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Phyto-Pharmacognostic Experimental Study of *Epimedium Sagittatum* and *Gloriosa Superba L.* for the Treatment of Hypogonadism

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Abstract:- The pharmacognostic investigation on Gloriosa superba L. and Epimedium sagittatum is covered in this research article. Physical-chemical parameters have been analysed using fluorescence, including ash and extractive values. The various extracts have also undergone preliminary phytochemical investigation and thin layer chromatographic behaviour. Research has demonstrated the anti-inflammatory, anti-cancer, anti-parkinsonian, adaptogen, antipyretic, anti-obesity, antibacterial, antioxidant, and hepatoprotective activities of Gloriosa superba L. and Epimedium sagittatum. Numerous other effects have also been investigated, including immunomodulation, hypolipidemia, antimicrobial activity, cardiovascular protection, sexual behaviour, tolerance, and dependence. Further research on this plant is required to validate these results and elucidate any further possible therapeutic properties in light of these extremely positive results. Clinical studies should employ Gloriosa superba L. and Epimedium sagittatum to treat a range of diseases.

Keywords:- *Epimedium sagittatum; Gloriosa superba L.; testosterone; adaptogen; phytochemical screening; physicochemical analysis.*

I. INTRODUCTION

The clinical disease known as male hypogonadism is characterised by the testes' failure to produce adequate levels of testosterone, which is mostly brought on by a dysfunction of the hypothalamic-pituitary-gonadal (HPG) axis [1]. Hypogonadism frequently manifests as male factor infertility, erectile dysfunction and diminished libido, obesity with decreased lean body mass, low bone density, lethargy, and depression [2]. Since ancient times, the numerous plant species in the genus Epimedium have been used in Traditional Chinese Medicine (TCM) to treat a wide range of human ailments. These plants also referred to as "horny goat weed" or "yin yang huo," have garnered attention due to their alleged effectiveness in the treatment of sexual problems.

A. Epimedium sagittatum:

Epimedium's icariin (ICA), a flavonol glycoside derived from the plant's aerial section, is thought to be the most metabolically active extract of these plants, according to recent studies into its qualities [3]. In vitro experiments have proven that ICA has inhibitory effects on phosphodiesterase type 5 (PDE5) [4,5]. The 80-fold increase in PDE5 inhibitory action caused by the addition of two hydroxyethyl ether moieties to native ICA is comparable to the amount seen with sildenafil [5]. It has been proposed that ICA contains testosterone-mimetic features in addition to an erectogenic function [6]. These outcomes support the use of ICA in the treatment of sexual dysfunction. In human endothelial cells, ICA has been shown to increase eNOS expression and NO generation while decreasing caspase-3 expression and cellular death in response to hydrogen peroxide [7,8]. Additionally, it has been shown that ICA increases the intracavernous pressure (ICP) response brought on by cavernous nerve stimulation in rats. Nitric oxide synthase and guanylate cyclase inhibitors reversed these effects [9]. Additionally, a rodent model's loss in penile neuron nNOS content and impairment of erectile function due to both castration-related and arteriogenic factors have been successfully treated with ICA [10,11]. To the best of our knowledge, icariin's potential for enhancing erection in an animal model of radical pelvic surgery has not been investigated. This is especially intriguing in light of recent research demonstrating that routine dosage therapy with commercially available PDE5 inhibitors, a standard treatment plan, may not improve results for erectile function following prostatectomy [12]. In mature male Sprague-Dawley rats, oral administration of icariin (50 and 100 mg/kg) for 35 days markedly raised testosterone levels. Icariin therapy significantly reduced malondialdehyde levels and increased superoxide dismutase activity [13]. In addition to icariin, four additional compounds (ES01, ES02, ES03a, and ES03b) from E. sagittatum have been found to have effects on PDE5 that are comparable to those of

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sildenafil and tadalafil. According to the study's findings, E. sagittatum can be utilised to extract natural chemicals that can be used as alternatives to synthetic medications [14]. Male ED is shown to be greatly improved and treated by E. sagittatum.

Fig. 1: Epimedium sagittatum

B. Gloriosa superba L.

The Liliaceae family includes Gloriosa superba L. As a medication, the plant has been used to treat gonorrhoea, rheumatoid arthritis, erectile dysfunction, dermatitis, leprosy, ulcers, gout, and snake bites [15]. Colchicine and colchicoside alkaloids, two important components purified from G. superba, treat gout and relax muscles, respectively [15,16]. In a pioneering investigation, Pare et al. [17] identified the aphrodisiac effect of G. superba tuber. The dried water, chloroform, and alcohol crude extracts of the stem and leaves of G. superba were resuspended in water and olive oil (chloroform and alcohol crude extracts). Male albino rats were given oral doses of aqueous and olive oil G. superba extract (100, 250, and 500 mg/kg body weight per day) for 15 days. This led to higher blood testosterone levels and improved copulatory function. Also identified from G. superba were bioactive substances as alkaloids, saponins, and steroids [17]. The safety and effectiveness of G. superba's aphrodisiac activity in enhancing testosterone production, penile erection, and sexual performance in men have not been supported by published clinical research.

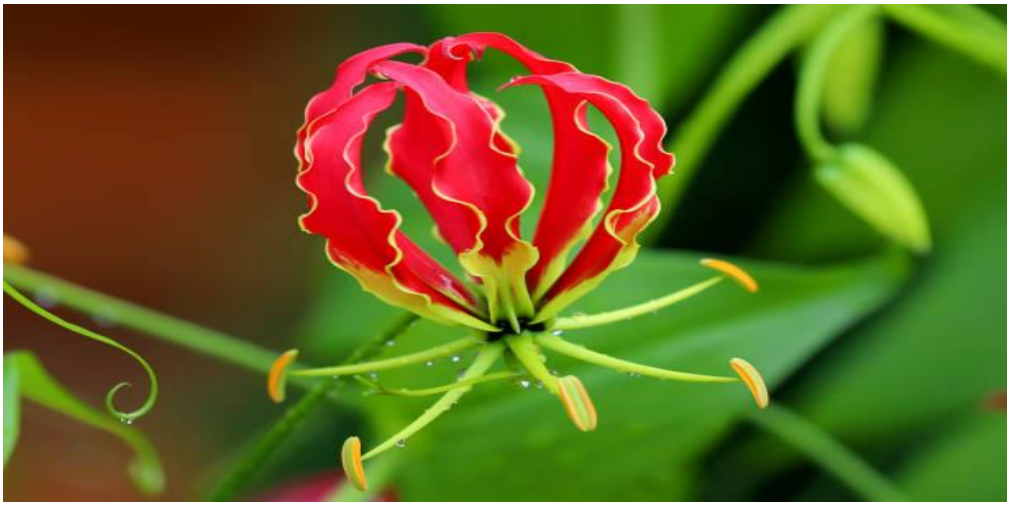

Fig. 2: Gloriosa superba L.

II. MATERIALS AND METHODS

A. Plant collection and authentication

In April 2023, fresh Epimedium sagittatum leaves and Gloriosa superba L. (stem and leaves) were taken from the Rishikul Ayurveda College herbal medicinal garden in Haridwar, India. Professor Dr. Vishnu Sharma of Rishikul Ayurveda College in Haridwar, India, recognised the specimens.

B. Uniformity of raw materials

For the organoleptic assessment and determination of Foreign Organic Matter of Raw Materials, the Ayurvedic Pharmacopoeia of India was used [18].

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C. Physicochemical studies

The ash levels, extractive values, and loss on drying tests were carried out in accordance with the Ayurvedic Pharmacopoeia of India's approved protocols [18].

D. Preliminary Phyto-chemical screening

Epimedium sagittatum leaf extract and Gloriosa superba L. (stem and leaves) extracts of one gramme each were dissolved in 100 ml of their respective mother solvents to produce a stock that contained 1 percent by weight of the substance. Then, it was tested whether this stock contained any alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, steroids, carbohydrate and phenolic compounds, and saponin [19].

E. Fluorescence evaluation

Fluorescence was examined in the incomplete medication under both ambient and UV lighting. Before and after the investigation, samples were treated with a mixture of 50% HCl and 50% NaOH, and the results were tabulated. [20].

F. Research into safety profiles

The safety profile characteristics, including those related to heavy metal analysis, pesticide residual analysis, and microbiological load analysis, were looked into in accordance with the official protocols provided in the Indian Ayurvedic Pharmacopoeia.

G. Heavy metals' quantitative estimation

Quantitative heavy metal estimation was done for the detection of arsenic, lead, cadmium, and mercury using Ayurvedic pharmacopoeia methods [21].

H. Calculating the amount of pesticide residues

Quantitative pesticide assessment was done for the detection of carbamates, organochlorine compounds, and organophosphorus compounds in accordance with the Ayurvedic pharmacopoeia protocols [22].

I. Microbial load analysis

Escherichia coli, Salmonellae, Pseudomonas Staphylococcus, Shigella, as well as the total aerobic viable count, yeasts, and moulds, were analysed to ensure that the raw material for the Bi-herbal capsules was safe to use [23].

III. PREPARATION OF EXTRACT

The parts of the chosen plants were sun-dried and kept in an airtight container. Each substance was then coarsely pulverised and extracted with hydro-alcoholic using the Soxhlet equipment (30:70). The produced hydro-alcohol extracts were concentrated at a temperature of 40°C in a revolving vacuum evaporator while under vacuum (removal of alcohol). At -20°C, the concentrated extracts were freeze dried. Until further use, the powders were kept in an airtight container in the desiccator.

IV. FORMULA OF MIXED HERBAL FORMULATION

The herbal composition contained, in a 1:1 ratio, hydro-alcoholic extracts of Epimedium sagittatum leaves and Gloriosa superba L. (stem and leaves).

V. FORMULA PREPARATION USING THE WET GRANULATION METHOD

Testing was done to create the formulation by choosing the quantity of lubricants and preservatives and adding various ratios of binders before the technique was fully optimised. Epimedium sagittatum and Gloriosa superba L. extracts were combined in a 1:1 ratio with 5% starch paste as a binder to make capsules using wet granulation. To create granules, the moist bulk was passed through sieve number 22. In a tray, the granules were dried at 45 °C [24].

VI. PRE-FORMULATION STUDIES

The final herbal granules underwent pre-formulation testing for bulk density, tap density, compressibility index, Hausner's ratio, and angle of repose; the best trial batch was selected for capsule filling and more research [25].

A. Standardization of herbal formulation

- **Capsule evaluation:** According to the standards of the Indian Pharmacopoeia, the herbal capsules were assessed for their description, average weight, weight fluctuation, moisture content, disintegration time, pH, and microbiological load [26]. Additionally, a preliminary screening of phytoconstituents and a quantitative calculation of phytoconstituents were performed.
- **Average weight:** The average weight of the twenty capsules was calculated after each one was weighed independently.
- **Weight variation:** The individual weight of each capsule should be between 90% and 110% of the average weight.
- **Moisture content:** Moisture content was determined using automatic Karl Fischer titration equipment.
- **Disintegration time:** The disintegration testing was conducted using a digital microprocessor-based disintegration test unit. Each tube received a disc and a capsule independently. 1000 cc of water were added to the beaker along with the assembly. The volume of water was at least 25 mm below the water's surface at its highest point and at least 25 mm above the beaker's bottom at its lowest position. With a 2°C accuracy, the equipment was run and kept at a temperature of 37°C.
- **pH value:** In order to calculate the pH of a 1 percent solution, a digital pH metre was employed.

B. Phytochemical screening:

Traditional techniques for phytochemical screening, such as the Mayers, Dragendroffs, and Borntragers tests, were used to conduct the preliminary phytochemical analysis of the ethanolic areal components extract. The alkaline test, the lead acetate test, the foam test, and the lead acetate test

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[27,28,29]. The two medicinal plants used in the current study have undergone preliminary phytochemical analysis, thin layer chromatographic studies, extractive values, weight loss on drying, moisture content, total ash, acid insoluble ash, water soluble ash, residue on igniting, and fluorescence analysis.Fruit and root samples, as well as their extracts in various solvents, underwent fluorescence examination. The Pharmacopoeia of India [30] was used to evaluate the ash content and extractive values of the fruit and root sample. The powdered, air-dried fruit and root samples underwent a series of extractions utilising Petroleum ether (60-800°C), benzene, chloroform, ethanol, and water. Following that, the extracts were used to conduct phytochemical testing.

C. Fluorescence analysis

Under both normal and UV light, we examined the crude drug for any colour changes. Samples were examined in the same way after being treated with a 50/50 solution of HCl and NaOH, and the results were tabulated. The samples were examined by fluorescence using 365 nm light (UV region).

D. Quantitative estimation of phytoconstituents

Estimates of the Bi-herbal formulation's alkaloids, phenolic compounds, flavonoids, and tannin content were made.

E. Microbial load analysis

The total aerobic viable count, yeasts, and moulds, as well as the bacteria Escherichia coli, Salmonellae, Pseudomonas Staphylococcus, and Shigella, were measured to ensure that the raw material for the Bi-herbal capsules could be utilised safely.

VII. RESULTS AND DISCUSSION

The standardisation process, which guarantees the formulation's quality, safety, and reproducibility, is its most crucial element. From procuring raw materials to creating the completed product, it covers every stage of the bio-prospecting process. To substitute the conventional liquid dosing form in the current trial, a standard bi-herbal mixture was turned into hard gelatine capsules. There are only two components in this bi-herbal mixture, and they come from two different families, morphological plant parts, and phytoconstituents.

Petroleum ether and benzene extract fluorescence may be seen in the long-UV range. Under UV light, the ethanol and aqueous extracts of Epimedium sagittatum glow a yellowish brown colour. When exposed to UV light (365 nm), the unprocessed drugs glow brown. They also glow brown after being treated with 1N NaOH and 1N HCl as well as in benzene and ethanol. Long-UV colours are red and yellow, while extracts in petroleum ether glow red orange. When dried, the sample lost between 3 and 7 percent of its weight. The mixture has the highest physicochemical characteristics, including large quantities of ash (16.57-6.03 percent). Fewer than 1.61 percent of the ash in these medications is acid-insoluble, less than 15 percent of the ash is water-soluble, and less than 7.43 percent of the ash is residual after ignition. The extractive values rise in tandem with the solvent's rising polarity. The water extract value is greater than the other extractive values. In the initial physiochemical analysis of crude medicines, the extracts from both samples frequently show the presence of saponins, reducing sugars, triterpenoids, steroids, tannins, and alkaloids. The sample's petroleum ether and chloroform extract both include flavonoids in them. The thin layer chromatographic behaviour of the numerous plant extracts used in the current investigation yields some incredibly intriguing results. The ethyl acetate: benzene (1:9) solvent system may exhibit the most spots in the sample's benzene extracts. For the purpose of precisely identifying the medication and determining if it has ever been adulterated, all pharmacognostic features can be employed as a diagnostic tool.

A. Fluorescence Analysis of Raw Materials

Fluorescence analysis was performed on raw materials, and the results are documented and summarised in Table 1.

Table 1: Fluorescence analysis

Sample	Light	Before Treatment	1N NaOH	1N HCl	1:1 H_2SO_4	1:1 HNO_3	Name of the extract				
							Ether	Benzene	CCl_4	Ethanol	Water
Epimedium sagittatum	Ordi-nary	Yellowish brown	Dark Yellow	Brown	Dark brown	Dark yellow	Dark yellow	Dark brown	Yellowish black	Yellowish green	Dark yellow
	Long-UV (366 nm)	Red	Dark Brown	Dark green	Brown	Black	Red	Red Orange	Red	Yellowish blue	Greenish yellow
	Short-UV (254 nm)	Yellowish Green	Yellow	Brown	Yellowish black	Dark green	Dark yellow	Yellowish green	Yellowish green	Dark green	Yellowish brown
Gloriosa superba L.	Ordin-ary	Crimson to dark brown	Light brown	Brown	Dark brown	Yellow	Dark yellow	Dark brown	Yellowish brown	Light brown	Darkish brown
	Long-UV (366 nm)	Dark Brown	Light brown	Dark brown	Black	Fluorescent green	Light brown	Red orange	Green	Red	Light Brown
	Short-UV (254 nm)	Brown	Dull Brown	Brown	Greenish black	Green	Dark yellow	Yellowish green	Yellowish brown	Brown	Yellowish brown

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B. Physicochemical Parameters

The bi-herbal formulation's herbal medications' physicochemical parameters were calculated in great detail. A list of several physicochemical parameters is provided in Table 2.

Table 2: Physicochemical parameters

Particulars	Epimedium sagittatum	Gloriosa superba L.
Loss of weight on drying	3.28%	9.12
Moisture content	10.71%	3.68%
Total ash	17.10%	8.46%
Water soluble ash	13.00%	2.74%
Acid-insoluble ash	2.11%	1.44%
Residue on ignition	9.23%	5.09%

Table 3: Extractive values

Solvents	Epimedium sagittatum	Gloriosa superba L.
Petroleum ether (60-800C)	4.77%	6.44%
Benzene	7.22%	6.99%
Chloroform	6.66%	8.96%
Ethanol	5.33%	5.74%
Water	10.95%	21.01%

Table 4: Epimedium sagittatum fruit thin layer chromatographic behaviour in ethyl acetate: Benzene (1:9) system of solvents

Name of the Extract	Rf value under UV light		Rf value in iodine chamber
	Long – UV 365nm	Short – UV 254nm	
Petroleum ether (60 – 800C)	*0.71	--	*0.70, @ 0.66
Benzene	@0.55, @ 0.49, * 0.83	--	*0.50, @ 0.44, * 0.73, *0.61, * 0.77,
Chloroform	--	--	*0.90, @ 0.54
Ethanol	@ 0.80	*0.55	@0.66, * 0.79, *0.88,
Water	*0.97	*0.72	*0.91, * 0.64

Table 5: Thin layer chromatographic behaviour of the fruit of *Gloriosa superba L* in Ethyl acetate: Benzene (1:9) Solvent System

Name of the Extract	Rf value under UV light		Rf value in iodine chamber
	Long – UV 365nm	Short – UV 254nm	
Petroleum ether (60 – 800C)	*0.58	--	*0.74, @0.66, *0.81
Benzene	*0.69, @ 0.43, * 0.91	--	@0.56, @ 0.48, * 0.69, *0.91, * 0.65, * 0.72
Chloroform	--	--	*0.93, @ 0.51
Ethanol	@ 0.72	*0.58	*0.97, @ 0.77, *0.96, *0.63
Water	*0.45	*0.72	*0.31, @ 0.25

@-Less intence *-more intense

C. Phytochemical Analysis

The raw materials were subjected to chemical analysis for a number of phyto components; the results are shown and explained in Table 6a.

Table 6(a): Preliminary phytochemical screening of Epimedium sagittatum

Extracts	Steroids	Triterpenoids	Reducing sugars	Alkaloids	Saponins	Tannins	Flavonoids
Pet. ether	+	-	-	-	+	+	-
Benzene	+	+	+	+	-	+	+
Chloroform	+	-	+	-	+	-	-
Ethanol	-	-	+	+	-	+	+
Water	+	-	+	+	+	-	+

Table 6(b): Preliminary phytochemical screening of Gloriosa superba L.

Extracts	Steroids	Triterpenoids	Reducing sugars	Alkaloids	Saponins	Tannins	Flavonoids
Pet. ether	+	-	+	-	-	-	+
Benzene	-	+	-	-	+	+	-
Chloroform	+	-	+	+	-	-	-
Ethanol	-	-	-	+	+	-	+
Water	-	+	+	-	+	+	+

D. Safety Profile Parameters Studies

Calculating the amount of heavy metals using heavy metal examination. The quantity of heavy metals in the raw materials was calculated, and the outcomes are shown in Table 6.

Table 7: Test for heavy metals

OBSERVATION (in ppm/ml)				
Plant name	**Arsenic (NMT 5)**	**Lead (NMT 10)**	**Cadmium (NMT 0.3)**	**Mercury (NMT 0.5)**
Epimedium sagittatum	0.005	0.088	0.004	0.018
Gloriosa superba L.	0.003	0.069	0.044	0.004

Table 8: Microbial load analyses

Parameters	Epimedium sagittatum	Gloriosa superba L.
Total aerobic count (NMT 1000 cfu/g)	645cfu/g	599cfu/g
Yeast and mould count (NMT 100 cfu/g)	NIL	Present
E. coli (To be absent)	Absent	Absent
Salmonella (To be absent)	Present	Absent
Pseudomonas (To be absent)	Absent	absent
Staphylococcus (To be absent)	Present	Present
Shigella (to be absent)	Absent	Present

Table 9: Evaluation of capsules

Parameter	Observation
Average weight	542.30 ±3.2mg
Weight variation	Within I.P. Limit
Moister content (LOD)	2.41±0.3 %w/w
Disintegration time	10.4±0.5(min)
pH (1% aqueous solution)	5.77± 0.77

Table 10: Fluorescence Analysis of Bi-herbal Capsule

Sample	Before treatment			After treating with 50 % HCl			After treating with 50% NaOH		
	Ordinary light	Short UV	Long UV	Ordinary light	Short UV	Long UV	Ordinary light	Short UV	Long UV
Bi-herbal formulation	Brownish yellow	Brown	Green	Brown	Greenish brown	Green	Greenish yellow	Brownish yellow	Dark green

VIII. CONCLUSION

The diagnostic traits developed as a result of this work will aid in the accurate identification and quality control of the crude medicament manufactured from the leaves of Gloriosa superba L. and Epimedium sagittatum (stem and leave). In addition to their traditional usage for treating conditions like spermatorrhoea and low libido power by increasing testosterone levels, it has been found that medicinal herbs contain a wide variety of therapeutically significant phytochemical groups. In addition to aqueous and alcoholic extracts, which demonstrated the highest extractive values for both plant sections, a hydro-alcoholic extract can be utilised to find new bioactive chemicals and to study their biological activity. It is possible to conduct additional scientific research on the chosen extract fractions from Gloriosa superba L. (stem and leaves) and Epimedium sagittatum leaves in order to create novel pharmaceuticals and establish this significant plant as a potential source of phytomedicines. Using the Indian Ayurvedic Pharmacopoeia as a guide helps standardise the job.

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