level of insulin remains in the blood, increased androgen will be produced.

• **Mood swings:** This causes the patient to lose their ability to concentrate, causing their mood to be disturbed while at work.

•**Breathing difficulty:** Patients with PCOD are more likely to develop heart failure and high blood pressure.

•**Stress:** PCOD has a negative impact on one's quality of life and may exacerbate anxiety and depression. Obese patients are more likely to experience depressive symptoms and mood disorders.

•**Hidradenitis Suppurativa:** It is a chronic inflammatory skin condition seen in women with PCOD. It is a pea-sized lump present in the skin which forms around the hair follicles. It may present underarms, thigh, groin, under breasts, and between buttocks (Arul, 2015).

1.5 EPIDEMIOLOGY OF PCOD

World Health Organization (WHO) estimates that PCOS has affected 116 million women (3.4%) worldwide in 2012. PCOS prevalence rates vary widely across the world, ranging from 2.2 percent to as high as 26 percent. Experts estimate that 10% of women in India are affected by PCOS, but there is no reliable statistical data on the prevalence of PCOS in India (Kabel, 2016). PCOD is expected to affect between 6% and 26% of the world's population. (*Jayasinghe et al., 2018*).

Randomly selected students (age group 18–20 years) from various disciplines made up the study population. The students' data was collected using a formal questionnaire based on Rotterdam's parameters, and anthropometric measurements were taken using standard methods. About 28% of college students were discovered to be at high risk of developing PCOD. More than 85 percent of PCOD patients had oligomenorrhea, 19 percent were hirsute, 41% had acne, 63% had mental distress, and 22% had a good family history. Furthermore, the body mass index of the PCOD students demonstrated a trend of Grade 1 obesity than the normal females of the same age group (*Wild, 2002*).

1.6 MENSTRUAL CYCLE IRREGULARITY

Among the PCOS students, 23 (85.18%) out of 27 students have an irregular menstrual cycle, whereas only 8 (8.42%) have menstrual irregularity among the non-PCOS group (*Franks, 2008*).

Body mass index

Participants weighed between 38 and 90 kilograms. Students with PCOS were found to be substantially heavier (72.62 kg) than those without the condition (54.77 kg) (*Vijayan and Sonia, 2013*). PCOS and non-PCOS students had the same height distribution. BMI showed a substantial difference between PCOS and non-PCOS females, suggesting slightly higher obesity in PCOS (30.47 2.72) relative to non-PCOS (23.25 3.19) females (95 percent confidence interval [CI]: 5.88–8.55, $t = 10.69$, $P = 0.0001$). (*Attlee et al., 2014*).

Hirsutism:

In this study, 18.51 percent of participants diagnosed with PCOS had hirsutism, compared to 7.36 percent of non-PCOS participants.

Acne:

Acne was observed in 40.74 percent of PCOS participants and 22.10 percent of non-PCOS cases.

Emotional issues

Emotional issues, such as feeling moody, irritable, or depressed, were seen in 63 percent of PCOS cases and 7% of cases without PCOS.

Family history of polycystic ovary syndrome

About 22% of cases showed to have a positive family history, 74% had no family history, and 4% were unaware of their family history regarding the occurrence of PCOD.

CHAPTER-2

REVIEW OF LITERATURE

People have created ancient medicine systems that have supplied mankind with modern remedies (gurib-fake,2006). The analysis examines the fragments of evidence that exist in the literature about the medicinal value of *Caesalpinia bonduc* (kalarchikkai) (Vetriselvi and Bharathajothi,2020).

Herbal medicine has grown in popularity as a means of treatment. Despite the fact that herbal medicine and traditional pharmacological therapies have many variations, herbal medicine can be evaluated for effectiveness using conventional trial methods. Several herbal extracts have been shown to be effective in treating particular ailments. Despite the fact that the public is often deceived into believing the all-natural treatments are inherently safe, herbal medicines do carry risks. Ultimately, we need to know which herbal remedies do more harm than good for which condition. Because of the current popularity of herbal medicine, research in this area should be intensified (Edzard Ernst,2005).

Natural-source medicines have become the foundation for prescription drugs. Traditional herbal medicines are plant-derived substances that have been used for the prevention and cure of a variety of diseases as well as as a nutraceutical. Toxicological research and testing aid in living safely and accurately anticipating the benefits of synthetic and natural substances while preventing damage. The toxicity analysis of different plant and herbal formulations is recorded to profile data and ensure the safety of herbal drugs. This analysis briefly addresses the need for toxicity research, plant toxicity, and traditional herbal medicine that is healthy (Sharwan et

Caesalpinia bonduc L. is a medicinal plant from the *Caesalpiniaceae* family. It's a prickly shrub found all over the world, but particularly in India, Sri Lanka, and the Andaman and Nicobar Islands in India. It is regarded as an essential remedy in Indian traditional plant medicine for the treatment of a variety of diseases. It is widely used in traditional medical systems such as Ayurveda, Siddha, Unani, and Homoeopathy. It is a very useful medicinal plant that is used in herbal medicine because all parts of the plant have medicinal properties. Antidiabetic, adaptogenic, anthelmintic, antiestrogenic, anti-inflammatory, antimalarial, antimicrobial, antifungal,

antispasmodic, antioxidant, antiproliferative, antipsoriatic, antitumor, larvacidal, muscle contractile, hepatoprotective, anticonvulsant, and antifilarial properties. *Caesalpinia bonduc* (L.) contains alkaloids, flavonoids, glycosides, saponins, tannins, and triterpenoids, according to a phytochemical study. This study aims to cover all of the literature on *Caesalpinia bonduc* (L.pharmacognostic)'s characteristics, chemical constituents, and a list of its various pharmacological activities. (Vibha Singh et al.,2012).

Caesalpinia bonduc (Fabaceae) ethanolic extracts yielded two new homoisoflavonoids, caesalpinianone and 6-O-methylcaesalpinianone, as well as five known natural products, namely hematoxylin, stereochoenol A, 6'-O-acetylloganic acid, 4'-O-acetylloganic acid, and 2-O—d-glucosyl Extensive NMR spectral experiments were used to deduce the structures of these compounds. The inhibitory and antifungal activities of glutathione S-transferase (GST) in all of these compounds were different (Athar Ata et al.,2009).

This research looked into the anti-inflammatory and analgesic properties of *Caesalpinia bonduc* seed coat extract (CBSCE). *C. bonduc* seeds are said to have anti-inflammatory and analgesic properties in folk medicine. We attempted to substantiate these claims in this study by conducting a systematic examination of *C. bonduc* seed coat extract. Before being tested for anti-inflammatory and analgesic efficacy, the seed coat of *C. bonduc* was extracted with 95 percent ethanol and condensed. Researchers used Carrageenan-induced Paw Edema, Egg albumin-induced Paw Edema, Eddy's Hot Plate Test, and Tail Immersion Process to demonstrate the acclaimed properties. The data were analyzed statistically by the Students 't to test. The results indicate that seed coat extract can decrease the induced inflammation at varying doses in the Carrageenan model as well as in the Egg albumin model in rats. The antinociceptive results indicate that the extract can increase the pain threshold of the animals and reduce the pain factor, thereby inducing analgesia. Thus, it can be concluded that CBSCE possesses analgesic and anti-inflammatory activity (Kannur et al.,2012).

The current study aims to evaluate the effect of ethanolic seed extract of *Caesalpinia bonduc* (ESECB) on altered hormonal levels in mifepristone-induced polycystic ovarian syndrome (PCOS) female rats. PCOS was induced in female rats by a daily

administration of mifepristone (4 mg/kg b.w.) for 8 days which was confirmed by the persistent estrous cycle. Once PCOS was induced, the rats were treated with ESECB 200 and 400 mg/kg b.w. p.o. for 28 days, and the hormonal parameters were analyzed. Metformin was used as a standard drug. A significant increase ($p < 0.01$) in the luteinizing hormone, Prolactin, insulin, testosterone, estrone, and estradiol levels and decrease in follicle-stimulating hormone and progesterone hormone levels in the PCOS rats were observed, which were normalized in the ESECB drug-treated animals. Among the two concentrations, 400 mg/kg b.w of ESECB drug treatment showed a significant effect in correcting this hormonal imbalance. Its effect was comparable to the positive control used in the present study. It can be concluded that the ESECB can be used as a potential ovulation inducer in the treatment of PCOS (Meera Murugesan *et.al.*,2020).

Plant and rhizobacterial interactions contribute partly to a plant's medicinal properties and are well studied through metagenomics. The genomic DNA obtained from the rhizosphere of *Caesalpinia bonducella*—a medicinal shrub commonly used to treat polycystic ovary syndrome—was used in this analysis to perform 16S rDNA, 18S rDNA, and ITS meta-sequencing (PCOS). 23.9 percent of the 665 Operational Taxonomic Units (OTUs) obtained from 16S rDNA sequencing belonged to microbes that increase plant therapeutic value (*Bacillus*, *Paenibacillus*), 6.4 percent to stress and drought tolerant microbes (*Pseudomonas*, *Rhizobium*, *Serratia*), 8% to plant-growth-promoting rhizobacteria—mostly *Proteobacteria*, and Firm Alpha diversity indexing by GAIA-metagenomics tool revealed the presence of a highly diverse group of microbes in the rhizosphere of *C. bonducella*; Chao.1 index (665), Shannon Weiner index (3.53), Simpson index (0.83), and Fisher index (106.13). The highly diverse microbes lingering around the roots of *C. bonducella* could be due to a strong symbiotic association with the plant; root exudates nourish the microbes and the microbes, in turn, enrich the medicinal value of the plant (Ramadurai.S and Balasundaram,2020).

After the NIH-sponsored conference on polycystic ovary syndrome (PCOS) in 1990, it has become clear that the syndrome covers a broader range of ovarian dysfunction signs and symptoms than those identified by the original diagnostic criteria. PCOS is a condition of ovarian dysfunction with the cardinal characteristics of hyperandrogenism and polycystic ovary (PCO) morphology, according to the 2003

Rotterdam consensus workshop. As a result, no particular diagnostic criteria (such as hyperandrogenism or PCO) are appropriate for the clinical diagnosis of PCOS. Menstrual abnormalities, symptoms of androgen excess, and obesity are some of the clinical manifestations. PCOS is associated with an increased risk of type 2 diabetes and cardiovascular events (The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group,2004).

Both genes and the environment contribute to PCOS. Obesity, exacerbated by poor dietary choices and physical inactivity, worsens PCOS in susceptible individuals. The role of other environmental modifiers such as infectious agents or toxins is speculative. Phenotype confusion has characterized genetic studies of PCOS. While several loci, including CYP11A, the insulin gene, the follistatin gene, and a region near the insulin receptor, have been proposed as PCOS genes, the evidence supporting linkage is not overwhelming. The area near the insulin receptor gene (but not including this gene) has the strongest case, as it has been discovered in two different studies and, perhaps most significantly, has yet to be debunked by larger studies. The responsible gene on chromosome 19p13.3, however, has yet to be identified. To date, no gene has been identified that causes or contributes substantially to the development of a PCOS phenotype (*Diamanti-Kandarakis et al.,2006*).

PCOS is a common, complex endocrine disorder characterized by polycystic ovaries, chronic anovulation, and hyperandrogenism, which causes irregular menstrual cycles, hirsutism, acne, and infertility. Evidence-based medical care for PCOS emphasizes a multidisciplinary approach, as traditional prescription treatment focuses on single symptoms, is often associated with adverse effects, and is ineffective in some instances. Women with PCOS have also shown a strong desire for non-traditional therapies. This review examines the reproductive endocrine effects in PCOS for an alternative treatment, herbal medicine. This review aimed to identify consistent evidence from both pre-clinical and clinical research, to add to the evidence base for herbal medicine in PCOS (and associated oligo/amenorrhoea and hyperandrogenism), and to inform herbal selection in the provided clinical care for these common conditions (*Arentz et al.,2014*).

Caesalpinia bonduc (Lin.) Roxb. is a known drug in Ayurveda to treat various diseases specifically tumors, cysts, and cystic fibrosis (CF). This study aimed to assess *in*

vitro as well as *in vivo* antimicrobial activity of *Caesalpinia bonduc* seeds. The *in vitro* antimicrobial activities of seed coat and seed kernel extracts were investigated by micro broth dilution assay. *In vivo* activities of hydro-alcoholic extracts were investigated in rat models of chronic *Pseudomonas aeruginosa* pneumonia mimicking that in patients with cystic fibrosis. Results showed that *Caesalpinia bonduc* may have the potential to be promising natural medicine, with other forms of treatments, for CF patients with chronic *Pseudomonas aeruginosa* lung infections.

Many herbal medicines have been used for the treatment and control of various diseases in various medical systems. The plant *Caesalpinia bonduc* has been used to treat diseases and illnesses in humans in a separate system of traditional medicine. *Caesalpinia bonduc* L., also known as Nata Karanja, is a prickly shrub found in India, Myanmar, and Sri Lanka's hotter regions. Anti-anaphylactic, antiviral, antiasthmatic, antiamebic, and anti-estrogenic properties have all been identified for the plant. *Caesalpinia bonduc*'s blood sugar-lowering behavior has been studied mainly in rabbit and rat models, with promising results.

It is reported to contain various Alkaloids, Glycosides, Terpenoids, and Saponins. It has been reported as an anti-asthmatic, anti-diabetic, anti-inflammatory, anti-oxidant, anti-bacterial, anti-filarial, anti-tumor, anxiolytic, immunomodulatory, hypoglycemic activity. This review is an effort to compile all the information reported on its phytochemical and pharmacological activities. Therefore, this information will be helpful to create interest in the plant and may be useful in developing new formulations (Shelar. P.A et al.,2014).

Polycystic ovarian syndrome (PCOS) is an endocrine disorder infertile woman, which seems to be adversely affected by associated thyroid dysfunction. Zinc methionine (ZM) has positive effects on PCOS, but its concerted effects on PCOS and thyroid function have not been investigated. We evaluated the effects of ZM on reproductive and thyroid hormones and the number of follicles in rats with PCOS. This study was conducted on 45 female rats, using sesame oil as control; PCOS animals administered with 0, 25, 75, and 175 mg/kg BW of ZM. Serum concentrations of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and thyroid function were investigated. Premature follicles (PMF), primary follicles (PF), preantral follicles (PAF), antralfollicles (AF), corpus luteum (CL), and cystic follicles (CF) were assessed. PCOS

decreased the concentrations of FSH and free T4 but increased the levels of LH, TSH, and LH/FSH ratio (ALL $P < 0.05$). ZM at a dose of 75 and 175 mg increased the level of FSH, free T4, and decreased LH, TSH, and LH/FSH ratio (ALL $P < 0.05$). PCOS induction reduced PMF, PF, PF, AF, and CL while increasing CF ($P < 0.05$). When compared to ZM 25 mg/kg and PCOS groups, PCOS treated groups (75 and 175 mg/kg) increased follicle numbers and decreased CF (both $P < 0.05$). Even though PCOS induction harmed reproductive and thyroid hormones, as well as follicle numbers, ZM therapy (75 and 175 mg/kg), mitigated the negative effects. The hormonal and cystic disruptions that occur in PCOS can be alleviated by taking a high dose of ZM (Fazel torshi et al.,2020).

This chapter evaluates the nutritional value of *Caesalpinia bonducella*, one of the most common herbs in folklore medicine, whose medicinal uses have been referenced in various Ayurvedic texts and scriptures. It is traditionally used to cleanse the uterus after childbirth and also serves as a uterine stimulant. During this time, it is considered to help with fever, edema, and abdominal pain. The seeds are used as anti-diabetics, and many scientific studies have confirmed their effectiveness. Anti-inflammatory, anthelmintic, and antimalarial properties have been demonstrated, as well as stomachic and digestive properties. They are used as a liver tonic in the treatment of jaundice and various liver disorders. In Indian traditional medicine, *Caesalpinia bonducella* is used in a vast range of diseases.

It is the best cure for abdominal pain due to flatulence. All extracts of *Caesalpinia bonducella* showed a significant decrease in the total cholesterol level and triglyceride level. The extracts of *Caesalpinia bonducella* seeds may stimulate the secretion of insulin by the beta cells and thus show a blood-glucose-lowering action. In particular, the kernel ethanolic extract (KEE) exhibited significant action. It also increased the HDL and was successful in suppressing the LDL level as compared to the standard drug. The properties possessed by *Caesalpinia bonducella* have significant commercial value and use. Investigations must be carried out in more depth to validate other claims as well as to find out the constituents responsible for its antidiabetic potential (Dayanand M. Kannur,2011).

Caesalpinia bonduc (L.) Roxb is traditionally used in Indonesia to treat various diseases, but still limited study about the different parts of this plant. This study aimed

to screen the phytochemicals, to evaluate the total flavonoid and total phenolic contents as well as antioxidant activity of ethanol extract of root, stem, leaves, and seed kernel of *C. bonduc*. Each part of the plant was extracted by reflux using 70% ethanol as the solvent for 2 h and repeated 3 times. Total flavonoid content was determined by aluminum chloride colorimetric assay on 415 nm. Using a microplate reader, total phenolic content was determined using Folin-Ciocalteu 1:4 at 765 nm. Free radical scavenger methods such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) were used to determine antioxidant activity.

Phytochemical screening showed that all of the samples positively contain flavonoids and saponin. Total flavonoid content was the highest in leaf and the lowest in root whereas total phenols content was highest in leaf and the lowest in seed kernel. The crude extracts displayed DPPH free radical scavenging activity with the highest value in leaf extract followed by root, stem, and seed kernel. The 70% ethanol leaf extract of *C. bonduc* showed the highest yield, total flavonoid content, and total phenolic content among other parts investigated. Moreover, leaf extract has the highest DPPH free radical scavenging activity (79.802 $\mu\text{g/ml}$) which could be related to its higher phenolic content.

Caesalpinia bonducella (Caesalpinaceae) is a plant well known for its medicinal value in Indian Ayurveda. However, to prove its efficiency for clinical utilization, more experimental data will be beneficial. The present study involved the investigation of immunomodulatory activities of ethanolic extract of *Caesalpinia bonducella* seeds. Neutrophil adhesion test, haemagglutinating antibody (HA) titer, delayed-type hypersensitivity (DTH) response, phagocytic activity, and cyclophosphamide-induced myelosuppression were determined by *in vivo* experiments. Oral administration of ethanolic seed extract of *Caesalpinia bonducella* (200–500 mg/kg) elicited a substantial increase in percent neutrophil adhesion to nylon fibers, as well as a dose-dependent increase in antibody titer values, and potentiated the delayed-type hypersensitivity reaction caused by sheep red blood cells, indicating immunomodulatory capacity. It also prevented myelosuppression in cyclophosphamide-treated rats and showed good phagocytosis response in a carbon clearance assay. The results obtained in this study indicate that *Caesalpinia bonducella* possesses potential immunomodulatory activity and has therapeutic potential for the prevention of autoimmune diseases (Shruti Shukla et al., 2009).

Leaves of *Caesalpinia bonduc* (L.) Roxb. (Caesalpinaceae) have been used by traditional Siddha healers of the Malabar region for psoriasis treatment. To test the antipsoriatic behavior of *Caesalpinia bonduc* decoction (CBD) and *Caesalpinia bonduc* hydroalcoholic extract (CBHA). The antipsoriatic activity was assessed using the mouse tail test for psoriasis. Swiss albino mice were given extracts at a dose of 500 mg/kg b.w. and fractions at a dose of 250 mg/kg b.w. Changes in epidermal thickness and percentage orthokeratotic values were studied in the mouse tail test. In vitro antiproliferative assays on HaCaT cell lines were also performed, as well as in vitro lipoxygenase inhibition. Butanol fraction of *Caesalpinia bonduc* hydroalcoholic extract (CBHAB) and water fraction of *Caesalpinia bonduc* hydroalcoholic extract (CBHAW) produced significant orthokeratosis ($p < 0.001$).

In relative epidermal thickness, a significant ($p < 0.05$) reduction concerning control was observed in groups treated with retinoic acid, CBD, butanol fraction of *Caesalpinia bonduc* decoction (CBDB), water fraction of *Caesalpinia bonduc* hydroalcoholic extract (CBHAW). Maximum antiproliferant activity was shown by CBHA (IC_{50} , $77.5 \pm 12.7 \mu\text{g/ml}$). In lipoxygenase inhibition assay, water fraction of *Caesalpinia bonduc* decoction (CBDW) showed maximum activity with an IC_{50} value of $164.71 \pm 4.57 \mu\text{g/ml}$. Among all the tested samples only CBHAW showed good activity in the mouse tail test, antiproliferant activity in HaCaT cells, and lipoxygenase inhibition assay. Other extracts and fractions showed varying degrees of activity. The present study supports the traditional use of *Caesalpinia bonduc* leaves for psoriasis treatment (Muruganantham. N et al.,2011).

Plant medicine is the oldest form of health care known to mankind, so research into its protection is critical for preventing adverse drug effects. *Caesalpinia bonduc* is one of many medicinal plants used in Benin as an aphrodisiac and to treat a variety of ailments, including prostatic hyperplasia. Despite its multiple ethnomedicinal benefits, toxicological data on its long-term use is currently lacking. As a result, the toxicity of an ethanolic root extract of *Caesalpinia bonduc* in Wistar rats was investigated in this study. Male Wistar rats were given *Caesalpinia bonduc* root extract by oral gavage at doses of 31.25, 125, and 500 mg/kg/day for 90 days, during which body weight changes, food intake, urinary parameters, hematological and blood biochemical parameters, organ weight changes, gross pathology, and histopathology of vital organs were evaluated.

There were no deaths or abnormal clinical signs associated with extract therapy, no major improvements in body weight gain or urinary parameters, and no changes in necropsy and histopathology findings of vital organs. However, in rats given high doses of the extract, some indices such as erythrocytes, total cholesterol, and aspartate aminotransferase, as well as the relative weight of testes, increased, accompanied by a decrease in food intake and prostate relative weight. The findings show that an ethanolic root extract of *Caesalpinia bonduc* has no major side effects and that it is tolerable at doses of up to 500 mg/kg per day for 90 days (Mariette Sindete, 2021).

This study aimed to see how *Caesalpinia bonduc* extract (HAECB) affected ovarian cysts in Wistar Female Rats with Polycystic Ovarian Syndrome.

Female Wistar rats were divided into 5 groups consisting of 6 rats each. PCOS was induced in all the animals except the normal group on the administration of letrozole (0.75mg/kg p.o) for 21 days daily. To ensure PCOS, the blood sample was collected through the cardiac puncture, and ovaries are excised for histopathology on the 22nd day. After 21 days, group III and group IV were orally administered 200 mg/kg and 400mg/kg of HAECB (Hydroalcoholic extract of *Caesalpinia bonduc* extract) respectively, Group V was treated with clomiphene citrate (CC) (1mg/kg.p.o) for 21 days

. During the induction and treatment period, estrus cycles have been evaluated. At the end of the experiment period, blood samples were obtained on 43rd (21 days for induction of PCOS after 21 days for treatment) day to evaluate serum levels of FSH, LH, testosterone, and estradiol. The ovaries were also removed and studied with light microscopy after cutting the tissues. In letrozole-induced PCOS decreased FSH and testosterone, an elevated level of LH and estradiol, the large size of cysts and an increased number of follicular cysts were seen.

The results of HAECB treatment revealed the normal cycle in the estrus cycle. FSH, LH, testosterone, and estradiol levels in group III and group IV showed a significant decrease compared to the PCOS group. Group III and group IV (HAECB treated groups) reverse the altered FSH LH, testosterone, and estradiol level near the control level and reduction in the size of cysts and less number of atretic follicles were seen when HAECB treated groups compared to the PCOS group. HAECB can improve

tissue symptoms and adjust the levels of sex hormones in polycystic ovary syndrome. Overall, from the obtained results, it is determined that the *Caesalpinia bonducella* extract (HAECB) exhibited a good antiandrogenic effect by reducing the letrozole levels in PCOS-induced conditions (*Divya. P et al.,2019*).

Siddha medicine is a treatment that is followed by Ancient Tamilians. It has no side effects and is cost-efficient. A polycystic ovarian syndrome is a hormonal disorder affecting many women nowadays. It is called a syndrome because it shows many symptoms. It is also named polycystic ovary disease, stein Leventhal syndrome, or hyper androgen anovulation syndrome. In this PCOS more than 12 tiny cysts present in the ovary. It may cause due to genetic problems or lifestyle factors. This review interrupts the solution to reduce the risk and ill effects of PCOS with help of Siddha medicine which is the best, nil side effects, and cheapest medicinal system (*Risvan MY et al.,2017*).

On treatment with graduated doses of alcohol seed extract of *Caesalpinia bonducella*, histological changes under a light microscope show follicular degeneration in the ovary, vacuolation, and moderate disorganization of the uterus. The length of the estrous cycle and mean ovarian weight both decreased significantly ($p<0.05$). There were no consistent differences in mean uterine weight, serum estradiol, or progesterone levels, however. The findings indicate that *C. bonducella* alcohol seed extract has antiestrogenic properties, likely by inhibiting estrogen secretion (*Kanchan R et al.,2011*).

To evaluate pharmacognostic, physicochemical, and preliminary phytochemical of investigation of root bark of *Caesalpinia bonducella*. The current research incorporates pharmacognostic criteria such as macroscopic examination, powder analysis, and histochemical evaluation, as well as physicochemical parameter examination, fluorescence analysis, and qualitative and quantitative chemical assays carried out according to World Health Organization methodology and standard laboratory procedures.

The research would serve as a reference point for correctly identifying the crude substance. Microscopy showed the presence of cork, cortex, calcium oxalate crystals in stone cell medullary rays, starch grains, tannins, and alkaloids. In aqueous and ethanol extracts, preliminary phytochemical screening showed the existence of

alkaloids, saponins, flavonoids, hormones, phytosterols, and carbohydrates. Thus, it was thought worthwhile to explore this plant based on its standardization parameters. The study will provide referential information for the correct Identification of the crude drug (*Ganesh H Wadkar,2007*).

In 1989–1995, it was proposed that polycystic ovary syndrome (PCOS) was caused by functional ovarian hyperandrogenism (FOH) caused by androgen secretion dysregulation. Subsequent research has backed up and expanded on this theory. Two-thirds of PCOS cases have functionally typical FOH, characterized by 17-hydroxyprogesterone hyperresponsiveness to gonadotropin stimulation when identified as otherwise unexplained hyperandrogenic oligo-anovulation. After the suppression of adrenal androgen activity, two-thirds of the remaining PCOS patients have FOH detectable by testosterone elevation.

An associated isolated functional adrenal hyperandrogenism affects about 3% of PCOS patients. The remaining PCOS cases are mild and show no signs of steroid secretory abnormalities; the majority of these people are obese, which we believe is the cause of their atypical PCOS. Subclinical FOH-related steroidogenic defects affect about half of the normal women with polycystic ovarian morphology (PCOM). In long-term culture, theca cells from polycystic ovaries of classic PCOS patients have an intrinsic steroidogenic dysregulation that may explain the steroidogenic anomalies seen in FOH. Most steroidogenic enzymes, especially cytochrome P450c17, are overexpressed in these cells. In normal theca cells, overexpression of a protein detected by genome-wide association screening that is differentially expressed in normal and neoplastic development 1A.V2 has reproduced the PCOS phenotype in vitro.

In around half of PCOS patients, a metabolic syndrome of obesity-related and/or intrinsic insulin resistance develops, and compensatory hyperinsulinism has tissue-selective effects, including aggravation of hyperandrogenism. PCOS appears to be a complex trait that develops as a result of the combination of various genetic and environmental factors. PCOM, hyperandrogenemia, insulin resistance, and insulin secretory defects are all heritable factors. Prenatal androgen exposure and low foetal growth are environmental causes, while acquired obesity is a significant postnatal cause. The syndrome's multifactorial existence and heterogeneity are shown by the

variety of pathways involved and the absence of a common thread. To optimally correct androgen levels, ovulation, and metabolic homeostasis, further research into the disorder's fundamental causes is needed (Rosenfield et al.,2016).

Caesalpinia bonducella (L.) Fleming (Syn. *Caesalpinia bonduc* (L.) Roxb, Syn. *Caesalpinia crista* Linn.), belonging to the family *Caesalpiniaceae*, is a prickly shrub widely distributed all over the world especially in India, Sri Lanka, and Andaman and the Nicobar Islands, in India especially found in tropical regions.

All parts of the plant have medicinal properties so it is a very valuable medicinal plant that is utilized in the traditional system of medicine. Alkaloids, flavonoids, glycosides, saponins, and tannins have been found to have anxiolytic, antinociceptive, antidiarrheal, antidiabetic, adaptogenic, anthelmintic, antiestrogenic, anti-inflammatory, antimalarial, antimicrobial, antifungal, antispasmodic, antioxidant, antiproliferative, antipsoriatic, antitumor, larvacide (khan et al.,2012).

CHAPTER- 3

AIM AND SCOPE

3.1 AIM

The present study was undertaken with the following objectives:

- Radical scavenging activity of the aqueous and the chloroform seed extract of *Caesalpinia bonduc* L.
- Radical reducing power (Electron donation ability) activity of the seed extract.
- Screening of pharmacological compounds for the prevention of PCOD.

3.2 SCOPE

- Studies support the relationship between genetic and environmental factors induced diseases with Traditional Medicine and Modern Medicine from Natural Products.
- By correlating the Polycystic ovarian disease (PCOD) and assessment of *Caesalpinia bonduc* L serve as an effective model.
- Addressing the metabolic deviations and inculcating lifestyle modifications.
- By profiling new information provides the diagnose/prevention of Polycystic ovarian disease (PCOD).

CHAPTER-4

MATERIALS AND METHODS

4.1 TYPES OF EQUIPMENT AND GLASSWARES

Centrifuge, Water bath, Eppendorf, Deep freezer, measuring cylinder, UV-Spectroscopy, cuvette, Soxhlet Apparatus, Thin layer chromatography, Autoclave, Grinder, and pestle, Weighing machine, Test tubes, Funnel, Beaker, UV Lamp, GC-MS, Personal protective equipment.

4.2 CHEMICALS

- Methanol
- Chloroform
- Hexane
- Ferric chloride
- Ascorbic acid.

4.3 REAGENTS

- 0.1mM DPPH[•] solution
- Phosphomolybdenum reagent- Ammonium molybdate (4 mM), sodium phosphate (28 mM), and sulphuric acid (600 mM).
- 1 mL of 1% (w/v) potassium ferricyanide [K₃Fe (CN)₆] solution
- 1 mL of phosphate buffer (0.2 M, pH 6.6)
- 500μL of 10% (w/v) trichloroacetic acid solution.
- 1 mL of 0.1% (w/v) FeCl₃ solution.
- PBS Buffer- 81 ml of Disodium hydrogen phosphate (Na₂HPO₄) and 19 ml of Sodium dihydrogen phosphate (NaH₂PO₄).

3.4 PREPARATION OF EXTRACT

(i) Aqueous Extraction

About 10 g of seed powder was boiled with 100 mL of distilled water in cookware and the supernatant was filtered and condensed in a hot plate at 50°C, which yields pale yellow gummy extract.

(ii) Soxhlet Extraction

About 25 g of crushed seed powder is loaded into the thimble, which is placed inside the Soxhlet extractor. The chloroform solvent in the round bottom flask is heated using the mantle and will begin to evaporate, moving through the apparatus to the condenser. The condensate then drips into the reservoir containing the thimble. Once the level of solvent reaches the siphon it pours back into the flask and the cycle begins again. The process should run for a total of 2 hours.

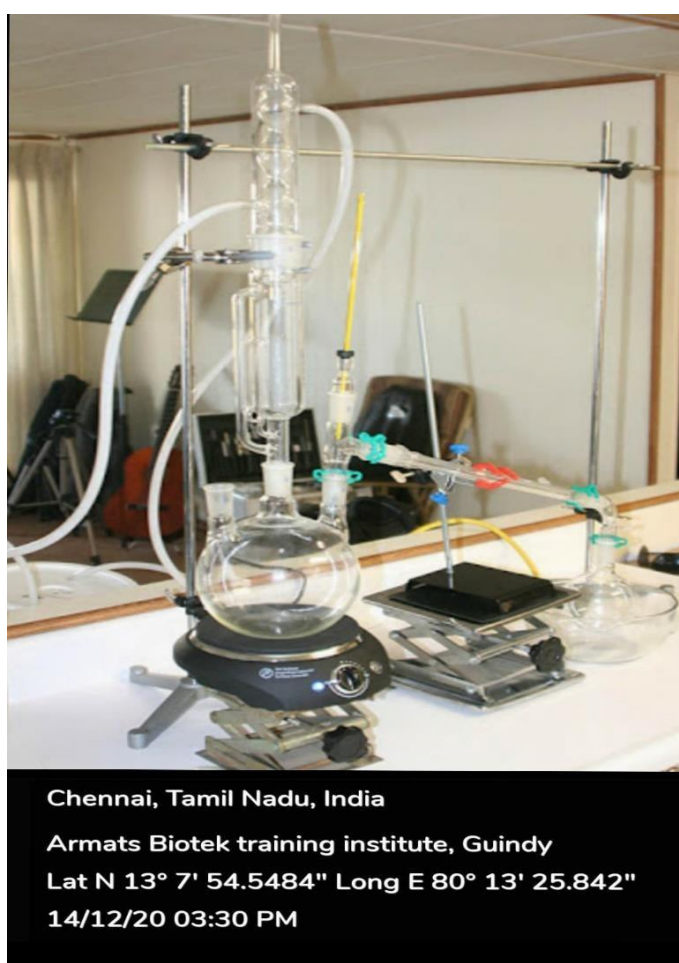


FIG 5: Soxhlet apparatus

***In vitro* antioxidant assays**

3.5 DPPH[•] RADICAL SCAVENGING ACTIVITY

The antioxidant activity of aqueous and chloroform seed extract of *Caesalpinia bonduc* L was measured by DPPH (1,1- diphenyl 2-picrylhydrazyl) free radical scavenging activity. One mL of 0.1 mM DPPH solution in methanol was mixed with 1 mL of various concentrations (20-120 µg/mL) of aqueous extract and chloroform extract. The mixture was then allowed to stand for 30 min incubation in dark. One mL of DPPH solution mixed with 1 mL of methanol was used as the control. The decrease in absorbance was measured at 517 nm. Ascorbic acid was used as the positive control. The percentage of inhibition was calculated using the following formula:

$$\% \text{ of DPPH}^{\bullet} \text{ radical inhibition} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

3.6 PHOSPHOMOLYBDENUM REDUCTION ASSAY

The antioxidant capacity of aqueous and chloroform seed extract of *Caesalpinia bonduc* L was assessed as described by Prieto *et al.* The extract was mixed with 1 ML of reagent solution containing ammonium molybdate (4 mM), sodium phosphate (28 mM), and sulphuric acid at various concentrations (20-120 g/mL) (600 mM). For 90 minutes, the reaction mixture was incubated in a water bath at 95°C. The coloured complex's absorbance was estimated at 695 nm. Ascorbic acid was used as the reference norm. The percentage of reduction was calculated using the following formula:

$$\% \text{ of Phosphomolybdenum reduction} = \left[\frac{\text{Sample} - \text{Control}}{\text{Sample}} \right] \times 100$$

3.7 FERRIC (Fe³⁺) REDUCING POWER ASSAY

The reducing power of aqueous and chloroform seed extract of *Caesalpinia bonduc* L was determined by the slightly modified method of Yen and Chen. One mL of extract with different concentrations (20 - 120 µg/mL) was mixed with 1 mL of 1% (w/v) potassium ferricyanide [K₃Fe (CN)₆] solution and 1 mL of phosphate buffer (0.2 M, pH 6.6). The mixtures were then incubated at 50°C in a water bath for 20 min. Five hundred µL of 10% (w/v) trichloroacetic acid solution was added to each mixture followed by 1 mL of 0.1% (w/v) freshly prepared FeCl₃ solution was added and shaken well. The absorbance was measured at 700 nm and ascorbic acid was used as the standard reference. The percentage of reduction was calculated using the following formula:

$$\% \text{ of Fe}^{3+} \text{ reduction} = \left[\frac{\text{Sample} - \text{Control}}{\text{Sample}} \right] \times 100$$

3.8 HEAT-INDUCED HAEMOLYSIS

The induced membrane lysis of chloroform seed extract of *Caesalpinia bonduc* L was determined by stabilization of the human red blood cell membrane. Extract with different concentrations (20-120µg/ml) and made up with 2ml Phosphate buffered saline (PBS buffer). It was combined with 200µl of RBC suspension (10% w/v). Incubate in a water bath at 50°C for 30 minutes. Centrifuge at 3000rpm for 5minutes. The absorbance of the supernatant was measured at 560nm and Ascorbic Acid was used as the standard reference. The percentage of inhibition was calculated using the following formula:

$$\% \text{ of Anti-haemolytic activity} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

3.9 COLUMN CHROMATOGRAPHY-SEPARATION OF ACTIVE COMPOUND

Sample preparation

The semi-solid extract of the drug (5 g) was weighed and ground with a small amount of silica gel (100-200 mesh), which was used for the column chromatography.

Column Packing

The silica gel (100-200 mesh) was activated in a hot air oven at 110°C for 1 h. The slurry of activated silica was made in hexane solvent at least 2 times the volume of solvent as silica, just measured out. The silica gel was thoroughly mixed by stirring vigorously to remove all the air from the silica. Before the addition of the slurry, a small piece of cotton was placed at the end of the column while the top of the knob of the column started. This is normally done by using a long stick or by a glass rod. The cotton should be compressed enough to support the column packing yet loose enough that the solvent flow will not be hindered. The cotton was placed to avoid slurry of the silica gel drain out while opening the knob of the column and allowed to pass through the solvent alone.

A funnel was placed on the top of the column and then, the slurry was poured into the column in small portions carefully and slowly, by keeping the knob open and with gentle tapping after each addition of the slurry, to ensure the uniform packing. Frequently pouring of the slurry into the column was stopped and to swirl the slurry so that the silica gel was evenly mixed. A small quantity of solvent was allowed to remain at the top of the column to avoid the drying or cracking of the column. Tapping is necessary to avoid the air bubble formation in the column during packing otherwise can interfere in separation. The column was run fast for some time with the hexane solvent system (low polar solvent only) to remove any impurities. The prepared sample

was then poured into the column by using a funnel and was allowed to settle. Then, it was eluted with the mobile phase starting from hexane to collect fractions of 15 mL each.

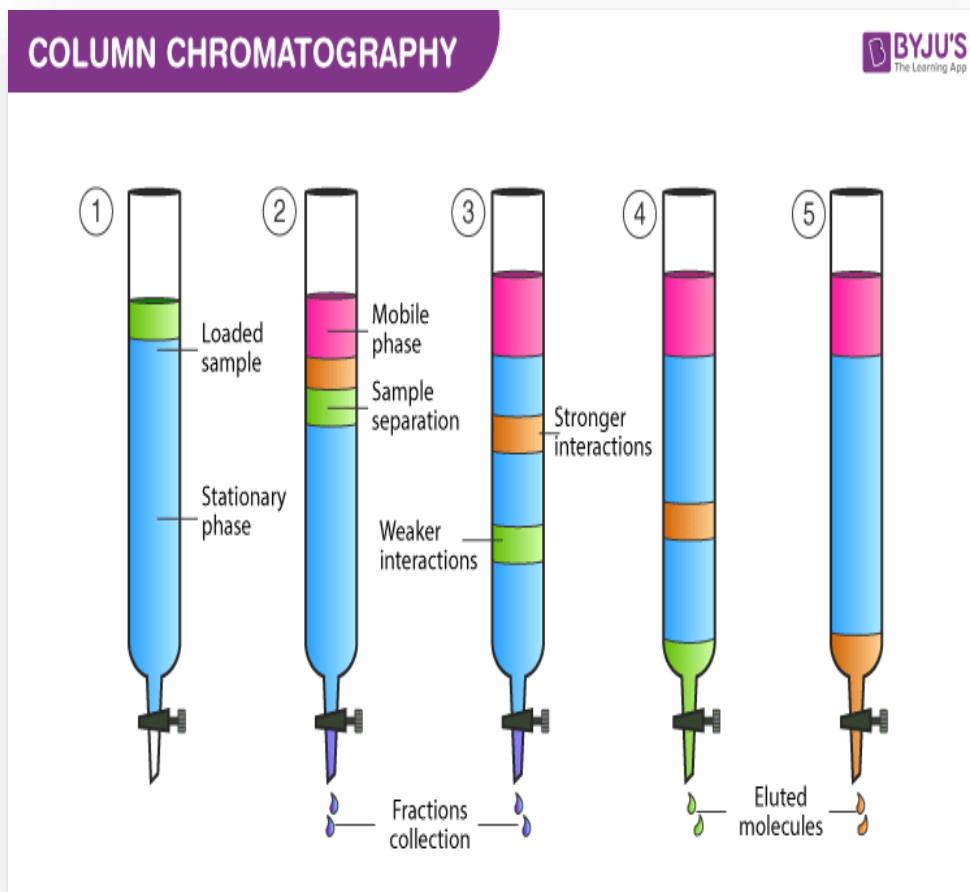


FIG 6: Column chromatography

3.9 GAS CHROMATOGRAPHY–MASS SPECTROMETRY (GC–MS)

The chloroform seed extract of *Caesalpinia bonduc* L was injected into an HP-5 column (30 m X 0.25 mm i.d with 0.25 μ m film thickness), Agilent technologies 6890 N JEOL GC Mate II GC-MS model. The following chromatographic conditions were used: helium as the carrier gas with a flow rate of 1 mL/min; the injector was set to 200°C; and the column oven temperature was set to 50-250°C with a 10°C/min injection mode. The following MS conditions were used: 70 eV ionization voltage;

250°C ion source temperature; 250°C interface temperature; 50-600 mass units mass range 13th. The mass spectrum of the GC-MS was interpreted using the National Institute of Standards and Technology (NIST) database.



FIG 7: GC-MS

CHAPTER -5

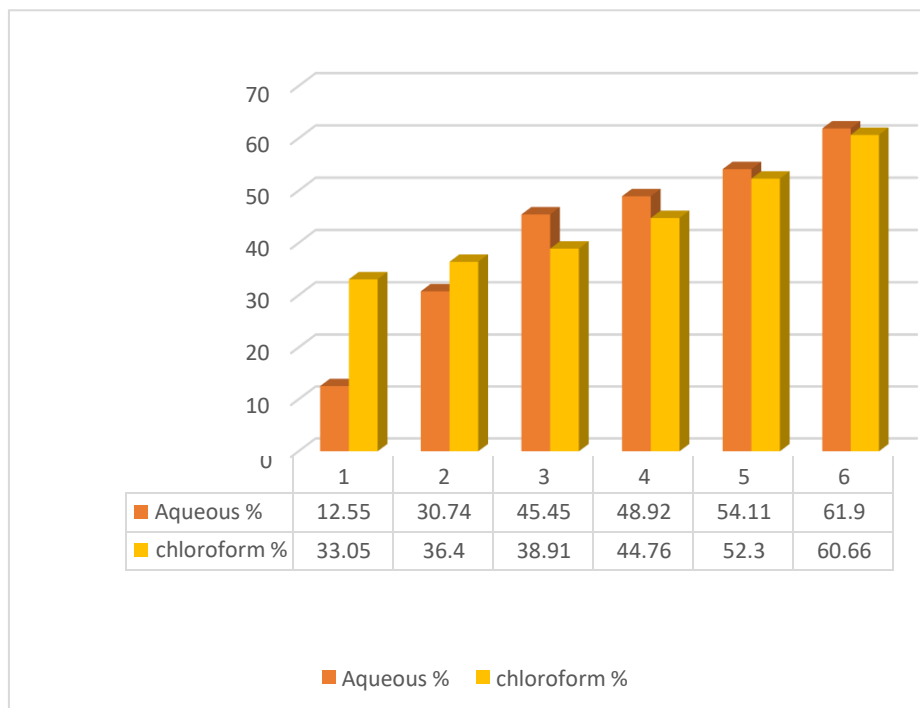
RESULT AND DISCUSSIONS

5.1 DPPH[•] RADICAL SCAVENGING ACTIVITY

Scavenging of DPPH free radicals is one of the popular antioxidant methods. DPPH radical scavenging assay is a decolorization essay that will measure the capacity of antioxidants to directly scavenge DPPH[•] radicals. The ability of aqueous and chloroform seed extract to scavenge free radicals was assessed by the DPPH method. The maximum DPPH[•] radical scavenging activity of aqueous seed extract was 61.90±0.08% at 120 µg/mL concentration and the IC₅₀ was 81.76 µg/mL concentration. The maximum DPPH[•] radical scavenging activity of chloroform seed extract was 60.66±0.09% at 120µg/ml concentration and the IC₅₀ was 95.60 µg/ml concentration. Inhibitory concentration at 50% of radical scavenging activity is the concentration of extract required to scavenge 50% of the radicals. It was compared with the standard of ascorbic acid (IC₅₀ = 11.98 µg/mL concentration). In this method, the purple color 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was reduced to yellow color 1,1-diphenyl-2-picrylhydrazine, and the reducing capacity increases with increasing concentration of the extract.

Table 1: DPPH[•] Radical scavenging activity

S. No	Concentration (µg/mL)	% of inhibition	
		Aqueous extract	Chloroform extract
1	20	12.55±0.20	33.05±0.16
2	40	30.74±0.16	36.40±0.15
3	60	45.45±0.12	38.91±0.14
4	80	48.92±0.11	44.76±0.13
5	100	54.11±0.10	52.30±0.11
6	120	61.90±0.08	60.66±0.09



Standard graph of DPPH' Assay

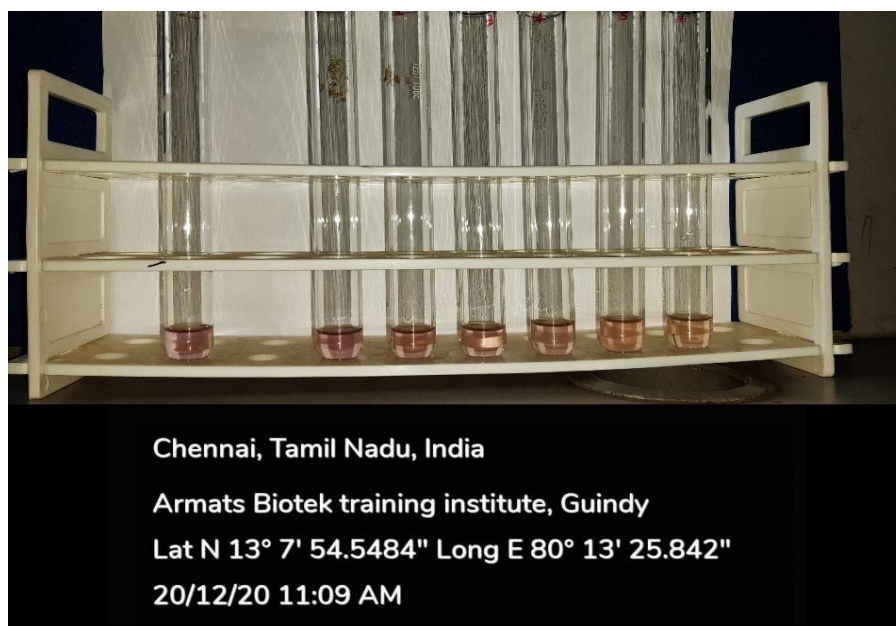


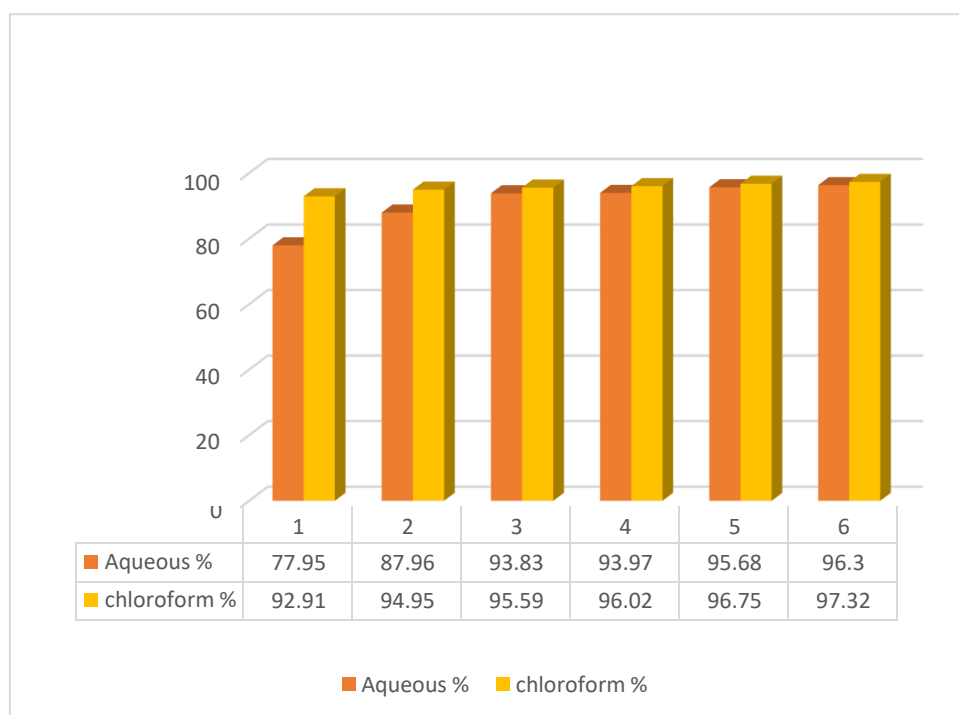
FIG 8: DPPH' Assay

5.2 PHOSPHOMOLYBDENUM REDUCTION ACTIVITY

The total antioxidant activity of aqueous and chloroform extract was measured by the phosphomolybdenum reduction assay method. In this method, Mo (VI) ion reduced to Mo(V) by the extract and the formation of green phosphate/Mo (V) complex at acidic pH. The maximum phosphomolybdenum reduction of aqueous extract was $96.30 \pm 1.51\%$ at $120 \mu\text{g/mL}$ concentration and the RC_{50} was $12.83 \mu\text{g/mL}$ concentration. The maximum phosphomolybdenum reduction of chloroform extract was $97.32 \pm 2.46\%$ at $120 \mu\text{g/mL}$ concentration and the RC_{50} was $10.76 \mu\text{g/mL}$ concentration. Reduction concentration at 50% of reducing activity is the concentration of extract required to reduce 50% of the radicals. It was compared with the standard ascorbic acid ($\text{RC}_{50} = 6.34 \mu\text{g/mL}$ concentration). PM assay is a quantitative method to investigate the reduction reaction rate among antioxidant, oxidant, and molybdenum ligand. It involves thermally generating auto-oxidation during the prolonged incubation period at a higher temperature.

Table 2: Phosphomolybdenum reduction Assay

S. No	Concentration ($\mu\text{g/mL}$)	PHOSPHOMOLYBDENUM ASSAY	
		% of reduction	
		Aqueous extract	Chloroform extract
1	20	77.95 ± 0.25	92.91 ± 0.931
2	40	87.96 ± 0.46	94.94 ± 1.30
3	60	93.83 ± 0.90	95.59 ± 1.49
4	80	93.97 ± 0.93	96.02 ± 1.66
5	100	95.68 ± 1.29	96.75 ± 2.03
6	120	96.30 ± 1.51	97.32 ± 2.46



Standard graph of PHOSHOMOLYBDENUM Assay

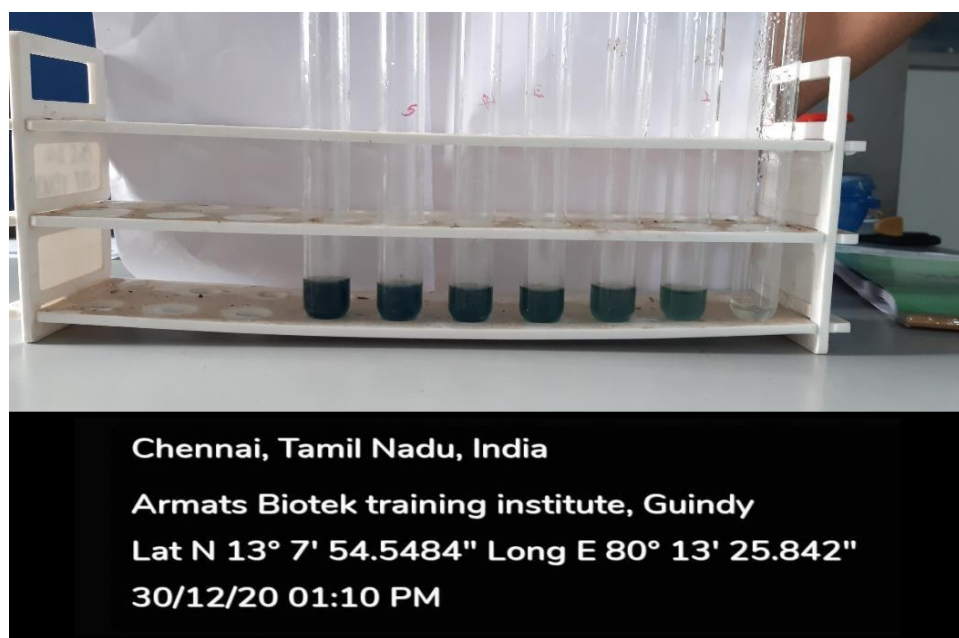


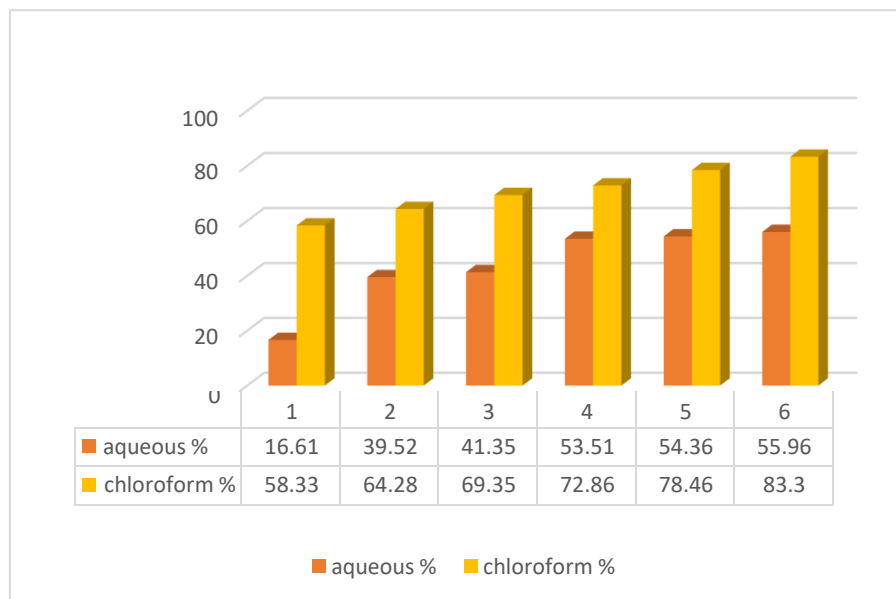
FIG 9: Phosphomolybdenum Assay

5.3 FERRIC (Fe³⁺) REDUCING POWER ACTIVITY

The reducing power assay was carried out by the reduction of Fe³⁺ to Fe²⁺ by the aqueous and chloroform seed extract and the subsequent formation of Ferro-ferric complex. The reduction ability increases with an increase in the concentration of the extract. The maximum Fe³⁺ reduction of aqueous extract was 55.96±0.57% at 120 µg/mL concentration and the RC₅₀ was 73.58 µg/mL concentration. The maximum Fe³⁺ reduction of chloroform extract was 83.30±0.56% at 120 µg/ml concentration and the RC₅₀ was 17.14 µg/ml concentration. Reduction concentration at 50% of reducing activity is the concentration of extract required to reduce 50% of the radicals. It was compared with the standard ascorbic acid (RC₅₀ = 7.72 µg/mL concentration). Also in this assay, higher absorbance of the reaction mixture indicates higher reduction potential and a significant indicator of its potential antioxidant activity. The reducing capacity of the extract was performed using Fe³⁺ to Fe²⁺ reduction assay as the yellow color changes to green or blue color depending on the concentration of antioxidants. The antioxidants such as phenolic acids and flavonoids were present, a considerable amount in the aqueous and chloroform extract, and showed the reducing capacity in a concentration dependant manner.

Table 3: Ferric reducing power Activity

S. No	Concentration (µg/mL)	Ferric reducing power activity	
		% of reduction	
		Aqueous extract	Chloroform extract
1	20	16.61±0.25	58.33±0.22
2	40	39.52±0.30	64.28±0.26
3	60	41.35±0.41	69.35±0.31
4	80	53.51±0.42	72.86±0.35
5	100	54.36±0.54	78.46±0.44
6	120	55.96±0.57	83.30±0.56



Standard graph of FERRIC REDUCING POWER Assay

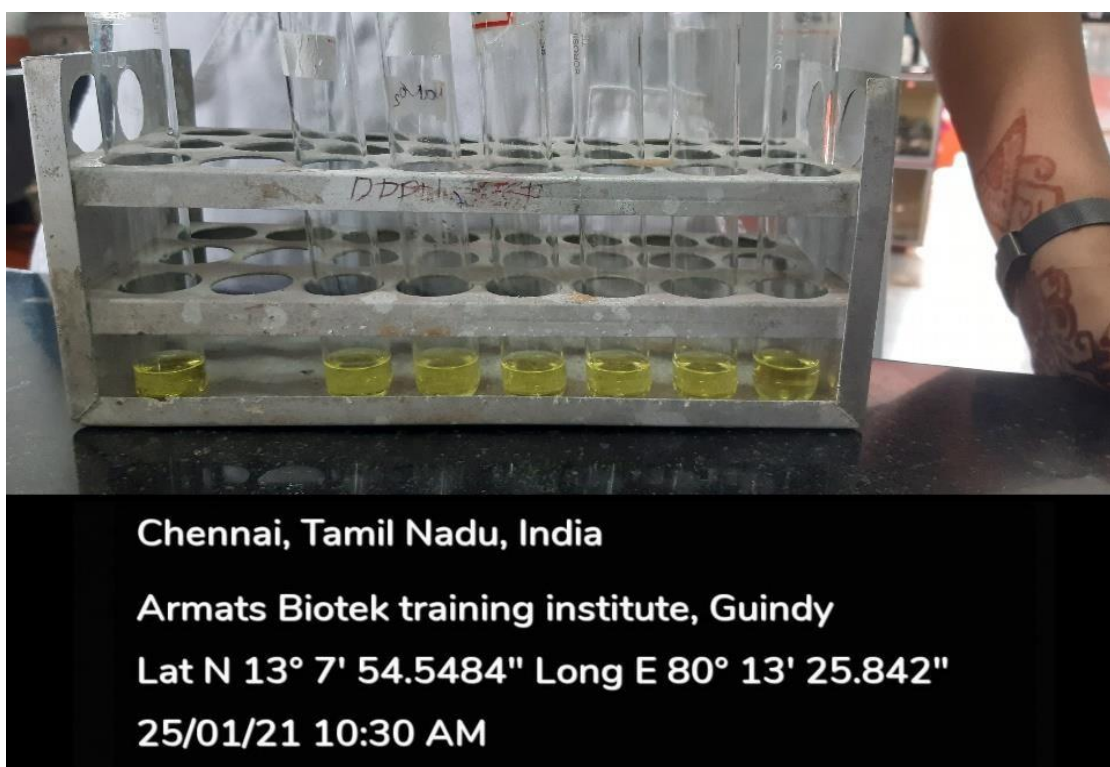


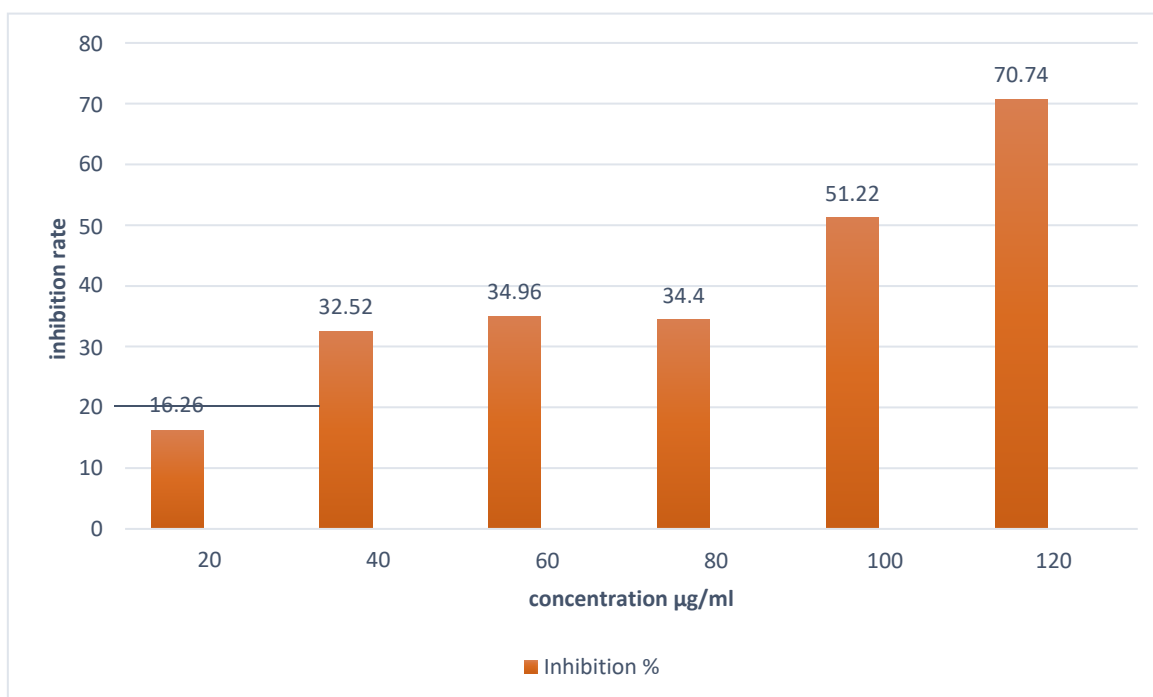
FIG 10: Ferric reducing power assay

5.4 HEAT-INDUCED HEMOLYSIS

Since the erythrocyte membrane is similar to the lysosomal membrane and its stabilization means that the extract can well stabilize lysosomal membranes, it has been used as a tool to research in-vitro anti-inflammatory activity. Stabilization of lysosomal constituents of activated neutrophils, such as bacterial enzymes and proteases, is crucial in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophils, such as bacterial enzymes and proteases, which causes additional tissue inflammation and damage when released extracellularly. The lysosomal enzymes released during inflammation produce various disorders. The extracellular activity of these enzymes is said to be related to acute or chronic inflammation. The non-steroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane. The maximum heat-induced hemolysis of chloroform seed extract was 70.74 ± 0.60 % at $120 \mu\text{g/ml}$ concentration. It was compared with the standard of ascorbic acid ($\text{IC}_{50} = 11.98 \mu\text{g/mL}$ concentration). The chloroform seed extract of *Caesalpinia bonduc* L was effective in inhibiting heat-induced hemolysis at different concentrations.

Table 4: Heat-induced hemolysis activity

CONCENTRATIONS ($\mu\text{g/ml}$)	INHIBITION RATE (%)
20	16.22
40	32.52
60	34.96
80	37.40
100	51.22
120	70.74



Standard graph of ANTI-HEMOLYSIS Assay

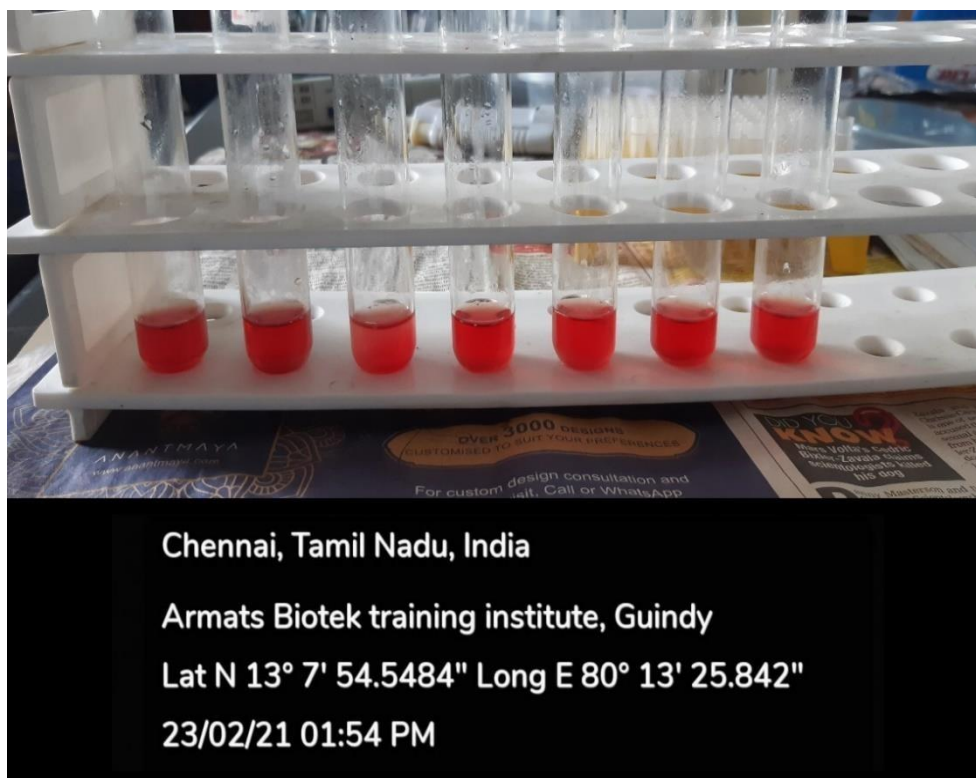


FIG 11: Anti- Haemolytic Activity



FIG 12: RBC Suspension

5.5 SEPARATION OF ACTIVE COMPOUND (COLUMN CHROMATOGRAPHY)

Chloroform extract

- The chloroform extract (5 g) of *Caesalpinia bonduc L* was subjected to column chromatography (15 mm column diameter) by increasing the polarity of solvents.
- The sample was loaded onto the silica gel packed column in a minimum volume of 100% hexane, then eluted sequentially by the following solvent systems:

Table 5: Column chromatography solvent system

Test Tube No	Solvent System
1	20 mL hexane
2	20 mL hexane
3	20 mL hexane
4	5 mL CHCl ₃ + 15 mL Hexane
5	10 mL CHCl ₃ + 10 mL Hexane
6	15 mL CHCl ₃ + 5 mL Hexane
7	15 mL CHCl ₃ + 5 mL Hexane
8	20 mL CHCl ₃
9	20 mL CHCl ₃

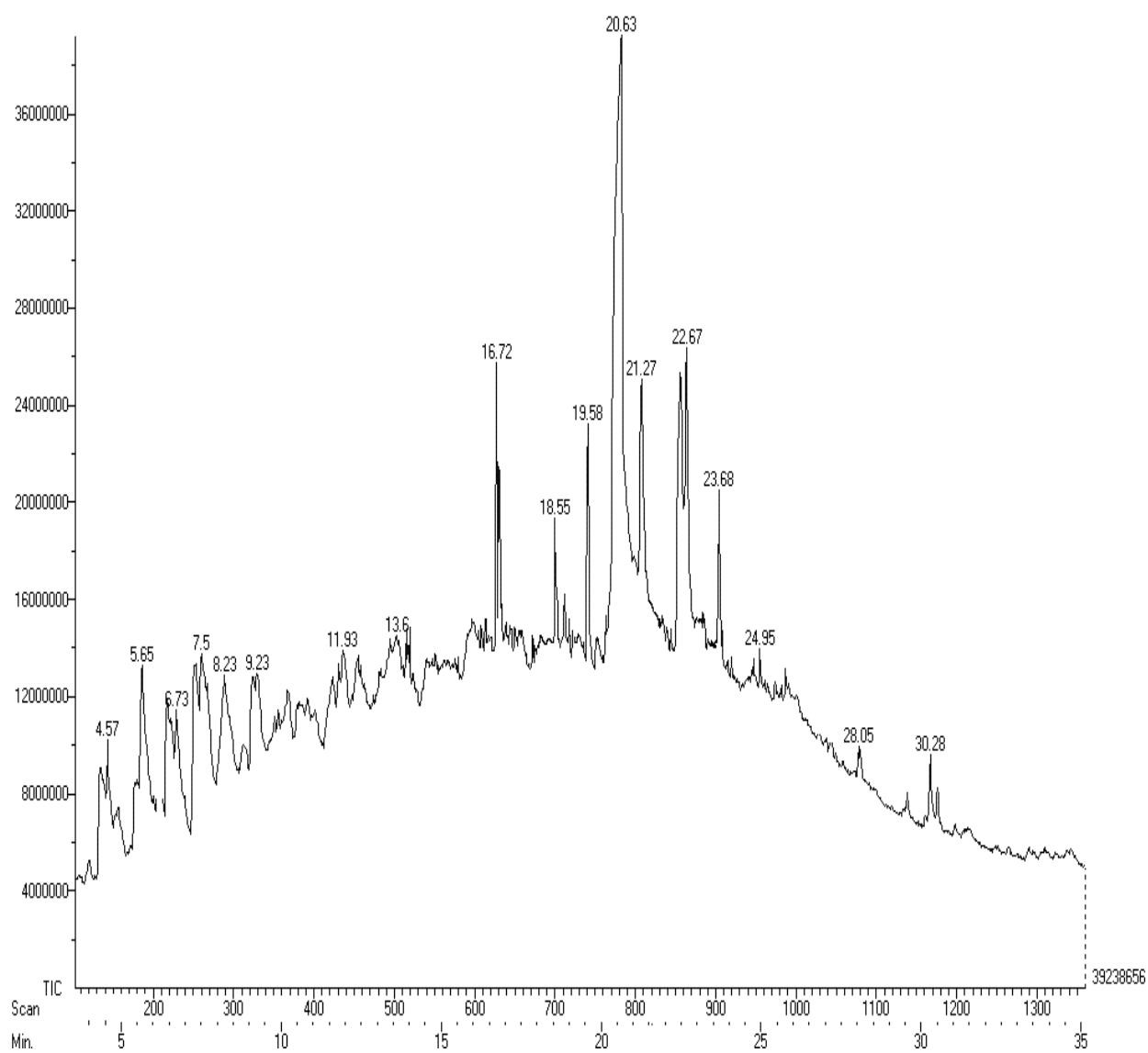


FIG 13: Column chromatography

5.6 IDENTIFICATION OF COMPONENTS (GC-MS)

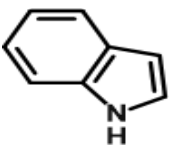
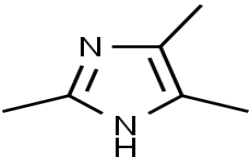
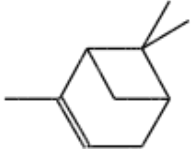
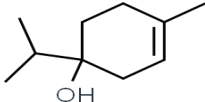
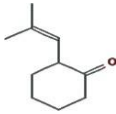
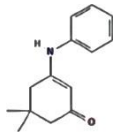
- For the interpretation of the mass spectrum of GC-MS, the National Institute of Standards and Technology (NIST) database of over 62,000 patterns was used.
- For the interpretation of the mass spectrum of GC-MS, the National Institute of Standards and Technology (NIST) database of over 62,000 patterns was used.

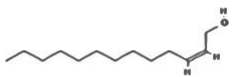
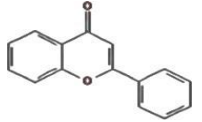
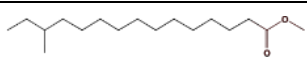
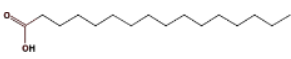
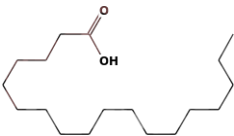
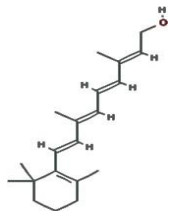
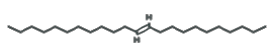
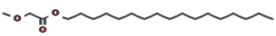
Aarthi



GC-MS Graph

Table 6: Identification of compounds through GC-MS

S. No	Compound Name	Molecular Formula	RT (Retention Time)	Molecular Weight	Compound Structure
1.	Indole	C ₈ H ₇ N	4.57	117.15	
2.	1-H Imidazole 2,4,5-trimethyl	C ₆ H ₁₀ N ₂	5.65	110.16	
3.	α Pinene	C ₁₀ H ₁₆	7.5	136.23	
4.	3-Cyclohexen-1-ol, 4-methyl 1(1-methylethyl)	C ₁₀ H ₁₈ O	8.3	154.24	
5.	2-(2-methyl-propenyl)- cyclohexanone	C ₁₀ H ₁₆ O	9.23	152.23	
6.	2-cyclohexen-1-one 5,5-dimethyl- 3-propenyl	C ₁₂ H ₁₈ O	11.93	215.29	

7.	Z-2 tridecen-1-ol	$C_{13}H_{26}O$	13.6	198.34	
8.	Flavone	$C_{15}H_{10}O_2$	16.72	222.23	
9.	E-6-tetradecen-1-ol acetate	$C_{16}H_{30}O_2$	18.55	254.40	
10.	Pentadecanoic acid,13-methyl-methyl ester	$C_{17}H_{34}O$	19.58	270.45	
11.	n-Hexadecenoic acid	$C_{16}H_{32}O$	20.63	254.42	
12.	Octadecanoic acid	$C_{18}H_{36}O_2$	22.67	284.48	
13.	Retinol	$C_{20}H_{30}O$	24.95	286.45	
14	11-tricosene	$C_{23}H_{46}$	28.05	322.62	
15	Methoxy acetic acid, hexadecyl ester.	$C_{20}H_{40}O_3$	30.28	328.5	

MASS SPECTROSCOPY

16	18-Nonadecenoic acid	C ₁₉ H ₃₆ O ₂	23.68	296.5	
----	----------------------	--	-------	-------	---

5.7 MTT ASSAY

5.7.1 Chemicals and reagents

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) Invitrogen, USA. Acridine orange was obtained from Sigma, USA. All other fine chemicals were obtained from Sigma, Aldrich.

5.7.2 Cell culture

HESCs were cultured in Rose-well Park Memorial Institute (RPMI) medium supplemented with 10% foetal bovine serum, penicillin/streptomycin (250 U/mL), gentamycin (100g/mL), and amphotericin B (1mg/mL) obtained from Sigma Chemicals, MO, USA. All cell cultures were held at 37°C in a humidified 5 percent CO₂ atmosphere. Before being used, the cells were allowed to expand to confluence for 24 hours.

5.7.3 Cell growth inhibition studies by MTT assay

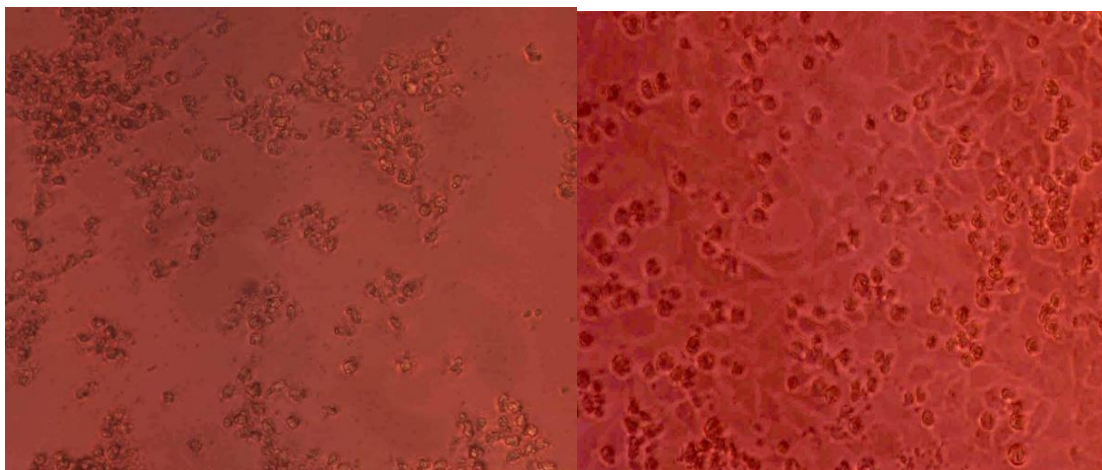
The cell viability was determined using the traditional MTT reduction assay, which was slightly modified. In 200L of RPMI with 10% FBS, Human Embryonic Stem cells were seeded at a density of 5103 cells/well in 96-well plates for 24 hours. The culture supernatant was then extracted, and RPMI with varying concentrations (20-120 g/mL) of *Caesalpinia bonduc* L chloroform fraction was added and incubated for 48 hours. After injection, the cells were incubated for 4 hours at 37°C with MTT (10L, 5mg/mL)

and then for 1 hour at room temperature with DMSO. A scanning multi-well spectrophotometer was used to read the plates at 595 nm. Data are represented as the mean values for three independent experiments.

$$\text{Cell viability (\%)} = \left[\frac{\text{Mean OD}}{\text{Control OD}} \right] \times 100$$

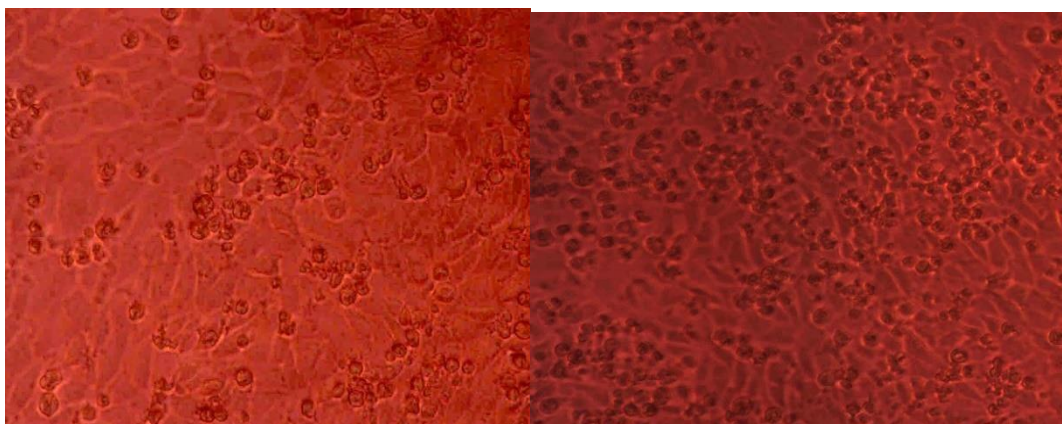
5.7.4 Research and development

The cell viability assay was carried out with Human Embryonic Stem cells (HESCs) by MTT assay. The percentage of cell viability was predicted with corresponding cell death, this could conclude how much the analyte is significant towards the cell mass. When the cell line was incubated with the extract, it induced cytotoxicity in a significant time which implicit the damage to the membrane integrity of the cell. Based on the cell disintegration, when the seed extract of *Caesalpinia bonduc L* was incubated with Human Embryonic Stem cell line and cell death was calculated. The percentage of cell death was tabulated. The maximum cell death percentage was 81.43±1.74 % at 250 µg/mL concentration. This is clearly stated that the selection of a Human Embryonic Stem cell line might provide a shred of good evidence for the treatment of Polycystic ovarian syndrome (PCOS).



250 µg/ml

50µg/ml



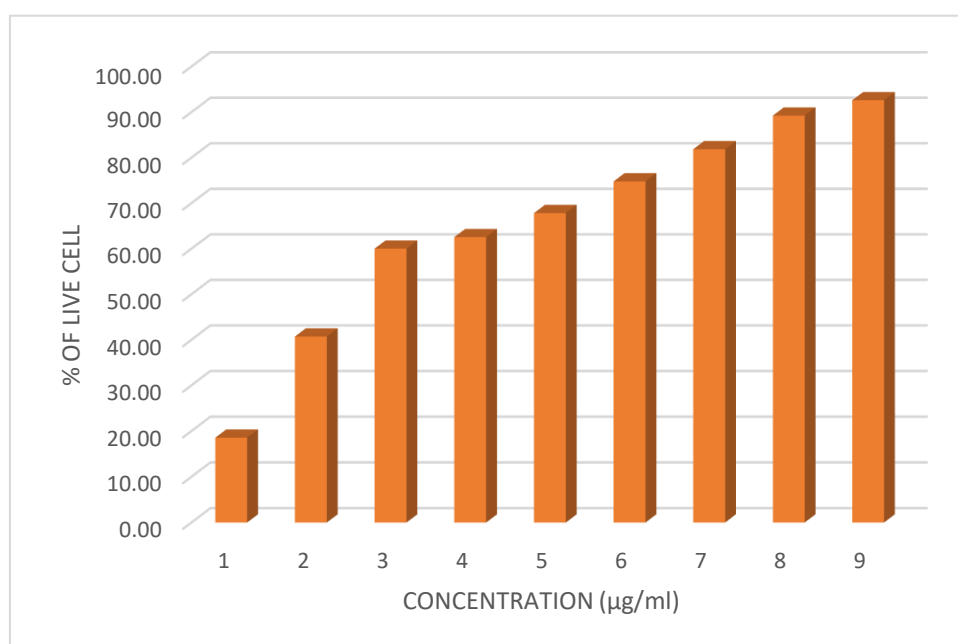
3.125µg/ml

CONTROL(Untreated)

FIG14: MICROSCOPIC STUDIES OF HESCs CELLS.

Table 7: Percentage of Live HESCs cells

CONCENTRATION ($\mu\text{g/ml}$)	% OF CELL DEATH			MEAN	SD	% OF LIVE CELL
250	79.43	82.28	82.59	81.43	1.74	18.57
100	58.23	58.23	61.39	59.28	1.83	40.72
50	40.51	37.97	41.46	39.98	1.80	60.02
25	37.97	37.97	36.39	37.45	0.91	62.55
12.5	32.28	31.96	32.28	32.17	0.18	67.83
6.25	26.27	26.58	22.78	25.21	2.11	74.79
3.125	22.47	15.82	16.14	18.14	3.75	81.86
1.562	16.14	9.81	6.33	10.76	4.97	89.24
0.781	8.54	8.23	5.38	7.38	1.74	92.62



CELL VIABILITY GRAPH.

DISCUSSION

Aqueous and chloroform extract of the plant species *Caesalpinia bonduc* L were prepared. They were screened for radical scavenging activity to determine the antioxidant capacities as well as the IC₅₀ values. The IC₅₀ value of aqueous extract was lower than the chloroform extract and by keeping this as evidence the chloroform extract was used for further analysis. Finally, this study is the first report that shows the fact that chloroform extract of *Caesalpinia bonduc* L has multifunctional therapeutic potential for the treatment of Poly Cystic Ovarian Disease. Further the cell viability was checked by using MTT assay.

Comparing the pros and cons of the treatment of PCOD, our treatment stands supreme. In other treatment, PCOD cysts are destroyed by laparoscopy paving the way for pregnancy. This is the temporary treatment wherein the patient seems cured for the short period. In reality, this problem persists and even continuous for long. On the contrary, Traditional system treats the patient paving the way for the permanent solution for the problem. Other systems lead to side effects which persist for very long time leading to recurrent illness and various ailments. Antioxidants analyses the root cause of PCOD and treat it in such a manner that the patient sign of relief the tissue damage. Thus, our system gives the complete cure to PCOD without any effects.

CHAPTER -6

SUMMARY AND CONCLUSION

SUMMARY

- The maximum DPPH[•] Radical Scavenging activity of aqueous and chloroform seed extracts of *Caesalpinia bonduc* L were 61.90±0.08% and 60.66 ±0.09% at 120 µg/ml concentration and the IC₅₀ for aqueous and chloroform seed extracts were found to be 81.76 µg/ml and 95.60 µg/ml concentration.
- The maximum Phosphomolybdenum activity of aqueous and chloroform seed extracts of *Caesalpinia bonduc* L were 96.30±1.51% and 97.32±2.46% at 120 µg/ml concentration and the RC₅₀ for aqueous and chloroform seed extracts were found to be 12.83 µg/ml and 10.76 µg/ml concentration.
- The maximum Ferric (Fe³⁺) reducing power activity of aqueous and chloroform seed extracts were 55.96±0.57% and 83.30±0.56% at 120 µg/ml concentration and the RC₅₀ for aqueous and chloroform seed extracts were found to be 73.58µg/ml and 17.14 µg/ml concentration.
- The maximum Heat-induced Hemolysis of chloroform seed extract was 70.74±0.60 % at 120 µg/ml concentration.

CONCLUSION

Antioxidants are the substance that significantly delays or prevent the oxidation of an oxidizable substrate when present in low concentrations. Plants are potential sources of invaluable anti-oxidants. The results of the present study indicate that chloroform seed extracts of *Caesalpinia bonduc* L have significant antioxidant activities to reduce the harmful effect of radicals and can be used as a potential drug in the management of PCOD.

REFERENCES

1. Abbott, J.A., Arentz, S., Smith, C.A, "Herbal medicine for the management of polycystic ovary syndrome (PCOS) and associated oligo/amenorrhea and hyperandrogenism", BMC Complement Altern Med volume 14, 2014, pp511.
2. Adam Gbankoto Anatole Laleye, Lamine Baba-Moussa, Mariette Sindee, Nounagnon Darius Tossavi Razack Ossein, Simon Azonbakin, "90-Day Oral Toxicity Study of an Ethanolic Root Extract of *Caesalpinia bonduc* (L.) Roxb. in Wistar Rats", Evidence-Based Complementary and Alternative Medicine, volume 11, 2021.
3. Ali A, Ashfaq M, Gouda S, Rao N, Shalam M, Shanta Kumar S, "Anticonvulsant Effect of Seed Extract Of *Caesalpinia Bonduc* (Roxb.)", International Journal of Pharmacology, volume5(2),2009, pp1531-2976.
4. Ali. L, Biswas. T.K, Chakrabarti.S, Mukherjee. B, Nahar. N, Seal.T, Rokeya. B " Review on Pharmacology and Phytochemistry of *Caesalpinia bonduc*", Journal of Ethnopharmacology, volume 9(1), 2005,117- 22.
5. Amudha. P, Divya. R, "Evaluation of *Caesalpinia bonduc* Extract (HAECB) on Ovarian Cysts in Polycystic Ovarian Syndrome-Induced Wistar Female Rats", Ijppr.Human, volume15(4) 2019, pp 183-199.
6. Amuthan, A., Anandh, S., Musharraf, S., Agarwal, N., Devi, V., Shenoy, R. P., & Nayal, B. (2015). Effect of Notch kudineer (*Vitex negundo* aqueous extract) on surgically induced Endometriosis in Sprague Dawley rats. International Journal of Pharmacology and Clinical Sciences], 4(3).
7. Anderson, H., Fogel, N., Grebe, S. K., Singh, R. J., Taylor, R. L., & Dunaif, A. (2010). Infants of women with polycystic ovary syndrome have lower cord blood androstenedione and estradiol levels. The Journal of Clinical Endocrinology & Metabolism, 95(5), 2180-2186.
8. Archana Mehta, Jinu John, Pradeep Mehta, Savita Shukla, Shruti Shukla, Suresh Prasad Vyas," Immunomodulatory activities of the ethanolic extract of *Caesalpinia bonduc* seeds", Journal of Ethnopharmacology, Volume 125, (2),2009, pp252-256.

9. Archana P, Chandra S, Lal J, Tandan SK, "Antipyretic and Analgesic Activities of *Caesalpinia bonducella* Seed Kernel Extract", *Journal of Phytotherapy Research*, volume19(5), 2005, pp376-381.
10. Arif T, Bhosale JD, Dabur R, Hole A, Kumar N, Lavekar GS, Mandal TK, Padhi MM, Sharma GL, "In vitro and in vivo antimicrobial activities of seeds of *Caesalpinia bonduc* (Lin.) Roxb.", *Journal of ethnopharmacology*, volume 123,2009, pp177-80.
11. Athar Ata, Elikana M. Gale, Radhika Samarasekera, "Bioactive chemical constituents of *Caesalpinia bonduc* (Fabaceae)", *Phytochemistry Letters*, Volume 2, 2009, pp106-109.
12. Attlee A, Nusralla A, Eqbal R, Said H, Hashim M, Obaid RS. Polycystic ovary syndrome in university students: Occurrence and associated factors. *Int J Fertil Steril* 2014; 8:261-6.
13. Bagul MS, Ragani. Phytochemical evaluation of classical formulation. A case study. *Indian Drugs*. 2005; 42:15-19.
14. Balagurusamy K, Risvan MY, Suresh S, "Siddha Elixir and Etiology of Polycystic Ovarian Syndrome", *Adv Tech Biol Med* volume 5,2017, pp 249.
15. Balasundaram, U, Ramadurai, S, "Rhizomicrobiomics of *Caesalpinia bonducella*, a wonder plant for PCOS treatment", *Physiology Mol Biology Plants*, volume 26, 2020, pp2453–2463.
16. Bansal, J., Kumar, N., Malviya, R., & Sharma, P. K. (2014). Hepatoprotective Models and Various Natural Product Used in Hepatoprotective Agents: a Review. *Pharmacognosy Communications*, 4(3).
17. Basavaraj KH, Dhanabal SP, Muruganantham N, Praveen TK, Rao KS, Shamasundar NM, "Screening of *Caesalpinia bonduc* leaves for antipsoriatic activity", *Journal of ethnopharmacology*, volume 133(2), 2011, pp897-901.
18. Berna Elya, Elin Novia Sembiring, Rani Sauriasari, "Phytochemical Screening, Total Flavonoid, and Total Phenolic Content and Antioxidant Activity of Different Parts of *Caesalpinia bonduc* (L.) Roxb." *Pharmacognosy Journal*, Volume, 10(1), 2018.
19. Bharathajothi. P, Vetriselvi. V, "Literature review on Kalarchi-*Caesalpinia bonduc* (L.) Roxb", *A Journal of Unani, Siddha, and Homeopathy*, Volume 9, 2020, pp7–11.
20. Billah, M. M., Islam, R., Khatun, H., Parvin, S., Islam, E., Islam, S. A., & Mia, A. A. Antibacterial, antidiarrhoeal, and cytotoxic activities of methanol extract and its

- fractions of *Caesalpinia bonducella* (L.) Roxb leaves. BMC complementary and alternative medicine, 13(1), 2013, pp1-7.
21. Chamani M, Fazel Torshizi F, Hejazi SH, Khodaei HR, Majidzadeh Heravi R Sadeghi AA, "The effects of zinc methionine on reproductive and thyroid hormones in rats with polycystic ovarian syndrome", Adv Biomed Res, volume 9, 2020, pp57.
 22. Chockalingam, V., Kadali, S. S., & Gnanasambantham, P. Antiproliferative and antioxidant activity of *Aegle marmelos* (Linn.) leave in Dalton's Lymphoma Ascites transplanted mice. Indian journal of pharmacology, 44(2), 2012, pp225.
 23. Dayanand M, Kannur, "Antidiabetic and antihyperlipidemic activity of *Bonducella* (*Caesalpinia bonducella*) seeds". Nuts and Seeds in Health and Disease Prevention, Academic Press, 2011, pp237-244.
 24. Dayanand M, Kannur, "Evaluation of *Caesalpinia bonduc* seed coat extract for anti-inflammatory and analgesic activity", Journal of advanced pharmaceutical technology & research, volume3, 2012, pp171-5.
 25. Diamanti-Kandarakis, E., Kandarakis, H. & Legro, R.S, "The role of genes and environment in the etiology of PCOS", Endocrine volume 30, 2006, pp19–26.
 26. Edzard Ernst, "The efficacy of herbal medicine", Wiley online library, volume 19, 2005, pp405.
 27. Ehrmann DA, Rosenfield RL, "The Pathogenesis of Polycystic Ovary Syndrome (PCOS): The Hypothesis of PCOS as Functional Ovarian Hyperandrogenism Revisited", Endocr Rev, volume 37(5), 2016, pp467-520. doi:10.1210/er.2015-1104.
 28. Fahim J Sayyad, Ganesh H Wadkar, "Pharmacognostic, Physicochemical and Phytochemical Investigation of Root Bark of *Caesalpinia bonducella*" International Journal of Pharmacognosy and Phytochemical Research, volume 9(1), 2017, pp26-30.
 29. Franks, S. Polycystic ovary syndrome in adolescents. International journal of obesity, 32(7), 2008, pp1035-1041.
 30. Gaur, R. L., Sahoo, M. K., Dixit, S., Fatma, N., Rastogi, S., Kulshreshtha, D. K., ... & Murthy, P. K. Antifilarial activity of *Caesalpinia bonducella* against experimental filarial infections. Indian Journal of Medical Research, 128(1), 2008, pp65.
 31. Gupta, M., Mazumder, U. K., Sambath Kumar, R., Sivakumar, T., Gomathi, P., & Rajeshwar, Y. Antioxidant defense system induced by a methanol extract of

- Caesalpinia bonducella. in rat liver. Pharmaceutical biology, 43(5), 2005, pp411-419.
32. Hari R, Meera Murugesan B, Muralidharan P, "Effect of ethanolic seed extract of Caesalpinia bonducella on hormones in mifepristone induced PCOS rats", J Appl Pharm Sci, volume10, 2020, pp072–076.
 33. Jabbar, A., Zaman, M. A., Iqbal, Z., Yaseen, M., & Shamim, A. Anthelmintic activity of Chenopodium album (L.) and Caesalpinia crista (L.) against trichostrongylid nematodes of sheep. Journal of Ethnopharmacology, 114(1), 2007, pp86-91.
 34. Jain P, Pandey R, Sharwan G, Shukla SS, "Toxicity profile of traditional herbal medicine", J Ayu Herb Med, volume 1, 2015, pp81-90.
 35. Jayakrishnan, B. M., Perumal, N., & Hashim, K. M. In vitro antioxidant studies and phytochemical screening on the seeds of Caesalpinia bonduc. European Journal of Experimental Biology, 4(4), 2014, pp47-51.
 36. Kabel, A. M. Polycystic ovarian syndrome: insights into pathogenesis, diagnosis, prognosis, pharmacological and non-pharmacological treatment. J Pharma Reports, 1(103), 2016, pp2.
 37. Kannur, D. M., Hukkeri, V. I., & Akki, K. S. Antidiabetic activity of Caesalpinia bonducella seed extracts in rats. Fitoterapia, 77(7-8), 2006, pp546-549.
 38. Khan, Neelam.D, Nazeerullah, Pal. S.R, Sunil, K, "A pharmacognostic and pharmacological overview on Caesalpinia bonducella" Research Journal of Pharmaceutical, Biological, and Chemical Sciences, volume 3, 2013, pp 440-496.
 39. Khedkar, A.S, Mandavkar, Y.D, Raje, V.N, Shelar, P.A, Thorat, M.B, "Review on Pharmacology and Phytochemistry of Caesalpinia bonduc", Journal of Current Pharma Research, volume 4, 2014, pp1309.
 40. Khot, B. M., Lad, M. D., Patil, A. J., & Kakad, A. C. Clinical efficacy of Ayurveda treatment on polycystic ovarian syndrome. IOSR Journal of Pharmacy, www.iosrphr.org, 3, 2013, pp21-25.
 41. Konan, A. B., Bléyééré, M. N., Amonkan, A. K., Bouafou, M. K., & Datté, J. Y. Why African traditional birth attendants used Caesalpinia bonduc leaves to facilitate childbirth in parturient women. International Journal of Pharmacy Review & Research, 4(1), 2014, pp11-16.
 42. Lilara, Marigoudar. R, Kanchan R. Salunke, R. Nazeer Ahmed, Shambanagouda, "Effect of graded doses of Caesalpinia bonducella seed extract on ovary and uterus in albino rats", Basic Clin Physiol Pharmacol, volume 22(2), 2011, pp49–53

43. M. Gupta, U.K. Mazumder, K.R. Sambath, T. Sivakumar, P. Gomathi, Y. Rajeshwar. *Pharm. Bio.*, 43, 5 (2005) pp411-19.
44. Patel Watson, Vinood B, "Nuts and Seeds in Health and Disease Prevention", Academic Press, 2011, pp 237-244.
45. Saeed, M. A., & Sabir, A. W. Antibacterial activity of *Caesalpinia bonducella* seeds. *Fitoterapia*, 72(7), 2001, pp807-809.
46. Saeed, M. A., & Sabir, A. W. Irritant potential of some constituents from the seeds of *Caesalpinia bonducella* (L.) Fleming. *Journal of Asian natural products research*, 5(1), 2003, pp35-41.
47. Sagar, M. K., Ashok, P. K., Chopra, H., Singh, M., & Upadhyaya, K. Analgesic and anti-inflammatory properties of *Caesalpinia* (bonduc) seeds. *The Pharma Research*, 1, 2009, pp54-59.
48. Sagar, V., & Ahamed, R. N. Antihyperlipidemic Effect of Alcoholic Seed Extract of *Caesalpinia bonduc* (Lin.) Roxb. In *Alloxan Induced Diabetic Male Albino Rats*. *International Journal of Diabetes and Endocrinology*, 1(1), 2015, pp1-9.
49. Sawant, A., Patil, S., & Shah, S. Review on PCOD/PCOS & its treatment in different medicinal systems—allopathy, Ayurveda, homeopathy. *Sci Jurno*, 1(1), 2017, pp1-16.
50. Shelar, P. A., Mandavkar, Y. D., Khedkar, A. S., Thorat, M. B., & Raje, V. N. Review on Pharmacology and Phytochemistry of *Caesalpinia bonduc*. *Journal of Current Pharma Research*, 4(4), 2014, pp1309.
51. Shukla, S., Mehta, A., John, J., Mehta, P., Vyas, S. P., & Shukla, S. Immunomodulatory activities of the ethanolic extract of *Caesalpinia bonducella* seeds. *Journal of ethnopharmacology*, 125(2), 2009, pp252-256.
52. Spritzer, P. M. (2014). Polycystic ovary syndrome: reviewing diagnosis and management of metabolic disturbances. *Arquivos Brasileiros de Endocrinologia & Metabologia*, 58(2), 2014, pp182-187.
53. Tanguturi, S. C., & Nagarakanti, S. Polycystic ovary syndrome and periodontal disease: an underlying links-a review. *Indian journal of endocrinology and metabolism*, 22(2), 2018, pp267.
54. The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group, "Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS)", *Human Reproduction*, Volume 19, 2004, pp41–47.

55. Varanasi, L. C., Subasinghe, A., Jayasinghe, Y. L., Callegari, E. T., Garland, S. M., Gorelik, A., & Wark, J. D. (2018). Polycystic ovarian syndrome: prevalence and impact on the wellbeing of Australian women aged 16–29 years. *Australian and New Zealand Journal of Obstetrics and Gynaecology*, 58(2), 2018, pp222-233.
56. Vijayan, C. P., & Sonia, A. Prevalence of Polycystic Ovary Syndrome among students of a teaching collegiate hospital, 2013.
57. Yadav, P. P., Maurya, R., Sarkar, J., Arora, A., Kanojiya, S., Sinha, S., ... & Raghubir, R. Cassane diterpenes from *Caesalpinia bonduc*. *Phytochemistry*, 70(2) 2009, pp256-261.

Urkund Analysis Result

Analysed Document: 2021-04-11 DIVYAARTHI FINAL THESIS.docx (D101259164)
 Submitted: 4/11/2021 6:20:00 PM
 Submitted By: masilamani.biotech@sathyabama.ac.in
 Significance: 6 %

Sources included in the report:

Thesis desdeepak 24102020.pdf (D82611214)
 182390825001_PCOS_Bhakti.docx (D75826924)
https://www.researchgate.net/publication/286546761_Anticonvulsive_effect_of_seed_extract_of_caesalpinia_bonducella_Roxb
<https://innspub.net/ijb/antispermatogenic-effects-seed-extract-caesalpinia-bonducella-swiss-mice/>
<https://www.hindawi.com/journals/ecam/2021/6620026/>
<https://www.thepharmajournal.com/archives/2018/vol7issue12/PartD/7-12-12-793.pdf>
https://www.researchgate.net/publication/47335777_Screening_of_Caesalpinia_bonduc_leaves_for_antipsoriatic_activity
[https://www.rjpbcs.com/pdf/2012_3\(1\)/59.pdf](https://www.rjpbcs.com/pdf/2012_3(1)/59.pdf)
<https://pubmed.ncbi.nlm.nih.gov/22865364/>
https://www.researchgate.net/publication/267097784_Review_on_pharmacological_properties_of_Caesalpinia_bonduc_L
https://www.researchgate.net/publication/343552465_Efficacy_of_extraction_methods_for_release_of_antioxidants_in_Caesalpinia_bonducella_seed_pods
<https://docplayer.net/195564467-Effect-of-ethanolic-seed-extract-of-caesalpinia-bonducella-on-hormones-in-mifepristone-induced-pcos-rats.html>
https://www.researchgate.net/publication/344452552_Polycystic_Ovary_Syndrome_PCOS_-_Hormonal_Change_In_Reproductive_Women

Instances where selected sources appear: