

1. INTRODUCTION

*“Communities and countries and ultimately the world
are only as strong as the health of their women”*

-Michelle Obama

Women are the primary caretakers in every country on the planet, and they are an important pillar of society (Mallika Singh, 2020). Women's health condition improves national productivity and enables up more resources for childcare, feeding, and education, all of which contribute to future productivity (WHO report, 2015).

Women's health is a complex and multifaceted area of study of the whole body. Number of factors like age, reproduction, health care, disease and deficiencies affect women health directly or indirectly. Women's health examines the biological characteristics unique to woman, the most obvious being the reproductive organs, difference in body structure, childhood development, reproductive hormones, genetics and brain chemistry (Alexander *et al.*, 2020).

Reproductive health is fundamental to social and economic development of a family, community as well as nation, and a key component of an equitable society. Reproductive health is important for gender equality and women empowerment and has been a great concern for every woman (Sivakumaret *al.*, 2020). Reproductive health refers to anything and everything that has to do with the reproductive system, its processes, and its functions defined as “state of complete physical, mental, and social well-being, just not the absence of disease or infirmity” (WHO, 2011).

Reproductive health is also recognized as an essential component of a person's total health status and a significant contributor to life quality (WHO, 2011). Human societies' reproductive health is recognized as a part of their human rights around the world (Zanjani and Jazaeri, 2015).

India is the world's second populous country having 1.3 billion people, but the unexpected problem faced by the young Indians are, they are unable to procreate. The 2017 revision also stated that the fertility rate of Indians has come down by more than 50% from 4.9 to 2.3 further it will be reduced to 2.1 during 2025 to 2030 and 1.8 from 2045 to 2050 and 1.7 from 2095 to 2100. Infertility affects 10 to 14 percent of the Indian population, with the rate being higher in urban areas, where one out of every six couples is affected. World Health Organisation (WHO) says that most of the

couples suffer from primary infertility (Sivakumar *et al.*, 2020). Infertility affects between 50 to 80 million people around the world. In general, one in ten couples experience primary or secondary infertility, but the rates vary from less than five percent to more than thirty percent in different countries (Mohanty and Kastor, 2017).

In Indian society, becoming a parent is regarded as one of the most significant life achievements. The importance of infertility as a public health problem affecting the individual and the family's mental and social wellbeing has resulted in its inclusion in the national program for reproductive and child health (WHO, 2011). A large number of couples in their reproductive ages are affected by infertility.

Infertility is defined as “a couple being clinically infertile if pregnancy has not occurred after at least twelve months of regular sexual activity without the use of contraceptives”, according to Family Health International. Primary infertility is defined as childlessness and secondary infertility as the inability to have an additional live birth for a parous woman. The well known biological causes for female infertility are divided into ovarian factor, pelvic factor, cervical factor, tubal factor, uterine factor, peritoneal factor and immunological factor (Parker, 2005).

Even though there has been significant progress in diagnosing and treating infertility in the last decade, the overall incidence has remained unchanged, with an estimated 40-50 percent of cases resulting from female-related factors. Unexplained infertility where the cause remains elusive, still accounts for up to 30% of cases (Showell, 2017). The prevalence of primary infertility is 8.9% in women of reproductive age group (15-49 years) in urban population of Central India (Katole and Saoji, 2019). According to the WHO (2015) report, the prevalence of primary infertility in India was 3.9% (age-standardized to 25-49 years) and 16.8% (age-standardized to 15-49 years) using the “age but no birth” definition. Adamson *et al.*, (2011) found a 12.6 percent occurrence of primary infertility in women aged 15 to 30 years in a study from South India. Shamila and Sasikala, (2011) made an effort to find out common factors responsible for female infertility in three study areas of Tamil Nadu and Kerala. The prevalence of female infertility was 45.6% in Kanyakumari, 44.2% in Thirunelveli, and 41.9% in Thiruvananthapuram and also found that PCOS being linked to an increased risk of infertility.

PCOS is the most common endocrinopathy in women of reproductive age, with a prevalence rate of 8-13 percent. It is associated with chronic anovulation or infrequent ovulation, obesity, hirsutism, hyperandrogenism and numerous follicular cysts in enlarged ovaries (Gunalan *et al.*, 2018). The term “Polycystic Ovarian Syndrome (PCOS)” was first described by Irving Stein and Micheal Leventhal as a Triad of ‘Amenorrhoea’, ‘Obesity’ and ‘Hirsutism’ in 1935. Hence, it is also known as the ‘Stein-Leventhal Syndrome’ or ‘Hyperandrogenic Anovulation’ (HA). The term polycystic means “composed of many cysts” which are the remains of follicles that never release mature eggs (Bozdag *et al.*, 2016).

Since PCOS plays an important role to the global disease burden, it is critical to detect it early in order to facilitate intervention strategies and avoid complications (Gibson- Helm *et al.*, 2017). Global prevalence of PCOS is estimated to be between 6% - 28% (Rao *et al.*, 2020). The prevalence rate of PCOS in India was determined to be 28% (Chatterjee and Bandyopadhyay, 2020).

The underlying cause of PCOS is unknown. According to (Muhas *et al.*, 2018) Lifestyle modification along with pharmacological therapies help to improve hyperandrogenism and improve insulin sensitivity, assisting regular menstrual cycle and increased fertility and preventing cardiovascular and other consequences. Oxidative stress is considered as a pathological characteristic of PCOS, and in women with PCOS, total antioxidant status declines.

Evidence indicates that the levels of reactive oxygen species (ROS) in ovarian tissue increase in PCOS, causing imbalance between oxidant and antioxidant systems (Tangvarasittichai, 2015). On the other hand, studies declare that reactive oxygen species (ROS) has been associated with PCOS and oxidative stress (OS) in excess level damage cellular functions and by influencing ovulation can affect female fertility (Agarwal *et al.*, 2004).

Complementary and alternative medicine (CAM) has been adjuvant medical management of PCOS (Raja-Khan *et al.*, 2011). For example, CAM in PCOS involves lifestyle modification, yoga, acupuncture, meditation, homeopathy, aromatherapy, ayurveda, herbal medicine, antioxidants especially vitamins and weight loss (Stener-Victorin *et al.*, 2013 and Nidhi *et al.*, 2013).

Use of antioxidants in the treatment of PCOS in women has prompted a lot of interest. Obesity, abdominal adiposity, accumulation of androgen, and insulin sensitivity are some of the characteristics of PCOS that can induce oxidative stress in PCOS patients (Murri *et al.*, 2013). PCOS is a condition which causes a significant drop in serum antioxidant and vitamin levels, putting these women at risk for oxidative stress (Al-kataan *et al.*, 2010).

Increased risk of cardiovascular disease, hypertension, insulin resistance, abdominal obesity and hyperlipidemia are linked with oxidative stress and antioxidant shortfall in PCOS women. Antioxidant supplementation has been shown to improve insulin sensitivity and other health threatening conditions in women with PCOS (Seknon *et al.*, 2010).

Antioxidants are substances that protect the body from the destructive impacts of highly reactive species and oxidants by transforming the free radical damage into more stable forms (Lobo *et al.*, 2010). Antioxidants can be generated endogenous (enzymatic) or received from foods or supplements (non-enzymatic) (Bell, 2010). Phytoestrogens and antioxidant compounds derived from medicinal plants can improve PCOS symptom and therefore be effectively used to treat this syndrome (Zahra Abasian *et al.*, 2018).

Clomiphene citrate, letrozole, metformin, troglitazone, and tamoxifene are some of the most well-known allopathic treatments for PCOS. All these medicines have mild to severe side effects including hot flushes, arthritis, joint or muscle pain and psychological side effects as irritability, mood swings, depression and bloating. Due to the adverse effects caused by the allopathic medicines, alternative medicines which have a safer profile are the areas of interest in the present situation (Khandelwal *et al.*, 2016).

Whole grain *Shashtika and Shaali* (Navara rice), green leafy vegetables rich in minerals, vitamins and nutrients, low glycaemic index whole fruits like apples, pears, grapes, oranges, plums, prunes etc., that contain fibre, vitamins, minerals and phytonutrients, bright coloured vegetables like carrots, capsicum, beet, salads etc., can be used as antioxidants that reduce the oxidative stress of Polycystic Ovarian Syndrome (PCOS). Phytoestrogens found in grain sprouts and whole grains lower oestrogen levels. To prevent PCOS, small, frequent and healthy meals with sufficient

water intake, and rich intake of vitamin B12 help to maintain glucose and fat metabolism, thyroid function and hormonal balance (Khandelwal *et al.*, 2016).

World Health Organization (WHO) recommends average daily intake of more than 400 grams of vegetables per person that protects against diet related chronic diseases (Gowthami *et al.*, 2016). Globally, next to China, India is the second largest producer of vegetable. Around, 175 major and minor vegetable crops are grown in India that includes 82 leafy vegetables to fulfill the preferred requirement by 2025. Green leafy vegetables are rich in nutrients and micronutrients and they are also recognized important for their non nutritive health promoting compounds such as antioxidants and phytochemicals. This would provide health benefits over and above basic nutrition, and is thus thought to boost human energy and dynamism. According to Prasad *et al.*, (2012), green leafy vegetables are seasonal in nature and thus available in abundance at lower prices during peak season.

Underutilized plants are domesticated plant species that have been used for centuries for fodder, fibre, food, medicinal properties or oil but have been reduced in importance over time (Padulosi, 2017). Various types of underutilized vegetable leaves are available seasonally in India but are underutilized despite their higher nutritive value thus, their utilization can be explored to overcome nutritional disorders. Leaves of Carrot, Radish and Beetroot come in this category of waste products where the vegetables are popularly consumed but the leaves are edible but remain underutilized due to lack of knowledge. They are discarded as waste and fed as fodder for animals.

Carrot leaves (*Daucus Carota*.L) are a biennial herb that belongs to the Apiaceae family and is grown all over the world. Carrots are high in beta-carotene, antioxidant, anti-cancer, cardiovascular protection, detoxification, prevention of cataract and liver defence properties (Luby, 2014). Carrot leaves have good mineral contents, antioxidant activities, fatty acid composition and chlorophyll contents (Almeida, 2009). When compared to carrot roots, high amount of essential oils are present in carrot leaves. Major components which are present in carrot leaves are β -farnesene, α -asarone, limonene, β -caryophyllene, β -myrcene, methyl isoeugenol and sabinene. Carrot leaves are wealthy in β -carotene and vitamin C nutrients, many minerals such as potassium, sodium, manganese, magnesium, calcium, phosphorus, zinc and fiber (Habegger and Schnitzler, 2012)

Greens of radish vegetable is also an underexploited by product. Prasad, (2014) reports that *Raphanus sativus* L. of Brassicaceae family commonly known as radish or mulangi is one of the widely cultivated most ancient, quick growing, annual or biennial, cold tolerant, cool season and heat sensitive vegetable root. The underground short season radish vegetable and the entire parts are eatable and used for the treatment of variety of illnesses. It is normally consumed as salad vegetable and leaf is not utilized. The use and importance of these underutilized vegetable leaves is widely reported in Ayurveda, which have therapeutic, preventive and curative properties.

Agarwal and Varma (2014) suggest that radish originated from both China and the Mediterranean area from where it spread to Japan and other parts of Asia. Its roots, fruits and leaves are edible. All parts of this plant have immense ethno medicinal uses. Abundance of research work has been carried out on different parts of radish with respect to its antioxidant and antimicrobial potential.

Beetroot (*Beta vulgaris* L.), a traditional vegetable in many countries, belongs to the family of Amaranthaceae. Its leaves, known as “beetroot greens”, are used as an organic manure, livestock feed, and conventional medicine on occasion (Amnah, 2013). Due to the lack of awareness or due to dietary habits, beetroot leaves are not commonly used as vegetables and considered as wastes (Biondo *et al.*, 2014).

Beet (*Beta vulgaris* L. *subsp. vulgaris*), is a plant originated from Northern Africa, Eastern and Southern Europe. *Beta vulgaris* L. is one of the most famous plants of the taxonomic family Amaranthaceae which has over a hundred genera (Sakhare *et al.*, 2019). It is dicotyledonous, biennial plant with tuberous root stocks. It is grown for food, not just for sugar and bioethanol production (Kale *et al.*, 2018).

Red beet plants are grown all over the world for their roots, but the beet leaves (BLs), which make up about half of the plant, are usually discarded as useless. Beet leaves constitute functional compounds (polyphenols and betalains) that can be recovered to improve plant utilisation efficiency and increase sustainability in production systems and the agro - food chain (Fernandez *et al.*, 2017). In fact, during special process, the extraction of phytochemicals from this fresh plant matrix was previously studied by optimising temperature, ratio, and ethanol concentration (Bengardino *et al.*, 2019).

Due to a major lack of knowledge about how to use them, beet root leaves *Beta vulgaris* L. are commonly cut off and discarded before using the bulb. Beet tops are a nutritious and versatile green leafy vegetable. Nonetheless, because they contain no cholesterol, are low in fat, and contain health-promoting vitamins, minerals, and antioxidants, they are one of the healthiest greens in the diet.

Beet greens have a high-quality nutritional profile that protects against anaemia, vitamin A deficiency, and heart disease. Beet root greens are loaded with vitamin A, C, K and some key B vitamins like folate. Calcium, iron, magnesium, and potassium are among the essential trace and major minerals found in it. The root portion is consumed, while the leaves are discarded. Beet root is mainly cultivated in Uttar Pradesh, Haryana, West Bengal, Himachal Pradesh, Maharashtra and also to some extent in parts of Tamil Nadu (Kaushik, 2020).

In traditional Indian medicine, several parts of the beetroot plant are used for a variety of therapeutic purposes. Leaves are tonic, diuretic and useful in alleviating inflammation, paralysis and disease of spleen and liver (Kritikar and Basu, 2005). Due to the high nutritional value, leaves are widely consumed as vegetables worldwide. The phytochemicals have been reported as a potent antioxidant (Lee *et al.*, 2009).

Beetroot roots and leaves have been used for various ailments since ancient times. Constipation and Pyrexia were treated with it by the ancient people. It has been recommended wound healing by Hippocrates. In the Medieval Era, beet juice was thought to be herbal remedy. Beetroot has also been used to treat a variety of systemic disorder which affects the liver, blood, lungs, heart, GI (gastrointestinal) system, and CNS (central nervous system) (De Azeredo *et al.*, 2009).

Fernandez *et al.*, (2017) found that the beetroot leaf and bulb are both good sources of nutrients, and that anti-nutritional factors like tannins, phytate, and oxalate in beetroot leaves are either the same as or lower than in other leafy vegetables like spinach (*Spinacia oleracea* L). Minerals, fibre, and vitamins such as cyanocobalamine, pyridoxine, calcium, and folic acid are found in beet greens, as well as phytochemical compounds such as phenolic compounds (kaempferol, quercetin, and rutin), plant pigments (carotenoids, chlorophylls, and betalains) and antioxidant

activity. Beetroot leaf is also high in omega-3 fatty acids like linolenic acid (Biondo *et al.*, 2014).

Due to the presence of biologically active substances such as cobalt, iron, zinc, copper, vitamin C, and folic acid beetroots are commonly used in medical nutrition (Konstantinova and Popova, 2020). The content of micronutrients and biomicroelements in fresh beet leaves has a greater biological value, according to Rajaeifar *et al.*, (2019).

Ghafurniya *et al.*, (2015) showed supplementation of green tea extract decreased the thickness of the follicular theca layer in PCOS rats, decreased hypertrophy of the layer and increased lipolysis. Due to this decrease, the androgens and steroids production decreased. Green tea extract increased the number of follicles and corpus luteum and decreased the number of cystic follicles in the ovary. Goshasbi *et al.*, (2010) proved that the extract of dried *Matricaria chamomilla* L. flowers induced PCO rats recovered, and regulated the secretion of luteinizing hormone and also increased dominant follicles. Another study showed that synbiotic yogurt decreased body weight and the testosterone levels, increased estrogen, progesterone, follicle stimulating hormone levels and ameliorated PCOS symptoms in PCOS mice (Tiangu Li *et al.*, 2022).

Need and Significance of the study

Polycystic ovarian syndrome is a common endocrine disorder in females of reproductive age and is now the leading cause of anovulation-related infertility. This syndrome is associated with wide spectrum complications in different aspects of health, including reproductive (hyperandrogenism, hirsutism, anovulation, infertility, and menstrual disturbance), metabolic (diabetes mellitus, cardiovascular risk and as well as obesity), and psychological features (decreased quality of life and mood disorders).

Numerous studies have reported an increase in the prevalence of PCOS in the past years. Oxidative stress may play a significant role in PCOS-related infertility in women. If the antioxidant system has been depleted due to excessive production of Reactive Oxygen Species (ROS), female genital tract function can be disrupted. This can alter oocyte maturation, steroidogenesis, ovulation in addition it can accelerate granulosa cells apoptosis, which is a naturally occurring phenomenon, involving programmed cell death.

Obesity and insulin resistance, both of which are common in PCOS patients, are likely to contribute to increased oxidative stress. Antioxidants can prevent DNA damage by acting as efficient scavengers of free radicals and oxidants.

Scientists are becoming more interested in the use of antioxidant properties in the therapies of PCOS. Antioxidants are found naturally in foods. There are number of underutilized greens especially that are used for medicinal purposes. Recent studies have shown that these underutilized greens can also serve as rich source of antioxidants and thus may provide health benefits. Green leafy vegetables are a low-cost but high-nutrient source of a variety of micronutrients and antioxidant phytochemicals (Devi *et al.*, 2020).

Beetroot is a food ingredient containing betalain pigments that show antioxidant activity. Beet greens, the leafy portion of the beetroot, have been shown in studies to be a good source of fibre, protein, antioxidants, vitamins A, C, and E, and polyphenols. Antioxidants and polyphenols are preferred as protective ingredients in pharmaceutical, food industries as food additives, preservatives and dietary supplements. Hence the present study was undertaken to assess the management of oxidative stress in PCOS induced rats by administration of Beet greens (*Beta vulgaris* L.) extract.

Operational Definitions

The key terms of the study are operationally defined with specific reference to the study is as follows:

- ***Beta vulgaris* L.:** Scientific name of the plant, Beetroot
- **Leaf :** Part of the plant *Beta vulgaris* L. used for the study.
- **Nutraceutical potential:** Nutrient content, phytochemical constituents and antioxidant potential of the *Beta vulgaris* L. leaf extract.
- **PCOS and Oxidative stress:** Potential of *Beta vulgaris* L. greens extract in controlling oxidative stress in Polycystic Ovarian Syndrome.
- **Polycystic ovarian rats:** Experimental Wistar strain Albino female rats which were induced polycystic ovarian by estradiol valerate.

Scope of the study

Recent research suggests that, in addition to their lipid-lowering properties, some vegetables and herbs can also reduce the production of reactive oxygen species (ROS) and increase the resistance of plasma lipoprotein to oxidation, possibly contributing to their effectiveness in preventing PCOS. PCOS is now regarded as a lifestyle disorder that affects young women of reproductive age. It is one of the leading causes of infertility or menstrual disorders in women and is increasing exorbitantly due to sedentary habits.

Medicines like metformin, tamoxifene, clomiphene citrate, letrozole, and troglitazone are among the most well-known allopathic treatments for PCOS. All these medicines have mild to severe side effects including hot flushes, arthritis, joint or muscle pain and psychological side effects as irritability, mood swings, depression and bloating. Because of the side effects caused by allopathic medicines, alternative medicines with a lower risk profile are becoming more popular these days.

Numerous studies have demonstrated that lifestyle intervention helps in reducing the symptoms of PCOS, although effective changes in dietary habits is difficult. There are only few scientifically supported dietary approaches that help in controlling PCOS. Studies have shown that calorie restriction, low-carbohydrate, low-fat, high protein, liberal vitamin and herbal supplementation can all assist to treat PCOS. However, the majority of trials on PCOS dietary supplement therapies are limited, nonrandomized, and uncontrolled. Future well-designed studies are needed to further evaluate the safety, effectiveness and mechanism of food supplements in treating PCOS. The scope of the study can be extended as per the following topics.

1. Efficacy of underutilized greens of carrot, radish and beetroot greens powder for analysis of total antioxidant activity.
2. Efficacy of Beetroot greens (*Beta vulgaris* L.) extract for antioxidant capacity and polyphenol for control of Polycystic Ovarian Syndrome condition.
3. Effect of *Beta vulgaris* L. leaf extract on Polycystic Ovarian Syndrome.

Objectives

Primary Objectives

- To select an antioxidant rich vegetable greens powder for administration on Polycystic Ovarian Syndrome induced female rats.
- To evaluate the effect of the selected antioxidant rich vegetable greens powder on Polycystic Ovarian Syndrome induced female rats.

Secondary Objectives

- To determine the total antioxidant, total phenol and total flavonoid in the selected vegetable greens powder.
- To screen the presence of pesticides, secondary metabolites, flavonoids, phytochemicals and anti-nutritional factors in the selected vegetable greens powder.
- To determine the nutrient content, beta-carotene, fat soluble vitamin A and E in the selected vegetable greens powder.
- To assess the effect of selected vegetable greens powder on the antioxidant status in Polycystic Ovarian Syndrome induced female rats.
- To evaluate the effect of administration of the selected vegetable greens powder on Polycystic Ovarian Syndrome induced female rats.

Hypothesis

It has been hypothesized that:

- The administration of *Beta vulgaris* L. leaf extract helps to reduce oxidative stress in Polycystic Ovarian Syndrome condition.
- Aqueous extract of *Beta vulgaris* L. leaf has positive effect on regulating the reproductive hormone level in Polycystic Ovarian Syndrome.