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Therapeutic Investigation of Ethanolic Extract of *Rubus ellipticus* for PCOS in Wistar rats

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Abstract: The most prevalent endocrinopathy that impacts women in their reproductive years, polycystic ovarian syndrome (PCOS), is not yet widely recognized as a serious health issue. Worldwide, 4% to 20% of women who are of reproductive age are affected. The majority of PCOS-affected women also have high body mass indexes or obesity, which increases androgen production while compromising metabolic and reproductive capabilities and contributes to the development of the PCOS.

Materials and procedures: 24 Albino Wistar rats, all female, were used for the study. For 21 days, letrozole (1 mg/kg) was administered orally to all groups to induce PCOS. After PCOS induction, rats were treated for 49 days with medication (Group II, Clomiphene citrate, 1 mg/kg) and different concentrations of extract (Group III, 200 mg/kg, and Group IV, 400 mg/kg). Regular vaginal smears were used to track the estrous cycle. Every week, the body weight was measured. On days 0, 21, and 49, blood samples were taken to measure the levels of hormones, lipid profiles, and fasting blood glucose.

Results: This study indicated that ethanolic extract of *Rubus ellipticus* 400mg/kg has anti-inflammatory and antihyperlipidemic activity also normalize hormonal levels and shows significant impact on lipid, cholesterol and triglyceride profile in PCOS rats.

Key Word: PCOS, reproduction, *Rubus ellipticus,* body mass index

INTRODUCTION:

Polycystic ovarian syndrome (PCOS) is a diverse disease associated with a female endocrine reproductive problem. It affects females aged 18 to 44. The constant hormonal imbalance leads to complexity such as many cysts and a cycle that is not regular, which ultimately cause female infertility [1]. Other names for this condition are Stein Leventhal Syndrome, Multi cystic Ovaries, and Schlerocystic Ovaries, which were coined by American gynecologists Irving F. Stein, SR, and Michael L. Leventhal [2Numerous 8 mm cysts are seen in the ovarian sacs of PCOS patients. Over 12 cysts are seen in the ovary. This disease renders around 70% of females sterile [3], [4]. Signs or symptoms of excess androgens, such as abundant facial or body hair, acne, hair loss on the scalp, or increased blood testosterone levels, irregularity or lack of

menstruation. Diabetes mellitus, obesity, dyslipidemia, hypertension, Depression and anxiety are more prevalent among PCOS-affected women [5]. Furthermore, an ultrasound scan may not reveal ovarian cysts, and the ultrasound image is not always visible.[6]. Although research indicates a significant genetic component that may be influenced by lifestyle factors, the gestational environment, or both, the origin of polycystic ovary syndrome remains unclear. [7]. Environment or lifestyle during pregnancy also resulted in elevated hormone levels. Immediate female relatives of the patient, such as daughters or sisters, have a one-in-two chance of getting PCOS. [8]. For the treatment of symptoms many medicines are there Metformin acts as a PCOS treatment by boosting the body's insulin sensitivity [9]. Gonadotropins: AI and SERM should consider this as a backup alternative [10]. Inositol also found as a dietary supplement, boosts insulin signaling. It can enhance ovulation and menstrual cycles, [11]. Although there are many allopathic medicines for PCOS, they have many side effects and may elevate other symptoms too. The utilization of lead compounds, spices, nutraceuticals, and therapeutic plants in traditional medicine has contributed. humans fight multiple health conditions. Many medicinal plants have been used since ancient times for the treatment of infertility, menstrual ir-regulation and hormonal imbalance, which are effective and have barely any side effects. Our study included the use of *Rubus ellipticus* plant extract for the treatment of PCOS. *Rubus ellipticus* is also referred to as, yellow Himalayan raspberry, golden Himalayan raspberry, and ainselu [12, 13]. The tinsel is a big shrub that reaches a maximum length of 4.5 meters (15 feet) with sturdy stems. Its leaves have long bristles and are toothed, trifoliate, elliptic, or obovate. Its leaves have a maximum length of 5 to 10 cm (2 to 4 in). It blooms in the Himalayas in February and April and has short, white flowers with five petals that grow in clusters [15].

Its fruit is tasty, separable, and highly prized by birds and elephants [16]. Native to India, Nepal, southern China, Assam, Myanmar, the Philippines, Sri Lanka, Thailand, Tibet, and Vietnam, the ainselu originated in the temperate Himalayas [17]. Several bioactive compounds have been found to have positive effects on a number of chronic diseases, including diabetes [18], hypercholesterolemia [19], high blood pressure [20], cirrhosis [21], cancer [23], Bacterial infections, age-related degenerative illnesses, and autoimmune disorders [24]. The leaves and fruits of *Rubus ellipticus* are used in conventional medicine to treat nausea and bronchitisa, ulcers, and diabetes. It also has antibacterial, tonic and carminative qualities. [25], [26]. The root bark is used as an emmenagogue and abortifacient, and it is used to heal shattered bones, diarrhoea, and dysentery [27]. Chemical constituent: This plant has a high concentration of

Polyphenols, flavonoids, anthocyanins, tannins, and terpenoids. It contains several plant compounds, kaempferol, gallic acid, ascorbic acid, and catechin, among others,

have been intensively examined for their possible health benefits [28].

Material and Methods:

Collection and authentication of Plant: Plant *Rubus ellipticus* were obtained from Himalayan region of Garhwal (Uttarakhand). The herbal plant *Rubus ellipticus* was authenticated by BSI-Botanical Survey of India, Dehradun (Uttarakhand).

Preparation of plant extract: Dried leaves of *Rubus ellipticus* were collected and grinded into coarse powder using grinding machine, stored in airtight container and herbal plant extract prepared by Soxhlet extraction method using ethanol as solvent. [29]

Experimental Animals: The Siddhartha Institute of Pharmacy & Research's Institutional Animal Ethics Committee (IAEC) granted consent for the study's use of animals under approval number SIP/IAEC/PCOL/03/2023. Female Albino. The study employed 24 female Wistar rats (aged 20 weeks) weighing between 150 g and 250 g.

Letrozole induced PCOS study

(a) Experimental animals: Rats, adult female Albino Wistar (150 and 250 grams) were utilized in the research. The animals were allowed to acclimate for two weeks. All animals in the research were caged and kept in a controlled environment with 22 ± 3 C temperature, 55 ± 5% humidity, and a 12 h light/dark cycle. The animals were fed a regular food and given free access to water as needed. The Institutional Animal Ethical Committee (IAEC) accepted the use of animals.

(b) PCOS induction: For 21 days, letrozole, dissolved in distilled water at a rate of 1 mg/kg, was given orally to all of the experimental animals.

(c) Study design: 24 female Albino Wistar rats were used in the investigation, and they were split equally into four groups:

Group I (control): letrozole (1 mg/kg) + normal saline

Group II (Standard): letrozole (1 mg/kg) + clomiphene citrate

Group III (EERE 1): letrozole (1 mg/kg) + EERE I (200mg/kg)

Group IV (EERE II): Letrozole (1mg/kg) + EERE II (400mg/kg)

Since a rat's cycle lasts five days, the research was planned to span four cycles, resulting in a 28-day course of therapy. Every animal's body weight was recorded at the start of the trial and then every week after that.Serum glucose, lipid profile were measured on days 0, 21 and 49. Hormone levels were assessed and the ovary was removed for histopathological analysis on the last day of the trial.[30]

(d)Evaluation parameters:

i] Physical parameter: Body weights and BMIwere recorded at starting and weekly intervals during the trial.

ii] Biochemical Parameters: testosterone, blood glucose level. [31]

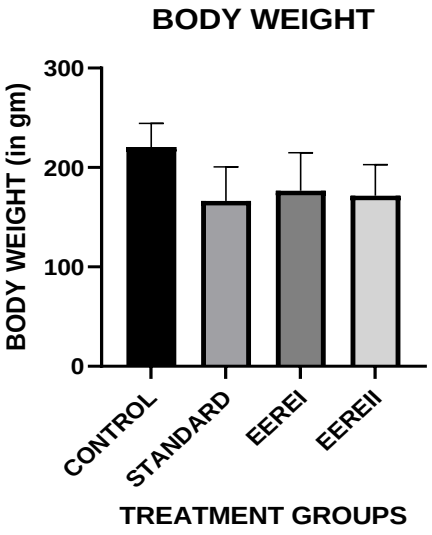
iii] Histopathology of ovary: On the last day of the investigation, each animal's ovaries were removed. It was taken out, wiped down, and weighed. The ovaries were embedded in paraffin blocks and preserved in a 10% formalin solution. After being sliced and dyed, tissue slices underwent a histopathological examination. The slides underwent examination under the microscope. Evaluation was done on the changes in the ovary, including the corpus lute, atretic, and cystic follicles.[32]

RESULT

Extraction of *Rubus ellipticus:* The thimble containing 30 gm of rude extract powder was put into the soxhlet chamber. The extraction procedure was started with 150 ml of Petroleum ether and then 250 ml methanol in Soxhlet assembly. After extraction, the solvent was recovered by using rota evaporator. The percentage yield of extract was found to be 50%.

Letrozole induced PCOS:

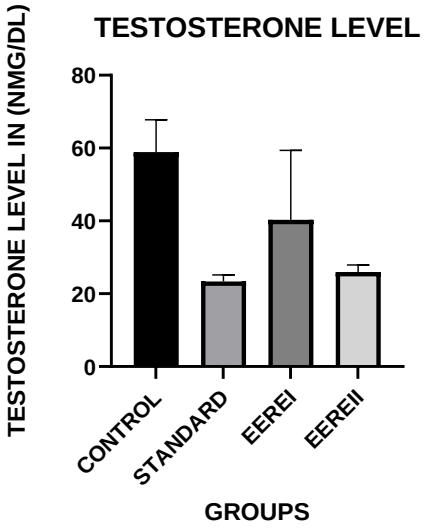
1. **Body Weight:**



Graph no 1: Showing the difference in body weight among various groups.

It was found that the body weight of the animals increased significantly in all groups when Letrozole was administered. Standard drug Clomiphene citrate was administered to the standard group which shows a reduction in body weight. In EEREI and EEREII the body weight was also decreased after treatment.

2.Testosterone Level:

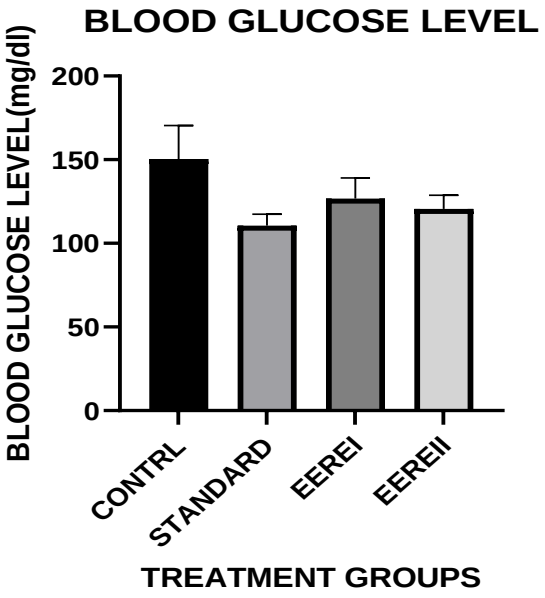


Graph no 02: Showing the difference in testosterone levels among various groups.

Testosterone levels increased significantly in all groups when Letrozole was administered. The testosterone level was decreased when the standard drug Clomiphene citrate was administered

to the standard group. In EERE I and EERE II, the level of testosterone was decreased significantly.

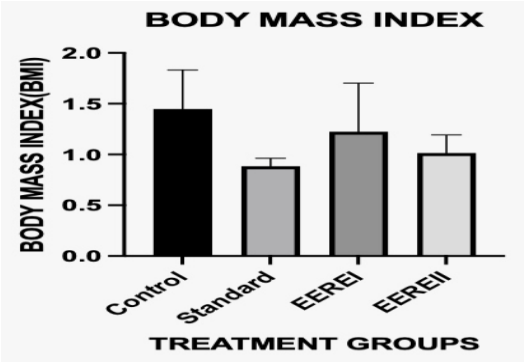
3. Blood Sugar Level:



Graph no 03: Showing the difference in Blood glucose levels among various groups.

Level of Blood Sugar increased significantly in all groups when Letrozole was administered. The Blood glucose level was decreased when the standard drug clomiphene citrate was administered to the standard group. In test EEREI and EEREII the Level of Blood Sugar was decreased significantly.

4. BODY MASS INDEX:



Graph no.04: showing the difference in body mass index among various groups.

Histopathological Analysis of Ovary:

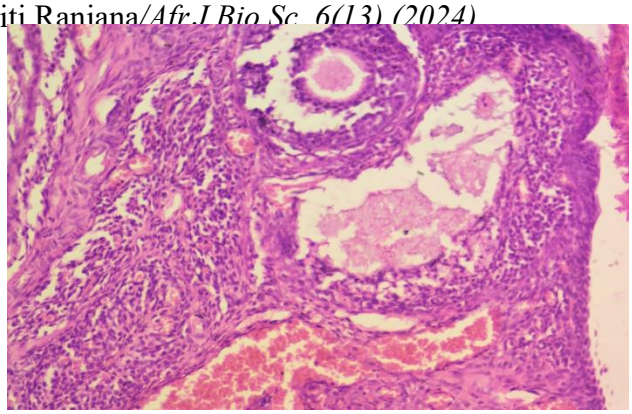


Fig. no. 1 Disease Control

Microscopy: Ovary sections showing preantral follicles, antral follicles, atretic follicles and corpus luteum, corpus albicans at various stages. Destrupted oocyte, tunica albuginea are also noted. Follicular cyst is increased. Ovary impression was essentially showing poly cystic ovary.

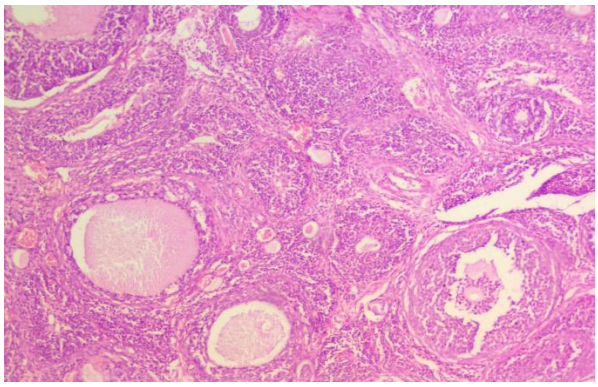


Figure no. 2: Standard Control

Microscopy: Ovary sections show modestly decrease in the numbers of cysts, Cystic follicles are modestly decrease in numbers. Corpus luteum are increased in numbers. Tunica albuginea moderately thickened. Mild vascular congestion was noted. Ovary impression was showing almost recovered Polycystic Ovarian Disease**.**

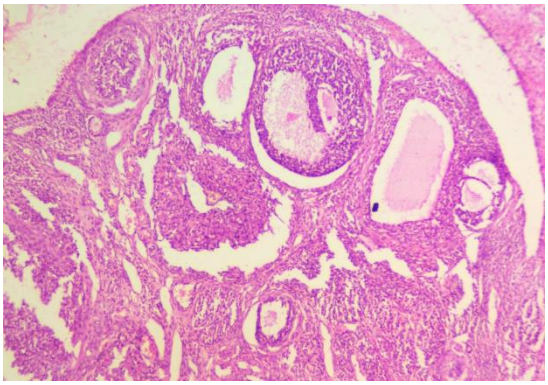


Figure no. 3: EERE I

Microscopy: Ovary sections showing multiple cysts. Cystic follicles are moderately increased in number. Corpus luteum and intact normal-sized oocyte are decreased. Tunica albuginea thickened. Vascular congestion along with fibrinous clot noted. And ovary impression was Polycystic Ovarian Disease with Mild Treatment Effect**.**

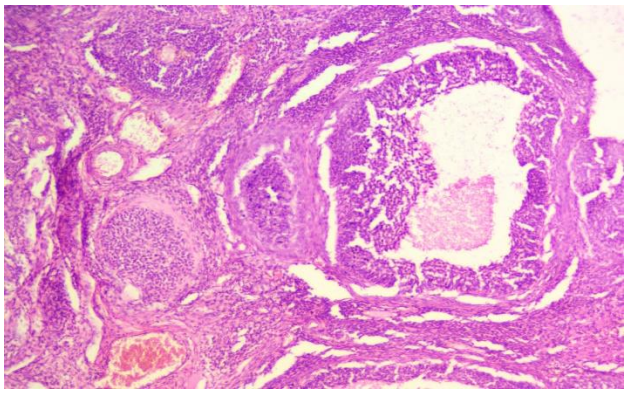


Figure no. 4: EERE II

Microscopy: Ovary sections showing moderately decrease in numbers of cystic changes in antral follicles. Cystic follicles are moderately decreased in number. Corpus luteum and intact normal sized oocyte are moderately increased. Tunica albuginea mildly thickened. Vascular congestion along with fibrinous clot was noted. Ovary impression was recovering polycystic Ovarian Disease.

Conclusion: The study concluded that the plant extracts of *Rubus ellipticus* have beneficial effects in treating PCOS by reducing blood glucose levels and improving BMI in Wistar rats. As per the literature, *Rubus ellipticus* has antihyperlipidemic, anti-inflammatory activity which helps in normalizing different hormone levels and shows significant impact on PCOS induced Wistar rats. Also, from histopathological reports of rats having poly cystic ovaries, it is concluded that cystic ovaries are recovering much when ethanolic extract of plant *Rubus ellipticus* was given in different doses (EERE II most effectively). In conclusion, the extract of *Rubus ellipticus* Showing pharmacological activities those are really beneficial in both treatment and management of PCOS and also maintaining the hormonal, blood glucose, and blood testosterone levels. The study can be a bases for furthermore exploration of the plant *Rubus ellipticus* which could also be a better possibility than hormonal replacement therapy.

REFERENCE

[1]. Ajmal N, Khan SZ, Shaikh R. (2019 Jun). Polycystic ovary syndrome (PCOS) and genetic predisposition. A review article EurJ Obstet Gynecol Reprod Biol X, doi: 10.1016/j.eurox.2019.100060. PMID: 31403134; PMCID: PMC6687436.

[2]. Legro R.S. (2009). Stein-Leventhal syndrome. Encyclopedia britannica. Retrieved from [Google Scholar] [Ref list]

[3]. Diamanti-Kandarakis E.D.A .(2012). Insulin resistance and the polycystic ovary syndrome revisited, an update on mechanisms and implications. Scopus preview ,Volume 33.p.g no 981-1030. Retrieved from View at publisher_CrossRefView in ScopusGoogle Scholar

[4]. Ajmal N, Khan SZ, Shaikh R. (2019 Jun). Polycystic ovary syndrome (PCOS) and genetic predisposition. A review article EurJ Obstet Gynecol Reprod Biol X, doi: 10.1016/j.eurox.2019.100060. PMID: 31403134; PMCID: PMC6687436.

[5]. Witchel, S.F., Teede, H.J. & Peña, A.S. (2020).Curtailing PCOS. Pediatr, ,volume 87, p.g no. 353–36. Retrieved from https://doi.org/10.1038/s41390-019-0615-1

[6]. WHO (world health organization). (28 june 2023).Retrieved from https://www.who.int/news-room/fact-sheets/detail/polycystic-ovary-syndrome

[7]. Dewailly D, Legro R. (2007 August 25). Polycystic Ovarian Syndrome (PCOS): A review article The Lacent. Retrieved from Doi: https://doi.org/10.1016/S0140-6736(07)61345-2.

[8]. Health direct https://www.healthdirect.gov.au/amp/article/polycystic-ovarian-syndrome-pcos#causes

[9]. Palomba S, Falbo A, La Sala GB. (2014). Metformin and gonadotropins for ovulation induction in patients with polycystic ovary syndrome: a systematic review with meta-analysis of randomized controlled trials. Reprod Biol Endocrinol. Doi 12:3. 10.1186/1477-7827-12-3

[10].Trent M, Gordon CM. (2020). Diagnosis and management of polycystic ovary syndrome in adolescents . Pediatrics. doi 145:S210-8. 10.1542/peds.2019-2056J

[11]. Melo AS, Dos Reis RM, Ferriani RA, Vieira CS.(2017) Hormonal contraception in women with polycystic ovary syndrome: choices, challenges, and non contraceptive benefits. Open Access J Contracept. 8:13-23. 10.2147/OAJC.S85543

[12].Aiselu Wine: A Community Project in Nepal". Aiselu Wine: A Community Project in Nepal. Retrieved 2020-06-1

[13]. Tsarong.Tsewang.j.(1994). Useful topical plants. Tibetian medicinal plants,India .

[14] USDA, NRCS (n.d.).(25October 2015) ."Rubus ellipticus" The PLANTS Database (plants.usda.gov). Greensboro, North Carolina: National Plant Data Team

[15]. David E. Boufford. (1815). "Rubus ellipticus Smith, 椭圆悬钩子 tuo yuan xuan gou zi". Flora of China, volume 9.

[16] Chen jin, Deng Xiaobao .(febuary 2006). Diet composition and foraging ecology of Asian elephants in Shangyong, Xishuangbanna, China.Acta ecologica sinica ,volume 26.pg no-309-316.Retrieved from https://doi.org/10.1016/S1872-2032(06)60006-1.

[17] "Rubus ellipticus Sm. | Plants of the World Online | Kew Science". Plants of the World Online. Retrieved 30 October 2023.

[18] Jugran, A. K., Rawat, S., Devkota, H. P., Bhatt, I. D., & Rawal, R. S. (2021). Diabetes and plant-derived natural products: From ethnopharmacological approaches to their potential for modern drug discovery and development. Phytotherapy Research, 35(1), 223-245.

[19].Fahed, A. C., Wang, M., Patel, A. P., Ajufo, E., Maamari, D. J., Aragam, K. G., Brockman, D. G., Vosburg, T., Ellinor, P. T., Ng, K., & Khera, A. V. (2022). Association of the Interaction Between Familial Hypercholesterolemia Variants and Adherence to a Healthy Lifestyle With Risk of Coronary Artery Disease. JAMA network open, 5(3),Retrieved from doi:10.1001/jamanetworkopen.2022.2687

[

[20]. Appel, L. J., Champagne, C. M., Harsha, D. W., Cooper, L. S., Obarzanek, E., Elmer, P. J., ... & Young, D. R. (2003). Effects of comprehensive lifestyle modification on blood pressure control: main results of the PREMIER clinical trial. Jama, 289(16), 2083-2093.[PubMed] [CrossRef] [Google Scholar] [Ref list]

[21]. Thoma, C., Day, C. P., & Trenell, M. I. (2012). Lifestyle interventions for the treatment of non-alcoholic fatty liver disease in adults: a systematic review. Journal of hepatology, 56(1), 255-266. Retrieved from [PubMed] [CrossRef] [Google Scholar] [Ref list]

[22]. Bourbeau, J., Nault, D., & Dang-Tan, T. (2004). Self-management and behaviour modification in COPD. Patient education and counseling, 52(3), 271-277. Retrieved from [PubMed] [CrossRef] [Google Scholar] [Ref list]

[23]. Anand, P., Kunnumakara, A. B., Sundaram, C., Harikumar, K. B., Tharakan, S. T., Lai, O. S., ... & Aggarwal, B. B. (2008). Cancer is a preventable disease that requires major lifestyle changes. Pharmaceutical research, 25(9), 2097-2116.

Retrived from [PMC free article] [PubMed] [CrossRef] [Google Scholar] [Ref list]

[24]. Khan, H., Sureda, A., Belwal, T., Çetinkaya, S., Süntar, İ., Tejada, S., ... & Aschner, M. (2019). Polyphenols in the treatment of autoimmune diseases. Autoimmunity reviews, 18(7), 647-657.Retrieved from [PMC free article] [PubMed] [CrossRef] [Google Scholar] [Ref list]

[25]. Subba, B. I. M. A. L. A., Gaire, S. A. N. J. A. Y., & Raj Sharma, K. (2019). Analysis of phyto-constituents, antioxidant, and alpha amylase inhibitory activities of Persea Americana Mill., Rhododendron Arboretum Sm. Rubus Ellipticus Sm. from arghakhanchi district nepal. Asian J Pharm Clin Res, 12(1), 301.Retrived from [CrossRef] [Google Scholar] [Ref list]

[26].Subba, B. I. M. A. L. A., Gaire, S. A. N. J. A. Y., & Raj Sharma, K. (2019). Analysis of phyto-constituents, antioxidant, and alpha amylase inhibitory activities of Persea Americana Mill., Rhododendron Arboretum Sm. Rubus Ellipticus Sm. from arghakhanchi district nepal. Asian J Pharm Clin Res, 12(1), 301.Retrieved from [CrossRef] [Google Scholar] [Ref list]

[27]. Kirtikar, K. R., Basu, B. D., & CS, I. (2001). Indian medicinal plants, oriental enterprises. Dehradun, 6, 2029-2035.Retrieved from [Google Scholar] [Ref list]

[28]. Lamichhane A, Lamichhane G, Devkota HP. (2023).Yellow Himalayan Raspberry (Rubus ellipticus Sm.): Ethnomedicinal, Nutraceutical, and Pharmacological Aspects. Molecules. 15;28(16):6071. doi: 10.3390/molecules28166071. PMID: 37630323; PMCID: PMC10458938.

[29]. Wei, Q., Yang, G. W., Wang, X. J., Hu, X. X., & Chen, L. (2013). The study on optimization of Soxhlet extraction process for ursolic acid from Cynomorium. Shipin Yanjiu Yu Kaifa, 34(7), 85-8.Retrieved from [Google Scholar] [Ref list]

[30]. Prajapati P, Patel M (2022). ,Beneficial effect of polyherbal formulation in letrozole induced Polycystic ovarian syndrome (PCOS). Journal of traditional and complimentary medicine . Pages 575-583 https://doi.org/10.1016/j.jtcme.2022.08.003

[31] Kakadia N, Patel P, Deshpande S, Shah G. Effect of Vitex negundo L. seeds in letrozole induced polycystic ovarian syndrome. J Tradit Complement Med.

[32]. Basheer M, Rai S, Ghosh H, Ahmad Haja Y.(2018). Protective role of seed extract of Tephrosia purpurea in letrozole induced polycystic ovary syndrome in wistar rats. J Biol Sci. https://doi.org/10.3923/jbs.2018.458.467