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CLINICAL EFFICACY AND SAFETY OF A POLYHERBAL SUPPLEMENT IN THE MANAGEMENT OF FEMALE REPRODUCTIVE HEALTH CONDITIONS

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ABSTRACT

Introduction: The current lifestyle preferences to natural alternatives for preventive health and the desire to manage chronic diseases through dietary means has increased the need for Dietary supplements exponentially. The supplements that could assist certain conditions like cardiovascular diseases, Cancer, Diabetes, Arthritis, Gestational trophoblastic diseases (GTD) etc. has been commercialized and proven useful by word of mouth and through scientific evidences. This article discusses the extraction of bioactive compounds from four plants: *Asparagus racemosus*, *Camellia sinensis*, *Daucus carota* and *Piper nigrum*. **Methods:** The ingredients of the capsule were analyzed using High pressure liquid chromatography (HPLC) to ensure quality and safety as well as tested for any heavy metal toxicity and microbial contamination. Random Controlled Trial (RCT) consisting of 20 patients was conducted to determine the efficacy of the product. **Results and Discussions:** The study investigated the effectiveness of these compounds in formulations for the treatment of female reproductive disorders, including irregular menstrual cycles, PCOS, and abnormal bleeding, pain in the vaginal tract etc. The study has reported the efficacy of the capsule through a Phase II clinical trial to validate their effect on patients. The results inferred from the reports show improvement in 70% of the patients with a reduction in the clinical symptoms. **Conclusion:** Thus, the product could be an effective supplement for women experiencing issues with their reproductive systems. In the future, Nutra-economics may play a very important role in health maintenance and preventive care.

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INTRODUCTION

Lifestyles have evolved significantly in recent years, reflecting changes in societal norms and technological advancements. The food consumption patterns have undergone significant changes, characterized by an increased junk food intake. This may lead to chronic diseases like obesity, Heart disease, Cancer, depression, poor dental health, digestive issues, skin problems, hormonal imbalances, impaired immune system, neuroinflammation, etc., and nutritional deficiencies, negatively impacting overall health (Murugesan and Mahendraprabu, 2024). An article by Pandey *et al.* surveyed the United Kingdom, Germany, and France and determined that consumers prioritize diet over exercise and hereditary factors to achieve optimal health (Pandey *et al.*, 2010). Dietary supplements have increasingly demonstrated efficacy in delivering specific dietary substances, effectively functioning as nutritional components.

These encompass isolated nutrients, dietary supplements, targeted dietary regimes, genetically engineered designer foods, and herbal products. This comprehensive analysis highlights the growing interest in the diverse nutritional options available in the contemporary market (Pandey *et al.*, 2010; Chopra *et al.*, 2022). The therapeutic potential of herbal plants plays a significant role in many nutraceutical formulations, which are intended to serve as supplements for prevention and relief, rather than to cure overall health conditions. They come in various forms, including tablets, capsules, syrups, powders, liquids, or gummies. This design facilitates the delivery of specific bioactive compounds extracted from natural sources, such as plants, foods, and probiotic bacteria, which are classified based on their chemical nature and pharmacological action (Donnoet *et al.*, 2018; Rajasekaran, 2017; Dikmen&Filazi, 2016). A review article by Bruno *et al.* had analyzed the filtered data of over 502 studies to determine whether nutraceutical products or active

ingredients like vitamin D, vitamin K, polyphenols, omega-3 fatty acids, probiotics and prebiotics can lead to an improvement in cardiometabolic diseases (CMD) and metabolic syndrome (MetS). The study concludes that natural bio-based formulation could be a new therapeutic strategy that could effectively alleviate diseased conditions, side effects, and cost-effectiveness rather than conventional pharmacological therapy. At the same time, it is crucial to consult and communicate the safety and care concerning the products with a licensed medical practitioner and pharmacist (Bruno et al., 2022). The dietary patterns and nutritional compounds are pivotal factors in the emergence of chronic diseases associated with alterations in physiological morphology (Velissaridou et al., 2024). Embracing a diverse and balanced diet is essential for safeguarding our health and promoting overall well-being. Low and middle-income countries have experienced a nutritional transition due to changes in dietary supplementation and energy expenditure, influenced by economic, demographic, and epidemiological shifts (Mathews et al., 2025). A case study conducted in ILBS, New Delhi, India by Pal et al. highlights the long-term use of Complementary and Alternative Medicine (CAM) therapies for patients with Chronic Liver Disease (CLD). It suggests that incorporating popular self-help practices such as yoga, meditation, and prayer may enhance the effectiveness of treatments. Similarly, a study by Abdel-Moneim et al. has utilized the use of traditional medicine as a complement to physician-prescribed drugs for patients with chronic hepatitis C (CHC) in Egypt (Abdel-Moneim et al., 2017). The dietary pattern associated with lower Systolic blood pressure (SBP) and diastolic blood pressure (DBP) among Southwest Chinese was partially explained by body composition (Chen et al., 2023). Thus, there is a need to promote healthy and traditional dietary patterns, along with physical activity. In recent years the change in lifestyle and the lack of time for physical exercise have made prioritizing overall well-being difficult. Hence, the need for functional food to reduce the risk of chronic diseases like Obesity, diabetes, blood pressure, stress, allergy, cardiovascular diseases, cancer, etc., has become popular. The female reproductive system is an integral physiology of women that enables reproduction, intercourse, and menstruation. The development of female secondary sex characteristics also depends on the sex hormones of the reproductive system.

Hormonal imbalance within the reproductive system could affect the ovulation cycle leading to irregular menstruation, and potentially altered blood flow volume causing adverse conditions like PCOS, thyroid imbalances, stress, weight change, or menopause (Ramirez-González et al., 2016; Robboyet et al., 2017; Boyd et al., 2018). Stress has also become one of the key reasons for inducing psychological, physiological and physical leading to changes in the hormonal balance especially in women. Hormonal dysbiosis could generate reactive oxygen species (ROS) intern causing Oxidative stress (OS) (Pandey et al., 2018). The product in this manuscript is targeted at female patients with an Irregular menstrual cycle (Upasravanaroha), Abnormal bleeding disorder (Rakthaasravaroha), Pain in the vaginal tract (Yonisoola), menstrual cramps, etc. due to polycystic ovarian syndrome (PCOS). The bioactive constituents from natural food sources commercialized in the form of nutraceuticals or Dietary supplements have gained massive attention (Shahgholian 2022). This study validates the efficacy of the Ayurvedic proprietary medicine, capsule, formulated with key ingredients such as *Asparagus racemosus*, β -carotene from *Daucus carota*, polyphenolic compounds from *Camellia sinensis*, and piperine from *Piper nigrum*, to strengthen and maintain a woman's health. It is primarily recommended to support the female reproductive system.

***Asparagus racemosus (Shatavari)*:** The species *Asparagus racemosus* also known as, Shatavari, a flowering plant native to India, has long been recognized as an Ayurvedic medicine. Shatavari has a tuberous root and is a much-branched, spinous, perennial climber found throughout the country notably presented in Ayurvedic Pharmacopeia of India (API) Vol 4. Its health benefits are due to its bioactive compounds, such as steroidal glycosides, saponins (Shatavarins I, II, III, and IV), polyphenols, flavonoids, alkaloids (racemosol), and vitamins (Akhtar et al., 2024). The medicinal plant from the Liliaceae

family is known for its pharmacologically active compounds, which are therapeutically used as a supplement for various health benefits (Sachanet et al., 2012; Hussain et al., 2015). The tuberous roots and tender shoots of Shatavari serve as the primary plant parts utilized in medicinal applications. The active compounds derived from root extracts have demonstrated a variety of pharmacological effects. These include the promotion of lactation (galactagogue effect), modulation of immune responses (immunomodulatory effects), enhancement of immunity (immunoadjuvant effects), and prevention of lithiasis (antilithiatic effect) (Alok et al., 2013). The roots of *A. racemosus* are rich in the active component steroidal saponin (Shatavarins I–IV) (Hussain et al., 2015). The phytochemical component Glycosides of quercetin, rutin, hyperoside and diosgenin, quercetin 3-glucuronide, sitosterol and stigmasterol saponin and its derivatives from the roots of Shatavari are noted for its Antineoplastic activity, anti-dysenteric activity, antifungal activity, antibacterial activity, anti-inflammatory activities, antiulcer activity, antioxidant activity, anti-abortifacient activity Antioxytoxic, Spasmodic activity affecting the uterus, along with hypoglycemic and hypertensive effects, and anticoagulant activity (Sachanet et al., 2012). A study by Greed et al. has brought to notice the positive effects of Shatavari on improving the skeletal muscle proteome, especially in women with estrogen deficiency at the post-menopausal stage (Greed et al., 2025). The supplementation of Shatavari in post-menopausal women with estrogen deficiency could help regulate muscle contraction (O'Leary et al., 2021). Data repositories highlight that Shatavari's roots, stems, leaves, and flowers can effectively alleviate issues related to the female reproductive system. Research on fortifying food with Shatavari suggests it could significantly improve nutritional value and help prevent various diseases (Akhtar et al., 2024). The roots of *A. racemosus* were administered in a randomized controlled trial involving sixty lactating mothers, whose ages ranged from twenty to forty years. This study was conducted at the Institute of Post-Graduate Ayurvedic Education and Research, located within the S. V. S. P. Hospital in Kolkata. The trial yielded positive results by measuring changes in prolactin hormone levels among the participants (Gupta and Shaw 2011).

Various reports suggest that Shatavari may enhance female reproductive health by addressing hormonal imbalances and polycystic ovarian syndrome (PCOS). It promotes follicular growth and improves oocyte quality, potentially reducing oxidative stress and increasing antioxidant levels in the body (Moiniet et al., 2019; Pandey et al., 2018). The phytoestrogenic effects of shatavari, the ancient Ayurvedic herb supports muscle and bone health in postmenopausal women. Additionally, it provides ergogenic benefits to help preserve muscle mass (Chauhan et al., 2022). A placebo-controlled trial conducted by Gudiseet et al. assessed the safety and efficacy of the Ayurvedic herb Shatavari in managing menopausal symptoms, comparing its effects to those of a placebo. The study found that the active ingredient significantly alleviated a range of symptoms, including hot flashes, night sweats, insomnia, anxiety, nervousness, vaginal dryness, and loss of libido. These findings suggest that Shatavaricould be a viable alternative therapeutic option for managing menopausal symptoms (Gudiseet et al., 2024). According to the Ayurvedic Formulary of India (AFI), the medicinal herb shatavari has been used as an ingredient in multiple formulations some of which are DhatriyadiGhrta AFI, Part-I, 6:21, PhalaGhrta AFI, Part-I, 6:30, and DhanvantaraTaila (Synonym BalaTaila) AFI, Part-I, 8:22. The ingredients shatavari was incorporated in these formulations essentially due to their main therapeutic property as per API-Vol:4/108 namely Sotha, Ksaya, Parinamasula, Gulma, Atisara, Raktatisara, Raktavikara, Mutrarakta, Amlapitta, Arsa, Vatajvara, Svarabheda, Naktandhya, Vatarakta, Raktapitta, Visarpa, SutikaRoga, StanyaDosa, StanyaKsaya. *Asparagus racemosus* is identified as a primary component in the formulation due to its therapeutic advantages, which encompass Raktavikara, Raktapitta, SutikaRoga, StanyaDosa, and StanyaKsaya.

***Daucus carota*:** The carotenoid terpene member β -carotene is recognized for its significant antioxidant properties and serves as a precursor to vitamin A. This compound plays a crucial role in

reducing oxidative stress and mitigating the risk of chronic diseases, including cardiovascular disease, ocular disorders, cancer, and reproductive health issues (Babar *et al.*, 2025). β -carotene is a polyene compound with an acyclic structure consisting of 40 carbon atoms and 11 conjugated double bonds (Laughlin *et al.*, 2002; Bogacz-Radomska and Harasym 2018).

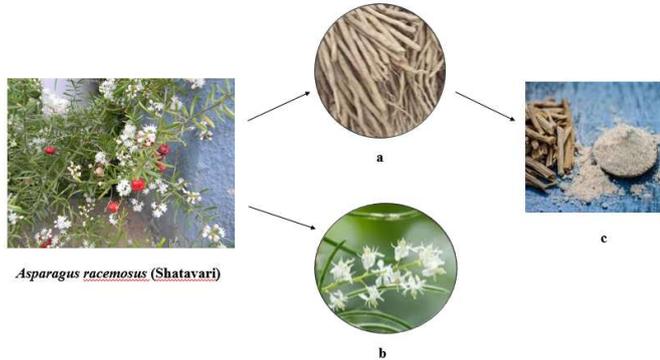


Figure 1. Illustration of the herbal plant shatavari a). Root of the plant b). Flowers of shatavari c). Powdered form of Shatavari root

In 'DravyagunaVijnana', an Ayurvedic pharmacology book, the tuberous root of *Daucus carota* (Garjara) is noted for its properties related to Rakta pitta, Arsas, Atisara, and Sotha. In vivo studies have demonstrated that the concentration of malonate dialdehyde, a byproduct of lipid oxidation, diminishes with the long-term consumption of lipid-soluble β -carotene. An experimental study evaluating the effects of carrot leaf extract on the reproductive and growth characteristics in rabbits has shown improved reproductive performance in female rabbits and combat against oxidative stress, even when facing external factors (Chongsiet *et al.*, 2024). A study in Nepal highlights the positive impact of vitamin A and β -carotene supplementation on maternal health, demonstrating a significant reduction in pregnancy-related mortality among women in Nepal (Olsen 1999). In Thailand, a randomized controlled trial was conducted involving lactating Holstein cows to evaluate the effects of synchronized ovulation and timed artificial insemination (TAI) accompanied by oral supplementation of β -carotene. The findings demonstrated that the probability of pregnancy in the cows increased by 44%, which correlated with elevated activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) (Khemarachet *et al.*, 2021). A study by Kramer *et al.* demonstrates that low alpha- and beta-carotene levels are associated with preterm birth outcomes (Kramer *et al.*, 2009). Recent studies suggested that prostaglandin D (PGD) synthase binds retinoic acid and retinal (Urade 2021). Prostaglandin D2 plays a significant role in promoting sleep, immunomodulation, regulation of body temperature, and the secretion of luteinizing hormone (Kadian and Garg 2012).

Camellia sinensis: *Camellia sinensis* is a species of plant that serves as a source of tea, a widely consumed beverage around the world. The various forms of the plant can be utilized to produce green tea, oolong tea, yellow tea, black tea, and dark tea. These beverages are distinguished by their rich content of phytochemicals, including phenolic acids, catechins, methylxanthines, flavonoids, tannins, amino acids, minerals, and proteins (Fernandes *et al.*, 2023). In Ayurveda, *Camellia sinensis* (tea) is commonly referred to as Syamaparni or Chaay and is addressed in various Ayurvedic texts for its numerous medicinal properties. The term Parishishta is referred from Bhavprakash Nighantu, an Ayurvedic text to understand Materia Medica as an additional commentary or supplementary section that elaborates on the uses, qualities, and therapeutic benefits of tea (Syamaparni) in Ayurvedic practice (Gajarmalet *et al.*, 2020). Catechins are polyphenolic flavonoids and the primary active compounds found in *Camellia sinensis*. Catechins are known for their ability to scavenge free radicals, and they possess significant antioxidant properties. The main catechin components include epigallocatechin, epicatechin, and epicatechin-3-gallate (Sutherland *et al.*, 2006). Epigallocatechin gallate (EGCG) is the predominant catechin present in tea, comprising approximately 50 to 80 percent of the total catechin content (Khan and Mukhtar, 2007). A study by Heber *et al.* involving

rats, administration of EGCG at a dosage of 85 mg/kg body weight is associated with a reduction in energy intake of approximately 15%. Additionally, this treatment decreases serum concentrations of leptin and luteinizing hormone. (Heber *et al.*, 2014) Polyphenols serve as powerful antioxidants, effective antibacterial agents, and skilled chelators of metal ions, showcasing their remarkable health benefits (Idowu 2017). The overproduction of reactive oxygen species (ROS) contributes to oxidative stress (OS). ROS plays a key role in anaerobic metabolism, mediating between redox signaling and regulating cellular metabolism (Lennicke and cocheme 2021). OS has become a cause of major concern mainly owing to its involvement in altering the reproductive physiology of females. Luteinizing hormone (LH) is a gonadotropin produced by the anterior pituitary gland. It plays a critical role in initiating ovulation as well as facilitating the development of the corpus luteum in females (Bhardwaj *et al.*, 2021). The metabolic disorder, polycystic ovarian syndrome (PCOS) is estimated to affect 6–13% of women of reproductive age, and up to 70% of cases are undiagnosed as per WHO Reports 2025. A review article has highlighted that green tea polyphenols (GrTPs) exhibit properties that enhance the quality of both male and female gametes, primarily through the ability of catechins to mitigate the production of reactive oxygen species (ROS). The antioxidant effects of GrTPs have been shown to positively impact key semen parameters, including sperm concentration, motility, morphology, DNA integrity, fertility rate, and overall gamete quality. These unique attributes of green tea catechins position them as a promising therapeutic avenue for improving reproductive health, making this an important area of ongoing research (Rahman *et al.*, 2018).

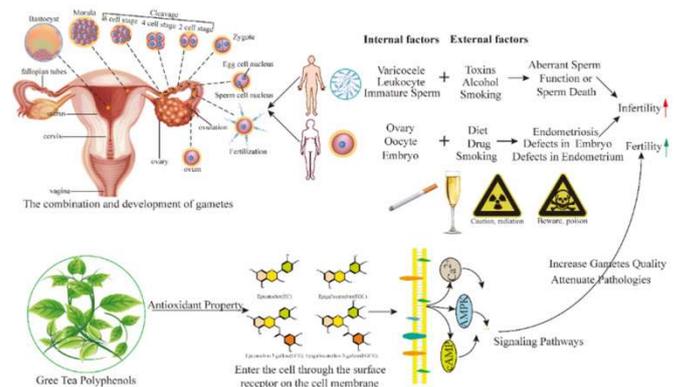


Figure 2. Effect of Green Tea polyphenols on the reproductive system (Rahman *et al.*, 2018)

Piper nigrum: The matured dried fruit of *Piper nigrum* Linn. Belonging to Family Piperaceae, known as Marica in Ayurveda is a climber found in the north Konkan Kerala and Assam. The mature fruits of Marica are harvested during the month of December to April depending on the climatic conditions. Piperine is used in many ayurvedic formulations essentially because of its Svasa, Sula, Krimiroga and Tvagroga therapeutic properties according to Ayurvedic pharmacopeia of India (API, Volume 3, 2023). It is renowned for its pharmacological activity due to specific phenolic components such as alkaloids, flavonoids, carotenoids, terpenoids, etc. Piperine, the alkaloidal bioactive component, is used as a medicine, flavoring agent, and preservative. Pepper has been used traditionally to treat colds, coughs, sore throat, dysentery, stomachaches, fever, etc. (Takooree *et al.*, 2019; Mehmood and Gilani 2010; Vijayan and Thampuran 2000). Piperine is known to enhance the bioavailability of various nutrients and pharmaceuticals by inhibiting crucial enzymes involved in drug metabolism within the intestines. This action effectively increases the absorption of these compounds into the bloodstream, thereby improving their overall efficacy (Srinivasan 2007; Kumar *et al.*, 2018; Bachar *et al.*, 2020; Ashokkumar *et al.*, 2021). A study by Antonelli *et al.* reported that administering piperine significantly improved kidney histopathology. Furthermore, it was observed that piperine administration led to reductions in the levels of blood urea nitrogen (BUN), creatinine, and malondialdehyde (MDA). Additionally, there was a notable increase

in the levels of antioxidant enzymes, specifically superoxide dismutase (SOD) and glutathione peroxidase (GPx), in the kidneys of rats treated with lead acetate (Antonelli et al., 2025). Piperine enhances the bioavailability of beta-carotene through several mechanisms of action. These mechanisms include the inhibition of various enzymes that are responsible for the metabolism of pharmaceuticals and nutritional substances, the stimulation of amino acid transporter activity within the intestinal lining, the inhibition of P-glycoprotein, a protein that eliminates substances from cells, the reduction of glucuronic acid production in the intestines, which facilitates a greater influx of active substances into the body. This multifaceted approach underlines the potential of piperine as an effective bioenhancer in nutritional science (Derosa et al., 2016).

MATERIALS AND METHODS

The dried extract of *Asparagus racemosus*, *Camellia sinensis*, *Daucus carota* and *Piper nigrum* was collected by washing and drying the roots/shoots of *Asparagus racemosus*, and leaves of *Camellia sinensis*, the powdered extract was obtained using a solvent extraction process and the concentrated extract was later dried with the help of a desiccator. In the case of *Piper nigrum*, the dried seeds from the fruit of the pepper plant are obtained and processed to extract its bioactive components.

Extraction and estimation of *Asparagus racemosus*: The sample weighing about 5 grams was taken in a round bottom flask and reflux with 50ml of 90% v/v Methanol for half an hour. The mixture was cooled and filtered into a dried 250ml round bottom flask. The above procedure was repeated till the alcoholic extracts appear colourless which was later placed on water bath and allowed to evaporate to a soft residue. This soft residue was treated with 25ml Petroleum ether (40-60) by refluxing for half an hour. The slurry was allowed to cool and the Petroleum ether layer was decanted. Again, this residue was treated with chloroform separately, as above i.e. each with 25ml by refluxing for half an hour. All the solvent layers were discarded. 20 ml 90% v/v methanol was added to dissolve the residue and precipitated with 100 ml acetone. The obtained precipitate was kept aside for 30 minutes and the acetone was decanted. The precipitate was dried for one hour at 105°C in an oven, cooled in a desiccator, and weighed.

Calculation

$$\% \text{ of Saponin} = \frac{\text{Wt. of the residue}}{\text{Wt. of sample}} \times 100$$

Validation of Beta-carotene Extract: The stock solution of beta-carotene was prepared by taking 0.1g of sample in a volumetric flask to which 0.5 ml of alkaline solution protease R and 5 ml of milli-Q water were added and sonicated at 55 to 65° C for 10 minutes. The solution was cooled using cold water till it reached room temperature. The volume is made up to 50 ml by adding ethanol and up to mark using Dichloromethane. 30 ml of the solution was then centrifuged at 3500 rpm for 5 minutes. The sample stock of 1 ml was pipetted out and diluted with 9:1 ratio of cyclohexane and ethanol. The sample absorbance was tested using a UV- spectrophotometer to determine the amount of beta-carotene present and to validate its quantity and quality.

Calculation

$$\text{Beta-Carotene content} = \frac{\text{Absorbance of the sample solution at 421.0 nm} \times 5000}{480 \times \text{Weight of the sample in g}}$$

Where, 5000 = Dilution factor.

1480=Reference A (1%1cm) for Beta-carotene in Cyclohexane

Estimation of Green Tea Polyphenols: The dry extract of green tea weighing about 0.5g was taken into a 1000 ml conical flask to which 100ml distilled water was added. The flask was vortexed till the

extract was dissolved. Another 650ml of distilled water was added to the solution to which 25 ml Indigo carmine sulphuric acid solution was added. The solution was titrated to a golden yellow colour with 0.1N potassium permanganate solution. A blank sample without active component was taken as standard. Here, 1ml of 0.1 N potassium permanganate is equivalent to 0.004157g of Polyphenols. The polyphenol extracted from green tea has been measured using the DPPH assay and estimated using the titration.

Calculations:

Total polyphenols (%) =

$$\frac{(V1-V2) \times \text{Normality of 0.1N KMNO}_4 \times 0.004157 \times 100}{0.1 \times \text{Weight of the Sample}}$$

0.1 X Weight of the Sample

V1= Volume of 0.1N potassium permanganate consumed for sample in ml.

V2= Volume of 0.1N potassium permanganate consumed for blank in ml.

Processing and standardization of Piperine: The dry powdered seed of *piper nigrum* bioactive compound piperine was estimated using High-Pressure Liquid Chromatography (HPLC). The HPLC instrument comprises a Phenomenex column 18 of 5μ and follows the isocratic system with a run time set up to 30 minutes. The flow rate for the solvent and sample to pass through is 1ml/minute while the detector wavelength is set at 345nm. The in-house 0.1% v/v phosphoric acid in water of 5 g (standard solvent) is taken in a flask and dissolved in Acetonitrile at the ratio of 60:40 to make up the volume. The same procedure is followed for the sample to be estimated. The sample and the standard are filtered through the injector and membrane filter and the calculated values are read using a computer.

Calculation

$$\frac{\text{Sample Area} \times \text{Standard Concentration} \times \text{Assay of Standard}}{\text{Standard Area} \times \text{Sample Concentration}}$$

RESULTS

The dried extracts and bioactive compounds used in the formulation underwent a comprehensive analysis to assess their quality and safety. The appearance of the extracts was examined to ensure consistency and purity, while their organoleptic properties (such as taste, odor, and texture) were evaluated to understand their sensory characteristics. Additionally, the samples were tested for heavy metal toxicity to ensure they met safety standards and did not contain harmful levels of toxic substances. Furthermore, the anti-microbial activity of the extracts was assessed to determine their effectiveness in inhibiting the growth of harmful microorganisms, confirming their potential therapeutic benefits (Table 1).

Asparagus racemosus

Physical Properties: The sample appears to be Brown to dark brown colour while the taste of *Asparagus racemosus* (Shatavari) root extract is notably bitter, with an earthy and slightly woody flavor. Its odor is similarly earthy, with a mild musky, herbal scent. Overall, both the taste and odor are strong and herbal. In the sieve analysis, 95% of the sample passed through a 40-mesh screen, indicating an appropriate particle size. The loss on drying is 1.18%, which is below the 2% limit, confirming the moisture content is within acceptable levels. The bulk density of the sample falls within the required range of 0.40 to 0.60 gm/ml. Additionally, the pH of a 1% aqueous solution is 4.45, which is within the specified range of 4 to 7. Overall, the sample meets all the required criteria for these physical properties.

Gravimetric analysis was carried out to measure saponin content, and the result shows that the sample contains 20.45% saponin, which is slightly higher than the required minimum of 20%.

Heavy metal toxicity Test: The heavy metal analysis conducted on the sample demonstrated that the concentrations of Arsenic (As), Lead (Pb), Mercury (Hg), and Cadmium (Cd) were within the prescribed safety limits, as specified in the standards. The Arsenic, Cadmium content and the Lead levels were well below the maximum permissible limit while Mercury was. These findings indicate full compliance with the established in-house standards for heavy metal contamination.

Anti-microbial Test: The microbiological analysis of the sample demonstrated its compliance with established safety standards. The Total Plate Count was recorded at 656 cfu/g, well below the maximum permissible limit of 10^5 cfu/g. The Yeast and Mold count was 58 cfu/g, which is within the acceptable threshold of 10^3 cfu/g. Additionally, the sample tested negative for both Salmonella and E. coli, confirming the absence of these harmful pathogens. These results are in full compliance with the specifications outlined in API-II, Vol.-1, 179, ensuring the microbiological safety and quality of the sample.

Beta Carotene

Physical Properties: The physical and chemical analysis of the sample confirms the samples compliance with the specified quality parameters. The sample is a reddish-brown colored, uniform dry powder, and dispersible in water ensuring its suitability for formulation in aqueous systems. The loss on drying is 3.0% w/w, well within the acceptable limit of Not More Than (NMT) 8.0% w/w, indicating appropriate moisture content for stability and storage. The bulk density of 0.55 g/ml falls within the prescribed range of 0.3 - 0.7 g/ml, demonstrating optimal compactness and flow properties. Additionally, the Beta Carotene content is 22.9% w/w, exceeding the minimum required Not Less Than (NLT) 20% w/w, indicating a high concentration of this bioactive compound. Thus, all parameters meet the established standards, confirming the quality and suitability of the sample for its intended use.

Heavy metal toxicity Test: The heavy metals analysis of the sample confirms its compliance with the established safety standards for toxic substances. The Arsenic content was found to be within the permissible limit. Similarly, the concentration of Lead adheres to the required safety threshold. The levels of Cadmium and Mercury were measured and noted to follow the regulatory limits. These findings ensure that the sample is free from harmful levels of heavy metals, confirming its safety for use or consumption.

Anti-microbial Test: The microbiological analysis of the sample demonstrates its high quality and safety. The Total Plate Count was found to be less than 10 CFU/g, which is well below the acceptable limit of 1000 CFU/g, indicating minimal microbial contamination. The Total Yeast and Mould count were also less than 10 CFU/g, significantly lower than the specified maximum of 100 CFU/g, ensuring that the sample is free from excessive fungal contamination. Additionally, both Salmonella and E. coli were absent in the sample confirming the sample's safety from these harmful pathogens. These results highlight the sample's microbiological purity and make it suitable for safe consumption or use.

Camellia sinensis

Physical Properties: The green tea powder is light to dark green and has a fresh, grassy aroma with subtle seaweed notes. Its taste is mildly bitter, vegetal, and slightly astringent due to catechins, with occasional sweetness. The water extractive value was 92.56% (w/w), and the methanol extractive value was 82.52% (w/w), exceeding the required 80% (w/w) for both solvents, indicating high solubility. The sieve analysis showed 95% passed through a 40-mesh screen, ensuring consistency in particle size. The loss on drying was 1.41% (w/w), within limits, and the pH of a 1% aqueous solution was 4.59. Polyphenol content was 50.55%, surpassing the minimum 50%,

confirming its antioxidant properties. The sample meets all quality standards for its intended use.

Heavy Metal Toxicity Test: The heavy metal analysis of *Camellia sinensis* showed Arsenic, Lead, and Cadmium all below permissible limits while Mercury was absent. These results confirm that the sample adheres to safety standards outlined in API-II, Vol.-1, 147, ensuring it is free from harmful heavy metals and is safe for consumption or use in products.

Anti-microbial Test: The microbiological analysis of *Camellia sinensis* showed a Total Plate Count of 656 cfu/g, which is well below the 10^5 cfu/g limit, indicating low microbial contamination. The Yeast and Mold count were 58 cfu/g, far below the 10^3 cfu/g limit. Both Salmonella and E. coli were absent, confirming that the sample meets safety standards and is free from harmful pathogens. These findings comply with API-II, Vol.-1, 179, ensuring the sample is microbiologically safe for its intended applications.

Piper nigrum

Physical Properties: The *Piper nigrum* sample is a light yellow, free-flowing powder with a characteristic odor, aligning with expected sensory properties. Identification using HPLC confirmed its authenticity. Loss on drying was 1.24% (w/w), well below the 5% limit, ensuring stability and shelf life. Residue on ignition was 0.77% (w/w), below the permissible 5% limit. The pH of a 1% solution was 6.2, within the 4.0 – 8.0 range, suitable for both oral and topical applications. The tapped bulk density was 0.6 g/ml, falling within the 0.30 – 0.80 g/ml range, and sieve analysis confirmed it passed through a 40-mesh screen, ensuring uniformity in particle size for formulations.

Heavy Metal Toxicity Test: The heavy metals analysis of *Piper nigrum* showed Lead, Arsenic, Cadmium, and Mercury all within permissible USP limits. These results, obtained through ICP-OES, confirm that the sample complies with the safety standards in USP 2232. The residual solvent analysis showed ethyl acetate content within the permissible 5000 ppm limit (USP 467), and pesticide residue analysis complied with USP 561 for botanicals, ensuring that the sample meets stringent safety guidelines for heavy metals, solvents, and pesticides.

Anti-microbial Test: The microbiological analysis of *Piper nigrum* revealed a Total Plate Count of 90 cfu/g, well below the USP limit of 5000 cfu/g, indicating minimal microbial contamination. The Yeast and Mold count were less than 10 cfu/g, well within the 100 cfu/g limit. No harmful pathogens such as E. coli, Salmonella, Staphylococcus aureus, or Pseudomonas aeruginosa were detected, confirming the sample's safety. These results comply with USP 2021 and 2022 standards, ensuring that the sample is microbiologically safe for its intended uses in both pharmaceutical and food applications.

The table summarizes the key physical, chemical, and microbiological properties of the extracts of *Asparagus racemosus*, Beta Carotene, *Camellia sinensis* (Green Tea), and *Piper nigrum* (Black Pepper) in terms of various quality parameters including appearance, loss on drying, heavy metals, microbial counts, and bioactive content. Each sample complies with the specified regulatory standards, confirming their suitability for use in formulations.

Clinical Validation of the Polyherbal Supplement: The study was conducted in vivo to determine the efficacy of encapsulated formulation and whether it could alleviate and benefit women suffering from reproductive disorders. The capsule was tested at the Ashram Siddha Research Institute in a Phase II-A, single-center, open-label, therapeutic exploratory clinical center for validation. The clinical research was conducted in Salem city with more than 10,00,000 population with an estimated prevalence rate of patients with the symptoms "Upasravanaroha (Irregular menstrual cycle), Rakthaasravaroha (Abnormal bleeding disorder), Yonisoola (Pain in vaginal tract), menstrual cramps, etc. due to polycystic ovarian syndrome (PCOS), to be more than 30% in the locality. The patients considered for this study were all females aged between 20 and 40

years suffering from the above clinical symptoms. The study was conducted for almost a month from 29.11.2024 to 31.12.2024 on 20 patients (Table 2).

significant improvement in their symptoms. Two patients had moderate improvement, two had mild improvement, and two showed no significant improvement.

Table 1. Analysis report of the key ingredients

Test/Parameter	<i>Asparagus racemosus</i> (Shatavari) Root Extract	<i>Daucus carota</i> Beta Carotene	<i>Camellia sinensis</i> (Green Tea)	<i>Piper nigrum</i> (Black Pepper)
Appearance	Brown to dark brown, earthy, slightly woody, bitter taste	Reddish-brown, uniform dry powder	Light to dark greenish color, fresh vegetal aroma	Light yellow, free-flowing powder, characteristic odor
Loss on Drying	1.18% (NMT 2%)	3.0% w/w (NMT 8%)	1.41% w/w (NMT 3%)	1.24% w/w (NMT 5%)
Bulk Density	Within range (0.40 to 0.60 gm/ml)	0.55 g/ml (0.3 - 0.7 gm/ml)	Within required range	0.6 g/ml (0.30 – 0.80 g/ml)
pH	4.45 (range 4.0 – 7.0)	-	4.59 (range 3 – 7)	6.2 (range 4.0 – 8.0)
Saponin Content	20.45% (min. 20%)	-	-	-
Extractive Value in Water	92.56% w/w (min. 80%)	-	92.56% w/w (min. 80%)	-
Extractive Value in Methanol	82.52% w/w (min. 80%)	-	82.52% w/w (min. 80%)	-
Sieve Analysis	95% passed through 40 mesh	-	95% passed through 40 mesh	100% passed through 40 mesh
Heavy Metals	Arsenic Lead, Cadmium: Below Detectable Levels Mercury: Absent	Arsenic, Lead, Mercury: Detectable Levels	Arsenic, Lead, Cadmium: Detectable Levels Mercury: Absent	Lead, Arsenic, Mercury, Cadmium: Below Detectable Levels
Microbial Analysis				
Total Plate Count	656 cfu/g,	<10 cfu/g,	656 cfu/g,	90 cfu/g,
Yeast & Molds	58 cfu/g	<10 cfu/g	58 cfu/g	<10 cfu/g
Salmonella	Absent	Absent	Absent	Absent
E. coli	Absent	Absent	Absent	Absent
Staphylococcus aureus	Absent	Absent	Absent	Absent
Pseudomonas aeruginosa	Absent	Absent	Absent	Absent
Beta Carotene Content	-	22.9% w/w (min. 20%)	-	-
Residual Solvents	-	Complies (Ethyl acetate NMT 5000 ppm)	-	-
Residual Pesticides	Complies (USP 561)	Complies (USP 561)	Complies (USP 561)	Complies (USP 561)

The patients with a history of osteoarthritis, psoriatic arthritis, pregnancy, drug addiction and alcoholism, diabetic complications, and any other complications, night/shift-work employees, and patients with ages above 45 years were excluded. A dose of 1 to 2 capsules was administered orally, dissolved in 100 ml of lukewarm water. The dose was administered twice a day, once in the morning and once in the evening. The ayurvedic herbal capsule was administered after food and before sleep. Each patient was dosed for 30 days to achieve the optimal effect on the outcome of the trial. The blood samples of the patients were collected after overnight fasting and analyzed using routine automatic techniques in a prescribed laboratory. The follow up visits for this trial were scheduled at the referral hospital by registered investigators. The tests were mainly conducted to determine the platelet count, the White Blood Cells (WBC) count, lymphocytes activity, the Erythrocyte Sedimentation Rate (ESR), the RBC count and the amount of hemoglobin. This is tested to determine the patient's condition before taking the capsule, to ensure that the patient does not exhibit any adverse effects from taking the capsule, and to check whether the capsule dosage is appropriate. The chronicity of the condition for patients aged between 21-30 years and 31-40 years was determined after blood test and diagnosis of which are mentioned in Table 1.

Table 2. Chronicity of the disease

Age group (years)	Number of patients		
	Total	Acute Onset (Within 3 months)	Chronic Onset (More than 3 months)
21-29	12	6	6
30-40	8	4	4
Total	20	10	10

The above table 2, provides the total number of participants between the ages 20 to 40 and their onset pattern. The patient's condition after consuming the capsule were re-tested after 30 days and the improvement in criteria before and after administering the intervention was noted. As depicted in Figure 3, after 30 days of treatment, the results showed that 14 patients (70%) experienced

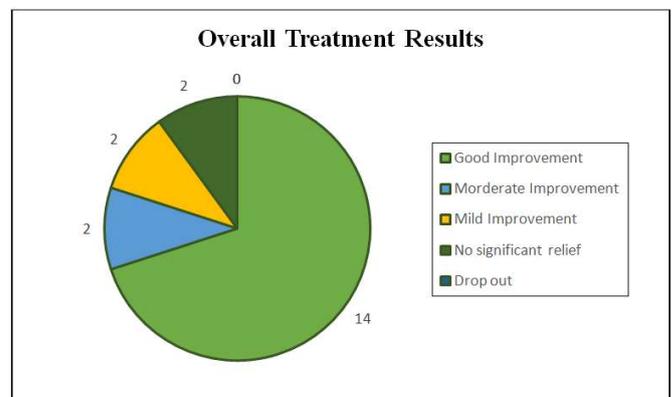


Figure 3. Depicts the number of patients improved in percentile

DISCUSSIONS

The evolving lifestyles in modern society, marked by increased consumption of processed and junk foods, have resulted in a significant rise in chronic diseases, especially among women. These diseases include obesity, cardiovascular diseases, diabetes, hormonal imbalances, and reproductive disorders like polycystic ovarian syndrome (PCOS) and infertility. Such conditions are frequently exacerbated by poor dietary habits, environmental stress, and insufficient physical activity, creating a complex interplay of factors that negatively impact overall health. Recent advancements in the use of herbal and naturally sourced food formulations—bioactive compounds derived from plants and foods—have garnered significant attention for their potential in preventing, alleviating, and managing these conditions (Pandey et al., 2010; Bruno et al., 2022). The therapeutic potential of natural bio-based formulations, particularly those derived from herbal plants, has emerged as an alternative or

adjunct to traditional pharmacological therapies. As highlighted by previous studies, herbs like *Asparagus racemosus* (Shatavari), *Daucus carota* (carrot), *Camellia sinensis* (green tea), and *Piper nigrum* (black pepper) contain bioactive compounds that contribute to a wide range of health benefits, especially in relation to female reproductive health and chronic disease prevention (Sachan et al., 2012; Akhtar et al., 2024; Heber et al., 2014). The combination of *Asparagus racemosus*, *Daucus carota*, *Camellia sinensis*, and *Piper nigrum* in the Polyherbal blend encapsulated presents a comprehensive approach to supporting female reproductive health. Each component contributes unique bioactive properties that, when combined, offer synergistic benefits. *Asparagus racemosus* addresses hormonal imbalances, *Daucus carota* provides antioxidant support to combat oxidative stress, *Camellia sinensis* offers additional antioxidant and anti-inflammatory effects, and *Piper nigrum* enhances the bioavailability of all these active compounds. This multi-pronged approach is particularly relevant in addressing the underlying causes of common reproductive disorders like PCOS, menstrual irregularities, and stress-induced hormonal imbalances. The therapeutic potential of this formulation could not only help manage these conditions but also offer a preventative measure against chronic diseases by improving overall reproductive health and boosting immunity.

This study aimed to evaluate the efficacy of the capsules in alleviating symptoms associated with reproductive disorders in women, particularly those suffering from conditions like irregular menstrual cycles, abnormal bleeding disorders, and pelvic pain, commonly seen in cases of polycystic ovarian syndrome (PCOS). The trial, conducted at the Ashram Siddha Research Institute, involved 20 female participants aged 20-40 years from Salem city, a region with a significant prevalence of these disorders. The participants were administered with the capsules for a period of 30 days, with the dosage regimen of 1 to 2 capsules twice daily. The results indicated a positive therapeutic effect, with 70% of patients experiencing significant improvement in symptoms after the 30-day treatment period. This suggests that encapsulated formulation may provide notable benefits for women suffering from reproductive health issues, especially those related to PCOS. The study's clinical measures, including routine blood tests like platelet count, WBC count, RBC count, ESR, and haemoglobin levels, showed no significant adverse effects, further indicating the safety of the product. Despite the overall positive outcome, two patients exhibited only mild improvement, and another two showed no significant change. This variability could be attributed to individual differences in response to the treatment, such as the severity of the condition or other underlying factors not accounted for in the study. Further research with a larger sample size, longer treatment duration, and more diverse patient profiles would be beneficial to confirm these findings and better understand the potential of this formulation as a treatment for reproductive disorders, particularly in relation to PCOS. As natural, bioactive compounds from plants and other sources continue to be explored, Dietary supplements offer an opportunity to develop more effective, affordable, and accessible solutions for managing health conditions. However, it is essential to approach their use with careful consideration and professional guidance to ensure safety and efficacy. As research progresses, Health-enhancing products could play an increasingly important role in promoting health and preventing diseases. The wellness product's economic impact is not widely known and studies promoting the same should be considered for future scope. The metabolomic study will likely reveal potential ways to produce more efficient and affordable formulations for use as a Pharmaceutical-grade nutritional product.

CONCLUSION

The growing interest in herbal formulations highlights their potential to complement conventional medical treatments by offering preventive health benefits, alleviating root causes, and improving overall well-being. Therefore, investigation of various naturally sourced bioactive compounds combinations and formulations for the

health benefits could show promising results. In this research article, the integration of herbal bio actives into the management of female reproductive health provides a promising alternative to conventional therapies. The combination of Shatavari, β -carotene, green tea polyphenols, and piperine formulated in the capsule exemplifies the therapeutic benefits of utilizing natural bioactive compounds to address reproductive disorders, hormonal imbalances, and oxidative stress. The preliminary evidence supporting the efficacy of these components is promising, furthermore the clinical trials and definitive therapeutic guidelines including safety profiles for their long-term consumption has clear indication for improving the female reproductive system. Given the growing interest in natural and plant-based health solutions, this formulation represents a significant step toward empowering women to manage their reproductive health more effectively, while also promoting holistic well-being.

Participant protections and ethics: To protect human participants, the protocol was written according to general ethical guidelines, such as the Declaration of Helsinki and Good Clinical Practice, approved by the institutional ethical committee of the organization. The study participant consent process includes information about potential risks, benefits, alternatives, and responsibilities during the trial. Before participants agree to participate in this trial, researchers will explain this information in detail in person.

Data and safety monitoring: Regular monitoring, as outlined in a standard operating procedure, will be performed to ensure high data quality. Monitors from IEC will assess whether the case report forms are completed accurately and if the recruiting and treatment procedures are executed correctly according to the protocol. Investigators will be contacted to discuss the need for revising the study protocol, inclusion criteria, and other critical issues. Both investigators and independent researchers will evaluate the clinical trial's progress, review any severe adverse events, and determine whether these events are acceptable or if modifications to the trial or a cessation is necessary. Case Sheets are prepared for each patient, and all records and vouchers will be kept at the treatment centre for three years.

Conflict of Interest: There is no conflict of interest.

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