Ethnobotanical and Phytochemical Analysis of Seeds of the Medicinal Plant *Bryonia laciniosa* Linn.

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Abstract: -

Ethnobotany deals with the direct traditional and natural relationship between human societies and plants. Ethnobotany and phytochemistry together plays a vital role in drug development and understanding traditional medicinal practices. Infertility is becoming a very serious problem worldwide. It has been said to approximately affecting 8 to 12 % couples worldwide and *Bryonia laciniosa* seeds could be the solution for this problem. The seeds of the medicinal plant *Bryonia laciniosa* (Shivlingi) appears like 'Shivling Symbol" of "Lord Shiva". It is used for treating infertility in men and women which is being its highlighting benefit. The present study reveals identification, estimation and quantification of various phyto-constituents present in *B. laciniosa*.

***Keywords*: -** *Bryonia laciniosa* Linn. Infertility, Phytochemical analysis, Ethnobotany

I. INTRODUCTION: -

The term ethnobotany emerged when the primitive man noticed animals eating certain plants e.g. Deer eating grass or even mongoose eating a certain type of plant after fighting with snake to get healed from snake poison etc. **Ethnobotany deals with the direct traditional and natural relationship between human societies and plants**. This knowledge gained broader application and success in experiments on humans which led out to recognize food and medicine while we are all familiar with the commonly used food crops like maize, rice, wheat and jowar and fruit trees like apple, orange, pear, mango, guava, etc. There are hundreds of other species that grows in the wild. Rural families also earn cash by collecting wild fruits, seeds, flowers, tubers, bark, leaves, fibers, gums, lac, honey, bees wax, herbal drugs etc. and selling them in local market. Ethnobotany and phytochemistry together plays a vital role in drug development and understanding traditional medicinal practices. Phytochemistry a conceptual framework in pharmacognosy progressing from a typical descriptive botanical subject to a more chemical or biological focus has gained relevance due to the increased use of herbal remedies in modern pharmacy practice. Phytochemistry, is the study of plant-procured chemicals, especially secondary metabolites, has gained a significant importance in recent decades (Egbuna et al., 2018). "This includes bioactive compounds such as flavonoids, alkaloids, and terpenoids which plays crucial role in plant defense and have numerous applications in medicine and industry (Egbuna et al., 2019; Prakash et al., 2012). Phytochemicals exhibit a wide range of therapeutic properties, such as anti-inflammatory, antioxidant, and anticancer effects, making them valuable in the prevention and treatment of various diseases". (Prakash et al., 2012; Nyamai et al., 2016).

The present study focuses on a life restoring pharmaceutical formulation and a blessing for men and women with infertility problem prepared from the medicinal plant i.e. *Bryonia laciniosa* an ethnomedicinal plant (climber) which is traditionally known as 'Shivlingi' or 'Vaduballi' in Marathi i.e. in local language, as the outer appearance of the seeds appears like 'Shivling Symbol" of "Lord Shiva" (Kadam et al., 2023). It belongs to the family "*Cucurbitaceae*". In Greek the meaning of *Bryonia* is 'to sprout'. It is an annual plant which is found all over India, Philippines and some parts of South Africa. "*Bryonia laciniosa*, also known as Shivlingi, is a medicinal plant from the Cucurbitaceae family with significant therapeutic potential" (Kaurav et al., 2021). "The ethnomedicine used in Ayurveda and as traditional medicine, is used for the treatment of various diseases such as cholera, bronchitis, diabetes, and snake bites" (Kaurav et al., 2021). Bryonin is the plant's main chemical constituent. (Kadam et al., 2023). *B. laciniosa* manifests antidiabetic, anti-inflammatory, antioxidant, spermatogenic and fertility-enhancing properties (Kaurav et al., 2021). It is used for the ailment 'Diminished Ovarian Reserve' i.e. DOR, a medical disorder in which woman ovaries loss their normal reproductive ability affecting the fertility. The seed powder made from the fruits of this medicinal plant acts as a uterine stimulant and helps improvement in conceiving pregnancy chances in women. Some references suggests that it can also help curing potency in men. Recent findings depict its antimicrobial, analgesic, antipyretic, anticonvulsant, anti-asthmatic and anticancer properties (Katiyar et al., 2022). The plant's pharmacological profile shows, proteins, carbohydrates, crude fats, calories, tannins, glycosides, steroids, starch, total phenolic compounds, flavonoids, saponins, terpenoids, and alkaloids, etc.

Table 1: - Classification of *Bryonia laciniosa* Linn.

Taxonomical Rank	**Taxon**
Kingdom	Plantae
Sub-kingdom	Tracheobionta
Super-Division	Spermatophyta
Class	Magnoliopsida
Subclass	Dilleniidae
Order	Violales
Family	*Cucurbitaceae*
Genus	*Bryonia L.*
Species	*Bryonia laciniosa* Linn.

II. MORPHOLOGY OF THE PLANT: -

The medicinal plant comes under the family '*Cucurbitaceae'*. According to the study conducted on the plant by various authors, Sud, K., & Sud, S. 2017, Kadam P. V. et al., 2023 and Kaurav, H. et al., 2023., the plant morphology shows it is an undershrub climber with various tendrils. The stem is sulcated, slender, glabrous and having many branches. The leaves of the plant are simple, alternate, 10-15 cm long, broad, membranous, smooth and green, deeply cordate at base, acute, and Lanceolate. Tendrils are striated, glabrous and slender. Petioles are 2.5-3.7 cm long. Both male and female flowers can be seen which seems like regular and green-pale-yellow. Male Flowers are quite distinct, contain 3 stamens and having 3-6 small Fascicles. Female flowers are small in number, in clusters and are solitary". Calyx is glabrous, Corolla is 3-5 mm long with fragments, oval, intense and presence of short and soft hairs (Sud, K., & Sud, S. 2017). Unripe fruits seem smooth, globose and sub-sessile, 1.8 to 2.5 cm in diameter and are green in colour with white stripes formed vertically and turn to red or orange color when the fruits are matured. The appearance of seeds is yellowish to brown in colour and they are 5-6 mm long. Seeds are yellowish cocoa in color and appear like "Shivling Symbol" of Lord Shiva and are 5-6 mm long.

Fig 1 & 2. *Bryonia laciniosa* Linn. Plant and Fruits

Fig 2. Medicine Man (*Vaidu*)

Fig 3. *Bryonia laciniosa* Linn. Sample Collection

Fig 4. Bryonia laciniosa Seeds

III. Geographical Distribution of the Plant: -

The plant is widely distributed in various countries like Sri-Lanka, Tropical Africa, Indonesia, India, Australia, Bhutan, China, etc. In India it is found in states like Maharashtra, Madhya Pradesh, Tripura, Rajasthan, Chhattisgarh, Kerala, etc.

IV. Review of Literature: -

Rasagna Yadavalli, et al., (2012), "Phytochemistry and Pharmacology of *Bryonia Lacinosa*: A Review" in their study revealed that, "The plant *Bryonia lacinosa* has been used in different system of traditional medication for the treatment of Diseases and ailments of human beings. Goniothalamin, punicic acid and lipids were previously isolated from the whole plant of *Bryonia laciniosa*. From leaves a bitter principle, bryonin, has been reported. Arabinoglucomannan, a Polysaccharide is present in the fruit which is mainly used for anti-microbial activity. The plant contains anti-inflammatory, analgesic, anticonvulsant, anti-microbial and cytotoxic properties.

Kadam, V and Deosthale, S. (2015) in their "Documentation of Traditional Ethnomedicinal Plants Used by Tribals from Painganga Forest Range in Umarkhed region of Yavatmal District of Maharashtra State" conclude that Painganga forest is a rich store house of medicinal plants.

Chavan S. A. et al., (2019) in their "Phytopharmacognostic Review on *Bryonia laciniosa* (Shivlingi Beej)" concluded that *Bryonia laciniosa* Linn. Commonly called as shivlingi is a medicinal plant belongs to the family Cucurbitaceae. It is a uterine tonic and improves the chances of conception in women suffering from infertility and its main chemical constituent is 'Bryonin'.

Beldar G. Vishal and Sidat S. Parin (2020) in their "Ethnomedicinal Plants used in Maharashtra for Treatment of Various Diseases and Disorders" described about reliance of peoples on nature for their daily needs.

Shinde D. Smita (2021) in their "Phytochemical Analysis of Few Plants from Trimbakeshwar and Surgana Forest, Nashik District, Maharashtra, India" reported about phytochemical constituents of eleven rare plants from Surgana forest of Nashik division and about the phytochemicals which are also the secondary metabolites present in smaller quantities in higher plants and they include the alkaloids, steroids, flavonoids, terpenoids, tannins and many others.

Kaurav et al., (2021) in their "An Ayurvedic Herbal Plant '*Bryonia laciniosa*' with its Ethnomedicinal Significance" illustrated about a medicinal plant Bryonia laciniosa which is mainly known for its seeds and its therapeutic uses in treating various diseases like cholera, bronchitis, Diabetes, Snake bites, Paralysis, etc. it's main highlight is that it is used to treat infertility in woman's as it acts as uterine tonic.

Kadam P. V. et al., (2023) in their "*Bryonia laciniosa*: A Ethnopharmacological Approach of Ayurvedic Shivlingi" stated that, all men or women have an innate, passionate desire to preserve their race. Becoming a mother is one of every woman's greatest love dreams. Herbal medicines are much cheaper and less difficult to obtain and have no known ill effects. Many herbs that promote infertility are also used in folk remedies that are mostly undiscovered. It is a little-known conventional drug that has been proven to be effective in treatment and that is where this plant *Bryonia laciniosa* "Shivlingi" which works as Uterine tonic for women and fertility booster for men.

V. Dosage (Formulation): -

The dosage (formulation) for Shivlingi Beej is as follows-

Crushed seeds powder is made and given to the patients having infertility problem (Dosage is same for both male and female) in milk twice a day, 3 hours after taking the meal. For weight loss the crushed seeds powder is taken with warm water.

VI. Safety Profile: -

The medicine is safe for all types of patients; it can also be given to the pregnant women's since there are no side-effects or allergies.

VII. Methodology: -

i. Sample (Fruit) Collection Site: -

The sample (freshly ripened fruits) were collected from the forest nearby Kelapur Taluka, Pandharkawada (Yavatmal District).

ii. Methodological Approach: -

For phytochemical analysis standard procedure (Harborne 1992:1998, Kokate 1994, Abulude 2001: 2007) is followed. Phytochemical analysis involves both qualitative and quantitative analysis. "Qualitative analysis is related with the presence or absence of a phytochemical compound, while quantitative analysis is concerned with the quantity or the concentration of the phytochemical compound present in the plant sample" (Panhekar, et al., (2019). Phytochemicals- Extraction, Separation & Analysis Techniques). There are various Techniques given for the Phytochemical Analysis from Collection of Medicinal Plants to Extraction of phyto-chemicals and to finally identification of those phyto- constituents whether they are present or absent, if the phytochemical compounds are found present then quantification is done for those phytochemicals by various methods.

Phytochemical analysis of *B. laciniosa*, involves collection of fresh ripened fruits of the *Bryonia laciniosa* plant (Shivlingi). Collected fruits were thoroughly washed with water. After washing they are dried by sun drying method for 6-7 days. Seeds are subsequently extracted from the dried fruits. The seeds are then grounded into fine powder. The seed powder is then added to ethanol in beaker (suitable solvent for *B. laciniosa*) and it is stirred rigorously with a stirrer for few minutes after that the beaker containing plant sample and the solvent is placed in Ultrasonicator. Ultrasonicator uses ultrasonic waves to disrupt the seed cell walls, facilitating the release of desired phytochemicals into a solvent. This technique is also known as ultrasonic-assisted extraction (UAE), this is an efficient method for improving extraction efficiency and yield as compared to the traditional methods, such as maceration or Soxhlet extraction. To homogenize the mixture well, 3 cycles of 45 minutes each is given to the sample in Ultrasonicator.

The beaker containing the sample is then poured into the rotating round-bottom flask of the rotary evaporator. The heating bath temperature is adjusted according to the boiling point of the solvent used in the experiment. As the solvent starts evaporating, it rises and enters into condenser, where it is cooled and condensed back into a liquid. The condensed solvent is then collected in a separate flask, while the concentrated crude extract remained in the rotating flask. The rotary evaporator removes the solvent, yielding a residue ready for analysis.

Qualitative phyto-chemical analysis was conducted by using the general techniques outlined by Banu, K. S., & Cathrine, L. 2015 and Panhekar, et al., 2019. For **Carbohydrates**- Molisch's Test, Fehling's Test, Benedict's Test, Iodine Test are conducted, for **Proteins and amino acids**: Biuret Test, Millon's Test, Ninhydrin Test, Xanthoproteic Test. **Lipids**: Solubility Test, Translucent Spot Test, Acrolein Test. **Alkaloids**: Wagner's Test, Mayer's Test, Hager's Test, Dragendorff's Test. **Flavonoids**: Shinoda Test, Alkaline Reagent Test, Lead Acetate Test, Zinc Hydrochloride Test. **Tannins**: Ferric Chloride Test, Lead Acetate Test, Gelation Test. **Saponins**: Foam Test,

Hemolytic Test. **Phenols**: Ferric Chloride Test. **Terpenoids**: Salkowski Test. **Glycosides**: Keller-Kiliani Test, Legal's Test, etc. are conducted. Other parameters such as Total Calories, Total Moisture, Total Ash, Total Crude Fat, etc. are calculated and found out.

For Quantitative analysis of the plant sample various techniques such as HPLC (High Performance Liquid Chromatography), GC-MS (Gas Chromatography – Mass Spectroscopy), HPTLC (High Performance Thin Layer Chromatography) are conducted. Detection of phyto-constituents involves using techniques like UV- Visible Spectroscopy. The techniques used for each of the phytochemicals is as follows-

Table 2: - Estimation of Phyto-chemicals by various Methods

Phytochemicals	**Name of Test/Method**
Protein	Kjeldahl method
Carbohydrates	FSSAI method
Crude Fat	Pet Ether extraction
Calories/Energy	By Calculation
Total ash	By Muffle furnace
Total Moisture	By Hot Air Oven
Saponins	Gravimetry
Total phenols	Spectrophotometer
Alkaloids	Spectrophotometer
Tannins	By HPLC
Tannic Acid	By HPLC
Glycosides	Spectrophotometer
Steroids	Spectrophotometer
Flavonoids	By Spectrophotometer

VIII. RESULTS: -

Table 3: - Sample Details

Sample Details	
Sample Quantity	50 gm
Sample Description	*Bryonia laciniosa* Linn. – Shivlingi (Seeds)
Sample Collected by	Mr. Sarvesh Bandu Raut
Sample Collection Date	26/01/2025
Sample Collection Site	Forest Nearby Kelapur Taluka
Sample provided by	*Vaidu* Shri. Namdeo Bawane
Phytochemical analysis initiation date	06/02/2025
Phytochemical analysis completion date	25/02/2025

Table 4: - Qualitative Analysis of *Bryonia laciniosa* Linn**.** (Seeds)

Compound	**Presence**
Anthroquinones	Absent
Quinones	Absent
Alkaloids	Present
Glycosides	Merely Present
Cardiac Glycosides	Absent
Phenols	Merely Present
Polyphenols	Absent
Tannins	Present

i) Tannic Acid (Sub-Class of Tannins)	Present
Flavonoids	Merely Present
Phytosterols	Absent
Phlobatannins	Absent
Saponins	Present
Steroids	Merely Present
Xanthoproteins	Absent
Chalcones	Absent
Terpenoids	Present
Anthocyanins	Absent
Coumarins	Present
Emodins	Absent

Table 5: - Quantitative Analysis of *Bryonia laciniosa* Linn.

Results				
Sr. No.	**Parameter**	**Result**	**Unit**	**Method of Analysis**
1	Protein	33.1855	%w/w	Kjeldahl method
2	Carbohydrates	3.0794	%w/w	FSSAI method
3	Crude Fat	29.0836	%w/w	Pet Ether extraction
4	Calories/Energy	476.7333	%	By Calculation Method
5	Total Ash	4.16969	%	By Muffle furnace
6	Moisture	59.56532	%	drying method, often in a hot air oven at a controlled temperature (e.g., 105°C)
5	Saponins	8.8038	%w/w	By Gravimetry
6	Total phenols	1.2530	%w/w	By Spectrophotometer
7	Alkaloids	5.2958	%w/w	By Spectrophotometer
8	Tannins	3.0698	%w/w	By HPLC
	i) Tannic Acid	2.813	%w/w	By HPLC
9	Glycosides	0.0943	%w/w	By Spectrophotometer
10	Steroids	1.1406	%w/w	By Spectrophotometer
11	Flavonoids	1.0917	%w/w	By Spectrophotometer
12	Starch	3.2078	%w/w	By Spectrophotometer

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Data file  : C:\CHEM32\1\DATA\14-02-2025_TANNIC ACID_036_042_ODS 2025-02->
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Sample Name     : WLS25_037_Shivlingi         Location   :     Vial 24
Acq Operator    :                             Inj. No.   :           1
                                              Inj. Vol.  :       20 µl

Acq. Method     : C:\Chem32\1\DATA\14-02-2025_TANNIC ACID_036_042_ODS ->
Analysis Method : C:\CHEM32\1\METHODS\TANNIC ACID T4_NEW.M
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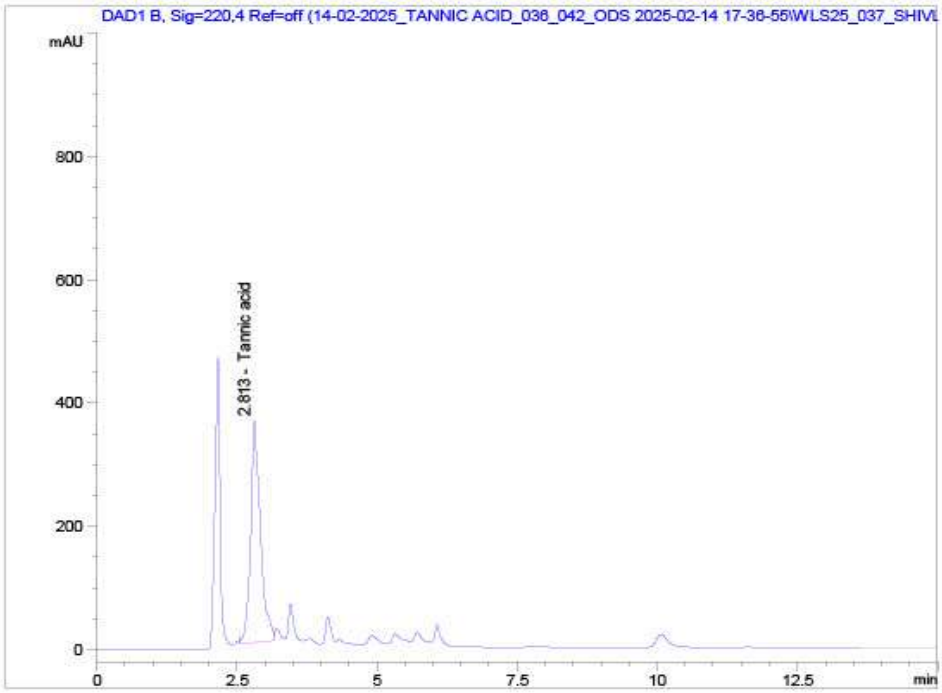


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Sorted By Signal
Calib. Data Modified  :  Wed, 26. Feb. 2025,06:32:39 pm
Multiplier            :  1.000000
Dilution              :  1.000000
Uncalibrated Peaks    :  not reported

Signal  1: DAD1 B, Sig=220,4 Ref=off

|Peak|   RT    | Type  | Width |   Area    | Area % |     Name      |
| #  | [min]   |       | [min] |           |        |               |
|----|---------|-------|-------|-----------|--------|---------------|
|   1|    2.813|VV     |  0.158|  4317.576| 100.000|Tannic acid    |
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Instrument 1     Wed, 26. Feb. 2025    07:16:09 pm          Page   1 of 1
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Chromatogram 1- Estimation of Tannic Acid in *Bryonia laciniosa* Linn. Seeds

IX. DISCUSSION: -

The present study is an honest attempt to put forth phyto-constituents and medicinal properties present in the seeds of the medicinal plant, *Bryonia laciniosa* Linn. And one such important medicinal property is infertility in men and women; the powder made from the seeds extracted from the dried fruits of this medicinal plant can acts as a fertility booster for men and women. Previous study by Kadam, P. V., et al. (2023) on seeds of the *Bryonia laciniosa* Linn. shows molecules such as saponins, flavonoids, phenolic acids, sugars, punicic acid, goniothalamin, and glucomannan. Another study by Kaurav et al., (2021) shows that the seeds of the plant contain saponin molecules, flavonoids, phenolic acids, sugars, punicic acid, goniothalamin and gluccomannan. The findings of this research on

phytochemical analysis of *Bryonia lacinosa* Linn. shows findings such that the seeds of this plant contain primary phyto-constituents like Carbohydrates, Proteins and Secondary Phyto-constituents such as, Alkaloids, Terpenoids, Phenols, Flavonoids, Coumarins, Tannic acid and Saponins. Other parameters such as Total moisture, Total Ash, Total Fat, Energy/calories are also calculated and found out by various established analytical methods.

X. CONCLUSION: -

Ethnobotany and phytochemistry together plays a vital role in drug development and understanding traditional medicinal practices. Phytochemistry a conceptual framework in pharmacognosy progressing from a typical descriptive botanical subject to a more chemical or biological focus has gained relevance due to the increased use of herbal remedies in modern pharmacy practice. Our traditional medicinal systems have remedies on most of diseases and disorders. Infertility is one such disorder which is becoming a very serious problem worldwide in men and women. Especially in Women with a condition called as 'Diminished Ovarian Reserve' a medical disorder where ovaries of a woman loss their normal reproductive potential which affects the fertility. The seed powder made from the fruits of this medicinal plant acts as a uterine stimulant and helps improvement in conceiving pregnancy chances in women. In men it served as a fertility booster, contributing to elevated testosterone levels and improved sperm count.

XI. ACKNOWLEDGEMENT: -

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XII. CONFLICT OF INTEREST: -

The authors declare that there are no conflicts of interest.

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