2. REVIEW OF LITERATURE

The review of literature pertaining to the study entitled **"*In-vivo* Effects of *Beta vulgaris* L. Leaf Extract on Polycystic Ovarian Syndrome Induced Adult Rats**" has been discussed under the following headings:

A. Polycystic Ovarian Syndrome – An Overview
B. Polycystic Ovarian Syndrome and Oxidative Stress
C. Role of Antioxidants in the Management of Polycystic Ovarian Syndrome
D. Awareness on consumption of Underutilized Vegetable Greens
E. Nutraceutical Potential and Antioxidant Effect of Beet Greens (*Beta vulgaris L*)

A. Polycystic Ovarian Syndrome – An Overview

Polycystic Ovarian Syndrome (PCOS) is a heterogeneous endocrine disorder distinguished by the manifestation of anovulation, ovarian cysts, and endocrine variation in woman (Escobar - Morreale, 2018). Also PCOS characterized by menstrual disorders (amenorrhea/oligomennorrhea), alopecia, hirsutism, acne, and biochemical examination results showed an increase in androgen (testosterone) (El Hayek *et al*., 2016 and Ilie, 2020).

According to Teede *et al.*, (2019) patients with Polycystic Ovarian Syndrome also exhibit high serum Anti-Mullerian hormone (AMH) and Luteinizing hormone (LH) levels. Abbott, (2019) illustrated multifactorial disorder is generally associated with major metabolic disturbances, such as insulin resistance (IR), abdominal adiposity, high cholesterol levels, and high blood pressure with obese Polycystic Ovary syndrome. In accordance with the DoHAD (Developmental origins of Health and Disease) cumulative evidence now indicates that reproductive and metabolic disorders observed in women with polycystic ovary syndrome might result from developmental alterations during fetal and pre-pubertal life (Dumesic *et al*., 2020). Polycystic Ovarian Syndrome related symptoms also have negative psychiatric effects, that impairs quality of life of the affected patients (Blay *et al*., 2016 and Rzonca *et al*., 2018).

(i) Pathophysiology of Polycystic Ovarian Syndrome

Polycystic ovarian syndrome is a complex genetic trait, where multiple genetic variants and environmental factors interact to foster the development of this disorder

(Branfield, 2019) as shown in Figure 1. Hyperandrogenism is evidenced by raised levels of free (unbound) testosterone in the blood stream, a key hormone contributor to the pathophysiology of polycystic ovary syndrome (Ibanez *et al*., 2017).

Predisposing risk factors, according to Van Hooff and Lambalk, 1998, include genetics, neuroendocrine and lifestyle/ environment. Obesity is a major higher risk factor for developing Polycystic syndrome in some women with dominant genes. Data on genome-wide association revealed that specific loci and alleles plays a major role in Polycystic Ovary Syndrome phenotype identification (Hayes *et al*., 2015; Shi *et al*., 2012; Dumesic *et al*., 2020) and androgen exposure can impede the hormone levels to increase the high pulse frequency of gonadotropin releasing hormone GnRH affecting the LH:FSH proportion and leads to follicular arrest and dysplasia (Dumesic *et al.*, 2020, Cheung and Cog, 2010). These factors lead to the cause of hyperinsulinemia, hyperandrogenism, oxidative stress, irregular periods eventually up surging the metabolic syndrome. Polycystic ovary syndrome was named so because it indicated multiple ovarian cysts (undeveloped follicles) on ultrasound examination.

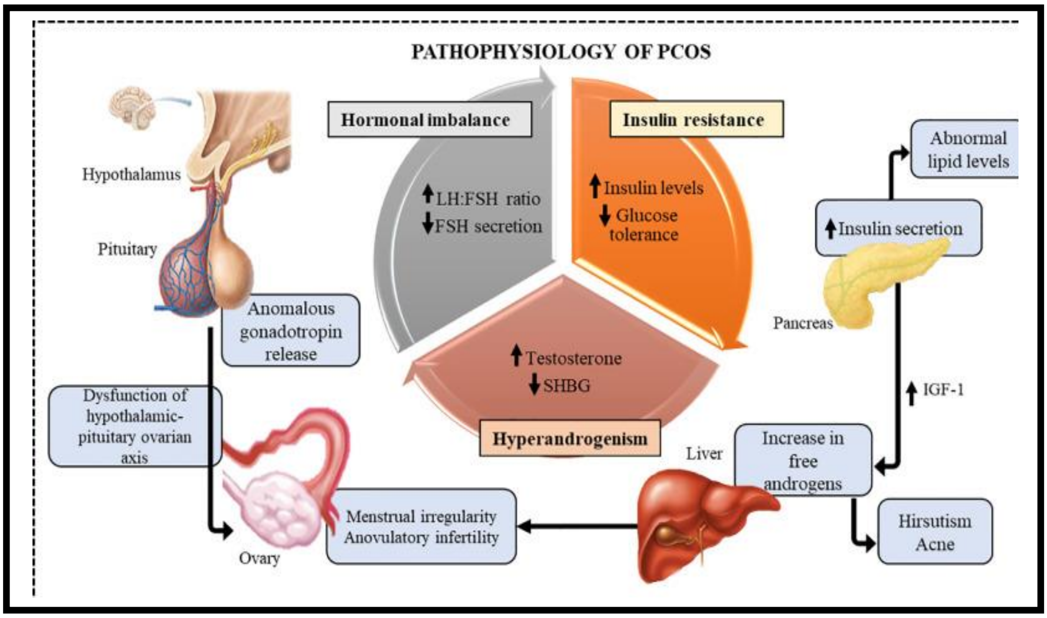

Figure 1: Pathophysiology of Polycystic Ovary Syndrome

(ii) Prevalence of polycystic ovary syndrome

According to the World Health Organisation (WHO) estimation revealed over 116 million women (3.4%) are affected by Polycystic ovary syndrome worldwide (Bharathi *et al.*, 2017). Based on the diagnostic criteria National Institute of Health 1990, the prevalence of Polycystic ovary syndrome is 6% to 10% of reproductive age women, but prevalence with Rotterdam criteria is twice as high (Hillman, 2018). Approximately 4-10% of the world population experience Polycystic ovary syndrome although the incidence rate is higher for India, rounding off to 20-26% (Radha *et al*., 2016). The estimated prevalence in women of reproductive age is 5-10%. Under the new criteria (Rotterdam- 2003), the prevalence among general female population will rise up to 10 %. Polycystic ovary syndrome prevalence is also notably higher in black and South Asian women than in white women (Ding *et al*., 2018).

According to Szczuko *et al*., (2021) and Ozcan Dag *et al*., (2017) the estimated prevalence of Polycystic ovary syndrome (PCOS) was 6% to as high as 26%. Chatterjee and Bandyopadhyay, (2020) indicated that 28% young women in the reproductive age group were diagnosed with Polycystic ovary syndrome in India, which indicated that 85% of the polycystic ovary syndrome cases had oligomenorrhea, 19% had hirsute, and 41% had acne and about 50% of the oligomenorrheic adolescents develop Polycystic ovary syndrome in adults. A pilot cross-sectional study conducted by Balaji *et al*., 2015 found a prevalence of 18% for Polycystic ovary syndrome in Tamil Nadu. Nidhi *et al*., 2011 prospectively studied 460 girls aged 15-18 years from a residential college in Andhra Pradesh, South India and reported a prevalence 9.1% of Polycystic ovary syndrome in Indian adolescents. Diagnosis based on hyperandrogenism and anovulation estimated 5-7 percent prevalence of Polycystic ovary syndrome among reproductive age women. However, the spectrum of this syndrome is still much more wider. Recent findings show that there is a mild form of Polycystic ovary syndrome that includes hyperandrogenism and polycystic ovaries but whose ovulatory function is maintained. Polycystic ovary syndrome (PCOS) is a serious health problem that usually strickes during puberty (Gill *et al*., 2012).

Nidhi and Padmalatha, 2011 studied 460 Indian adolescents and found out that one (0.2%) had olio/amenorrhea with clinical hyperandrogenism, 29 (6.3%) had oligomenorrhea with polycystic ovaries, one (0.2%) had polycystic ovaries with clinical hyper androgenism and 11 (2.3%) had oligomenorrhea with polycystc ovaries in the presence of clinical hyperandrogenism. The finding confirmed that 42 (9.1%) girls satisfied Rotterdam's criteria for polycystic ovary syndrome. This draws more attention to the issue of early diagnosis in adolescent girls. Vijaya and Bharatwaj (2014) studied the prevalence of Polycystic ovary syndrome among medical undergraduate young women in a teaching hospital in Pondicherry and found out that prevalence rate was 11.7%. Gill *et al.*, 2012 at Lucknow, North India showed that prevalence of polycystic ovary syndrome in women between the ages of 18-25 years was 3.7 percent.

(iii) Etiology and Symptoms of Polycystic ovary syndrome

Though the etiology of polycystic ovary syndrome is unclear, but thought to develop from the interaction of genetic, fetal and environmental factors.

Genetics and Androgen Excess

Many studies have showed that impaired folliculogenesis is the result of surplus androgens that disrupt normal androgen synthesis. The excess androgens promote the development of primordial follicles and increase in the antral follicles at the early gonadotropin stage (Centeno, 2016). The secretion of GnRH from the hypothalamus will activate the gondotropin hormone release from the pituitary. Luteinizing hormone activates the LH receptor to promote androgen production in ovarian theca cells, and the follicular stimulating hormone acts on the FSH receptor simultaneously in the ovarian granulosa cells to transform the androgens to estrogens, which promote the follicle growth (Ashraf *et al*., 2019). It has been assumed that the dysregulation in the neuroendocrine system results in an imbalance of the hypothalamic-pituitary-ovarian axis leading to a surplus level of gonadotropin. The rise in the GnRH promotes the production of LH over FSH, resulting in a marked hormonal increase in the LH:FSH ratio in polycystic ovary syndrome (Walter *et al*., 2018).

High GnRH pulse frequency increases luteinizing hormone (LH) and decrease follicle-stimulating hormone (FSH), which promotes androgen production and interferes with normal follicular development. Besides, there is evidence of resistance to the effects of FSH at the follicular levels in the ovaries of polycystic ovary syndrome women, possibly in part secondary to excess local anti-mullerian hormone

(AMH) production (Azziz *et al.,* 2016 and Paulson *et al* ., 2020). Furthermore, studies (Tsiami *et al.,* 2021 and Sun *et al.*, 2020) found that excessive intrauterine exposure of female foetuses to androgens, including testosterone, may contribute to the reproductive and metabolic consequences of polycystic ovary syndrome.

Obesity

According to Messinis *et al*, (2020) the most likely environmental factor affecting the development of polycystic ovary syndrome is diet and its association with obesity. Obesity is linked to hyperinsulinemia which further increase the lipid profile, glucose intolerance in polycystic ovary syndrome patients. Obesity, according to Glueck and Goldenberg, (2019), increases androgen production by stimulating LH, which leads to hyperandrogenism.

Leptin, an appetite-controlling adipokine has a direct impact on the neuroendocrine and reproductive function of obese polycystic ovary syndrome women (Rojas *et al*., 2014). Furthermore, hyperlipidemia may hinder ovarian follicular growth (Barber *et al*., 2006). So, decreasing the visceral fat would control the appetite, glucose levels, lipolysis, and increase the SHBG, thereby regulating the androgen action in the ovary (Bulsara *et al*., 2021).

Age at onset

Some research suggests that girls who develop premature pubarche (pubic hair early most often before the age of eight), have many of the signs and symptoms of Polycystic ovary syndrome. Another study done on pre-pubescent girls throughout puberty showed that premature pubarche resulted in excess testosterone production and irregular periods consistent with Polycystic ovary syndrome lead researchers to conclude the premature pubarche may be an early form of Polycystic ovary syndrome (Witchel, 2006).

Insulin resistance

Zeng *et al*. (2020) pointed out that, patients with Polycystic ovary syndrome were hyper-insulinemic in response to an oral glucose tolerance test, a condition which is known as insulin resistance (IR). Studies have found that 50-70% of women with polycystic ovary syndrome showed clinically measurable insulin resistance, that determined beyond their body weight (i.e. degree of obesity) (Conway *et al.,* 2014).

The causes for the increased insulin resistance and, consequently, the hyperinsulinism in Polycystic ovary syndrome remains unclear. A post-binding defect in receptor signaling that selectively affects metabolic, but not mitogenic, pathways in classic insulin target tissues (e.g. adipose tissue, muscle, and possibly the ovaries). These defects seem to affect glucose transporter-4 (GLUT4) expression. Additionally, epigenetic dysfunction may play a role in the insulin resistance of Polycystic ovary syndrome (De Medeiros *et al*., 2018).

According to (Puttabyatappa and Padmanabhan, 2018 and Barber *et al*., 2016) suggested that hyperinsulinemia is the root cause of excess androgens because insulin directly stimulates the action of LH and raise the GnRH indirectly.

Hyperandrogenemia favors excess abdominal fat deposition, exacerbating insulin resistance and hyperinsulinemia in women with polycystic ovary syndrome, and further enhance the secretion of ovarian androgens. Insulin decrease the sex hormone binding globulin (SHBG), a main circulatory protein controlling the testosterone levels. As a result, lowering SHBG causes an increase in freen androgens, which cause clinical manifestations such as hirsutism, alopecia and acne (Rojas *et al*., 2014).

Polycystic ovary syndrome are at high risk for cardiovascular disease and diabetes due to insulin resistance can cause dyslipidemia (Rocha *et al*., 2019). According to National Institute of Health (NIH) criteria, the prevalence of polycystic ovary syndrome is 19 - 41% in women with type I diabetes (Escobar-Morreale and Roldan-Martin, 2016). Several studies revealed that controlling insulin resistance in polycystic ovary syndrome women, eventually would decrease the excess androgens and improve the condition (Ashraf *et al*., 2019 and Goodarzi, 2011).

Other risk factors also play a role in pathogenesis of polycystic ovary syndrome include chronic inflammation (Dhesi and Morelli, 2015) exposure to endocrine disrupting chemicals (Takeuchi, 2004) use of medications that increase prolactin production (Hernandez, 2000) and autoimmune disorders, especially those involving the ovaries, pancreas, thyroid and adrenal glands (Petrikova, 2010). The syndrome is associated with numerous morbidities, including infertility, obstetrical complications, type 2 diabetes mellitus, cardiovascular disease, mood and eating disorders.

Women with Polycystic ovary syndrome are present with diverse symptoms including irregular menstrual cycles, sub-fertility, excessive hair growth, and pregnancy complications. Moreover, it is associated with psychological issues such as anxiety, depression, and distributed bodily images with reduce self-esteem. With increasing of age, the syndrome evolves from a reproductive disease to a metabolic disorder include insulin resistance, impaired glucose tolerance, type 2 diabetes mellitus (DM2), dyslipidemia and cardiovascular risk factors (Meier, 2018).

Biochemical (elevated circulating androgen concentrations) and clinical (hirsutism, acne and alopecia) lead to Hyperandrogenism. Hirsutism is defined as the presence of excessive terminal hair growth in androgen-dependent regions. In the general population, it is estimated that 5-15% of the female population suffers from hirstutism (Yildiz *et al*., 2012). However, 80-90% of patients with hirsutism will have an androgen excess disorder (Azziz *et al*.,2016).

A recent study by Ollila *et al*. (2016), showed that weight gain in early adulthood is significantly associated with symptoms or diagnosis of Polycystic ovary syndrome. Depending on Body Mass Index (BMI) obviously (33-88%) of Polycystic ovary syndrome is associated with overweight and obesity (Barber *et al*., 2006). Obesity aggravates the reproductive and metabolic phenotype in polycystic ovary syndrome women (Yildiz *et al.*, 2008).

Subfertility was found 15-fold higher in women reporting Polycystic ovary syndrome compared with controls independent of Body Mass Index(BMI) (Joham *et al*., 2015). A meta-analyses study done by Christ *et al*., (2019) reported women with polycystic ovary syndrome had an increased risk for gestational diabetes mellitus (GDM), gestational hypertension, pre-eclampsia (PE), and cesarean section during delivery. Some studies report a higher risk of miscarriage (Khomami *et al*., 2019).

(iv) Diagnostic Criteria of Polycystic ovary syndrome

Women with polycystic ovary syndrome have recently been identified as a potentially high-risk group for COVID -19 complications. According to a study conducted from January to July 2020 that included 21,292 women with polycystic ovary syndrome and 78,310 control groups, 52 percent of women with polycystic ovary syndrome had an increased risk of COVID- 19 infection (Subramanian *et al*., 2021).

In 1990, a group of researchers at an NIH –sponsored conference defined polycystic ovary syndrome (PCOS) as hyperandrogenism and/or hyperandrogenemia (HA) with oligoanvulation, excluding other endocrinopathies (based on a consensus questionnaire) (Zawadzki, 1992).

However in 2003, Rotterdam consensus (based on closed session consensus among primarily European and American investigators), expanded the diagnostic criteria to in which any two of the following three conditions need to be fulfilled for the inclusion: (i) Oligo- and/or anovulation (i.e. less than 9 menstrual periods in a year or menstrual cycles more than 35 days in length), (ii) Clinical hyperandrogenism (i.e. acne or hirsutism; modified Ferriman-Gallwey scores $\geq$ 8) or biochemical hyperandrogenism –[i.e. free testosterone (FT) $\geq$ 7.0 pg/ml), and (iii) Ultrasonographic findings of polycystic ovarian morphology (presence of $\geq$ 12 follicles in each ovary measuring 2-9 mm in diameter).

The Rotterdam criteria for the diagnosis of polycystic ovary syndrome are based at least two of the following three features: (1) an irregular ovulatory function (oligomenorrhea or amenorrhea), (2) the evidence of either biological or clinical hyperandrogenism, and (3) the presence of a polycystic ovarian morphology (Fauser *et al*., 2012). National Institute of Health recommended clinicians to use Rotterdam criteria for diagnosis of PCOS (Wolf *et al.*, 2018).

(v) Hormonal Changes in Polycystic Ovary Syndrome

A study conducted in China with 677 Polycystic ovary syndrome patients confirmed INSR gene had a risk factor for the development of polycystic ovaries (Bassiouny *et al*., 2014). Based on Restriction Fragment Length Polymorphism (RFLP) analysis among Chinese population, it was found that there was an increased risk of insulin resistance and associated polycystic ovary syndrome in lean patients (Bassiouny *et al*., 2014; Carolyn, 2020, Eriksen *et al*., 2013).

Similar research conducted on Indian women by Kaur *et al.*, (2018) reported that there is an association of INSR gene with the incidence of polycystic ovary syndrome. Different studies conducted among Madurese tribe of Madura the northeastern coast of Java also confirmed that the C allele to be higher in polycystic ovary syndrome patients than in healthy women (Belani *et al*., 2018 and Zainiyah, 2019).

In a study using colour Doppler blood flow within the ovarian stroma was recorded and serum concentrations of FSH, LH and testosterone were measured for 52 women with polycystic ovary syndrome before and after LOD, the results showed a significant correlation between hormonal and ovarian stromal blood-flow changes. The changes in the Doppler indices were significantly higher for women who ovulate. The measurement of ovarian stromal blood flow correlated to the changes in androgen markers of polycystic ovary syndrome (Gadalla, *et al*., 2020).

Androgen exposure can impede the hormone levels to increase the high pulse frequency of GnRH affecting the LH:FSH proportion and leads to follicular arrest and dysplasia (Dumesic *et al.*, 2020 and Cheug, 2010). Serum anti-Mullerian hormone (AMH), originating mostly from granulose cells of large preantral/small antral follicles (Baskind and Balen, 2016) has emerged as a possible surrogate marker of Polycystic ovaries (Balen *et al*., 2003 and Dewailly, 2011). They are positively correlated with LH and T levels and negatively predicted by BMI (Piouka *et al*., 2009 and Homburg *et al*., 2013). A novel finding by Schmidt *et al*., 2020 showed that Polycystic ovary syndrome women had higher free androgen index but lower FSH and SHBG than controls.

(vi) Therapeutic management of Polycystic Ovary Syndrome

According to (Rotterdam ESHRE/ASRM- sponsored polycystic ovary syndrome consensus workshop group 2004) there is no pharmacological therapy that can cure the Polycystic ovary syndrome, but some interventive medications are used to treat the clinical symptom of polycystic ovary syndrome. (Ndefo *et al*., 2013). Pharmacological therapies along with a change in the lifestyle ameliorate the overall condition.

The treatment strategy varies according to the clinical symptoms and underlying cause which can be divided by treating ovulatory dysfunction, hyperandrogenism, improving insulin resistance and infertility (Zimmerman *et al*., 2019).

Oral contraceptives (OCPs)

The OCPs are divided into progesterone-only pills and combined pills containing both estrogen (estradiol dose up to 50µg) and progesterone (norethisterone, desogestrel). They are first-line therapy for women who do not want to ovulate and are facing menstrual irregularities. OCPs decrease the circulating androgens by raising the SHBG (Geller *et al*., 2011). The use of OCPs do not affect insulin resistance but show variability in lipid profiles which may lead to metabolic disturbances (Geller *et al*., 2011 and Halperin *et al*., 2011). So, the usage of OCPs should be according to the risk grade and stopped immediately if any contradiction occurs.

Insulin sensitizers

This class of drugs is generally used to treat polycystic ovary syndrome-associated metabolic co-morbidities by decreasing insulin resistance and normalizing insulin levels. By lowering the IR, the associated androgen level will decrease resulting in improvement in the menstrual cycle (Geller *et al*., 2011).

Metformin

Metformin is a large-scale manufactured biguanide used to treat insulin resistance and reinstate the menstrual irregularities in polycystic ovary syndrome (Lauretta *et al*., 2016). Metformin increase the glucose uptake and its utilization which ameliorates insulin resistance in polycystic ovary syndrome patients (Geller *et al*., 2011 and Moghetti *et al*., 2000). It regulates the glucose level, unlike other insulin-regulating drugs which lead to either hypoglycemia or hyperglycemia as its side effect (Sivalingam *et al*., 2014).

Thiazolidinediones (TZDs)

This class is commonly named glitazones comprising of rosiglitazone, pioglitazone which decrease the 11-β-HSD enzyme activity responsible for conversion of cortisol (Lauretta *et al*., 2016). They are the second-line choice of drugs for treating polycystic ovary syndrome women who are resistant to insulin (Stout and Fugate, 2005).

Ovulation inducing agents

Clomiphene citrate (CC) is the prime choice of drug for treating anovulatory sterile women (Thessaloniki ESHRE/ASRM –Sponsored polycystic ovary syndrome Consensus Workshop Group 2008). Clomiphene citrate (CC) increases the FSH level by inhibiting the estrogen receptor through a negative feedback mechanism (Badawy and Elnashar, 2011).

Tomaxifen acts similarly to clomiphene which is used to treat anovulation in patients who fail or not respond to clomiphene citrate, combined studies of clomiphene and tamoxifen revealed a marked increase in pregnancy rate (Borenstein *et al.*, 1989 and Dhaliwal and Shepherd, 2021).

Letrozole is an off-label aromatase inhibitor, that obstructs the androgen to estrogen conversion pathway and aid in folliculogenesis by stimulating FSH (Kar, 2013). Studies suggest that letrozole is more effective in anovulatory infertility than CC in polycystic ovary syndrome patients (Setji and Brown, 2014).

Gonadotropins such as recombinant FSH, human menopausal gonadotropin (HMG) are the second-line choice of treatment for anouvulatory infertile polycystic ovary syndrome women (de Melo *et al.*, 2015 and Louwers and Laven, 2020).

Lifestyle Intervention

Most women with polycystic ovary syndrome, regardless of weight, have a form of insulin resistance that is intrinsic to the syndrome. For this reason, lifestyle changes that improve insulin sensitivity should be the first line of treatment for women with polycystic ovary syndrome, particularly for those who are overweight. Lifestyle interventions should also accompany pharmacological treatment (de Medeiros *et al.,* 2018 and Moran *et al.*, 2019). The dietary management of polycystic ovary syndrome should focus on weight reduction for those who are overweight (Zeng *et al.,* 2020).Women with polycystic ovary syndrome may need to restrict energy intake significantly to maintain a normal weight (Linden *et al.,* 2004).

Lifestyle modifications, including diet, exercise, and weight loss; are effective for women with polycystic ovary syndrome, with a reduction in weight of as little as 5% of total body weight having been shown to reduce insulin levels, improve menstrual function, reduce testosterone levels, improve symptoms of hirsutism and acne and increase ovulation and fertility (Moran *et al*., 2019). Furthermore, research suggests that diet and lifestyle changes may be more effective for women with polycystic ovary syndrome than the insulin-lowering medication metformin (Kim *et al.,* 2020).

Regular exercise have been shown to significantly lower plasma homocysteine concentrations in young overweight or obese women in polycystic ovary syndrome (Parker *et al.,* 2020 and Woodward *et al*., 2020). Studies revealed that change in the lifestyle, including diet, exercise, and attitude have a positive impact on body weight, insulin resistance, and testosterone levels in polycystic ovarian women (Moran *et al*., 2019).

Herbal Intervention

Single herbs (e.g. *Salvia officinalis, Cimicfuga racemosa* and *Coptidis rhizome)* combined with conventional ovulation induction drugs (e.g., letrozole and clomiphene citrate) also showed many superiorities such as ameliorated ovulation, increased pregnancy rates thickened uterine wall thickness, and live birth rate, as well as regulated menstruation (Jamilian *et al*., 2020; Shahin and Mohammed., 2014).

Pueraria normalized menstrual cycle, IR markers, and serum level of triglyceride when used for 12 weeks in polycystic ovary syndrome patients (Raoofi, 2018). Cinnamon and Curcuma showed similar therapeutic effects on glucolipid profile, such as reducing FBG, IR, TC, and TG and enhancing HDL-C level in Polycystic ovary syndrome patients (Khan and Begum, 2019).

Ayurveda

Studies showed that Aleo vera gel formulation exerts a protective effect against the polycystic ovary syndrome phenotype by restoring the ovarian steroid status and altering key steroidogenic activity (Maharjan *et al*., 2010). Ayurveda theories of *Shodhana, Shamana* and *Tarpana* for 40 subfertility patients with polycystic ovary syndrome, the results revealed that, sub fertility due to polycystic ovary syndrome can be cured successfully by using this Ayurveda treatment regimen (Siriwardene *et al*., 2014). Bala *et al.,* 2020, revealed 87% of polycystic ovary syndrome patients were successfully cured by non surgical and non hormonal treatment and by using only ayurvedic treatment regimen.

Acupuncture

An animal experiment conducted by Zhang, (2010) showed that acupuncture could significantly reduce the number of cystic expanded follicle, increase corpus luteum and significantly improve the ovarian morphology. In addition, the studies have also shown that acupuncture can reduce the incidence of polycystic ovary syndrome by changing the distribution of specific intestinal flora, increase the content of beneficial bacteria, and also maintain the balance between the internal and external environment of the patients (Ding *et al.,* 2020).

Siddha

The classical Siddha Poly-mineral drug *Panchalvana Thiraavagam* a liquid form of medicine indicated for polycystic ovary syndrome as per traditional Siddha literature Kannusamiyam Ennum Vaidhiya Saegaram (Gnanasundari, 2014). The ovulation inducing activity of *Navachara Churnam* was carried out in rats and the results revealed that significant influence at the dose level of 50mg/kg and this marked effect was ensured with the histological evaluation of ovary of experimental rats also. Hence it may be concluded that the *Navanchara Churnam*is an excellent traditional medicine in the treatment of anovulatory conditions like polycystic ovary syndrome and the effect may be attributed to the elevation of the ovulation stimulatory hormones in animal model (Leelavathi *et al* ., 2013).

Yoga

A study conducted by Shanthi, (2013) showed that yoga can help control endocrine function and relieve symptoms of polycystic ovary syndrome. A study conducted among adolescent girls with polycystic ovary syndrome found that yoga was more effective than conventional physical exercises, it helped to improve glucose, lipid, and insulin values, including IR values (Nidhi *et al*., 2012). Patel *et al*., 2020 confirmed that regular yoga practice could reduce serum androgen in polycystic ovary syndrome patients, which is a useful complementary therapy.

Lifestyle Management

In polycystic ovary syndrome, obesity, central obesity and insulin resistance are strongly implicated. Short-term weight loss has been consistently successful in reducing insulin resistance and restoring ovulation and fertility. Hence, current conservative treatment should emphasize on sustainable weight loss through dietary modification and exercise. Modifying additional lifestyle factors, including psychosocial stressors, alcohol consumption, and smoking are also crucial in long-term treatment of polycystic ovary syndrome (Norman *et al*., 2002).

Studies have shown that dietary restriction alone and combined with exercise had similar benefits for improving depression and health-related quality of life (HRQOL) scores in overweight and obese women with polycystic ovary syndrome (Thomson *et al*., 2009).

Nutrition Management

As food and medication are the same in terms of nature, origin, taste and function, they are equally important in preventing and curing diseases (Zou, 2016) Dietetics therapy plays an important role in auxiliary treatment for polycystic ovary syndrome (Qi, *et al* ., 2019). The latest findings of Abdolahian *et al*., (2020) showed that green tea consumption has a significant effect on overweight and obese women with polycystic ovary syndrome, leading to weight loss and a drop in fasting insulin and free testosterone level.

Choudhary *et al*. (2012), opines that safe and effective treatment of polycystic ovary syndrome is a change in lifestyle, a low-carbohydrate diet, leafy vegetable, and high fiber content in diet. Omega 3 oils and a good multi vitamin with regular exercise are also useful. In recent years, complementary and alternative drugs has made some progress in the treatment of polycystic ovary syndrome, such as probiotics, melatonin, fish oil, fatty acid, vitamin D, vitamin K, carnitine, chromium, selenium. They are found to have certain intervention effects on polycystic ovary syndrome (Patten *et al*., 2020).

Probiotic foods showed that Lactobacillus could alleviate the symptoms of polycystic ovary syndrome in letrozole model rats. Lactobacillus play an important role in improving polycystic ovary syndrome by regulating and controlling the sex hormone-related microorganisms (Wang *et al.*, 2020 and Lei, 2021).

Vitamin D and Calcium have a direct effect on the ovarian and/or adrenal steroid genesis pathway. Three months supplementation with vitamin D and Calcium can reduce androgens (Pal *et al*., 2012). Krul- Poel *et al*., 2018 confirmed that vitamin D is a significant and independent factor predicting IR, and vitamin D status is closely related to the metabolic disorder of polycystic ovary syndrome. Vitamin D supplementation can reduce abnormally elevated serum AMH levels, and the reduction of serum AMH levels may improve follicular formation by reducing androgens in the ovaries of women with polycystic ovary syndrome and increasing the sensitivity of follicles to FSH (Irani *et al*., 2014). Jafari *et al*., (2018) confirmed that vitamin D supplementation and low-calorie diet improved the menstrual frequency in polycystic ovary syndrome patients.

Polycystic ovary syndrome patients provided with vitamin D deficiency supplements including calcium, vitamin D, and vitamin K for 8 weeks showed that insulin metabolism, serum triglyceride, and very low-density lipoprotein cholesterol levels improved (Rahmani *et al*., 2018).

Vitamin E plays a vital role in the entire reproductive process. It can antagonize the oxidative stress caused by oxygen free radicals and antioxidative imbalance and regulate the normal physiological functions of the reproductive system (Chen *et al*., 2020). The combined treatment of magnesium and vitamin E for polycystic ovary syndrome for 12 weeks has benefits for hirsutism, serum hs-CRP, plasma NO, and TAC levels (Shokrpour and Asemi, 2019). Treatment of vitamin E in patients with polycystic ovary syndrome showed a significant effect by significantly reducing TG and serum total cholesterol and improve IR, T, and free testosterone index in patients with polycystic ovary syndrome. Izadi *et al*., 2019 and Rahmani *et al*., 2017 confirmed that vitamin E can improve the endometrial thickness of patients with unexplained infertility.

Polyunsaturated fatty acids (PUFA) decrease insulin resistance and androgen excess in polycystic ovary syndrome (Dandona*et al*., 2010).Oner and Muderris, (2013) showed omega-3 also may be effective in decreasing hirsutism, BMI, LH, testosterone, insulin , Homeostatic model assessment (HOMA) levels, and increasing sex hormone-binding globulin (SHBG) and TNF-α in women with polycystic ovary syndrome. Fish oil can significantly improve the expression of PPAR-γ, IL-1, and IL-8 genes related to polycystic ovary syndrome; omega -3 fatty acids can reduce C-reactive protein and increase adiponectin levels to reduce the inflammatory state of women with polycystic ovary syndrome (Yi *et al*., 2021).

Folic acid is one of the supplements which have a well-known physiological effect on Homocysteine (Hcy) reduction. Polycystic ovary syndrome is one of the conditions that are associated with elevated homocysteine levels. Hence, folic acid supplement can reduce homocysteine levels. (Grodnitskaya and Kurtser, 2012).

Zinc supplementation has some beneficial effects on cardio metabolic risk factors in polycystic ovary syndrome women (Tabrizi *et al*., 2010). Several amino acids have been studied as a dietary antioxidant supplement to alleviate oxidative stress in polycystic ovary syndrome (Liu *et al*., 2021 and Wang *et al*., 2022).

Melatonin, an endogenous indoleamine synthesized from l-tryptophan, is mainly secreted by the pineal gland. Melatonin has certain effects on reducing oxidative stress, and acts as an antioxidant, anti-inflammatory, and antidepressant agent in polycystic ovary syndrome patient (Hu *et al*., 2020).

B. Polycystic Ovarian Syndrome and Oxidative Stress

It is clear that oxidative stress (OS) is involved in polycystic ovary syndrome disorder. Oxidative stress is defined as “a disturbance in the prooxidant/antioxidant balance in favor of the former, potentially leading to damage” (Sies, 2019). Oxidative stress discloses when the levels of oxidants significantly overcome the antioxidant defenses (Pisoschi and Pop, 2015). Both genetic and environmental factors can exacerbate Oxidative Stress (Ganie, *et al*., 2019).

Among the cellular components involved in regulation of OS levels, mitochondria play an important role, dysfunctions at this level have been demonstrated to assist in the pathogenesis of several diseases, including polycystic ovary syndrome, metabolic syndrome, and diabetes mellitus, cardiovascular disease, and cancer (Zhang *et al*., 2019 and Papalou O *et al*., 2016). In this regard, as a result of mitochondrial dysfuntions, systemic increase of OS occurs in patients of reproductive age who develop symptoms of polycystic ovary syndrome, and have found to have elevated serum levels of inflammatory markers such as C-reactive protein, interleukins, and proinflammatory cytokines, increased cell counts of leukocyte series such as lymphocytes and monocytes, change in tumor necrosis factor, as well as increase in some metabolites resulting from the processes of carbonylation and oxidation of proteins and lipids (Khashchenko *et al* ., 2020).

Polycystic ovary syndrome is also associated with oxidative stress which leads to increased androgen production (Zhang, 2012). In polycystic ovary syndrome, increased formation of lipid hydroperoxides and high levels of malondialdehyde (MDA) could be due to the increased oxidation of biomolecules leading to extensive lipid peroxidation in protein, membranes and genes. The high levels of MDA are indicator of free-radical mediated damage to the tissues (Akkol *et al.,* 2015).

Oxidative stress (OS) as one of the main causes of molecular damage to cellular is increased in women with the polycystic ovary syndrome. The circulating markers of oxidative stress are abnormal in women with polycystic ovary syndrome.

Several factors such as formation of reactive oxygen species (ROS) are involved in low-quality of embryos following in vitro culture. ROS blocks meiosis in oocytes and prevents embryonic development and induces cell death. Different antioxidants are applied to mitigate the negative effects of ROS in embryos (Sak *et al*., 2018).

Oxidative stress and inflammation play critical roles in pathophysiology of polycystic ovary syndrome (Mohammadi, 2019 and Schmidt *et al*., 2016). A high level of oxidative stress in patients with polycystic ovary syndrome has harmful effects on oocyte maturation and embryo development.

Obesity and insulin resistance play a vital role in the pathogenesis of polycystic ovary syndrome and subsequently increased OS in these patients (Al-kataan *et al*., 2010). Body iron stores reflected by serum ferritin levels are increased in non-obese women with polycystic ovary syndrome (Supriya *et al.*, 2012).

Numerous investigations have revealed that oxidative stress level is significantly increased in patients with polycystic ovary syndrome compared with the normal ones. Desai *et al*., (2014) displayed that the OS is also present in non-obese women with polycystic ovary syndrome

Oxidative stress and antioxidant decrease may lead polycystic ovary syndrome women to increased risk of cardiovascular disease, insulin resistance, hypertension, central obesity and dyslipidemia (Gupta *et al.*, 2010). Therefore, antioxidant supplementation may be effective in these patients. Antioxidants are considered significant agents in the healthy body. Many studies have displayed that the use of antioxidant, as well as herbal agents, might help to reduce OS (Bi., 2015).

C. Role of Antioxidants in the management of Polycystic Ovary Syndrome

Antioxidants are substances capable of scavenging free radicals and prevent them from causing cell damage. Antioxidants provide protection by neutralizing free radicals, which are toxic byproducts of natural cell metabolism (Infusino, 2020).

The non-enzymatic antioxidants include nutrients that are not produced by the body, and thus need, to be included through the diet. Nutrient antioxidants are found in fruits, vegetables, and fish, and are extremely important because each one of them has a role in oxidative stress neutralization (Pisoschi, 2015 and Liakopoulos *et al*., 2019). The human body naturally produces antioxidants but the process is not 100 percent effective in case of overwhelming production of free radicals and that effectiveness also declines with age. Increased intake of antioxidants can prevent diseases and lower the health problems (Infusino, 2020).

Food may possibly enhance antioxidant level because they contain a lot of antioxidant substances. Fruits and vegetables are rich in key antioxidants such as vitamin A, C, E, beta-carotene and important minerals, including selenium and zinc. Natural products, mainly obtained from dietary sources provide a large number of antioxidants (Saikat *et al*., 2021). Specifically, natural extracts, such as polyphenols, exert an antioxidant activity that includes suppression of ROS formation by either inhibition of enzymes involved in their production, like NOX2 (Carnevale *et al*., 2019), scavenging of ROS (Aboul-Enein, 2007), or up regulation or protection of antioxidant defenses (Li*et al*., 2006).

Medicinal herbs are expected to have a similar degree of efficacy without the side effect related to conventional medication. Different flavonoids and polyphenols isolated from cinnamon source have free-radical-scavenging activities and antioxidant properties. These compounds have been revealed to decrease oxidative stress in a dose-dependent manner through the inhibition of 5-lipoxygenase (Ranasinghe *et al*., 2013). Oral administration of lutein and nettle extract, alone and in combination, improved reproductive function by improving antioxidant activity in polycystic ovary syndrome induced mice (Bandariyan *et al*., 2021).

Azam Borzoei *et al.* (2018), confines that cinnamon supplementation had oxidative capability and improved oxidative stress in polycystic ovary syndrome women. Antioxidant supplementation has been shown to improve insulin sensitivity and other health threating conditions in women with polycystic ovary syndrome (Sekhon, 2010). The *Vitex agnus-castus* VAC plant extract effectively reversed the polycystic ovary syndrome induced changes in hormones, lipid profile, oxidative stress and glycemic status (Hamza *et al*., 2019). Combined use of carnitine and chromium can improve the mental health indicators, serum total T, high-sensitivity C-reactive protein , Total Antioxidant Capacity (TAC), and Malondialdehyde (MDA) levels of women with polycystic ovary syndrome (Karamali *et al*., 2017).

A study by Masjedi *et al*., (2019) demonstrated a significant reduction in superoxide dismutase (SOD) activity in serum and follicular fluid of patient with polycystic ovary syndrome. A recent cohort study exhibited that, a short-term supplementation of vitamin E to polycystic ovary syndrome patients can reduce oxidative stress, and reduce exogenous human menopausal gonadotropin dosage in the ovulation induction cycle (Chen *et al*., 2020).

Liang *et al*.(2020) revealed that considerable changes in the composition of gut microbiota have been observed in patients with polycystic ovary syndrome. Xue *et al*., (2019) suggested that restoration of gut microbiota dysbiosis can be done through dietary probiotic inulin supplementation or α-linolenic acid-rich flaxseed oil contributes to the amelioration of polycystic ovary syndrome (Wang *et al*., 2020).

Changes in serum vitamin D levels have also been investigated in polycystic ovary syndrome patients (He*et al*., 2015). Vitamin D plays a critical role in the regulation of inflammation and oxidative stress (Wimalawansa, 2019). Vitamin D supplementation to polycystic ovary syndrome patients improved some markers like C-Reactive Protein, malondialdehyde, and total antioxidant capacity, but was ineffective on others like nitric oxide and glutathione, according to a recent meta analysis (Foroozanfard *et al*., 2015; Razavi *et al*., 2016 and Fatemi *et al*., 2017).

Some studies support the effect of vitamin D deficiency on pathophysiology of polycystic ovary syndrome and even insulin resistance (Liu *et al*., 2017). Pal *et al*., 2012 found that 3 months supplementation with Calcium (Ca) and vitamin D can reduce androgens. Calcium and vitamin D have a direct effect on the ovarian and/or adrenal steroid genesis pathway. Firouzabadi *et al*., (2014) also found calcium and vitamin D supplementation can make a positive effect on weight loss, follicle maturation, menstrual regularity and improvement of hyperandrogensim, in infertile women with polycystic ovary syndrome.

Oner and Muderris (2013) showed omega-3 also may be effective in decreasing hirsutism, LH, testosterone, insulin, BMI, Homestatic model assessment (HOMA) levels, and increasing sex hormone-binding globulin (SHBG) and TNF-α in women with polycystic ovary syndrome.

Rafraf *et al*., (2012) in their study found that supplementation of omega-3 fatty acids for 8 weeks can decrease total cholesterol (TC), low density lipoprotein (LDL), triglyceride (T), malonodialdehyde (MDA), and increase high density lipoprotein (HDL), but make no changes in total antioxidant capacity (TAC). The researchers concluded that omega-3 fatty acids are useful for polycystic ovary syndrome women in order to reduce lipids and lipid peroxidation levels.

Mohammadi *et al*., (2019) also found 8 weeks supplementation with omega-3 fatty acids can reduce TC/HDL-C and LDL-C/HDL-C ratios and increase paraoxonase – 1 (PON1) activity in comparison with placebo.

Gayatri *et al.*, (2010) showed significant effects of NAC (N-acetyl-cysteine) on the clinical features, biochemical markers of insulin resistance, anovulations, hormonal levels and oxidative stress inhibition in polycystic ovary syndrome women. Tabrizi *et al.*, (2013) in a randomized, double- blind, placebo-controlled parallel groups trial on 65 polycystic ovary syndrome women showed that 8 week supplementation with 50 mg of zinc in the zinc sulphate form can rise serum zinc significantly and also reduce homeostasis model of assessment-insulin resistance score, fasting serum total cholesterol, triglyceride, LDL-C, testosterone, and TG/HDL-C ratio in comparison with placebo. They believed zinc supplementation for polycystic ovary syndrome women has some beneficial effects on cardio metabolic risk factors.

Patrick *et al*., (2012) found that 3 months supplementation with vitamin D and Calcium can reduce androgens and had a direct effect on the ovarian and/or adrenal steroid genesis pathway. Firouzabadi *et al*., 2014 also found calcium and vitamin D supplementation made positive effect on weight loss, follicle maturation, menstrual regularity, and improvement of hyperandrogenism, in infertile women with polycystic ovary syndrome. One study on calcitrol supplementation showed an increase in PTH, systolic BP, and ovulation (Bonakdaran *et al*., 2012). Rashidi *et al.*, 2014 suggest combination of calcium-vitamin D therapy increases therapeutic effects of metformin treatment.

Polycystic ovary syndrome is one of the conditions that are associated with elevated homocysteine levels (Han *et al.*, 2015). Folic acid is one of the supplements which have a well-known physiological effect on homocysteine reduction. So it can improve endothelial function either due to its effect on homocysteine or even via other mechanisms that are not associated to homocysteine (Moens *et al*., 2007 and Antoniades *et al*, 2009). According to Palomba *et al*., (2010) six months treatment with metformin and folic acid, could cause a significant improvement in all the markers of structure and function of the vascular endothelium and this improvement was significantly different between folic acid supplementation and placebo groups.

D. Awareness on consumption of Underutilized vegetable greens

The term “underutilized vegetables” refers to vegetable crop that are neither commercially grown nor widely traded. Despite the fact there are 7,000 edible crop species, only about 30 of them provide 95 percent of the household’s food. Many crop species have been neglected as a result of modern agricultural practices, and their genetic pools have been eroded to the point where they are now considered lost crops (Chable *et al.,* 2020).

(i) Global scenario on the awareness and consumption of underutilized vegetable greens

India’s climate permits the growth of a variety of range of improved crop production that will give national security with many essential nutrients. Although, India has diverse agro climatic conditions which is suitable for growing more than 60 varieties of cultivated crops and about 30 lesser known vegetable crops, but not much attention is given on underutilized vegetables. (Kumar *et al*., 2018). Though the growth of crops has increased the need to focus on providing a balanced meal, but much attention is not given on the ways of utilization of underutilized vegetables. In general, edible green leafy vegetables appear to be under-utilized by regional-specific peoples or throughout the world (Mathew, 2013).

For food welfare and livelihoods around the world, vegetable play a crucial role. As animal foods are often too costly for low-income families, various underexploited vegetables can be a replacement for vitamins, micronutrients, and secondary plant metabolites that promote health (Tripathi *et al*., 2020)

In comparision to China, India is well- known for its vegetable cultivation. The average Indian citizen consumes only 175 grams of vegetables per day. Green Leafy Vegetables (GLV) play an important role in Indian cuisine because of their high nutritional, vitamin, mineral, and dietary fibre content, as well as lysine and tryptophan, medicinal value, nutraceutical, pharmaceutical, and functional properties (Bhavithra *et al*., 2020).Besides, leafy vegetables are rich in antioxidants that helps to scavenge free oxygen radicals in the body and protect against many disease (Law-Ogbomo *et al*., 2017).

Kuhnlein *et al.*, (2013) also defines underutilized or neglected plants are those edible plants that remain neglected, though they possess good potential nutrients, to contribute to food security and poverty alleviation. These plants are referred to as underutilized or neglected plants. Durst and Bayasgalanbat, (2014) presented a paper in the regional symposium stated that traditional foods like forest fruits, sago palm, medicinal wild plants and edible insects are neglected traditional foods in Asia.

Ahmad and Javed (2012) conducted a research on exploring the economic value of underutilized plant species in Ayubia National Park, Pakistan stated that six most prominently used medicinal and food species viz., *Adhatoda vasica, Artemisia scoparia, Galium aparine, Amaranthus viridus, Hedera nepalensis* and *Utrica dioica* that gained little attention by scientific research and local community. They were investigated in detail for exploring their economic values. Better communication with local communities improved awareness, importance and preservation of the diversity of these 6 underutilized species and build capacity among stakeholders.

Addis *et al*., (2013) opines that except in few cases of South Ethiopian communities consider consumption of underutilized edible plant as a sigh of poverty, largely a reflection of lack of knowledge on their nutritional benefits.

Buragohain *et al*. (2013) in his study collected and evaluated twenty five underutilized leafy begetables from the state of Meghalaya showed that these leafy vegetables play an important role in the life of rural people. They form an important part of food and nutrition for their utilization in terms of medicinal, therapeutic and nutritional values since time immemorial and are consumed either as raw or as cooked vegetables as traditional delicacies.

Pandey *et al.* (2014) examined that underutilized vegetables are embedded with rich nutrient potentials may prove boon to all concerns –growers, consumers and environmentalists, provided that they are tamed properly. The possible reasons for the low utilization of underutilized vegetables is lack of information on how they can fit into production systems and non-viability of indigenous vegetable production like the major cultivated species of vegetables such as tomato, pepper, eggplant, cauliflowers, cabbage, etc. Lamo *et al.* (2012) also observed that long before the new vegetables were introduced, Ladakhi people survived on the traditional wild vegetables that grew almost everywhere in their region.

Ballabh and Chaurasia, (2009) has also reported utilization of Ethno-medicinal properties of various underutilized greens. Mitra, (2010) reports that underutilized plants are well adapted to different environmental conditions and can withstand biotic and abiotic stresses.

Salvi and Katewa, (2016) conducted an ethnobotanical study in southern Rajasthan with local indigenous communities who used herbs as primarily as leafy vegetable. The study showed that majority of species are edible after cooking and among that 28 percent were eaten raw. The study suggests that these underutilized plants may play important role in national food security policy and health care, and awareness should be made to the younger generation on the importance and uses of these underutilized plants.

(ii) Nutrient and Phytochemical constituents present in Underutilized vegetable greens

Plants contain various bioactive components, including alkaloids, flavonoids, gums, coumarins glycosides, polysaccharides, phenols, tannins, terpenes, and terpenoids, which possess various biological activities that benefit health. These effects encompass antioxidant, antidiabetic, anti-obesity, diuretic anticancer and anti-inflammatory activities, which enhance the immune system (Hasrini *et al*., 2017).

Emerging as Nutraceuticals, Phytochemicals are bioactive compounds which are obtained from plants including luteins, diallysulfides, isothiocyanates, antioxidants polyphenols, carotenoids, vitamins, glucosinolates, phytoestrogens, anthocyanins and flavonoids. Mostly found in vegetables, they have many potential health benefits (Capels *et al*., 2015, Rios-Chavez *et al*., 2019; Yalcin and Capar, 2017). Underutilized green leafy vegetables (GLVs) which not only increase iron and serum retinol concentrations but also help in lowering cholesterol and control blood glucose levels (Sahu*et al.*, 2020). There are green leafy vegetables which are underutilized in many locations of India that can be exploited for natural antioxidants and antinutritional factors (Subhashree *et al*., 2009).

Natesh *et al*., (2017) opines that green leafy vegetables have an important place in our diet due to their high micronutrient content and the presence of anti-nutritional factors should not hinder their consumption. It was observed in the following study that the anti-nutritional factors assessed have little or no impact on the absorption of nutrients and in turn, they help enhance the phytochemical profile of the vegetables. Different processing techniques have an impact on the anti-nutritional factors in foods and thus can be utilized to decrease the amounts of undesirable facts.

Leafy vegetables have been reported to be a significant source of antioxidants, vitamins, dietary fibers, and essential amino acids (Nkongho *et al*., 2014). Sheela *et al*., (2004) opine that underutilized greens are found to possess ample amount of vitamins and minerals. Gupta *et al*., (2013) substantiated the same that underutilized green leafy vegetables were good source of many nutrients like iron, calcium, carotene, ascorbic acid that could help in overcoming malnutrition at a negligible cost. The conservation and survival of these species is a must for sustainable development in order to meet up increasing demands and targeting the future potential demand.

Saha *et al*., (2015) contends that various types of underutilized plant parts which are available seasonally have no available information on the nutritional value. Sankhala (2019) observed that underutilized green leafy vegetable generally grow in arid, hilly and tribal areas. They are rich sources of vitamins and minerals and other nutrients that can provide a solution to the problem of malnutrition to a great extent provide the masses.

Pandey *et al*., (2014) opines that leafy vegetables like *Laminum album* used by tribals of Gurez valley of Kashmir are very rich in minerals (20.4 percent) and proteins (26 percent). Seeds of *Cicer soongaricum,* a wild plant of Ladakh area, contain high protein and phospholipids that contains 1% lecithin, as is present in soybean.

Abuajah *et al*., (2015) suggest that pytochemicals, plant-based non-nutritive compounds contribute toward biological activity, aid in protecting the body against non-communicable diseases. An approximately 120g portion of fruits and vegetables provides 100 different phytochemical (Srividya *et al*., 2010), among which phenolic compounds are the most abundant functional compounds. African traditional leafy vegetables are rich in vitamin A and meet more than 75% of the recommended dietary allowance (RDA) (Jaarsveld *et al*., 2014). Phenolic compounds positively correlated with antioxidant activity (Katerere *et al*., 2012). Current consumer trend is to replace synthetic antioxidants with natural dietary antioxidants for health benefits. Vitamin E (tocopherols) is important for disease prevention by preventing the breakdown of polyunsaturated fatty acids in membrane lipids and alleviating the oxidative stress (Lee *et al*., 2015).

Joshi and Mathur, (2010) investigated the nutritional potential and acceptability of leaf mixtures (LM) prepared from the less utilized greens of beet root (*Beta vulgaris),* carrot (*Daucus carota),* cauliflower (*Brassica oleracea)* and turnip (*Brassica rapa)* which are usually discarded or are used as animal fodder in a definite ratio (1:2:1:1). The Leaf Mixture (LM) was analyzed for the proximate composition, mineral composition (Ca, P, Fe, Mn, Cu, Zn, and Mg) and antinutritional composition (oxalate and phenols). The findings showed that the LM was rich in micronutrients like iron and calcium and provided good amounts of energy and protein. High protein diets help in better absorption of iron and thus became a concentrated source of almost all the nutrients. Iron and calcium content of the recipes was directly proportional to the level of LM incorporated. Thus, it was suggested that these leaves can be used for the preparation of value added products rich in micronutrients.

Saha *et al.* (2015) demonstrated a study on the nutritional, anti nutritional and mineral compositions of eight locally available underutilized green leafy vegetables of Sonitpur district, Assam. Among the underutilized green leafy vegetables namely*Basella rubra, Basella alba, Diplazium esculentum, Chenopodium album, Amaranthus viridus, Brassica nigra, Brasscica juncea. Basella rubra* had high fiber (8.5 g/100g) compared with *Moringa oleifera* and *Amaranthus viridus* (0.2g/100g). Ascorbic acid was higher in *Diplazium esculentum* (23.5 mg/100 g) and lower in *Brassica nigra (*8.5 mg/ 100 g), beta-carotenoid ranged between 4.65 – 18.90 mg/100 g in all the green leafy vegetables. Antioxidant and phenolic contents of the green leafy vegetables were almost similar. *Brassica nigra* had the highest iron content (241.2 mg /100g) and *Moringa oleifera* recorded the lowest iron content (29.4 mg /100 g). Calcium, potassium was almost were in the same range in all the leafy vegetables, whereas sodium varied from 29.0 – 116.6 mg/100 g respectively. Highest zinc content was recorded in *Brassica juncea* and *Chenopodium album*(7.50 mg /100 g) and lowest zinc content was found in *Moringa oleifera* (1.50 mg /100 g). Based on the analysis performed in this study it can be concluded that the green leafy vegetables, which are mostly neglected, have a good potential in terms of food value and can serve as an easily accessible food resources.

Singh *et al.* (2015) conducted research to formulate and analyse value-added products made from underutilized fresh Indian sorrel leaves *Oxalis corniculata*, also known as wood sorrel. Value added products were analyzed for the proximate constituents of minerals and vitamin C. Products enriched with fresh Indian sorrel leaves were rich in energy, carbohydrate, protein, iron, calcium and vitamin C. Idliincorporated with Indian sorrel leaves seemed to have fat (18.7±0.5), fiber(24.1±1.3), protein(1.2±2.4), energy(91.2±54.4), carbohydrate(20.0±11.7) and vitamin C (26.1±3.1). The study concludes that incorporating Indian sorrel leaves into a variety of foods can improve nutritional quality. Similar study was also done by Umar *et al*., (2015) on the leaves of *Ipomoea aquatic.*

Pertus, (2014) reported that highest phenolic content in multivitamin greens in *Acalyphia indica L, Alternanthera brasiliana L, Alternathera sessilis L, Cardiospermum halicacabum L, Celosia argentea L, Chenopodium album L, Centella asiatica L, Coccinia garandis L, Hisbiscus cannabinus L, Ipomoea aquatic Forssk, Pisonia alba Span, Sauropus androgynus L, Talium fruticosum L, Solanum trilobatum L* weed that are beneficial to humans to prevent oxidative stress to muscles.

Sahu *et al.*, (2020) stated that ascrobic acid was maximum in *Acalypha indica* among the fifteen underutilized leafy vegetables evaluated. A study on underutilized green leafy vegetables viz., *Ipomoea aquatic Forssk*, *Sauropus androgynus* L, *Alternanthera brasiliana* L., are having a fair amount of Phosphorus in descending order (Bhavithra *et al*., 2021). The high amount of potassium increases iron utilization and also beneficial to the people taking diuretics to control hypertension (Archana *et al*., 2012).

Verma Anisha and Neeru Bala, (2014) observed that in India various types of underutilized foods are available seasonally but are not utilized to the extent they should be in spite of their higher nutritive value and utilization of underutilized foods can be explored to overcome nutritional disorders.

Ebert, (2014) examined that the two traditional vegetable crops Amaranth and Drumstick tree are considered underutilized for its low global production. Amaranth is a very nutritious leafy vegetable, both in raw and cooked form. The nutritional value of this crop is comparable to spinach, but much higher than cabbage and Chinese cabbage. Moringa has a high nutrient density and is rich in many essential micronutrients and vitamins as well as antioxidants and bio available iron. It excelled among 120 species of Asian traditional vegetables tested for their content of micronutrients and phytochemicals antioxidant activity (AOA), and traditional knowledge of their medicinal uses.

Gupta *et al.,* (2005) undertook an investigation with the objective of exploring the lesser-known underutilized GLV grown in and around Mysore district of Karnataka state, South India. The nutrient composition of underutilized GLV revealed to be good sources of many nutrients like iron, calcium, beta-carotene and ascorbic acid that could help in overcoming micronutrient malnutrition at a negligible cost. They also had a high fiber content, moisture, ash and ether extract of the greens were in the range 73-95 g/100g, 0.7-3.5 g/100h and 0.2-0.9g/100g, respectively. Four GLV had high iron content (13.1-17.7 mg/100g) while the rest had lower levels (2.6-9.8 mg/100 g). Beta-carotene was 13-25% of total carotene in all greens.

Radish leaves (*Raphanus raphanistrum subsp.sativus)* is consumed worldwide though we quit stopping at the roots, the radish leaves have an abundance of high levels of vitamins and mineral. It is also a very good detoxifer which helps to increase immunity and reduces fatigue. It is naturally diuretic with antiscorbutic properties (Mishra, 2020). Jing *et al.,*(2016) reports that *Cypercus esculentus* is an nderutilized plant which is effective in the treatment of spleen, liver and stomach related disorders. Thus, it is clear from the available literature that underutilized plants are good bio resource that can be utilized properly.

E. Nutraceutical Potential and Antioxidant Effect of Beet greens (*Beta vulgaris L*)

Beetroot (*Beta vulgaris* L), a traditional vegetable in many countries, belongs to the family of Amaranthaceae. Its leaves, known as “beetroot greens”, are occasionally utilized as an organic fertilizer, animal feed, and traditional medicine (Amnah, 2013). Due to the lack of awareness or 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 Southern and Eastern Europe and Northern Africa. The world beet root production in 2017 reached 301 million tons with a harvested area of 4.8 million hectares. Europe ranks the first in beet root production (207.9 tons), representing 69% of world beet production, followed by Asia (42.7 tons), America (34.3 tons) and Africa (15.9 tons) (FAOSTAT, 2019).

Beet root leaves *Beta vulgaris* are commonly cut off and discarded before using its bulb due to lack of knowledge of how to use them. Beet tops are one of very versatile, nutritious green leafy vegetables. Nonetheless, they are one of the healthiest greens recommended in the diet for their low fat, no cholesterol but health benefiting minerals, vitamins, and antioxidants. High quality nutrition profile of beet greens offer protection from vitamin A deficiency anemia and believed to protect from cardiovascular disease. Beet root greens are loaded with vitamin A, C, K and some key B vitamins like folate. It contains essential trace and major minerals including iron, calcium, potassium and magnesium (Kaushik *et al*., 2020).

Due to the high nutritional value, leaves are widely consumed as vegetables world wide. These phytochemicals have been reported as a potent antioxidant (Lee *et al*., 2017). Since traditional times roots and leaves of beet root have been used for different ailments. It was used by ancient Romans for treating pyrexia and constipation. It has been used for wound healing by Hippocrates. Beet juice was considered as aphrodisiac in middle ages. Various systemic disorders of blood, liver, heart, lung, gastrointestinal and central nervous system have been treated using beetroot (Azeredo *et al*., 2009).

Fernandez *et al*. (2017) demonstrated that beetroot leaf and its bulb are both valuable sources of nutrients, besides the values of anti-nutrition (tannins, oxalate and phytate) in beetroot leaves are either equal or lower than that of some other leafy vegetables like spinach (*Spinacia oleracea* L). Beetroot leaf contains protein, fiber, minerals and vitamins like pyridoxine, cyanocobalamin, folic acid and calcium, phytochemical compounds including phenolic compounds (quercetin, kaempferol and rutin), plant pigments (chlorophylls, carotenoids and betalains) and consequently antioxidant activity. Moreover, beetroot leaf is a rich source of omega-3 such as linolenic acid (Biondo *et al*., 2014).

It is known that beet roots are widely used in medical nutrition due to the presence of such biologically active substances as iron, cobalt, copper, zinc, vitamin C, folic acid. Along with this, there are studies of scientists in which it is proved that the content of vitamins and biomicroelements in fresh beet leaves have a higher biological value (Rajaeifar *et al*., 2019).

Beetroot greens (*Beta vulagris*) are packed with antioxidants, just like the red tuberous part of the plant. Beet greens (*Beta vulgaris*) contain large amount of nitrates which convert to a compound called nitric oxide in the body, nitric oxide has a positive effect as vasodilation and helps to lower blood pressure (Konstantinova and Popova, 2020). The foliage of the red beet is a delicious green vegetable, with higher content of various nutrients than in the roots. Beet foliage is rich in carotenoids (beta carotene, lutein and zeaxanthin), flavonoids and vitamin C, which are strong antioxidants and folic acid (a component of vitamin B complex, which is needed to release energy) and is useful in the functioning of the nervous and immune system and haemopoiesis (Neelwarne *et al*., 2013).

Fernandez, (2017) demonstrated that beetroot leaf and its bulb are both valuable sources of nutrients, besides the values of anti-nutrition (tannins, oxalate and phytate) in beet root leaves are either equal or lower than that of some other leafy vegetables like spinach (*Spinacia oleracea* L).Beetroot leaves extract has no harmful effects even at a higher dose of 2000mg/kg and apparently shows several health benefits (Abd El-Ghffar *et al*., 2019).

The oxalate content in beet leaves was 8328.6 ±122.2 mg kg-1 in fresh tissue (FT). It is well known that beet is one of the plants with higher content of oxalates (Simpson*et al*., 2009), especially when considering that the content of this anti-nutrient is usually similar to that found in other leafy vegetables commonly consumed in fresh salads. Particularly, oxalate content in spinach was extensively studied presenting values in the range of 4000-17650 (Shepherd *et al.*, 2019). Therefore, the oxalate content of beet leaves does not constitute a constraint for the development of a product for fresh consumption. In this case, like for other leafy vegetables, the only restriction would be associated with the recommendation of American Dietetic Association that establish a limit of dietary oxalate intake of 40 to 50 mg per day (ADA Nutrition care Manual, 2006) for people who have a tendency to form kidney stones.

Tannin content present in beet leaves were 84.6±9.4 mg kg-1 in fresh tissue(FT). This is a low value compared with those reported in other leafy vegetables consumed in a fresh way. In this sense, Amalraj and Pius, (2015) informed that tannin content were between 860 and 4240 mg kg-1 in a variety of green leafy vegetables commonly consumed in India.Phytate content in beet greens was 34.3±4.0 mg kg-1 in fresh tissue (FT). This is also a low value compared with those reported for other leafy vegetables (Singh *et al*., 2015). Ndlovu *et al*., (2021) found phytate content of 1171.6 and 1464 mg kg-1 in coriander, cabbage and spinach, respectively.

The antioxidant capacity of beet green (*Beta vulgaris*) was very high, placing them among the vegetables with the highest antioxidant capacity. Due to its antioxidant properties, the consumption of beet leaves helps to slow down the body aging process (Konstantinova *et al*., 2020).

Among phytochemical components, phenolic compounds constitute an extended group with health beneficial action mainly related to its antioxidant activity. Epidemiological studies correlate its ingestion with a lower incidence of chronic diseases such as cardiovascular disease, diabetes and cancer (Lima *et al*., 2014). The total phenolic content in beet greens resulted higher than that found for other leafy vegetables (Llorach *et al*., 2008).

The identification of polyphenols by TLC reveals the presence of quercetin, kaempferol and rutin in the enriched methanolic extract of beet leaves. The subsequent HPLC analysis revealed that the main polyphenol on beet leaf (61% of total polyphenols) is Rutin, with a concentration of 9.7 mg kg-1, while the other two were found in low concentrations (0.012 and 0.001 mg kg-1 for quercentin and kaempferol, respectively), Rutin (quercetin -3-O-rutinoside) is a flavonoid ubiquitously found in plants (Horcajada *et al*., 2015). The proximate composition of beet greens as well as its phytochemical composition revealed them to be good sources of many nutrients like iron, polyphenols and betalains that could help in overcoming micronutrient malnutrition at negligible cost (Fernandez *et al*., 2017). Beetroot leaves are an excellent source of omega-3, in addition to having significant antioxidant activity and amounts of total phenolic compounds, macro and micro mineral (Biondo *et al*., 2014).

The nitrate, phytate and oxalate content of the leaves would decrease by boiling it in water and then in 5% sodium chloride solution. Therefore, boiling the leaves of *Beta vulgaris* in 5% NaCl solution would increase its carotenoid contents only more than the boiling water (Singh *et al*., 2015).

Beetroot leaf contains protein, fiber, minerals and vitamins e.g. pyridoxine, cyanocobalamine, folic acid and calcium, phytochemical compounds including phenolic compounds (quercetin, kaempferol and rutin), plant pigments (chlorophylls, carotenoids and betalains) and consequently antioxidant activity. Moreover, beetroot leaf is a rich source of omega-3 such as linolenic acid (Biondo *et al*., 2014). The antioxidant capacity of beet greens was very high, placing them among the vegetables with the highest antioxidant capacity.

Beetroot leaves (*Beta vulgaris*) are used to reduce blood pressure, as a tool to fight against cancer, to regulate digestion and to fight against skin discomfort and dandruff. Beet leaves are rich source of potent nutrient potassium and vitamin A, K and C, which are important for cardiovascular health. In day today life the young leaves of beetroot is good for health, it is rich with high amount of nutrients and minerals when compared with matured leaf (Suganya *et al*., 2017). The use of beet stalks and leaves can help reduce the liver damage caused by a high fat diet and improve the alterations in metabolic parameters due to the presence of flavonoids, such as vitexins (Lorizola *et al*., 2018). The dried beet greens exhibits anti-anemic activity (Elaby *et al*., 2018). The *Beta vulgaris* leaves and stalks showed strong hemanitic and anti-anemic potential (Gheith *et al*., 2018).

The iron content in beet greens is higher compared to spinach. The content of vitamin A in 100 g of fresh beet greens is 35% of the daily requirement for humans. Vitamin C content in beet greens is 33% of the daily requirement. Beet greens contain up to 300% of vitamin K, which helps to prevent osteoporosis. In addition, vitamin K has the ability to keep the human brain neurons in a stable position, which eliminates the likelihood of developing Alzheimer’s disease. (Konstantinova *et al*., 2020).

Beetroot leaves (*Beta vulgaris*) extract had no harmful effects even at a higher dose of 2000 mg /kg and apparently showed several health beneficial effects, such as immune modulatory, antioxidant, hepatoprotective, hyperglycemic and lipid lowering effects against alloxan-induced diabetes and hepatotoxicity in a rat model. It is to consider *Beta vulgaris* leaves as a dietary adjunct for the control of metabolic syndrome in diabetes patients (Adb El-Ghffar *et al*., 2019).

The content of vitamin A in 100 g of fresh beet greens is 35% of the daily requirement for humans. Vitamin A is responsible for maintaining the reproductive function. Beetroot leaves are a source of folic acid, a substance that is extremely necessary for the female body during the period of fetal formation (Konstantinova *et al*., 2020). Beet greens are rich in vitamin B9, folate which is necessary for both adults and infants as it regenerates red blood cells in the immune system.

Food is the main source of principal antioxidants represented by phenolic compounds. They exhibit anti-inflammatory and anticancer properties, which has been well documented (Shahidi *et al*., 2018). One of the plants with a high level of phenolic compounds are beetroots and Swiss cards, which have been undervalued in terms of their pro-health influence to date (Clifford *et al*., 2015). Neverthless, the prominent properties of beetroot as a promising preventive or therapeutic agent in oxidative stress and inflammatory conditions are reported. In particular, there is ample evidence now that the contribution of beetroot in cardiovascular disease was likely due to its ability to diminish no bioavailability and improve endothelial function. Given the high level of phenolic compounds and antioxidant potential of Beetroot leaves, inclusion thereof in the diet may bring natural protective effects against so called disease of affluence, eg., cancer or cardiovascular disease and other free radical related disease (Pyo *et al*., 2004).

Red beetroot (*Beta vulgaris rubra*) as a source of nitrate, provides a natural means of increasing *in vivo* nitric oxide (NO) availability. It is also being considered as a promising therapeutic treatment in a range of clinical pathologies associated with oxidative stress andinflammation. The betalain pigments, display potent antioxidant, anti- inflammatory and chemo-preventive activity *in vitro* and *in vivo*. It helps in prevent cardiovascular disease,cancer and it also helps to reduce blood pressure, attenuate inflammation, avert oxidative stress, preserve endothelial function and restore cerebro vascular haemo dynamics. Beetroot is an excellent food which plays a very important role for the development and growth of human body. It also acts as fruit as well as vegetable. Fresh form of beet root consumed generally as a salad. Other than as a food, it play another role as a natural colorantin textile industries and as a medicinal plant to cure the various illness. The *Beta vulgaris, L.* is a traditional and most popular vegetable in many parts of the world. Beet root leaves are rich source of potent nutrient, potassium and vitamin A, K and C, which are important for cardiovascular (CVD) health. Beetroot leaves are used to reduce blood pressure and also as a tool to fight against cancer. The beetroot leaves can be advised to children, adults, pregnant women and diabetes patients (Prasuna *et al*., 2008).

Maleki *et al.* (2021) indicated that green tea extract (GTE) supplementation has potential beneficial effects on PCOS induced rats. Despite a lack of human studies on ovarian histology, animal studies support the impact the GTE improved ovarian function and histology. Moreover, GTE, could lead to improving glycemic control in PCOS and may decrease body weight, Leutinizing hormone, and androgens in PCOS induced rats. A study done by Datu Agasi Mohd Kamal *et al*., 2021 found catechins of green tea possess health benefits and effects against endometriosis, PCOS and dysmenorrheal in animal studies.

Peppermint (*Menthapiperita*) supplementation of 40 wistar rats with letrozole-induced PCOS caused improvement of ovarian cysts, necrosis of stromal mesenchymal cells and hyperplasia of luminal epitherlial cells (Amoura *et al*., 2015). Another study by Bardei, 2015 showed supplementation of Raspberry (*Rubusidaeusstrigosus*) for 120 wistar rats with estradiol valerate induced PCOS and the treatment groups treated with 100, 150, and 200 mg/kg of the extract for 60 days resulted decrease in testosterone, estradiol, lutenizing hormone, and C-reactive protein (CRP) and increase in progesterone and follicle stimulating hormone.

Fennel supplemented in doses of 250, 500 and 1000 mg/kg of the extract for 30 rats with estradiol valerate for 60 days showed increased concentration of follicle stimulating hormone and decreased lutenizing hormone and testosterone levels (Rajan and Balaji, 2017). Aleovera extract administered for 60 days for letrozole induced PCOS rats showed decrease in the levels of testosterone and insulin and improved levels of progesterone and estradiol (Radha and Laxmipriya, 2016).

Phytoesterogen rich foods including fruits like plum, pear, apple, grapes, berries, vegetables like beans, sprouts, cabbage, spinach, soybeans, grains, hops, garlic, onion, wine tea, help in reducing testosterone and increased insulin level in PCOS patients. The current study, therefore, aimed to formulate and evaluate affordable underutilized beet greens for treatment with PCOS.