4. RESULTS AND DISCUSSION

The results of the study entitled "**"*In vivo* Effects of *Beta vulgaris* L. Leaf Extract on Polycystic Ovarian Syndrome Induced Adult Rats"** are discussed under the following headings.

Phase I: Determination of total antioxidant, total phenol and total flavonoid in the selected underutilized green leafy vegetable powder

A. Determination of total antioxidant in the selected underutilized green leafy vegetable powder
B. Determination of total phenol in the selected underutilized green leafy vegetable powder
C. Determination of total flavonoid in the selected underutilized green leafy vegetable powder
D. Justification for selecting *Beta vulgaris* L. leaf powder for further study

Phase II: Screening of phytochemicals, antioxidant activity and nutrients content of *Beta vulgaris* L. leaf extract

A. Pesticide screening in *Beta vulgaris* L.leaf powder
B. Phytochemical screening in *Beta vulgaris* L. leaf extract
C. Quantitative analysis of anti nutrients in *Beta vulgaris* L. leaf powder
D. Determination of antioxidant activity in *Beta vulgaris* L. leaf extract
E. Screening of nutrients in *Beta vulgaris* L. leaf powder

Phase III: Screening of flavonoids, secondary metabolites and fat soluble vitamin A and E of *Beta vulgaris* L. leaf extract

A. Screening of flavonoids in *Beta vulgaris* L. leaf extract by HPLC method
B. Screening of Secondary Metabolites in *Beta vulgaris* L. Leaf Extract using GCMS
C. Screening of fat soluble vitamin A and E in *Beta vulgaris.* L. leaf extract by HPLC

Phase IV: Acute toxicity study of *Beta vulgaris* L. leaf aqueous extract on adult female rats

A. Acute Toxicity study of *Beta vulgaris* L. leaf aqueous extract on adult female rats
B. General observation of acute toxicity study of *Beta vulgaris* L. leaf aqueous extract on adult female rats

C. Observation of Gross Behavior of *Beta vulgaris* L. leaf aqueous extract on adult female rats

Phase V: *In-vivo* effects of *Beta vulgaris* L. leaf aqueous extract on polycystic ovarian syndrome (PCOS) in adult female rats

A. Evaluation of Estrous Cyclicity of Experimental Animals

B. Evaluation of the Effects of *Beta vulgaris* L. Leaf Aqueous Extract on PCOS Adult Rats in Association with Physical Parameters

C. Evaluation of the Effects of *Beta vulgaris* L. Leaf Aqueous Extract on PCOS Adult Rats in Association with Biochemical Parameters

D. Evaluation of the effects of *Beta vulgaris* L. Leaf aqueous extract on PCOS adult rats in association with reproductive hormones

E. Evaluation of the effects of *Beta vulgaris* L. Leaf aqueous extract on PCOS adult rats in association with hepatoprotective and oxidative stress

F. Histopathology Results of the PCOS Control Adult Rats Ovary

Phase I: Determination of Total Antioxidant, Total Phenol and Total Flavonoid in the Selected Underutilized Green Leafy Vegetable Powder

Green leafy vegetables are high in nutrients and health-promoting bioactive metabolites like antioxidants, phenolic compounds, and flavonoids, which help to prevent the spread of non-communicable disease (Moyo *et al.,* 2020). They are inexpensive and readily available, and they help to improve the micronutrient quality and diversity of local diets, as well as reduce the burden of 'hidden hunger,' thereby improving health (Icard-Verniere *et al*., 2015 and Uusiku *et al*., 2010). Hence, total antioxidants, total phenol and total flavonoid capacity were analyzed in the selected underutilized vegetable greens powder namely carrot, radish and beetroot.

A. Determination of total antioxidant in the selected underutilized green leafy vegetable powder

Green leafy vegetables contain high antioxidant capacity (AC) pigments such as betaxanthins, carotenoids, betacyanins, betalains, anthocyanins, and chlorophyll. Antioxidant compounds quench reactive oxygen species (ROS) in the human body and protect against diseases such as cardiovascular disease, cancer, retinopathy, emphysema, arthritis, atherosclerosis, and neurodegenerative diseases

(Sarker *et al.*, 2020). Antioxidants, particularly natural antioxidants that inhibit and prevent damage caused by free radicals and reactive oxygen species (ROS), are receiving a lot of attention (Hossain *et al*., 2017). Despite synthetic antioxidants available in the market owing to the carcinogenic effects, antioxidants of natural origin have drawn significant attention from society (Chandra *et al*., 2014). Hence, total antioxidants capacity analyzed in the selected underutilized vegetable greens powder namely carrot, radish and beetroot are statistically presented in Table III and Figure 6.

Table III

Total Antioxidant Present in the Selected Undertutilized Green Leafy Vegetable Powder

Underutilized greens powder	Total Antioxidants(%)
Carrot greens (*Daucus carota*.L)	24.70±0.73
Radish greens (*Raphanus stivus*.L)	23.07±0.30
Beetroot greens (*Beta vulgaris*.L)	42.06±0.94

Values are mean ± SD n=3

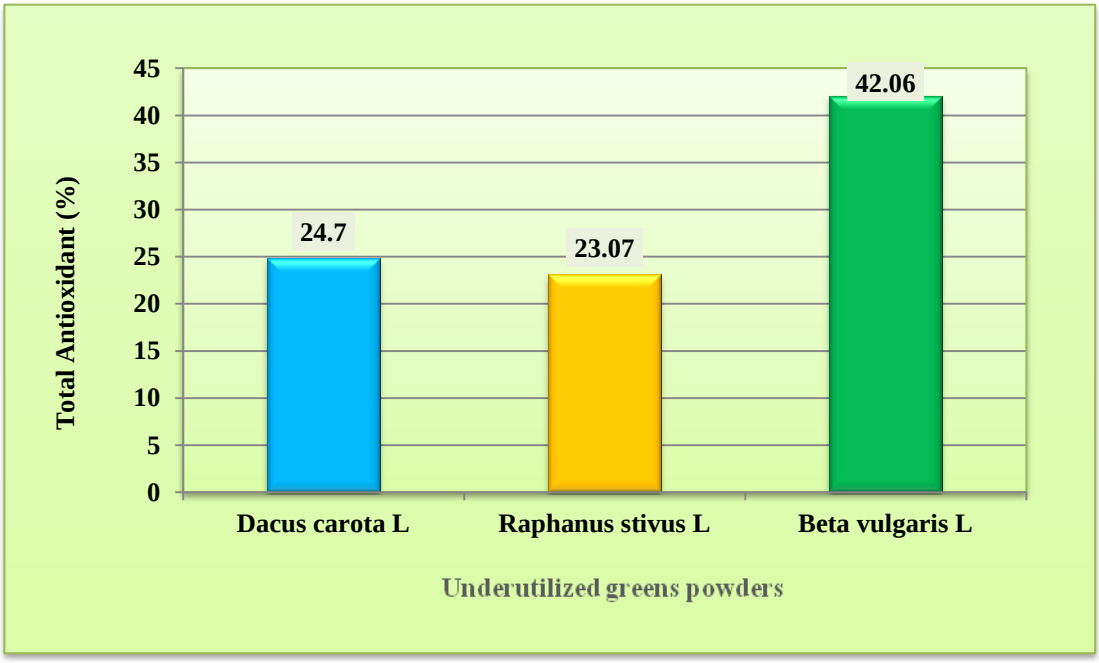


Figure 6: Total Antioxidant Level of the Selected Underutilized Green Leafy Vegetable Powder

Table III revealed that, all the three underutilized greens powder had effective scavenging capacity to fight against free radicals

From Table III it was evident that *Beta vulgaris.*Lpowder showed the highest free radical scavenging activity (42.06±0.94%) followed by *Daucus carota*L. powder (24.70±0.73%) and the least in *Raphanus stivus*L powder (23.07±0.30%). Hence, the result showed tremendous antioxidant potential in the greens of commonly available vegetables namely carrot, radish and beetroot of its easy reach of hands though underexploited in most cases. It can be identified as a powerful source of natural antioxidants that help to prevent degenerative diseases.

B. Determination of total phenol in the selected underutilized green leafy vegetable powder

In the plant kingdom, phenolic compounds are important, and it is well established among the Amaranthaceae species(Saha *et al*., 2015). Figure 7 depicts the total phenols estimated in the selected underutilized vegetable greens powder.

Table IV

Total Phenols Present in the Selected Underutilized Green Leafy Vegetable Powder

Underutilized greens powder	Total Phenols (mg of Gallic acid equivalents/100g)
Carrot greens (*Daucus carota.*L)	118.82±0.6
Radish greens (*Raphanus stivus.*L)	390.66±12.80
Beetroot greens (*Beta vulgaris.*L)	346.60±1.30

Values are mean ± SD n=3

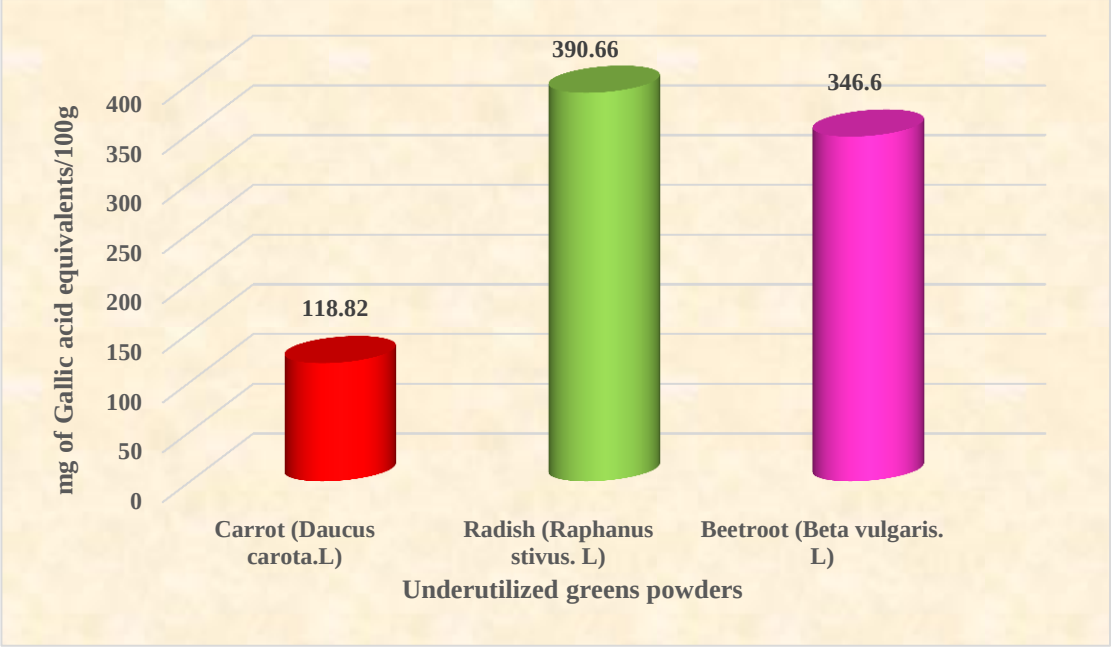


Figure 7: Total Phenol in the Selected Underutilized Green Leafy Vegetable Powder

Table IV shows that the phenol content of the analysed underutilised vegetable greens powder. The analyzed results reveal that phenols are found to be superior in *Raphanus stivus*.L(390.66±12.80 mg) and *Beta vulgaris* .L powder (346.60±1.30 mg) and significantly low for *Daucus carota L* powder (118.82±0.6mg). Polyphenolic compounds, which are secondary metabolites of plants with antioxidant properties, are abundant in vegetable resources (Munekata *et al* ., 2020).

As a result of the above findings, it can be concluded that the underutilised *Daucus carota* L., *Raphanus stivu*. L. and *Beta vulgaris* L, are noted for their potent antioxidant activity with respect to phenols.

C. Determination of total flavonoid in the selected underutilized green leafy vegetable powder

Flavonoids and phenolic acids are the main classes of phenolic compounds found in plants (Molina *et al*., 2010). Flavonoids and flavones are widely distributed secondary metabolites with antioxidant and antiradical properties (Oke and Hamburger, 2002). Phenolic acids and flavonoids are two types of naturally occurring polyphenols.Flavonoids are a type of secondary plant metabolites that is also known as Vitamin P and plays an important role in plant colour, according to Crozier *et al*.,

(2006).Flavonoids are readily ingested by humans and seem to display important anti-allergic, anti-inflammatory and anti-cancer activities. The amount of total flavonoids of the underutilised vegetable greens powder is shown in Table V. Figure 8 shows the total flavonoids found in a powder made from underutilised vegetable greens.

Table V

Total Flavonoids Present in the Selected Underutilized Green Leafy Vegetable Powder

Underutilized vegetable Greens	Total Flavonoids (mg Quercetin Equivalents/100g)
Carrot greens (*Daucus carota*.L)	845.01±13.07
Radish greens (*Raphanus stivus*.L)	381.8±7.03
Beetroot greens (*Beta vulgaris*.L)	2145±21.06

Values are mean ± SD n=3

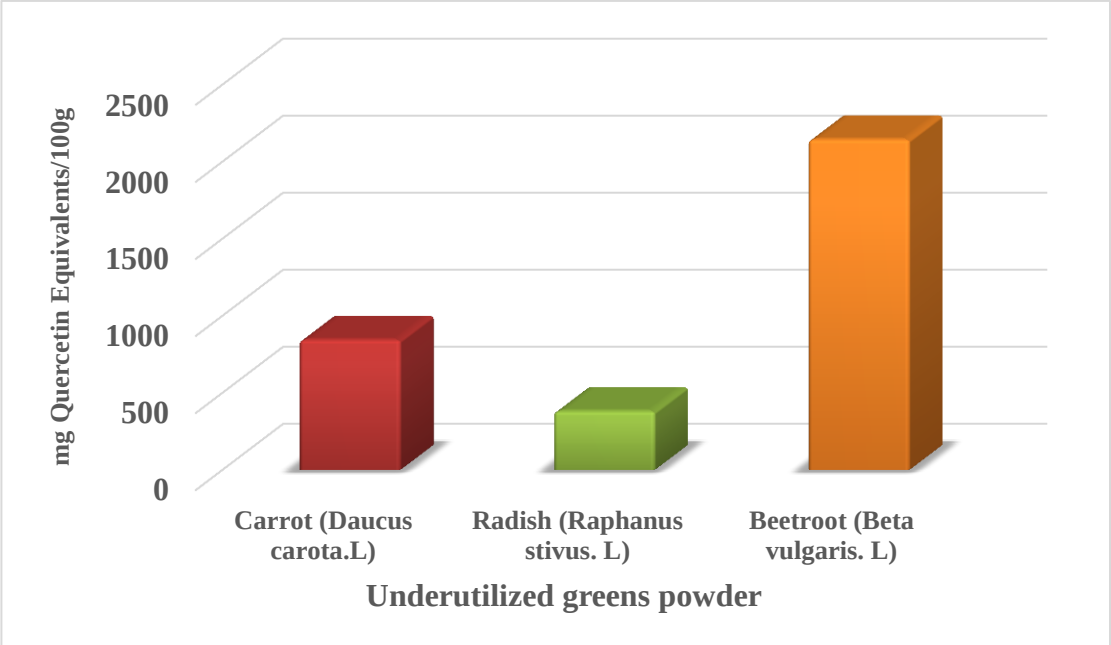


Figure 8: Total flavonoid in the Selected Underutilized Green Leafy Vegetable Powder

From Table V and Figure 8, it was statistically found that flavonoid content in *Beta vulgaris* L.was double compared to *Daucus carota* L.and *Raphanus stivus* L.

Antioxidant compounds, such as phenolic compounds (e.g., flavonoids, phenolic acids, stilbenes, quinines, lignans, tannins, coumarins), nitrogen compounds (betalains, amines, alkaloids), endogenous metabolites, terpenoids, and vitamin (C,E), are found in plants (vegetables, fruits, medicinal plants, herbs, etc.,). According to a study by Pourcel *et al*., (2007), greens with higher total flavonoid content have higher nutritional value because flavonoids have strong antioxidant activity and help to prevent oxidative stress. The study conducted by Şengül, (2014) reported total polyphenol and flavonoid content ranged from 44.94 to 340.94µg RAE/mg in raw green leafy vegetables. Dasgupta and Nirali Patel (2021) reported in a study of four green leafy vegetables *Amarantus viridis* L., *Raphanus sativus* L., *Chenopodium album* and *Spinacia oleracea* L. have fairly high phenol, and flavonoid content with appreciable antioxidant activities. The flavonoid content in selected underutilised vegetable greens confirms the presence of polyphenols (flavonoids), an essential bioactive compound for overall health.

D. Justification for selecting *Beta vulgaris* L. leaf powder for further study

Table III, IV and V depicts the statistical values of the analyzed results with respect to total antioxidant, total phenol and total flavonoid of the underutilized vegetable greens powder of *Daucus carota* L., *Raphanus stivus* L.,and *Beta vulgaris* L. powder respectively.

Beta vulgaris L. powder scored significantly higher total antioxidant (42.06±0.94%) and total flavonoids (2145±21.06mg) among the analysed underutilised vegetable greens powders. The total phenolic content of *Beta vulgaris* L. powder was lower (346.60±1.30 mg) than *Raphanus stivus* L. powder (390.66±12.80 mg). *Beta vulgaris* L. powder scored high in total antioxidants and flavonoids when total antioxidants, total phenols, and total flavonoids were compared. Beet greens contain apigenin, vitexin, vitexin-2-O-xyloside and vitexin-2-O-rhamnoside, vitexin-2-O xyloside, in combination with betaxanthins and betacyanins, exerts antiproliferative activity in breast, liver, colon and bladder cancer cell lines (Ninfali *et al*., 2017). Flavonoids are polyphenolic antioxidants and the most well-known polyphenols. According to the statistical findings of the experimental study,

the leaves of *Beta vulgaris* L. (Beetroot greens) contain a high amount of antioxidants and flavonoids, which can be used to reduce oxidative stress in PCOS patients.The flavonoids present in fruits and vegetables of plants, nuts, plant-derived beverages, traditional eastern medicines, and herb-containing dietary supplements are known to be antioxidants that can protect the cell from oxidative stress (Nassiri-Asl *et al*., 2016). Because the leaf parts of beetroot, cauliflower, and broccoli crops have higher total flavonoid content, their greens can be used as an alternative source of flavonoids, which are responsible for a variety of health benefits.Provitamin A carotenoid, vitamin K , vitamin B5, vitamin C present in *Beta vulgaris* L. leaf play a major role in protecting the body against oxidative stress damage (Sinbad *et al*., 2019).

On par of the above views an attempt was made to administer *Beta vulgaris.*Lpowder in PCOS condition to study its effect as an antioxidant to reduce the oxidative stress. Before administration in rat models the selected *Beta vulgaris* L.was screened for the presence of pesticide residue, nutrient analysis,phytochemicals, flavonoids, secondary metabolites, beta carotene, vitamin A and C using standard procedures.

Phase II: Screening of Phytochemicals, Antioxidant Activity and Nutrients content of *Beta vulgaris* L. Leaf Powder

A. Pesticide screening in *Beta vulgaris* L. leaf powder

In many developing countries, agricultural pesticide use is rapidly increasing, posing enormous challenges in managing the associated risks to people and the environment.Skretteberg *et al*., (2015) studied and found pesticide residues above maximum residue limits in 33% of fruit and vegetables from Southeast Asia.On reviewing the health hazards of pesticide exploited vegetables for safe consumption, screening of pesticide was done. Table VI shows the list of pesticides screened in the *Beta vulgaris.*L greens powder.

Table VI

Pesticide Screening in *Beta vulgaris* L. Leaf Powder

Insecticides	Amount detected (µg/g)
Organophosphates	BDL<0.05
Synthetic Pyrethroids	BDL<0.1
Organochlorine Compounds	BDL<0.01

BDL – Below Detection Level

The analysed Beetroot greens (*Beta vulgaris* L.) powder contains no detectable pesticide residues, as shown in Table VI. The absence of pesticide residues in the screened *Beta vulgaris* L. leaf powder was due to the fact that the leaves were grown organically, with the use of eco-friendly manures such as good quality compost mixture, neem cake, trycoderma and pseudomonas sprayed on the land prior to cultivation rather than on the leaves. As a result, the powdered beetroot greens (*Beta vulgaris* L.) was found to be free of pesticide hazards.

B. Phytochemical screening in *Beta vulgaris* L. leaf extract

Table VII brings out the presence of phytochemical compounds in *Beta vulgaris* L.greens powder.

Table VII

Phytochemical Screening in *Beta vulgaris* L. Leaf Extract

Phytochemical compounds	Ethanol extract	Methanol extract	Aqueous extract
Alkaloids	+ +	+ +	+
Flavonoids	+ + +	+ + +	+ + +
Amino acids	+	+	+
Proteins	+	+	+
Phenols	+ + +	+ + +	+ + +
Tannins	+	+ +	+
Cardiac Glycosides	+ +	+ +	+ +
Fats and Oils (fixed)	-	-	-
Terpenoids	-	-	-
Saponins	+ +	+ +	+ +
Sterols	+	+	+
Oxalate	+	+	+

'+' stands for presence, '-' stands for absence

From Table VII, it can be inferred that phytochemical screening in ethanol, methanol and aqueous extracted *Beta vulgaris* L. leaf extract showed the presence offlavonoids,alkaloids, tannins, phenols, quinines, glycosides, sterols and saponins. Yadav and Tiwari, (2021) define that naturally in all parts of the plant body phytochemicals are bioactive chemicals synthesized. Phytochemical analysis of plants confirms the presence of cardiac glycosides, carbohydrates, tannins, saponins, terpenoids, carotenoids, phytosterols and resins (Sood *et al*., 2012).

Plant species that accumulate high levels of this carbon dicarboxylic acid anion are known to contain oxalate. Oxalate accumulates primarily as insoluble calcium oxalate, soluble oxalate, or a combination of these two forms, depending on the species.Soluble oxalates include oxalic acid, sodium, potassium, and ammonium oxalates. Plants which contain insoluble oxalates are those that produce crystalline needles of calcium oxalate called raphides (Prasad and Shivay, 2017).

Saponins are used as natural cleanser and cardiac glycosides are used for ulcer and diabetic treatment (Karunyadevi *et al*., 2009). According to the findings, ethanol, methanol, and aqueous extracts of *Beta vulgaris* L. leaf powder appear to contain promising phytochemicals such as phenols, flavonoids, and saponins which have the potent to act as natural antioxidants in the food and pharmaceutical industries to improve people's health. According to Odriozola-Serrano *et al*., (2009), tannins are polyphenolic compounds with a complex mixture found in many plants. Because of the presence of various vital compounds for good health, consuming beet greens in any form has a better protective effect on health than single phytochemicals.

C. Quantitative analysis of anti nutrients in *Beta vulgaris* L.leaf powder

Table VIII shows the quantitative analysis of the anti nutrients in *Beta vulgaris* L.leaf powder, to ensure its administration.

TableVIII

Quantitative Analysis of Anti-Nutrients in *Beta vulgaris* L. Leaf Powder

Anti-nutrient Parameters	**Composition / 100 g**
Total Alkaloid(mg)	68.8 ± 0.08
Total Saponin (mg)	20.91±0.01
Total Oxalate(mg)	3.60
Total Tannin (mg)	7.76

Values are in mean ± SD (n=3)

Beta vulgaris L. leaf powder contains total alkaloid 68.8 mg, total saponin 20.91mg, total oxalate 3.60mg, and total tannin 7.76mg per 100g, according to TABLE VIII. The findings show that even though anti-nutrients were present in small amounts, they had no negative effects on human health. These anti-nutritional factors are known as “secondary metabolites” in plants, and they have a wide range of nutritional and pharmacologically active applications.

Massey (2007) found that oxalate content in beet leaves 760±6750 mg kg^{-1} in fresh tissue is similar to the content found in other leafy vegetables, particularly less than the oxalate content in spinach 4000-17650 mg kg^{-1}. Similarly, tannin content in *Beta vulgaris* L. was found to be 7.76 mg per 100 gram. Gupta and Rolletschek, (2013) found that tannin content resulted in fresh tissue of beet leaves was 84.6 ±9.4mg kg^{-1}. This level was low to the variety of green leafy vegetables commonly consumed in India ranged 860 and 4240 mg kg^{-1}. Flavonoids and saponins, have found wide applications in the fields of medicine, pharmacy and food industries as pharmacologically active principles, in food, drink and beverage industries, as antioxidants, preservatives and flavouring agents (Waldron *et al*.,1993).

D. Determination of antioxidant activity in *Beta vulgaris* L. leaf extract

Antioxidant activity of methanol extract of *Beta vulgaris* L. leaf were analyzed by DPPH (2, 2-diphenyl-1-picrylhydrazyl) method with standard ascorbic acid.

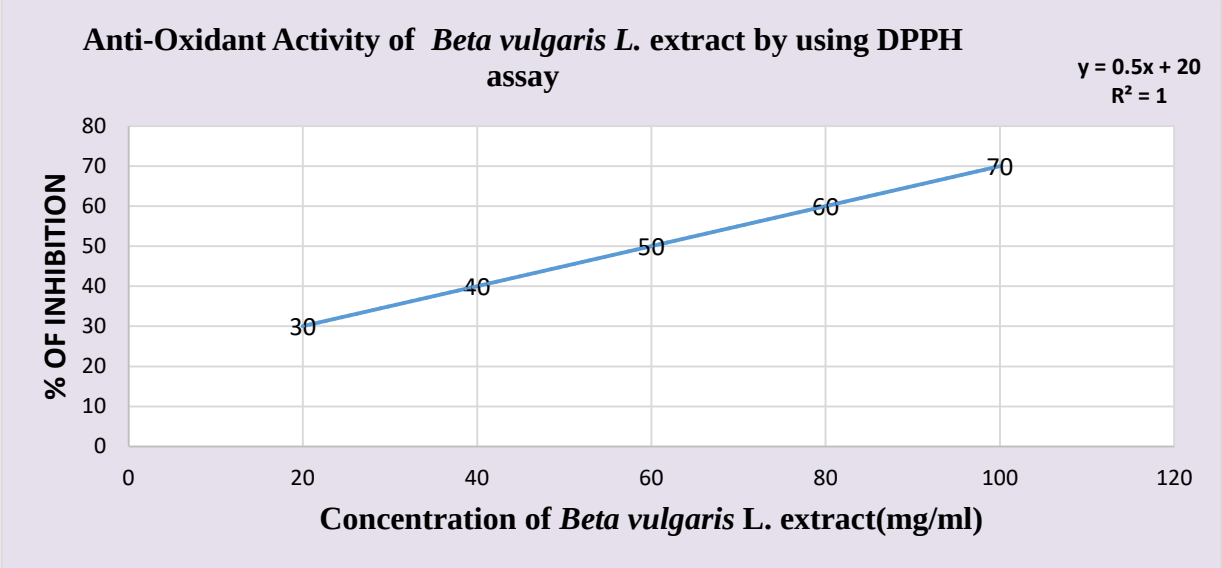
(i) DPPH (2,2-diphenyl -1-picrylhydrazyl) Assay

Table IX and Figure 9 shows the percentage of scavenging activity of *Beta vulgaris* L. leaf extract.

Table IX

Anti-Oxidant Activity of Leaf Extract of *Beta vulgaris* L. by DPPH Assay

	Concentration of *Beta vulgaris L* (mg/ml)	**% of Inhibition**	**Ascorbic acid**
DPPH ASSAY	20	30	56
	40	40	65
	60	50	70
	80	60	76
	100	70	80
IC_{50} Value		**60**	**8.5**



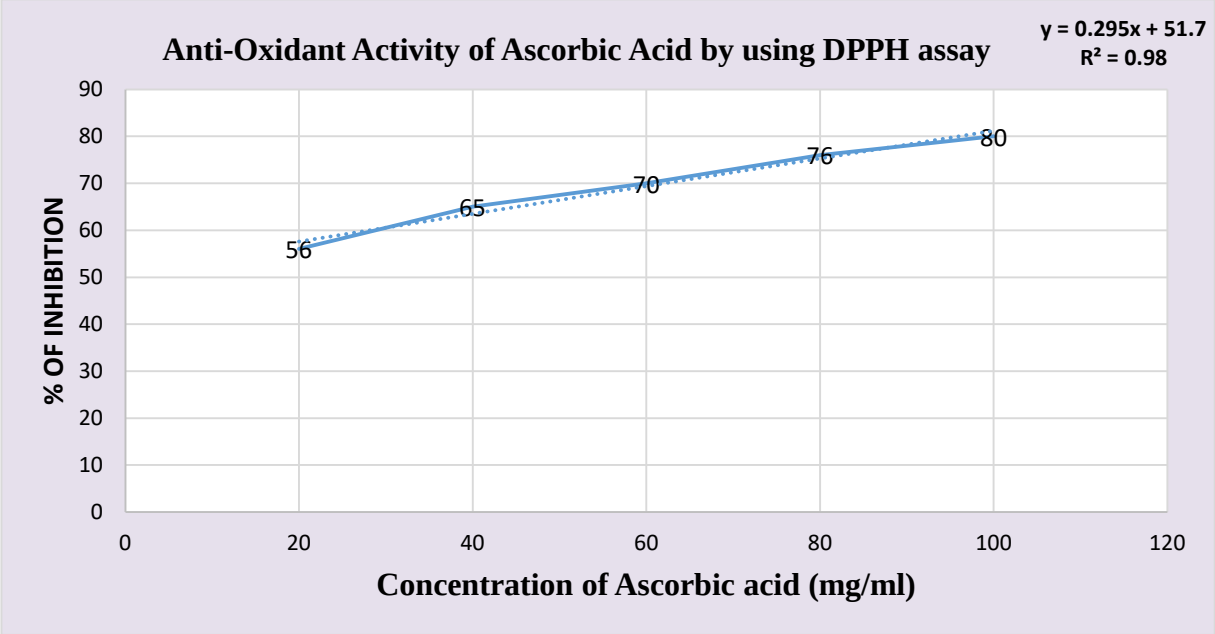


Figure 9: Antioxidant Activity of Beta vulgaris L. Leaf Extract by DPPH Assay

Table IX and Figure 9 showed that increasing the concentration of both standard and *Beta vulgaris* L. leaf extract from 20 to 80 mg/ml increased the percentage of scavenging effect on the DPPH radical.For *Beta vulgaris* L., the percentage of inhibition ranged from 30, 40, 50, 60, 70 mg/mL to 56, 65, 70, 76, 80 mg/mL, with an IC50 value of 60 mg/ml for *Beta vulgaris* L. and 8.5 mg/ml for standard. The antioxidant activity was attributed to some phytochemical constituents of the *Beta vulgaris* L. ion extract. Flavonoids and bioflavonoids are a class of

polyphenolic compounds found in most plants, primarily in the seed, fruit skin or peel, flowers, and bark. Flavonoids have been shown in numerous studies to have anti-oxidant properties, including the ability to scavenge hydroxyl radicals, superoxide anions, and lipid peroxy radicals.

The DPPH radicals were scavenged by ethanol extract of *Beta vulgaris* L.leaf in concentration dependent manner. DPPH is one of the free radicals widely used for testing the preliminary radical scavenging activity of the plant extract. The inhibition of lipid peroxidation is linked to the scavenging of DPPH radicals (Chand *et al*., 2012). DPPH is a substance that is commonly used to test anti-oxidant activity (Choi and Chung, 2003).

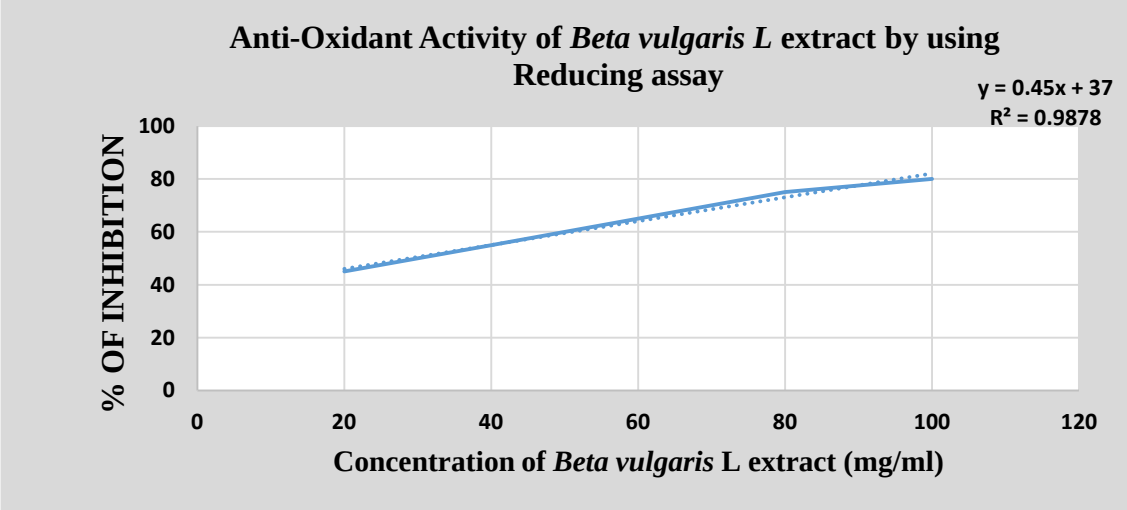
Reducing Assay

Antioxidant activity of ethanol extract of *Beta vulgaris*L. leaf powder was analyzed by Reducing Assay with standard ascorbic acid. Table X and Figure 10 shows the percentage of scavenging activity of *Beta vulgaris* L. leaf extract.

Table X

Antioxidant Activity of Leaf Extract of *Beta vulgaris* L. by Reducing Assay

	Concentration of *Beta vulgaris L* extract(mg/ml)	**% of Inhibition**	**Ascorbic acid**
REDUCING ASSAY	**20**	**45**	**50**
	40	**55**	**55**
	60	**65**	**65**
	80	**75**	**70**
	100	**80**	**80**
IC_{50} Value		**28.8**	**22.6**



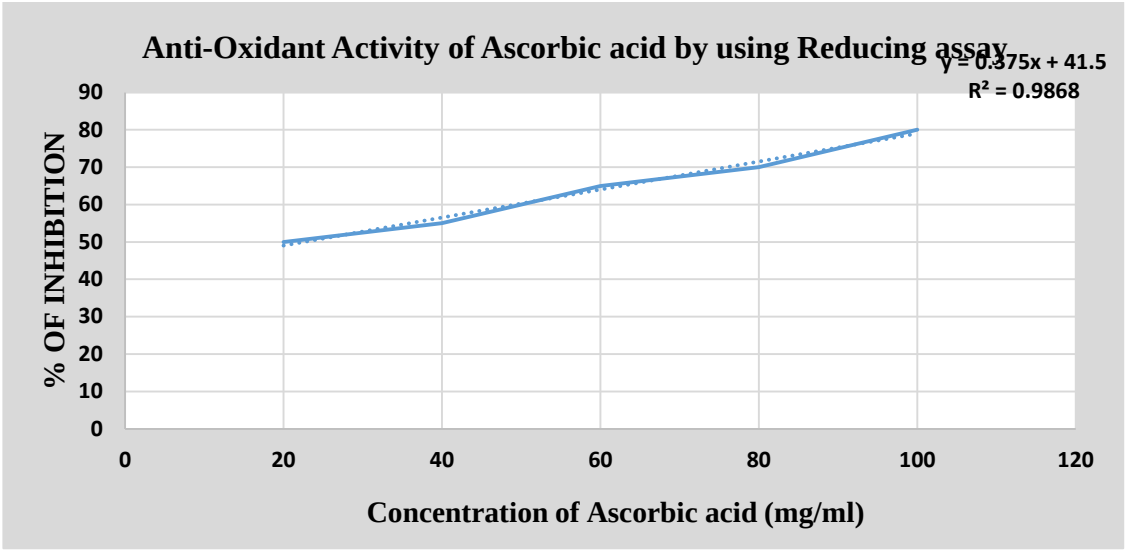


Figure 10: Antioxidant Activity of Beta vulgaris L. Leaf Extract by Reducing Assay

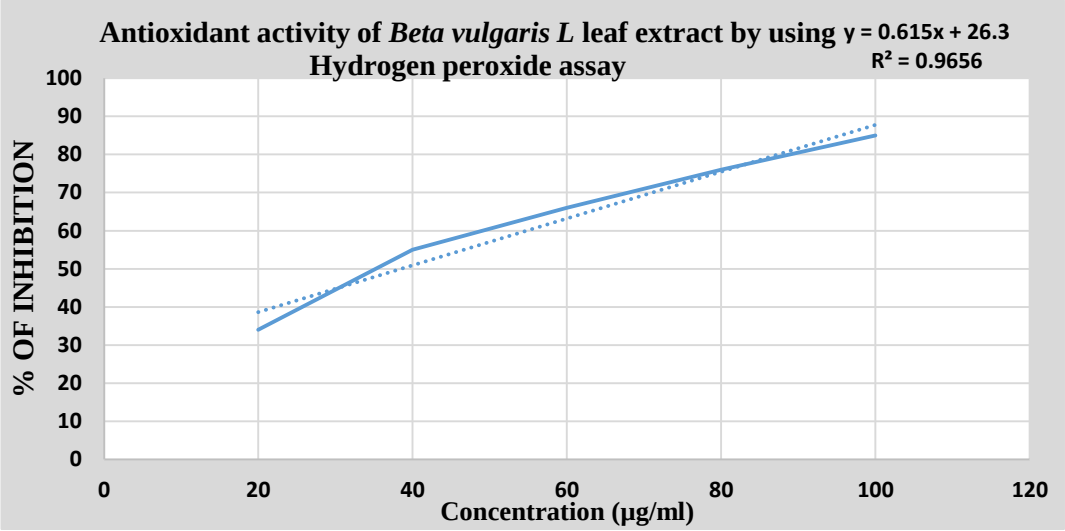
The Reducing assay percentage of inhibition was 45, 55, 65, 75, 80 at 20, 40, 60, 80, 100 mg/ml concentrations, and the IC50 value was for *Beta vulgaris* L., as shown in Table X and Figure 10. The concentration of *Beta vulgaris* L. was found to be 28.8 mg/ml, whereas the concentration of the standard drug was 22.6 mg/ml.The in vitro antioxidant activity of *Cacia fistula* Linn was similar to the results of the reducing assay scavenging activity. (Danish *et al*., 2011).

(ii) Scavenging of Hydrogen peroxide

Hydrogen peroxide assay with standard ascorbic acid was used to assess the antioxidant activity of an ethanol extract of *Beta vulgaris* L. leaf powder. Table XI and Figure 11 show the percentage of scavenging activity of leaf extract.

Table XI
Anti-Oxidant Activity of Leaf Extract of *Beta Vulgaris* L. by Hydrogen Peroxide Assay

	Concentration of plant formulation	% of inhibition of plant extract	% of inhibition of Ascorbic acid
H_2O_2 ASSAY	20	34	46
	40	55	65
	60	66	76
	80	76	86
IC_{50} Value		38	20



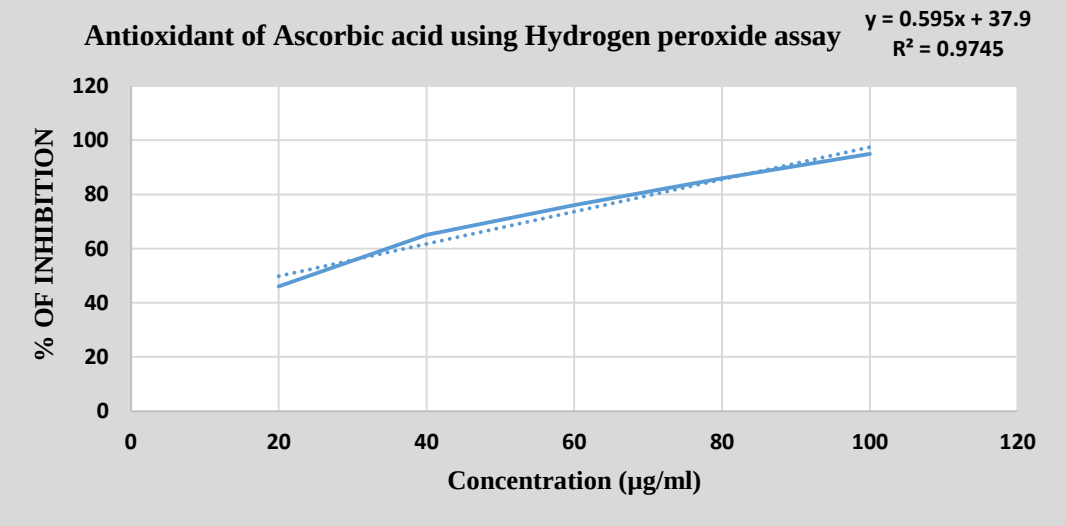


Figure 11: Antioxidant Activity of Beta vulgaris L. Leaf Extract by Hydrogen Peroxide Assay

From the Table XI and Figure 11 in H_2O_2 assay the percentage of inhibition of 34, 55, 66, and 76 at 46, 65, 76 and 86 mg/mL concentration respectively. *Beta vulgaris* L. ion had an IC_{50} of 38 mg/ml, whereas the standard drug had an IC_{50} of 20 mg/ml. The H_2O_2 scavenging activity of *Beta vulgaris* L. leaf is similar to the antioxidant activity of *Cinnamomun verum*in vitro (Mahmood *et al.,* 2019). The assay proves that the inhibition of hydroxyl radical production from Hydrogen peroxide in a dose dependent manner. The cell membranes are easily penetrated by hydrogen peroxide. These molecules will be transformed into hydroxyl radicals, which will cause cell damage. Anti-oxidants are chemical compounds that donate electrons to H_2O_2. By converting them into water, the donating electron reacts with H_2O_2 and neutralises it.Compounds with anti-oxidant activity, functions as free radical scavengers, complexes of pro-oxidant metals, reducing agents and quenchers of single-oxygen formation or reactive oxygen species, thereby protecting the body from degenerative diseases such as cancer. The reactive oxygen species (ROS) are harmful by products generated during normal cellular metabolism or from toxic insult (Alemdar *et al.,* 2009).

E. Screening of nutrients in *Beta vulgaris* L. leaf powder

Table XII shows the mean nutrient composition of *Beta vulgaris.* L. leaf powder per 100 gram.

Table XII

Nutrient Composition of *Beta vulgaris* L. Leaf Powder

Nutrient Parameter	Composition / 100 g
Ash (%)	1.4±0.01
Moisture (%)	9.13±0.8
Carbohydrate (g)	1.030±0.09
Protein (g)	2.4±0.03
Total Fat (g)	0.79±0.03
Dietary Fibre (g)	2.93±0.03
Energy (Kcal)	21.14±0.04
Iron (mg)	2.54±0.02
Calcium (mg)	52.50±0.73
Vitamin C (mg)	382±4.68

Values are mean ± SD (n=3)

From Table XII, it is evident that the moisture content of the analyze *Beta vulgaris* L. Leaf powder is 9.13 %. Regarding the ash content, it was 1.4 per cent, indicating high mineral content in the analyzed *Beta vulgaris* L.leaf powder. Beetroot greens (*Beta vulgaris* L.), a cheap, seasonal, nutritious green leafy vegetable in dehydrated form contains 2.4 g of protein in the analysis, which is very similar to the study reported by Konstantinova and Popova, (2020) that 100 gram of fresh beet greens contains 4.3 g of carbohydrates, 2.2 g of protein, 0.1 g of fat, 91 g of water and 2.4 g of ash. It is clear that the analyzed *Beta vulgaris* L. leaf powder contains high dietary fibre of 2.93 grams, and is closely related to a study by Orisa *et al.,* (2020) showed that the dietary fibre in *Spinacia oleraceae* ranged from 4.03 -23.12 per cent.

Leafy vegetables have low energy densities,making them ideal for weight loss (Nwanekezie and Obiarkor, 2014). Similarly, Beetgreens (*Beta vulgaris* L.) powder contains 1.030g of carbohydrate, representing a low carbohydrate level. Regarding fat content, Beetgreens (*Beta vulgaris* L.) powder contains a very low fat content of 0.79g which is related to the experimental study by Kaushik, (2020) on fat analysis of cauliflower leaf powder was 1.28% and amaranth leaves powder was 0.82%. The values obtained were similar to the reported values of food composition values of Indian foods (Gopalan *et al*., 2007).

Beetgreens (*Beta vulgaris* L.) powder contains total energy of 21.1 Kcal obtained by multiplying the percentage of protein, fat and carbohydrate. Beet root greens are an excellent source of iron, vitamin B1, B6 and pantothenic acid as well as phosphorus, protein and a good source of zinc, folate and vitamin B3. On analyzing the mineral content, 2.54 mg of iron and 52 mg of calcium were present.Rajaeifar, *et al*., (2019) and Vargas-Ramirez *et al*., (2017) proved that the content of vitamins and biomicroelements in fresh beet leaves have a higher biological value.

Vitamin C in dehydrated greens powder of *Beta vulgaris*L. had 382 mg per 100 g of vitamin C, is a desirable value and considered as a concentrated source of micronutrient. Olaynika *et al.,* (2012) investigated that higher blanching time up to 20 minutes and high temperature affects the antioxidant contents and antioxidant components are affected therefore 1 minute of blanching time is recommended for blanching vegetables. Therefore it was assumed that blanching of *Beta vulgaris* L. leaves for 10 seconds did not affect the vitamin C content. Beet greens can be used as

a source of vitamin C (100g of the product has 33% of the daily value), which helps in boosting the body's resistance to disease.Vitamin C help in the absorption of iron in the body, as well as a lack of it results in the destruction of blood vessel walls. Vitamin C boosts capillary elasticity and lowers the risk of seasonal depression (Konstantinova and Popova, 2020). Hence, Table XII clearly depicts that *Beta vulgaris.* L leaves in fresh or dried form can be considered as a good source of antioxidants and vitamin C that can be included in regular diets to eradicate deficiencies.

Phase III: Screening of Flavonoids, Secondary Metabolites and Fat Soluble Vitamin A and E of *Beta vulgaris* L. Leaf Extract

A. Screening of flavonoids in *Beta vulgaris* L. leaf extract by HPLC method

Table XIII presents the screening of flavonoids using High Performance Liquid Chromatography (HPLC). Figure 12 shows the graphical representation of flavonoids in *Beta vulgaris* L.leaf extract.

Table XIII
Screening of Flavonoids in *Beta vulgaris* L. Leaf Extract

Ret. Time (min)	Area %	Compound Name	Biological Activities
4.145	1.722	Ellagic acid	Antibacterial, Antidiarrheic, Antidysenteric, Antihepatoxic, Antiviral, Antigastric, fungicide, cycloxygenase inhibitor and lipoxygenase
5.321	49.075	Gallic acid	Antifungal activity, anticancer, antioxidant, antitumoral, anti-inflammatory activities
7.155	49.203	Catechin	Antioxidant, Pro-oxidative, Antiradical, Anticancer, Antimicrobial activity, Anti-inflammatory, immune and epigenetic modification

Source : Dr. Duke’s Data base (1994)

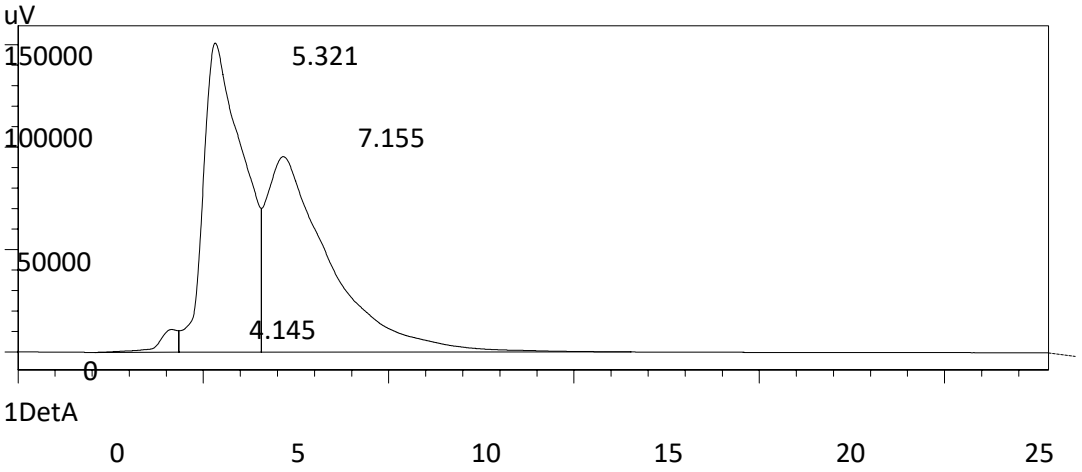


Figure 12: HPLC Screening for Flavonoids of Beta vulgaris L. Leaf Extract

The presence of three flavonoid compounds was detected in the HPLC chromatogram of ethanol extracted *Beta vulgari*s L. leaf, as shown in Table XIIIwith a peak percentage of ellagic acid (1.72 %), gallic acid (49.07%) and catechin (49.20%).

Ninfail and Angelino, (2013) opines that the flavonoids and glucosinolates (GLS) such as catechin and gallic acid in beet stalks and leaves have been the focus of much research, due to their potential as health promoting phytochemicals. Seal, (2016) in his experimental study on gallic acid observed that gallic acid that remains both in free state or in the combination form as ester, acts as a powerful antioxidant. It was encouraging to note that compounds such as ellagic acid , gallic acid and catechin that are present in tea leaves were also noted in the leaf powder of *Beta vulgaris* L.

Iranshahy *et al.,* (2017) has also reviewed that these bioactive compounds contained a variety of pharmacological and physiological properties that stimulate the central nervous system, induce gastric secretions, and act as a diuretic. Peng *et al.,* (2008) investigated that major phenolic compounds such as gallic acid and naturally occurring catechins (C), epicatechin (EC), galloactechin (GC), epigallocatechin (EGC), catechin gallate (CG), gallocatechin gallate (GCG), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG). Tapas *et al.,* (2008)

observed that the catechins and flavones were found to be the most powerful flavonoids that helps to protect the body against free radical formation.

Hence Table XIII concludes that the presence of gallic acid and phenolic compounds are considered as strong antioxidants, might be the major contribution of antioxidant activity in the analyzed *Beta vulgaris* L. leaf powder with respect to flavonoids. Owing to the presence of health promoting flavonoid compounds, *Beta vulgaris* L. leaf powder can be introduced in pharmacological, functional food and nutraceutical preparations.

B. Screening of Secondary Metabolites in *Beta vulgaris* L. Leaf Extract using GCMS

Table XIV shows the identification of compounds present in the *Beta vulgaris* L. leaf extract using Gas Chromatography Mass Spectrometer. Figure 13 shows the graphical representation of secondary metabolites in *Beta vulgaris* L. leaf extract.

Table XIV

Screening of Secondary Metabolites in *Beta vulgaris* L. Leaf Extract

RT (min)	Compound Name	Molecular Formula	Molecular Weight	Peak Area %	Common Name	Biological Activity
5.02	1,3,5,7-Tetroxane	$C_4H_8O_4$	120.10	73.1%	Hetero compound, Oxane	Antimalaria, antipyretic, antiinflammatory, non-central analgesic
7.39	Decane	$C_{10}H_{22}$	142.29	83.1%	Alkanes hydrocarbon	Antibacterial and neurotropic properties
11.07	Azulene	$C_{10}H_8$	128.17	73.8%	Aromatic hydrocarbon	Anti-inflammation, anti-cancer, anti-diabetes, anti-retroviral, antimicrobial, and antifungal
17.22	4-Hepten-2-one, 5-ethyl-3,3,4-trimethyl-	$C_{12}H_{22}O$	182.30	71.7%	Ketone	Antioxidant, antimicrobial, and antibacterial properties
18.68	6-Amino-1,3,5-triazine-2,4(1H,3H)-dione	$C_3H_4N_4O_2$	128.09	65.1%	Amino diol	Antibacterial, antifungal, and cancer-fighting properties Anti-inflammatory, anti-diabetic, anti-hyperlipidemic, and anti-tubular activity are all properties of analgesics.

22.00	Phthalic acid, 4-bromophenyl ethyl ester	$C_{16}H_{13}BrO_4$	349.17	83.7%	Acid	Antibacterial and antifungal properties
25.09	Neophytadiene	$C_{20}H_{38}$	278.5	93.1%	Hydrocarbons	Antipyretic, analgesic, antimicrobial, antioxidant, anti-inflammatory, and antipyretic
25.62	Neophytadiene	$C_{20}H_{38}$	278.5	88.2%	Hydrocarbons	Antipyretic, analgesic, anti-inflammatory, and antimicrobial are all terms that can be used to describe a substance.
26.19	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45	84.8%	Amino compound	anti-androgenic, hypocholesterolemic, lubricant, nematicide, flavouring agent, pesticide
26.76	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	84.3%	Palmitic acid ester	Antioxidant, lubricant, hypocholesterolemic, antiandrogenic, hemolytic
28.51	Phytol	$C_{20}H_{40}O$	296.53	86.0%	Diterpene	Antifungal against S.typhi, diuretic, headache, hernia, anticancer, resistant gonorrhoea, joint dislocation, stimulant, and antimalarial
28.84	9-Octadecenoic acid, (E)-	$C_{19}H_{36}O$	296.48	88.6%	Polyenoic fatty acid	Antiviral, anti-eczemic, hepatoprotective, antihistaminic, hypocholesterolemic
30.51	2-Hexadecen-1-ol,3,7,11,15-tetramethyl-,acetate, [R-[R*,R*-(E)]-	$C_{20}H_{40}O$	296.53	64.0%	Alkanes	Antioxidant, Hemolytic, Hypocholeserolemic, flavouring, nematicide, anti-androgenic, antibacterial, and antifungal
32.28	1-Tricosene	$C_{23}H_{46}$	322.6	72.3%	Alkene	Anticancer and anti-inflammatory properties
34.10	17-Pentatriacontene	$C_{35}H_{70}$	490.9	65.6%	Alkene	Antimicrobial, anti-inflammatory, and cancer-fighting properties

Source : Dr.Duke's Data base (1994)

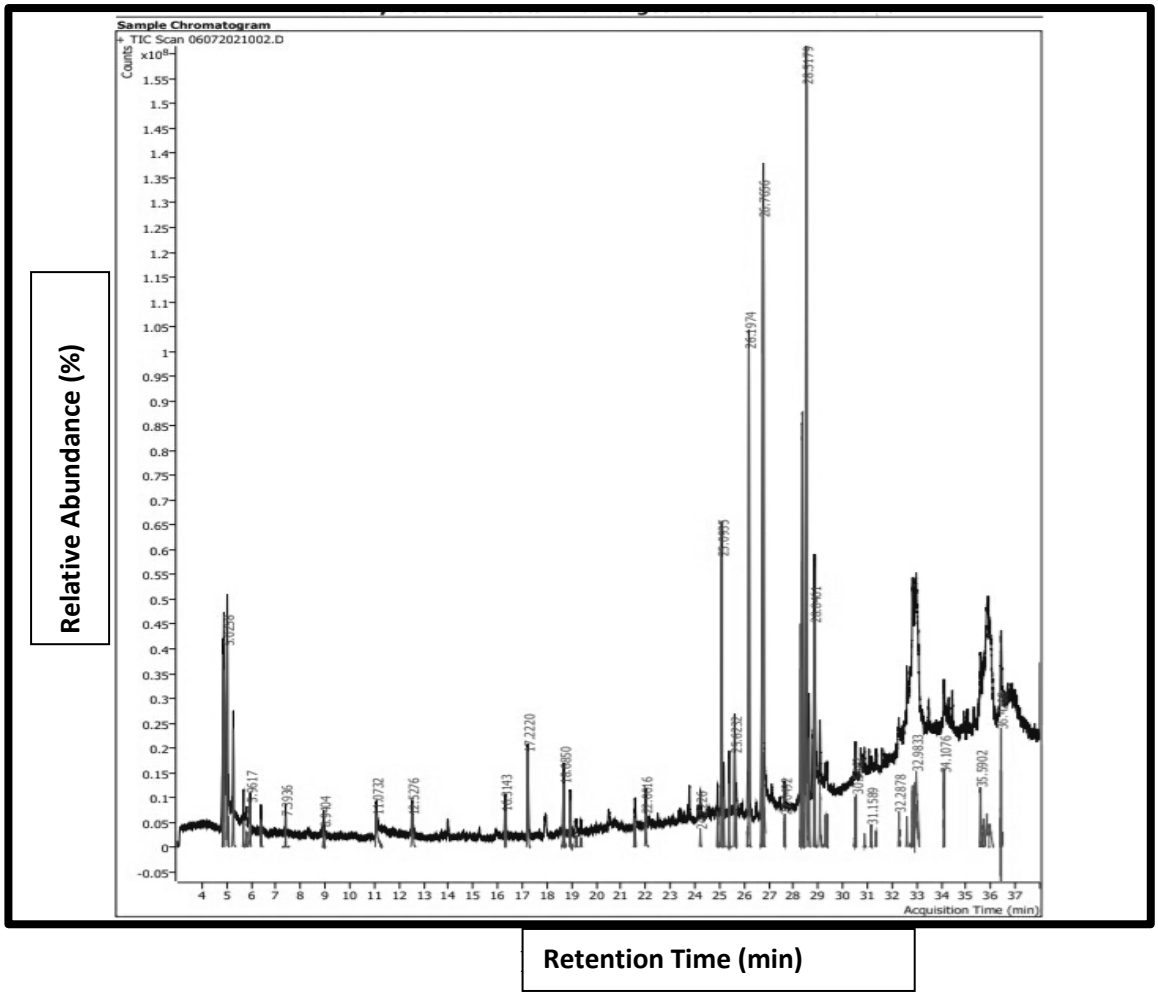


Figure 13: GCMS Screening for Secondary Metabolites of Beta vulgaris L. Leaf Extract

4-Hepten-2-one, 5-ethyl-3,3,4-trimethyl, Neophytadiene, Hexadecanoic acid, methyl ester, n-Hexadecanoic acid, 2-Hexadecen-1-ol,3,7,11,15-tetramethyl-acetate [R-[R*,R*-(E)] are antioxidant, antimicrobial, and anti-inflammatory among the identified compounds.In the leaves of *P.stratiotes* and *E.crassipes*, n-Hexadecanoic is a common compound. Hexadecanoic acid, 2-hydroxyl-1-(hydroxymethyl) ethyl ester found in leaf extract has antifungal, anti-tumor, and anti-bacterial properties, and Hexadecanoic acid, 2-hydroxyl-1-(hydroxymethyl) ethyl ester has hemolytic, pesticide, flavour, and antioxidant properties (Duke's Phytochemical and Ethnobotanical Databases, 1992-1996).

Similarly, research into the bioactivity of n-hexadecanoic acid (also known as palmitic acid) discovered that it has potent antioxidant and pesticidal properties (Dardiry, 2018). Antimalarial, antipyretic, and anti-inflammatory properties have

been reported for 1,3,5,7-Tetroxane. In the same way, 1,3,5,7-Tetroxane was found in the methanolic extract of *Jatropha curcas* L. (Pavithra*et al.*,2018). Azulene has antibacterial, antifungal, anticancer, analgesic, anti-inflammatory, anti-diabetic, anti-hyperlipidemic, and anti-tubular activity, according to studies. Antipyretic, analgesic, anti-inflammatory, anti-microbial, and antioxidant properties have been reported for Neophytadiene. Hashim *et al*., (2021) discovered the Azulene compound in GCMS hydrosol extract of *Aquilaria* (Agarwood) species, and leaves and flowers of Amaranthus were found to have the highest antioxidant activity (Peter and Gandhi, 2017). Antimicrobial, anti-inflammatory, diuretic, anticancer, and antimalarial properties were found in phytol. Phytol has been found to have good anti-arthritis, preventive, and therapeutic effects. Reactive oxygen species were found to promote a new class of pharmaceuticals for the treatment of rheumatoid arthritis and possibly other chronic inflammatory diseases, according to the findings (Ogunlesi*et al*., 2009). Anti-cancer, anti-inflammatory, and anti-cancer activity was found in 1-Tricosene and 17-Pentatriacontene. Verma *et al*., (2015) reported the pesticidal potential of 1-tricosene, (Z)-and [1,1' – bicyclopropyl]-2-octanoic acid, 2'hexy10 methyl ester.

In this lineof research, GC-MS analysis of leaves of *Beta vulgaris* L. leaf extract reveals the existence of various bioactive compounds possessing antioxidant and antimicrobial properties which could be introduced in developing safe functional health food products and in the preparation of pharmaceutical drugs.

C. Screening of fat soluble vitamin A and E in *Beta vulgaris.* L. leaf extract by HPLC

Vitamin A is a broad term for a number of similar compounds, according to the National Institute of Health (2016). Retinoids (from animals) and Provitamin A carotenoids (beta-carotene) from plants are the two main categories, which differ depending on whether the it comes from either an animal or a plant.Table XV shows HPLC screening for vitamin A and E in *Beta vulgaris* L.leaf extract.

Table XV

HPLC Screening of Vitamin A and E in *Beta vulgaris* L. Leaf Extract

Retention Time (min)	Parameters	Concentration (mg/kg of sample)
1.5	Vitamin A (Beta carotene)	3.5 µG/100g
5.6	Vitamin E	< BDL

It is evident from Table XV, that *Beta vulgaris* L.leaf extract contains an appreciable amount of vitamin A in the form of provitamin A carotenoids, since it has been obtained from plants.*Beta vulgaris* L. leaf powder screened for beta carotene confirms the presence of the antioxidant beta carotene. The results reveal that the dried leaf of *Beta vulgaris* L. lesser quantity of β-carotene but still has positive effects on health. Thus, the available inexpensive Beet greens (*Beta vulgaris* L.) in dehydrated form contains potent antioxidant benefits, and readily availablevitamin A in desirable amount can be used for scavenging free radicals owing to their antioxidant action.Vitamin E was found to be below the detection level in HPLC screening with a retention time (RT) 1.5 min was observed.

Vegetable greens like beet greens could be exploited to combat vitamin deficiencies and with the focus of its antioxidant potency it could be used as natural antioxidants to combat degenerative diseases.

Phase IV: Acute Toxicity Study of *Beta vulgaris* L. Leaf Aqueous Extract on Adult Female Rats

A. Acute Toxicity study of *Beta vulgaris* L. leaf aqueous extract on adult female rats

An acute toxicity study was conducted with twelve Wistar strain adult Albino female rats of 6 weeks old weighing an average of 120 g body weight (BW). The control group received standard rat chow and experimental groups received aqueous extract of *Beta vulgaris* L. leaf with different doses from 50 mg, 300 mg and 2000 mg

/kg body weight. The rats were observed for a period of 14 days, with special attention for the first 30 minutes, four hours and next 24 hours after supplementation.

B. General observation of acute toxicity study of *Beta vulgaris* L. leaf aqueous extract on adult female rats

Table XVI represent the general observation of acute toxicity study of the animals.

Table XVI

General Observation of the Acute Toxicity Study of the Animals

General observation	Control group N=3	Experimental group (50mg/kg BW) N = 3	Experimental group (300mg/kg BW) N=3	Experimental group (2000mg/kg BW) N=3
Breathing	Normal	Normal	Normal	Normal
Food Intake	Normal	Normal	Normal	Normal
Changes in skin and fur	No changes	No changes	No changes	No changes
Body weight	Normal	Normal	Normal	Normal
Drowsiness	Not present	Not present	Not present	Not present
Sedation	Not present	Not present	Not present	Not present
Coma	Not present	Not present	Not present	Not present
Alive/Dead	Alive	Alive	Alive	Alive

Source: OECD 423

Control Group - Standard rat chow

Experimental Group – *Beta vulgaris* L.leaf extract

After supplementation of a single high dose of *Beta vulagris* L. leaf aqueous extract of (2000 mg/kg/bw) showed that none of the animals in the experimental study showed toxicity signs in their general behavior. Rats of the toxicity study group showed normal breathing, normal food intake, no changes in skin and fur and normal body weight.

After supplementation of a high dose of aqueous extract of *Beta vulgaris* L. aqueous extract of 2000 mg/kg body weight for complete observation period of 14

days showed no toxic effects such as drowsiness, sedation and coma. Till the end of the observation period, all the animals of the toxicity study groups were alive, which indicated the safety of extracts for supplementation study.

C. Observation of Gross Behavior of *Beta vulgaris* L. leaf aqueous extract on adult female rats

(i) Observation of central nervous system stimulant activities

Observation of Central Nervous System (CNS) stimulant activities in association with acute toxicity study are expressed in Table XVII.

Table XVII

Observation of Central Nervous System Stimulant Activities

Gross behavior	Control Group N=3	Experimental group (50mg/kg BW) N = 3	Experimental group (300mg/kg BW) N=3	Experimental group (2000mg/kg BW) N=3
Irritability	x	x	x	√
Hyperactivity	x	x	x	√
Convulsions	x	x	x	x
Analgesia	x	x	x	x
Stereotype	x	x	x	x
Tremour	x	x	x	x
Stub Tail	x	x	√	√

Source: OECD 423
√- Present
X- Absent
Control Group - Standard rat chow
Experimental Group – *Beta vulgaris* L.leaf extract

Central Nervous System stimulant activities, such as stub tail was observed in the experimental group administered with aqueous extract of *Beta vulgaris* L. leaf extract of 300 mg/ kg body weight. Similarly, irritability and hyperactivity were exhibited by rats after the supplementation of aqueous extract of *Beta vulgaris* L. leaf with high dose of 2000 mg/ kg body weight. All the central nervous system stimulant activities such as stub tail, irritability and hyperactivity were exhibited immediately after supplementation of *Beta vulgaris* L. extractand got subsided after 3-4 hours.

(ii) Observation of Central Nervous System (CNS) Depression Activities

Observation of Central Nervous System (CNS) Depression activities in association with acute toxicity study are expressed in TABLE XVIII.

Table XVIII

Observation of Central Nervous System Depression Activities

Sl.	Gross Behavior	Control group N=3	Experimental group (50mg/kg BW) N = 3	Experimental group (300mg/kg BW) N=3	Experimental group (2000mg/kg BW) N=3
1	Hypo activity	x	x	x	x
2	Passivity	x	x	x	x
3	Relaxation	x	x	x	x
4	Necrosis	x	x	x	x
5	Ataxia	x	x	x	x

Source: OECD 423

√- Present

X- Absent

Control Group - Standard rat pellet

Experimental Group – *Beta vulgaris L* leaf extract

The Central Nervous System (CNS) depression activities observed in association with acute toxicity study showed none of the activities namely hypoactivity, passivity, relaxation, necrosis and ataxia were absent in the control and experimental groups after supplementation of *Beta vulgaris* L. leaf aqueous extract.

(iii) Observation of Autonomous Nervous System (ANS) activities

Activities of the Autonomous Nervous System (ANS) observed in association with acute toxicity study are expressed in Table XIX.

Table XIX

Observation of Autonomous Nervous System Activities

Gross Behavior	Control group N=6	Experimental group (50mg/kg BW) N = 3	Experimental group (300mg/kg BW) N=3	Experimental group (2000mg/kg BW) N=3
Salivation	x	x	x	√
Urination	x	x	x	√
Ptosis	x	x	x	x
Exophthalmia	x	x	x	x
Lacrimation	x	x	x	x

Source: OECD 423

√- Present

X- Absent

Control Group - Standard rat chow

Experimental Group – *Beta vulgaris* L.leaf extract

Autonomous Nervous System (ANS) activities such as urination and salivation were observed in the group supplemented with aqueous extract of *Beta vulgaris* L. leaf of a high dose of 2000 mg/kg body weight. Autonomous Nervous System (ANS) activities such as urination and salivation after supplementation of high dose of *Beta vulgaris* L. aqueous extract of 2000 mg/kg bodygot diminished after 3-4 hours.

Since a single high dose (HD) of 2000 mg/kg body weight of *Beta vulgaris* L.leaf extract supplementation did not exhibit high toxicity effect and mortality in any group, hence it was decided to supplement 400 mg/kg body weight/ animal /day dose extract for experimental study.Jain and Singhai, (2012) observed similar effect in acute oral toxicity studies of *Beta vulgaris* L. extract that did not show any sign and symptoms of toxicity and mortality up to 2000 mg/kg dose. Similar study was observed by Sulakhiya *et al*., (2016) with the administration of *Beta vulgaris* L. leaf of 200 mg/kg showed positive effect against anti-depressant behavior and oxidative damage in mice.

Phase V: *In vivo* Effects of *Beta vulgaris* L. Leaf Aqueous Extract on Polycystic Ovarian Syndrome (PCOS) in Adult Female Rats

A. Evaluation of Estrous Cyclicity of Experimental Animals

Throughout the experiment, the estrous cycle was studied through regular monitoring and the collection of vaginal smears from all of the experimental groups. The control group used to have a normal estrous cycle of 4-5 days with all four phases in order, whereas the PCOS induced groups' estrous cycle was infrequent and lengthy.Metformin treated Group III and *Beta vulgaris* L. leaf extract treated groups IV, V and VI showed restoration of estrous cycle. Jahan *et al*., (2018) observed Quercetin and metformin-treated groups with PCOS showed restoration of the estrous cycle. Rajan *et al.,* (2017) found that soy isoflavones 50 and 100 mg/kg resulted in a dose-dependent ($p< 0.001$) decreasein the percentage of dioestrous days in comparison to PCOS group.

B. Evaluation of the Effects of *Beta vulgaris* L. Leaf Aqueous Extract on PCOS Adult Rats in Association with Physical Parameters

(i) Effects of *Beta vulgaris* L. leaf aqueous extract on the changes ofthe body weight of the PCOS control and treatment groups

Mean body weight gain of PCOS induced control and treatment groups on *Beta vulgaris* L. leaf supplementation is presented in Table XX.

Table XX
Mean Body Weight of PCOS Control and Treatment Groups

Groups	Initial Weight (g)	Final Weight (g)	Mean Difference	't' Value	P value
I	125.67±2.9	159.83±4.0[c]	34.17	15.77**	0.000
II	130.17±2.6	107.50±4.4[bd]	22.7	9.66**	0.000
III	125.67±3.4	156.17±3.2[c]	30.50	14.25**	0.000
IV	126.00±2.8	138.50±2.8[bcd]	12.5	5.26**	0.001
V	128.5±3.4	143.33±2.9[bcd]	14.83	6.75**	0.01
VI	124.17±2.8[a]	152.83±2.6[bc]	28.67	21.11**	0.000

**** Significance at p<0.01**

Group I normal control
Group II Estradiol valerate induced PCOS standard control
Group III PCOS induced + Metformin standard control
Group IV PCOS induced + *Beta vulgaris* L. leaf extract 100mg
Group V PCOS induced + *Beta vulgaris* L. leaf extract 200mg
Group VI PCOS induced + *Beta vulgaris* L. leaf extract 400mg

a- Significant (P = < 0.05) – with PCOS standard control (Group II)
b- Significant (P = <0.01) – with normal control (Group I)
c- Significant (P = <0.01) – with PCOS standard control (Group II)
d- Significant (P = <0.01) – with PCOS + metformin standard control (Group III)

The rats were monitored for changes in body weight from the beginning to the end of the experiment. The animals included in the study showed approximately equal initial body weight. However, final body weights showed significant differences in PCOS induced group III when compared with normal control and other treatment groups IV,V and VI. After 21 days of *Beta vulgaris* L. leaf extract, PCOS treatment groups IV (100 mg/kg BW), V (200 mg/kg BW), and VI (400 mg/kg BW) showed more weight gain of 138.50±2.8 g,143.33±2.9g, and 152.83±2.6g respectively, compared to PCOS control group II, which received estradiol valerate intramuscular with a mean bodyweight of 107.50±4.4g. Food intake did not differ between the normal control group, PCOS control group or in any of the treatment groups.

However, rats exposed to estradiol valerate, PCOS control group II exhibited lower locomotor activity than the normal control group I administered with saline. At the end of the intervention, administration of *Beta vulgaris* L. leaf extract on female rats for 21 days resulted in a significant increase in final body weights of the PCOS group as compared to thePCOS control group ($p<0.05$).Treatment with *Beta vulgaris* L. leaf 200 mg/kg exhibited a significant increase in body weight ($p<0.01$), in comparison to vehicle treated PCOS group, at the end of the treatment period. Similar results were observed in treatment with soy isoflavones and chamomile tea extract in PCOS rats exhibited significance ($p<0.05$) in comparison to vehicle treated PCOS group (Romualdi *et al*., 2008). Another study by Wang*et al*., (2020) observed dramatically increase in body weight in the pair-fed with letrozole –induced PF/MOD group compared to the pair-fed control group PF/CON group (P = 0.0013) after 11 weeks of intervention with flaxseed oil (FO). Also, the study by Ghafurniyan *et al*., (2015) reported a statistically significant weight decrease in PCOS model rats after administration of green tea extract for 10 days (P < 0.05).

(ii) Effects of *Beta vulgaris* L. leaf aqueous extract on the changes of the ovary weight of the PCOS control and treatment groups

Mean ovary weight gain of PCOS induced control and treatment groups on *Beta vulgaris* L. leaf supplementation is presented in Table XXI.

Table XXI

Mean Ovary Weight of PCOS Control and Treatment Groups

Groups	Ovary Weight (g)	
	Mean ± SD	**SE**
I	0.96 ± 0.05^{c}	.02124
II	1.31 ± 0.03^{ad}	.01138
III	1.01 ± 0.02^{c}	.01014
IV	1.26 ± 0.02^{abd}	.00843
V	1.18 ± 0.02^{acd}	.00882
VI	1.06 ± 0.03^{ac}	.01065
'f' VALUE	**125.09 P=0.000****	

**** Significance at p<0.01**

Group I normal control
Group II Estradiol valerate induced PCOS standard control
Group III PCOS induced + Metformin standard control
Group IV PCOS induced + *Beta vulgaris* L. leaf extract 100mg
Group V PCOS induced + *Beta vulgaris* L. leaf extract 200mg
Group VI PCOS induced + *Beta vulgaris* L. leaf extract 400mg

a -Significant (P = <0.01) – with normal control (Group I)
b - Significant (P = <0.05) – with PCOS standard control (Group II)
c - Significant (P = <0.01) – with PCOS standard control (Group II)
d - Significant (P = <0.01) – with PCOS + Metformin standard control (Group III)

The results obtained from Table XXI regarding the ovarian weight showed, that ovarian weight increased after in all the groups II, III, IV, V and VI after induction of polycystic ovary syndrome.Following a 21-day administration of metformin and *Beta vulgaris* L. leaf aqueous extract, animal ovary weight decreased in treatment group with metformin control group III (1.01 ± 0.02g) and treatment group IV of *Beta vulgaris* L. 100 mg (1.26 ± 0.02g), treatment group V of *Beta vulgaris* L. leaf extract of 200mg (1.18 ± 0.02g), treatment group VI of *Beta vulgaris* L. leaf extract of 400 mg (1.06 ± 0.03g).The administration of metformin and *Beta vulgaris* L. leaf extract reversed the effect of estradiol valerate as it decreased animal ovary weight by significance (p <0.01), as compared with the Control Group II

(1.31 ± 0.03g). There was a significant difference noted in weights of both left and right ovaries among the control and treatment groups. Ndeingang *et al*., (2019) examined that ovarian weight reduced in the experimental group receiving aqueous extract of *Phyllanthus muellerians* plant extract (30 mg/kg; $P < 0.05$; 60 mg/kg: $P < 0.01$) and the large size of the polycystic ovaries compared to the PCOS control group ovaries. According to Jahan *et al*., 2016 observed no significant difference in weights diameter and ovarian organ index among metformin and rutin groups. (Ullah *et al*., 2020) reported no significant difference noticed in ovarian length in all treatment groups given metformin (2mg/kg), GABA (100 mg/kg/day) and GABA (500 mg/kg/day) along with letrozole.

(iii) Effects of *Beta vulgaris* L. Leaf extract on the changes of the liver weight of the PCOS control and treatment groups

Table XXII shows the effect of supplementation of *Beta vulgaris* L. leaf aqueous extract of *Beta vulgaris* L. leaf extract on liver weight of PCOS control and treatment groups.

Table XXII

Mean Liver Weight of PCOS Control and Treatment Groups

Groups	Liver Weight (g)	
	Mean ± SD	**SE**
I	5.86 ± 0.21^{bd}	0.09
II	4.46 ± 0.23^{ad}	0.09
III	5.54 ± 0.14^{ab}	0.06
IV	4.93 ± 0.15^{abd}	0.06
V	5.24 ± 0.07^{abc}	0.03
VI	5.40 ± 0.04^{ab}	0.01
'f' VALUE	**59.42P = 0.000****	

**** Significance at p<0.01**

Group I normal control
Group II Estradiol valerate induced PCOS standard control
Group III PCOS induced + Metformin standard control
Group IV PCOS induced + *Beta vulgaris* L. leaf extract 100mg
Group V PCOS induced + *Beta vulgaris* L. leaf extract 200mg
Group VI PCOS induced + *Beta vulgaris* L. leaf extract 400mg

a -Significant (P = <0.01) – with normal control (Group I)
b - Significant (P = <0.01) – with PCOS standard control (Group II)
c - Significant (P = <0.05) – with PCOS + Metformin standard control (Group III)
d - Significant (P = <0.01) – with PCOS + Metformin standard control (Group III)

Examining the changes in the liver weight from Table XXII depicteda decrease in liver weight in PCOS induced control group II (4.46±0.23 g). After administration of *Beta vulgaris* L. leaf extract for 21 days of 100 mg of *Beta vulgaris* L. leaf extract in experimental group IV increased in liver weight (4.93±0.15 g), 200 mg of *Beta vulgaris* L. extract in experimental group V (5.24±0.07 g) and 400 mg of *Beta vulgaris* L. extract in experimental group VI (5.40±0.04 g) respectively. At the end of the intervention, administration of *Beta vulgaris* .L. leaf extract on female rats for 21 days resulted in a significant increase in liver weight of the PCOS group as compared to the PCOS control group (p<0.01).

Wang *et al.,* (2021) observed that supplementation of omega-3 PUFAs sources had healthy impacts on liver weight, lipid profile, blood glucose and body weight of PCOS rats when compared with rat fed positive control group with PCOS.

C. Evaluation of the Effects of *Beta vulgaris* L. Leaf Aqueous Extract on PCOS Adult Rats in Association with Biochemical Parameters

(i) Effects of *Beta vulgaris* L. leaf aqueous extract on blood glucose level of PCOS control and treatment groups

Blood glucose level of PCOS control group and *Beta vulgaris* L. leaf aqueous extract treated groups are shown in Table XXIII.

Table XXIII

Blood Glucose Level of PCOS Control and Treatment Groups

Groups	Initial Glucose (mg/dl)	Final Glucose (mg/dl)	Mean Difference	"t" Value	P value
I	88.00 ± 3.03^{c}	94.50 ± 4.09^{cd}	6.50	5.17**	0.004
II	92.83 ± 2.56	156.67 ± 4.89^{bd}	63.83	39.38**	0.000
III	97.00 ± 5.29^{b}	103.83 ± 4.12^{bc}	6.8	2.12	0.088
IV	93.33 ± 2.25	142.00 ± 3.41^{bcd}	48.67	46.17**	0.000
V	94.00 ± 3.03^{a}	125.17 ± 1.72^{bcd}	31.17	23.94**	0.000
VI	96.16 ± 3.06^{b}	116.50 ± 3.62^{bcd}	20.33	9.57**	0.000

**** Significance at p<0.01**

Group I normal control
Group II Estradiol valerate induced PCOS standard control
Group III PCOS induced + Metformin standard control
Group IV PCOS induced + *Beta vulgaris* L. leaf extract 100mg
Group V PCOS induced + *Beta vulgaris* L. leaf extract 200mg
Group VI PCOS induced + *Beta vulgaris* L. leaf extract 400mg

a- Significant (P = <0.05) – with normal control (Group I)
b- Significant (P = <0.01) – with normal control (Group I)
c- Significant (P = <0.01) – with PCOS standard control (Group II)
d- Significant (P = <0.01) – with PCOS + *Beta vulgaris* L. treatment group (Group V)

Insulin resistance aggravates the metabolic consequences of PCOS, leading to glucose intolerance, dyslipidemia, chronic inflammation and hypertension (Barry *et al.,* 2014). Blood glucose levels as determined at day 1 of the experiment

groups IV,V,VI treated with *Beta vulgaris* L. leaf extract with 100 mg, 200 mg and 400 mg were 93.33±2.25 mg/dl, 94.00±3.03mg/dl and 96.16±3.06 mg/dl, showed similar levels in all the groups. But final blood glucose levels increased in PCOS induced control group II (156.67±4.89mg/dl). Treatment with metformin treatment group III reduced to (103.83±4.12mg/dl) and *Beta vulgaris* L. leaf extract treatment group IV, V, VI at 100mg, 200mg and 400 mg reduced to 142.00±3.41 mg/dl, 125.17±1.72mg/dl, and 116.50±3.62mg/dl. There was a significant increase ($P < 0.01$) observed in blood glucose of PCOS induced group as compared to the normal control group I (94.50 ± 4.09 mg/dl). In metformin and *Beta vulgaris* L. leaf extract treatment groups also showed significantly different ($P<0.01$) with the control in the blood glucose level. Ullah *et al.,* (2020) showed a significant difference in PCOS group blood glucose as compared to the metformin treated group ($P<0.01$), Gamma-Aminobutyric acid (GABA) 1 and GABA 2 treated group ($P>0.05$). Sherafatmanesh *et al*., (2020) found both HOMA-IR and FBS levels exhibited a significant increase ($p< 0.001$) in the PCOS control group in comparison to the sham group. All the treated rats showed significant decrease in the FBS level ($P < 0.001$) when compared to the rats in the PCOS control group. Treatment with naringenin (20 mg/kg) in the letrozole and naringenin-treated rats (Group IV) resulted in significantly reduced plasma glucose levels when compared with the rats treated with letrozole only (Group III) (Hong*et al*., 2019).

(ii) **Effects of *Beta vulgaris* L. leaf aqueous extract on lipid profile of PCOS control and treatment groups**

Total Cholesterol, HDL-C, LDL-C and triglyceride levels were quantified by following the protocols provided with (Merck) kits. The serum lipid profile was analyzed before supplementation of *Beta vulgaris* L. on day1 and at the end of the experiment after 21 days, of experiment to analyze the hypolipidemic effect of *Beta vulgaris* L. leaf extract on PCOS condition.

Total cholesterol, Triglycerides, HDL-C and LDL-C levels of control and treatment group before and after supplementation of *Beta vulgaris* L. leaf aqueous extract are presented in Table XXIV.

Table XXIV

Total Cholesterol, Triglyceride, HDL-C and LDL-C Levels of PCOS Control and Treatment Groups

Groups	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL –C (mg/dl)	LDL –C (mg/dl)
I	154.67±3.67^{b}	74.66±3.78^{bc}	70.33±2.16^{bc}	58.27±2.79^{b}
II	415.33±3.55^{ac}	264.83±3.87^{ac}	28.66±2.50^{ac}	104.77±2.67^{ac}
III	159.17±3.92^{b}	95.50±4.63^{ab}	65.16±2.40^{ab}	55.97±1.84^{b}
IV	375.83±3.71^{abc}	190.00±3.40^{abc}	37.50±1.87^{abc}	73.31±1.92^{abc}
V	297.50±3.61^{abc}	152.33±3.01^{abc}	48.33±2.16^{abc}	61.89±0.22^{bcd}
VI	196.33±3.93^{abc}	124.50±2.66^{abc}	60.33±1.63^{abc}	57.97±1.42^{b}
f value	**5.50** **P = 0.000****	**2.21** **P = 0.000****	**352.1** **P = 0.000****	**522.9** **P = 0.000****

**** Significance at p<0.01**

Group I normal control
Group II Estradiol valerate induced PCOS standard control
Group III PCOS induced + Metformin standard control
Group IV PCOS induced + *Beta vulgaris* L. leaf extract 100mg
Group V PCOS induced + *Beta vulgaris* L. leaf extract 200mg
Group VI PCOS induced + *Beta vulgaris* L. leaf extract 400mg
a - Significant (P = <0.01) – with normal control (Group I)
b - Significant (P = <0.01) – with PCOS standard control (Group II)
c - Significant (P = <0.01) – with PCOS + *Beta vulgaris* L. treatment group (Group VI)
d-Significant (P = <0.05)- with normal control (Group I)

The levels of Total cholesterol, triglycerides, and LDL-C increased while the HDL-C decreased significantly in the PCOS induced control group II (415.33±3.55 mg/dl, 264.83±3.87 mg/dl, 104.77±2.67 mg/dl) as compared to the normal control group I (28.66±2.5 mg/dl). Total cholesterol decrease in *Beta vulgaris* L.leaf extract treatment group IV, V and VI with 100 mg, 200mg and 400 mg were (375.83±3.71mg/dl, 297.50±3.61mg/dl and 196.33±3.93 mg/dl), Triglyceride levels decreased in *Beta vulgaris.*L leaf extract treatment group IV, V and VI were (190.00±3.40 mg/dl, 152.33±3.01 mg/dl, and 124.50±2.66 mg/dl). High Density

Lipoprotein Cholesterol increased in *Beta vulgaris* L. leaf extract treatment group IV, V and VI were (37.50±1.87 mg/dl, 48.33±2.16 mg/dl, and 60.33±1.63 mg/dl). Low Density Lipoprotein Cholesterol decreased in *Beta vulgaris* L. leaf extract treatment group IV, V and VI were (73.31±1.92 mg/dl, 61.89±0.22 mg/dl and 57.97±1.42 mg/dl). However, the levels of total cholesterol, triglycerides, and LDL-C significantly ($P< 0.01$) decreased and HDL-C was increased to near-normal levels in *Beta vulgaris* L. treated groups V and VI. As shown in Table XXIV *Beta vulgaris* L. leaf aqueous extract treated group VI reduced the level of the triglyceride by 124.50±2.66 mg/dl as compared to metformin treated group III 95.50±4.63 mg/dl. Total Cholesterol level and low density lipoprotein is also profoundly reduced as 196.33±3.93 mg/dl, 57.97±1.42 mg/dl in treatment group VI, while the level of high density lipoprotein was increased significantly ($p<0.01$) in both metformin treated group III and *Beta vulgaris* L. leaf extract treated group VI.

As a common metabolic disorder in PCOS, dyslipidemia appears to be caused by hormonal imbalance and insulin resistance (Uno *et al*., 2006). It was found that *F.deltoidea* restored the levels of total cholesterol, triglycerides, LDL-C and HDL-C to normal levels in PCOS rats that improvement in hormonal profile and the estrous cycle. The data support the relationship between lipid profile and sex hormones (Haslan *et al.,* 2021). Phytoestrogens in herbal extracts reduce the synthesis of gonadal steroids such as testosterone through the reduction of cholesterol, which agrees with the findings of this study. Based on the present study, the anti-hyperlipidemic potential of *Beta vulgaris* L. leaf extract can contribute to its capacity in correcting hyperinsulinemia and hyperandrogenemia in PCOS induced rats.

D. Evaluation of the effects of *Beta vulgaris* L. Leaf aqueous extract on PCOS adult rats in association with reproductive hormones

(i) Effect of *Beta vulgaris* L. leaf aqueous extract on Leutinising hormone, Follicle stimulating hormone and Estradiol hormone on PCOS control and treatment groups

The effect of the *Beta vulgaris* L. leaf aqueous extract on the reproductive hormones such as Luteinizing hormone, Follicle stimulating hormone and Estrogen hormone on PCOS induced female rats by ELISA test are discussed as follows in Table XXV and Figure 14, 15 and 16.

Table XXV

Reproductive Hormone Levels of PCOS Control and Treatment Groups

Groups	Leutinising Hormone (mIU/ml)	Follicle Stimulating Hormone (mIU/ml)	Estradiol Hormone (pg/ml)
	Mean± SD	**Mean ± SD**	**Mean ± SD**
I	102.33±5.68^{bc}	38.18±0.66^{b}	0.74±0.02^{b}
II	763.00±7.87^{ac}	9.05±0.37^{ac}	1.32±0.01^{ac}
III	123.00±4.47^{ab}	36.96±0.63^{b}	0.77±0.03^{b}
IV	633.00±5.65^{abc}	20.25±0.17^{abc}	1.10±0.24^{abc}
V	441.67±6.97^{abc}	25.16±0.87^{abc}	1.01±0.01^{abc}
VI	148.17±5.45^{abc}	34.61±1.20^{abc}	0.76±0.01^{b}
f Value	**1.32 p=0.000****	**1.45 p = 0.000****	**30.1 p = 0.000****

**** Significance at p<0.01**

Group I normal control
Group II Estradiol valerate induced PCOS standard control
Group III PCOS induced + Metformin standard control
Group IV PCOS induced + *Beta vulgaris* L. leaf extract 100mg
Group V PCOS induced + *Beta vulgaris* L. leaf extract 200mg
Group VI PCOS induced + *Beta vulgaris* L. leaf extract 400mg

a- Significant (P = <0.01) – with normal control (Group I)
b - Significant (P = <0.01) – with PCOS standard control (Group II)
c - Significant (P =<0.01) – with PCOS + metformin standard control (Group III)

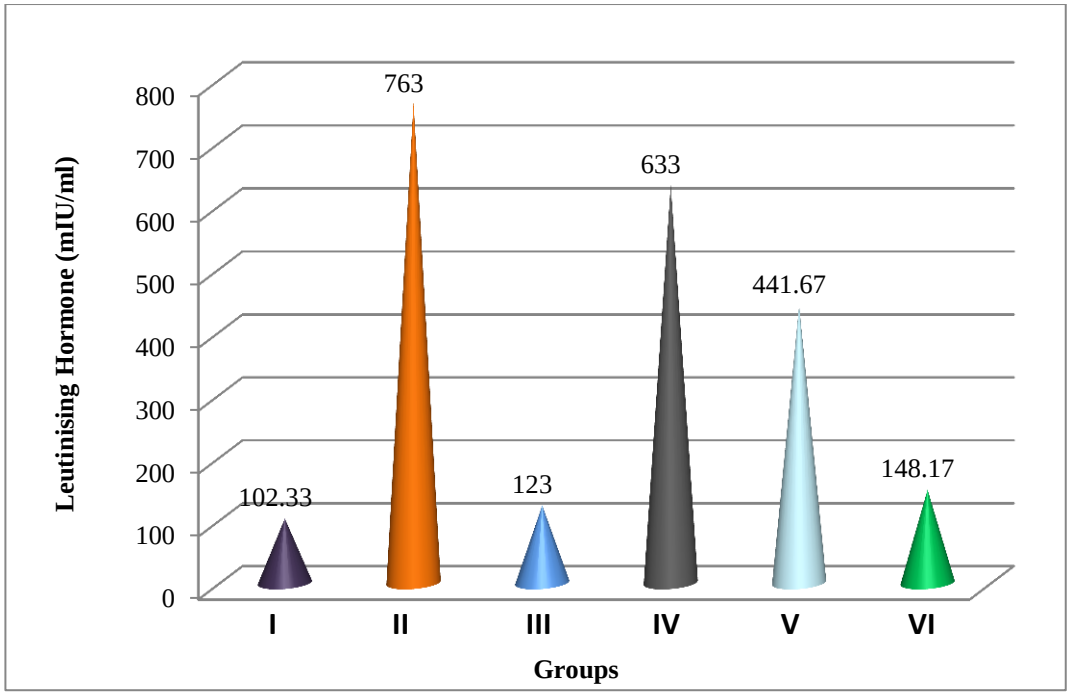


Figure 14: Lutenising Hormone Level of PCOS Control and Treatment Groups

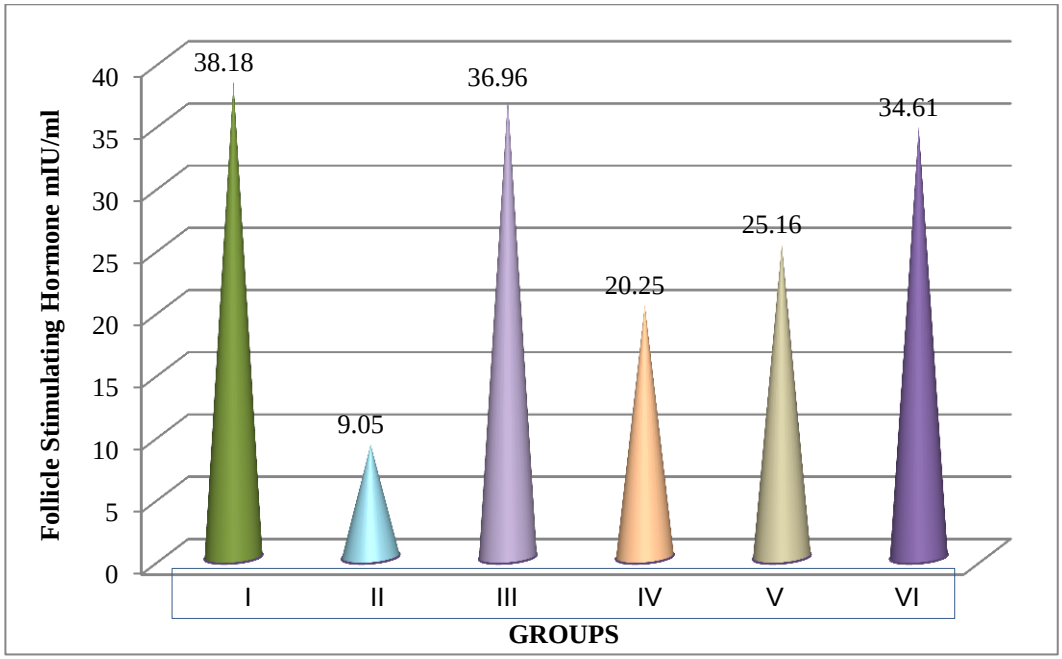


Figure 15: Follicle Stimulating Hormone Level of PCOS Control and Treatment Groups

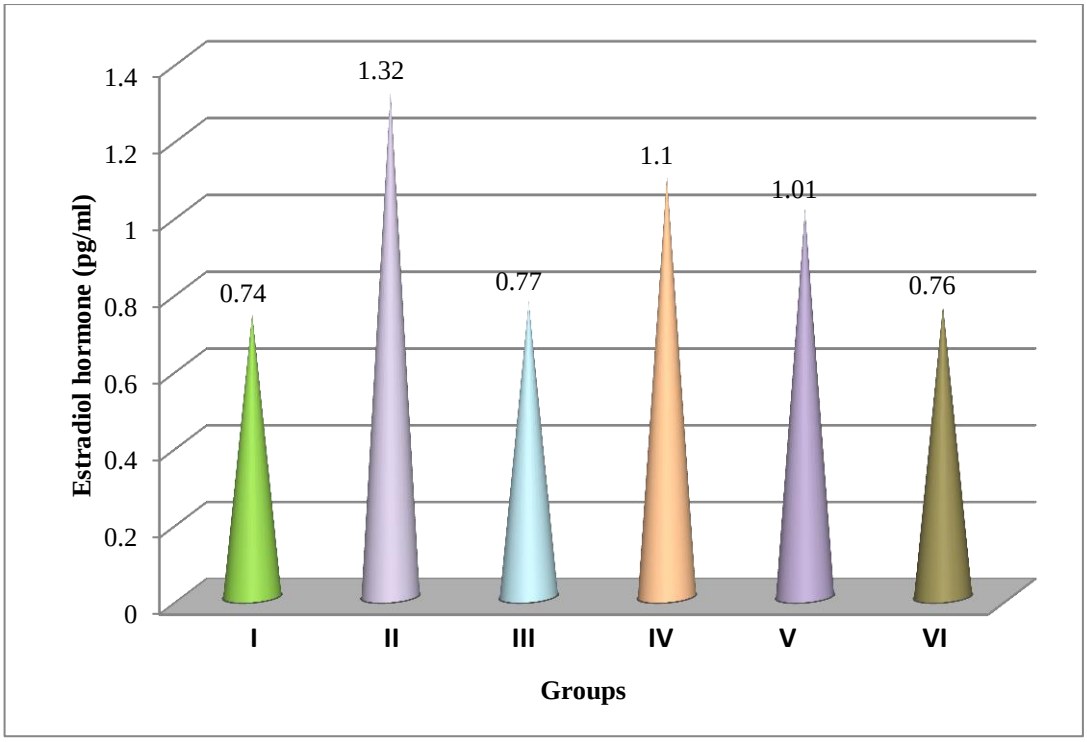


Figure 16: Estradiol Level of PCOS Control and Treatment Groups

Table XXV and Figure 14 clearly shows that luteinizing hormone was significantly higher at (p=<0.01), in PCOS induced group II (763.00±7.87mIU/ml) compared to the normal control group I (102.33±5.68mIU/ml). However, administration of *Beta vulgaris* L. leaf extract in treatment group VI of 400 mg for 21 days recovered those abnormal changes such as decrease in the leutinizing hormone (148.17±5.45mIU/ml) reduced the pathogenesis of PCOS. A similar effect was observed by Raja*et al*., (2021) administration of curcumin decreased Lutenizing hormone levels from 0.695 mIU/mL (PCOS group) to 0.560 mIU/ml, after treatment of PCOS rats with synthesized curcumin nanoparticles. Follicle stimulating hormone levels in the PCOS induced control group II (9.05±0.37mIU/ml) were significantly lower compared to the treatment group IV, V and VI (20.25±0.17mIU/ml, 25.16±0.87 mIU/ml and 34.61±1.20 mIU/ml) respectively. FSH level was significantly higher in metformin treatment group II (36.96±0.63mIU/ml). Administration of *Beta vugaris.*L leaf extract in treatment groups V and VI showed a significant increase (p=<0.01) in FSH hormone level in Figure 15. Ab Hamid, (2020) identified the significant increase of FSH hormone with the administration of royal jelly at 200mg and 400 mg/.kg BW in PCOS induced female Sprague Dawley rats.

Furthermore, estradiol level was significantly higher in the PCOS induced group II (1.32±0.01pg/ml) compared to the normal control group I (0.74±0.02pg/ml). Estradiol levels in *Beta vulgaris* L. leaf extract treatment groups IV, V and VI (1.10±0.24 pg/ml, 1.01±0.01pg/ml and 0.76±0.01pg/ml) were significantly decreased (p=< 0.01) compared to the PCOS induced control group II (1.32±0.01pg/ml) as shown in Figure 16. Serum levels of estradiol and gonadotropins, LH and FSH significantly decreased in the administration of *Chamomile* flower alcoholic extract in PCOS induced rats (Zanageneh *et al.,* 2010).

E. Evaluation of the effects of *Beta vulgaris* L. Leaf aqueous extract on PCOS adult rats in association with hepatoprotective and oxidative stress

Hepatoprotective effect of *Beta vulgaris* L. leaf aqueous extract was assessed by comparing the changes in lipid peroxidation in the liver. Non- enzymatic and enzymatic antioxidants were measured in liver homogenate levels and compared with PCOS control and treatment groups. All biochemical parameters were assessed at the end of 21 days.

(i) Effect of supplementation of *Beta vulgaris* L.leaf aqueous extract on lipid peroxidation level of PCOS control and treatment groups by TBARS assay

The effect of the *Beta vulgaris* L. leaf aqueous extract on the lipid peroxidation level on PCOS induced female rats by TBARS assay are discussed as follows in Table XXVI and Figure 17.

Table XXVI

Lipid Peroxide Level in Liver of PCOS Control and Treatment Groups

Groups	Lipid Peroxide (mM MDA/g)	
	Mean ± SD	**SE**
I	54.33±6.97[b]	2.84800
II	136.00±3.09[ac]	1.26491
III	56.66±3.26[b]	1.33333
IV	99.00±2.09[abc]	.85635
V	80.66±3.26[abc]	1.33333
VI	60.00±2.82[b]	1.15470
'f' Value	**398.6 P = 0.000****	

**** Significance at p<0.01**

Group I normal control
Group II Estradiol valerate induced PCOS standard control
Group III PCOS induced + Metformin standard control
Group IV PCOS induced + *Beta vulgaris* L. leaf extract 100mg
Group V PCOS induced + *Beta vulgaris* L. leaf extract 200mg
Group VI PCOS induced + *Beta vulgaris* L. leaf extract 400mg

a - Significant (P = <0.01) – with normal control (Group I)
b - Significant (P = <0.01) – with PCOS standard control (Group II)
c - Significant (P =<0.01) – with PCOS + metformin standard (Group III)

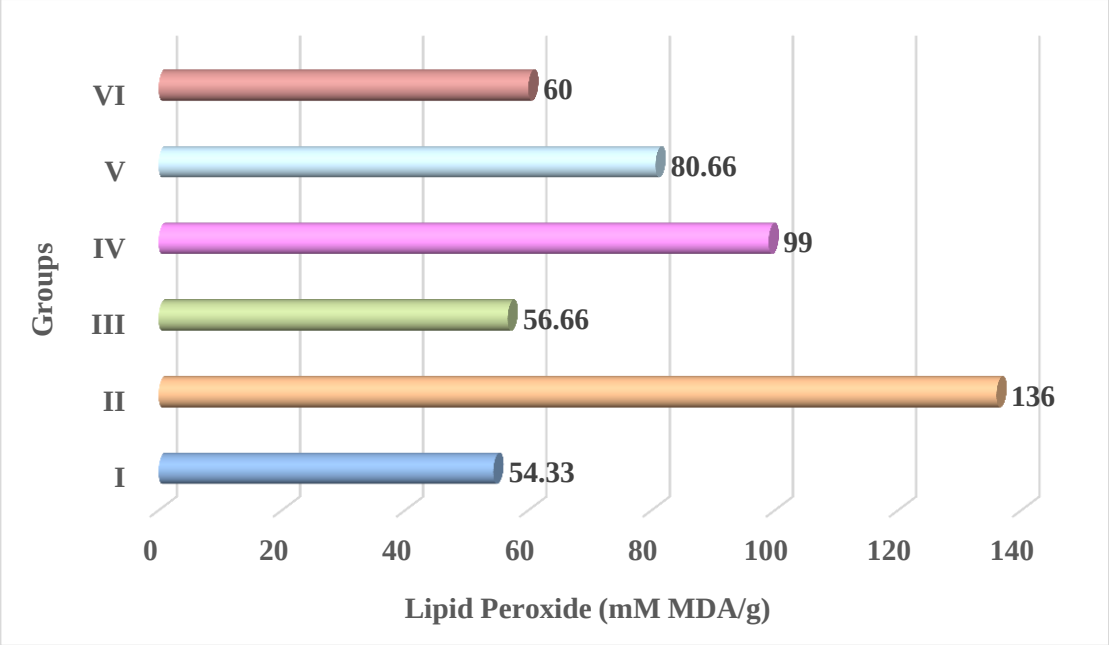


Figure 17: Lipid Peroxidation Level of PCOS Control and Treatment Groups

In comparison to normal control group I (54.33±6.97mM MDA/g), PCOS induced group II (136.00±3.09mM MDA/g) exhibited a significant elevation ($p < 0.01$) in lipid peroxide level. *Beta vulgaris* L. leaf aqueous extract 100 mg administered group IV (99.00±2.09mM MDA/g), 200 mg administered group V (80.66±3.26mM MDA/g) and 400 mg administered group VI (60.00±2.82mM MDA/g) significantly decreased ($p <0.01$) the lipid peroxidation levels in comparison to the metformin treated group III (56.66±3.26mM MDA/g). Momin and Yeligar, (2019) observed a similar effect in *Coccinea grandis* stem extract administered decreases the LPO levels in diabetic rats compared with the standard drug glimepride. Lee*et al.*, (2009) confirmed that red beet leaf extract decreased lipid peroxidation in mice fed high fat and high cholesterol diet.

(ii) Effect of supplementation of *Beta vulgaris* L. leaf aqueous extract on enzymatic antioxidants of PCOS control and treatment groups

Effect of supplementation of *Beta vulgaris* L. leaf aqueous extract on enzymatic antioxidants namely superoxide dismutase (SOD) and catalase (CAT) of PCOS induced control and treatment groups were assessed and interpreted as follows. Enzymatic antioxidants namely super oxide dismutase (SOD) and catalase (CAT)

were analyzed in ovarian homogenate after 21 days of the experimental period are given in Table XXVII and Figure 18 and 19.

Table XXVII

Superoxide Dismutase (SOD) and Catalase Level of PCOS Control and Treatment Groups

Groups	SOD (mg epinephrine oxidized /g)	Catalase (mg H_2O_2 hydrolyzed/g)
	Mean ± SD	**Mean ± SD**
I	11.99 ± 1.79^{b}	24.20 ± 0.97^{cd}
II	3.65 ± 0.67^{ac}	7.20 ± 1.56^{be}
III	11.05 ± 1.05^{b}	22.40 ± 0.48^{ac}
IV	6.58 ± 1.10^{abc}	12.50 ± 0.70^{bce}
V	11.63 ± 0.66^{b}	15.00 ± 0.53^{bce}
VI	11.09 ± 0.85^{b}	21.40 ± 0.90^{bc}
'f' VALUE	**58.32 P = 0.000****	**302.8 P = 0.000****

**** Significance at p<0.01**

Group I normal control
Group II Estradiol valerate induced PCOS standard control
Group III PCOS induced + Metformin standard control
Group IV PCOS induced + *Beta vulgaris* L. leaf extract 100mg
Group V PCOS induced + *Beta vulgaris* L. leaf extract 200mg
Group VI PCOS induced + *Beta vulgaris* L. leaf extract 400mg

a - Significant (P = <0.05) – with normal control (Group I)
b -Significant (P = <0.01) – with normal control (Group I)
c - Significant (P = <0.01) – with PCOS standard control (Group II)
d- Significant (P = <0.05) – with PCOS + metformin standard control (Group III)
e - Significant (P = <0.01) – with PCOS + metformin standard control (Group III)

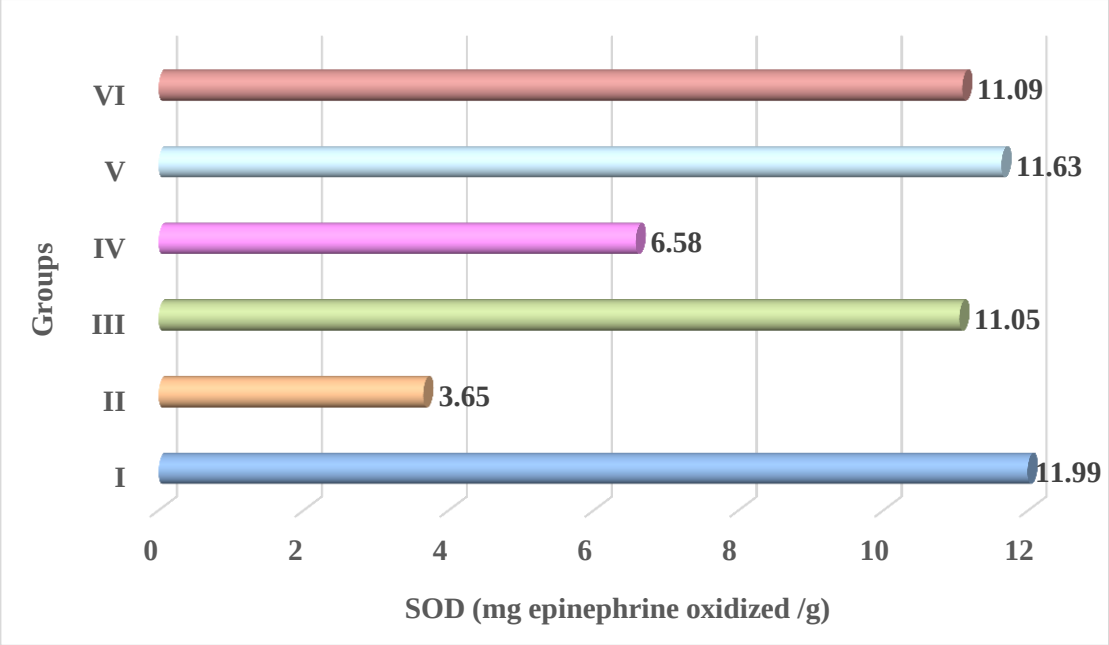


Figure 18: Superoxide Dismutase Activity of PCOS Control and Treatment Group

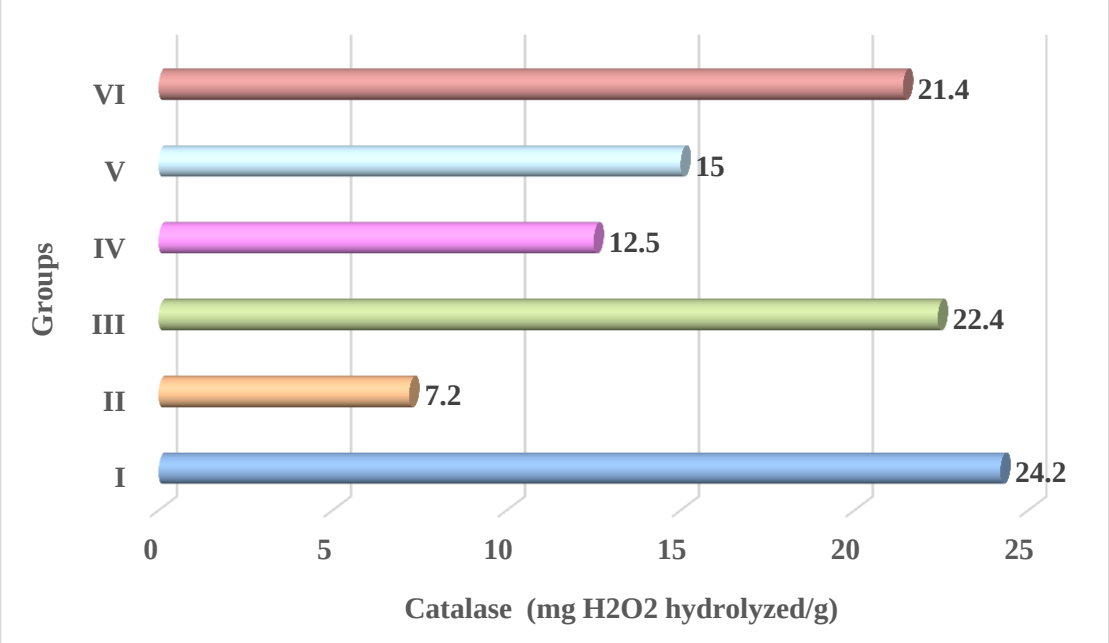


Figure 19: Catalase Level of PCOS Control and Treatment Group

In comparison to the normal control group I (11.99±1.79mg/g), PCOS induced control group II (3.65±0.67 mg/g) of estradiol valerate significantly decreased ($p<0.01$) the superoxide dismutase activity in the ovary. *Beta vulgaris* L. leaf aqueous extract (200 mg) treatment significantly increased ($p < 0.01$) the superoxide dismutase activity in the ovaries in comparison to the metformin treated control group III

(11.05±1.05 mg/g) rats as shown in Figure 18. In comparison to normal control group I (24.20±0.97 mg/g) rats , administration of estradiol valerate significantly decreased ($p < 0.01$) the catalase levels. Treatment with *Beta vulgaris* L. leaf extract 100mg, 200mg and 400 mg in treatment groups IV, V and VI reversed the estradiol valerate effect as (12.50±0.70mg/g, 15.00±0.53mg/g and 21.40±0.90 mg/g) evidenced by a significant increase ($p< 0.01$) in catalase activity, in the ovarian homogenate in comparison to PCOS induced group II (7.20±1.56 mg/g) rats (Figure 19). A high dose of curcumin (100 mg/kg) potentially augmented its after effect by increasing the activity of SOD ($p<0.05$), catalase ($p< 0.05$) and lipid peroxidation ($p< 0.01$). High dose of curcumin (200 mg/kg) potentially augmented the antioxidant enzyme activity significantly ($p<0.01$) and reduced lipid peroxidation level ($p<0.001$) when compared to the control group (Reddy *et al*., 2016).

(iii) Effect of supplementation of *Beta vulgaris* L. leaf aqueous extract on non-enzymatic antioxidants of PCOS control and treatment groups

Effect of supplementation of *Beta vulgaris* L. leaf aqueous extract on non enzymatic antioxidants namely Glutathione (GSH) content in PCOS induced control and treatment groups were assessed and interpreted as follows in Table XXVIII and Figure 20.

Table XXVIII

Glutathione Stimulating Hormone (GSH) Level of PCOS Control and Treatment Groups

Groups	Glutathione Stimulating Hormone (GSH) (mg/g)
	Mean ± SD
I	17.41±1.31[b]
II	3.50±0.78[ad]
III	16.33±1.16[b]
IV	5.25±1.21[ad]
V	10.66±1.50[abd]
VI	14.08±1.16[abc]
'f' Value	137.8 P = 0.000**

**** Significance at p<0.01**

Group I normal control

Group II Estradiol valerate induced PCOS standard control

Group III PCOS induced + Metformin standard control

Group IV PCOS induced + *Beta vulgaris* L. leaf extract 100mg

Group V PCOS induced + *Beta vulgaris* L. leaf extract 200mg

Group VI PCOS induced + *Beta vulgaris* L. leaf extract 400mg

a - Significant (P = <0.01) – with normal control (Group I)

b - Significant (P = <0.01) – with PCOS standard control (Group II)

c - Significant (P = <0.05) – with PCOS + metformin standard control (Group III)

d - Significant (P = <0.01) – with PCOS + metformin standard control (Group III)

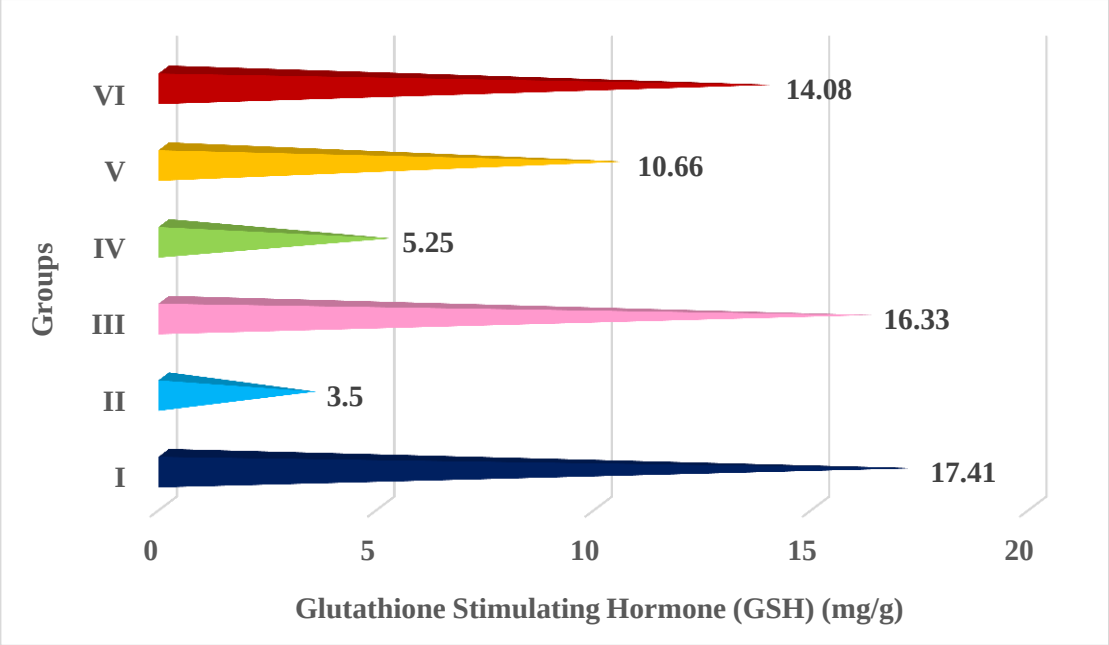
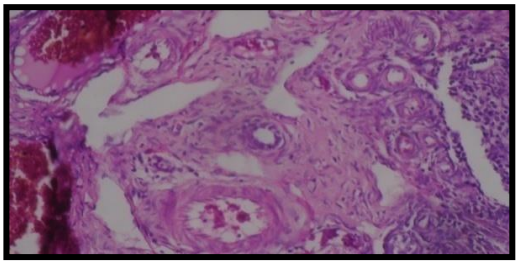


Figure 20: Glutathione Stimulating Hormone of PCOS Control and Treatment Group

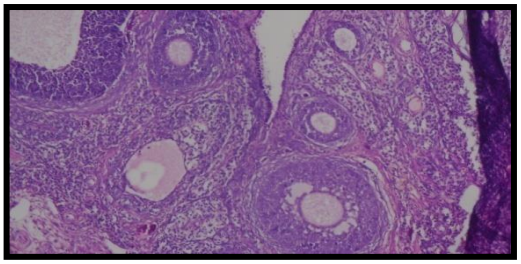
Glutathione stimulating hormone levels were significantly decreased ($p<0.01$) in PCOS induced group II (3.50±0.78 mg/g) in comparison to normal control group I (17.41±1.31 mg/g). *Beta vulgaris* L. leaf aqueous extract treatment at 100 mg (5.25±1.21 mg/g), 200 mg (10.66±1.50 mg/g) showed significance at ($p < 0.01$) and 400 mg (14.08±1.16mg/g) showed significance at ($p<0.05$) resulted in a significant increase in the Glutathione stimulating hormone levels in comparison to the metformin treatment group III (16.33±1.16 mg/g) as shown in Figure 20. In therapeutic groups given *Apium graveolens* and *Cinnamon zeylanicum* extracts, SOD and GPX levels in ovarian tissue increased significantly ($p<0.05$) when compared to the control group PCOS rats (Khodaeifar *et al.*, 2019).

F. Histopathology Results of the PCOS Control Adult Rats Ovary

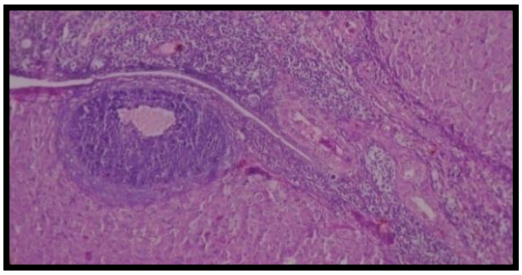
Histopathological examination of ovaries tissue harvested from control and treatment groups are given in Plate 10.



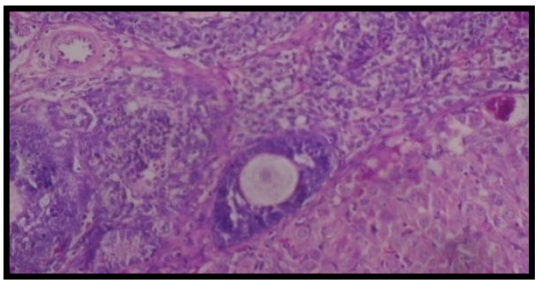
Normal Control Group I



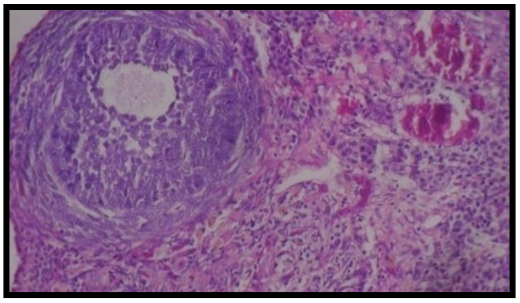
PCOS induced Standard Control Group II



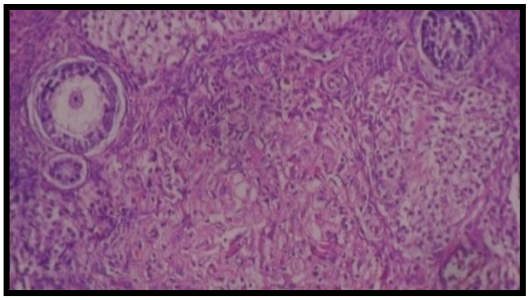
Standard Control Group + Metformin



Treatment Group I – BV (100 mg/kg bw)



Treatment Group II – BV (200 mg/kg bw)



Treatment Group III – BV (400 mg/kg bw)

Group I (Normal Control)
Group II (PCOS induced control)
Group III (PCOS + Metformin)
Group IV (PCOS + *Beta vulgaris* L. leaf aqueous extract 100 mg)
Group V (PCOS + *Beta vulgaris* L. leaf aqueous extract 200 mg)
Group VI (PCOS + *Beta vulgaris* L. leaf aqueuous extract 400 mg)

Plate 10: Histopathological Examination of Ovarian Tissue

Follicles with oocytes at various stages of development were visible in sections of ovaries from the normal control group I. PCOS induced standard control group II, which was induced by estradiol valerate, had numerous subcapsular cysts with a very thin or no granulose layer.Anovulation was indicated by the absence of corpora lutea. At this stage of development, only a few follicles were visible. They were also associated with atretic follicles with a fluid-filled antrum and a higher frequency of pyknotic granulosa cells. Control group III received metformin treatment, which resulted in the disappearance of cysts and the appearance of healthy

follicles and corpora lutea. In treatment group IV, sections from low doses of *Beta vulgaris* L. leaf extract (100 mg/kg) revealed larger follicles and fewer corpora lutea.In a section from treatment group V given 200 mg/kg of *Beta vulgaris* L. leaf extract, cysts were absent and normal sized healthy follicles at various developmental stages with oocytes were discovered.Many corpora lutea and antral follicles with clearly differentiated oocytes, granulosa cell layer, corona radiate, cumulus oophorus, and thecal cells were also observed with the administration of a high dose of*Beta vulgaris* L. treatment group VI. These morphological aspects were in accordance with pervious report by Brawer *et al.,* 1986. Curcumin (100mg/kg) showed similar results of few corpora lutea and high dose of curcumin (200mg/kg) showed the absence of cysts and normal sized healthy follicles (Reddy *et al.*, 2016).