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Estimation of Phytochemical Constituents in *Selaginella repanda* Collected from the Gautala Wildlife Sanctuary, Jalgaon Region

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Abstract: ***In traditional medicine some varieties of Selaginella extracts have been shown to have anti-inflammatory effects, antimutagenic effects, antispasmodic effects and cytotoxic effects. We collect the Selaginella repanda sample from the Gautala wildlife reserve in the Jalgaon area. The quantitative phytochemical examination of Selaginella repanda showed that all of the secondary metabolites that were looked at were there, albeit in different amounts Saponins had the highest average concentration, followed by tannins and glycosides and phenols. Flavonoids and alkaloids were found in moderate amounts, whereas terpenoids were found in the lowest amounts. The statistical analysis showed that the concentrations of phytochemicals were not all the same. The Shapiro–Wilk test showed that the data were normally distributed (p > 0.05). Pearson's correlation analysis showed that distinct phytochemicals were linked to each other, which suggests that they were made or stored in a coordinated way.***

Keywords: Sanctuary, Phytochemical**,** secondary metabolites, Pearson's correlation

1. Introduction

Traditional medicine, which is primarily composed of plant-based ingredients, is the main primary source for medical care for the majority of population living in underdeveloped nations, as stated by the World Health Organization. The phytochemical content of traditional plants are responsible to the medical efficacy of selected medicinally important plants. These compounds are responsible for eliciting unique effects of pharmacological agents on human physiology. Research has shown that both Ayurvedic medicine and the Unani School of medicine make use of pteridophytes for therapeutic purposes. It has been shown that pteridophytes are not vulnerable to microbial diseases, which may be a crucial factor contributing to their evolutionary success and their survival for over 350 million years. A variety of ferns were utilized in the practice of traditional Indian medicine. Sushruta, who lived about the year 100 A.D., and Charaka, who also lived around the same time, both advocated for the therapeutic application of specific ferns in their respective Samhitas. A variety of ferns have been utilized by Unani practitioners in western Asia and India.

In traditional medicine, *Sellaginella sp.* has been used to treat a wide range of conditions, such as wounds, fever, cancer, tonsils, kidney stones, headache, hepatitis, fever, skin diseases, bone fractures, jaundice, toothache, blood coagulation, diarrhoea, gastric ulcers, asthma, backache, blood purification, exhaustion, and to counteract the venomous effects of snake and scorpion bites. Among the secondary metabolites that can be discovered in *Selaginella spp.,* some examples include bioflavonoids, glycosides, alkaloids, and selaginellallin. There are numerous varieties of *Selaginella* that are utilized in traditional plant- based medicine in the world for the purpose of treating a wide variety of diseases. These diseases include cancer, hepatitis, diabetes, heart problems, skin problems and urine infections. Some varieties of *Selaginella* extracts have been shown to have antinociceptive effects, anti-inflammatory effects, antimutagenic effects, antispasmodic effects and cytotoxic effects.

2. Material and Method

Sample Collection and Powder Preparation

We got the *Selaginella repanda* sample from the Gautala wildlife reserve in the Jalgaon area. We collected the plant pieces, washed them under running water, and then autoclaved them to get rid of any final particles of dirt. Fresh plant leaves were let to dry at room temperature for ten days before being ground up in an automatic grinder. The plant powder that didn't have any dust in it was kept in an airtight container at 4 °C for later use (Mehra and De, 2017). After they dried, the materials were milled into a fine powder.

Quantitative determination of secondary metabolites:

Determination of Alkaloids: We used the Mbaeyi-Nwaoha and Onwuka (2014) approach to figure this out. We used 20 millilitres of 20% H_2SO_4 in ethanol (1:1) to dissolve one gram of each of the powdered samples. Then we filtered them. Five millilitres of 40% H_2SO_4 were added to two test tubes, each with one millilitre of leaf and bark filtrate. The two test tubes were then mixed together very well. The mixture was covered and let to settle for four hours before the measurement was taken. Using a spectrophotometer, we examined the two samples (leaf and bark) at 568 nm. Values were written down after the process was done three times.

Determination of Flavonoids: This was figured out using the method of Mbaeyi-Nwaoha and Onwuka (2014). One gram of each powdered substance was dissolved in 200 millilitres of ethyl and then filtered. Five millilitres of the leaf and bark filtrates were put into two different test tubes. Then, five millilitres of diluted ammonia were added, stirred well, and left to settle for a few hours. After that, a spectrophotometer was used to find the absorbance at 490 nm (Mbaeyi-Nwaoha and Onwuka, 2014). After the

operation was done three times, the values were written down.

Determination of Tannins: This was found out using the method of Mbaeyi-Nwaoha and Onwuka (2014). A separate conical flask was filled with one gram of each sample. The flask was shaken for 30 minutes, with breaks every five minutes, and then 10 millilitres of water were added and the mixture was filtered. We filled two different conical flasks with 2.5 ml of each filtrate. Then, one millilitre of FollinDenis reagent and Na2CO3 were added, and everything mixed thoroughly. After the combination had been sitting at room temperature for 90 minutes, a spectrophotometer was used to measure the absorbance at 720 nm. After doing the process three times, the values were written down.

Determination of Saponins: In separate conical flasks, 1g samples were mixed with 10 ml of petroleum ether following the Mbaeyi Nwaoha and Onwuka (2014) method. After being drained and dried, this was combined with ten milliliters of petroleum ether. Measurements were collected at 550 after adding 5 ml of ethanol to the dry mixture and completely mixing it. Each sample received around 2 ml of the mixture, which was then placed in two separate test tubes and left to settle for 30 minutes. The values were recorded after the process was carried out three times.

Determination of Glycosides: The approach of Mbaeyi-Nwaoha and Onwuka (2014) was used to determine this. In test tubes, 1 g of materials was thoroughly mixed with 2.5 ml of 15% lead acetate before being filtered. After carefully mixing and letting it settle, two milliliters of chloroform were added to the filtrate. The lower part was gathered and dried by evaporation. After adding three milliliters of glacial acetic acid, 0.1 milliliters of 5% ferric chloride, and 0.25 milliliters of concentrated H_2SO_4, the dried lower part was thoroughly mixed and allowed to sit for three hours. A spectrophotometer was used to measure the absorbance at 568 nm. The values were recorded after the process was carried out three times.

Determination of Total terpenoid content: The terpenoid content was calculated using the Ghorai et al. (2012) method. After adding 1.5 mL of chloroform to 200 µL of plant extract, the mixture was thoroughly vortexed. Following the remaining three minutes, 100 µL of concentrated sulfuric acid was added, and the mixture was left to sit at room temperature in the dark for one and a half to two hours. A reddish-brown precipitate formed after incubation, and the supernatant was carefully removed from the solution. The precipitate was thoroughly blended with 1.5 mL of methanol. Using a spectrophotometer, absorbance was measured at 538 nm, with linalool serving as the standard. The amount of terpenoid was stated in milligrams of linalool equivalents per gram (LE/g). of sample in dry weight.

Determination of Total Phenol content: One milliliter of 80% ethanol was mixed with 0.5 grams of plant extract to quantify total phenolics. After that, the mixture was centrifuged at 12,000 rpm for 15 minutes. The supernatant was then stored in a test tube, and the procedure was carried out six times. Following collection, the supernatant was put in a water bath to dry. The supernatant's volume was increased to three milliliters by adding distilled water. This solution was supplemented with 2 milliliters of 20% Na_2CO_3. 0.5 ml of Folin Ciocalteau regent was added to this followed by the addition of 2 ml of (Na_2CO_3) from 20% Na_2CO_3 solutions after 5 minutes. After thoroughly mixing the solution, the test tube was placed in the water bath with boiling water. Their absorbance was measured at 650 nm. As a benchmark, the Catechol was employed (Hagerman et al., 2004).

Statistical analysis: M.S. Excel was used for all statistical analyses. The phytochemical concentrations in *Selaginella repanda* were subjected to descriptive statistics, which included a mean. The Shapiro-Wilk test for a normal distribution of data was used to evaluate the data's normality. The association between various phytochemicals was assessed using Pearson's correlation analysis. The significance level for all statistical tests was set at 5% ($\alpha = 0.05$).

3. Observation Table

Table 1: Shows the phytochemical concentrations of secondary metabolites

Plant	Mean concentration of phytochemicals in mg/100 gm						
	Alkaloid	Flavonoid	Terpenoids	Phenols	Saponins	Tannins	Glycosides
S. repanda	0.82	0.98	0.67	1.96	2.3	2	2

4. Result and Discussion

The quantitative phytochemical examination of *Selaginella repanda* showed that all of the secondary metabolites that were looked at were there, albeit in different amounts (mg/100 g). Saponins (2.30 mg/100 g) had the highest average concentration, followed by tannins and glycosides (2.0 mg/100 g each) and phenols (1.96 mg/100 g). Flavonoids (0.98 mg/100 g) and alkaloids (0.82 mg/100 g) were found in moderate amounts, whereas terpenoids (0.67 mg/100 g) were found in the lowest amounts. The statistical analysis showed that the concentrations of phytochemicals were not all the same. The Shapiro–Wilk test showed that the data were normally distributed ($p > 0.05$). Pearson's correlation analysis showed that distinct phytochemicals were linked to each other, which suggests that they were made or stored in a coordinated way.

The present findings demonstrate that *Selaginella repanda* contains a variety of phytochemicals, such as saponins, tannins, glycosides, and phenolic compounds. Researchers have detected similar categories of bioactive chemicals in *Selaginella repanda* before. This verifies the presence of flavonoids, alkaloids, terpenoids, and phenolics (Rameshkumar et al., 2021). The somewhat elevated phenolic content identified in this study corroborates prior

research indicating that phenolic compounds are crucial for antioxidant activity due to their ability to alter their oxidation state and sequester free radicals (Rice-Evans et al., 1997). Rameshkumar et al. (2021) assert that phenolic acids and flavonoids constitute the principal components of *Selaginella* species that mediate biological activities. This investigation identified an increased concentration of saponins, corroborating previous findings that demonstrate their antibacterial and membrane-permeabilizing properties (Saxena et al., 2013). Tannins, when present in significant amounts, exhibit antibacterial properties via inducing protein precipitation and inhibiting enzymatic activity (Cowan, 1999). The moderate levels of flavonoids and alkaloids correspond with previous studies that identified their pharmacological properties, which encompass antioxidant, anti-inflammatory, and antibacterial activities (Trease & Evans, 2009). Even though terpenoids were found in lesser amounts, their presence is in line with what other research have found about *Selaginella* species and their involvement in fighting bacteria (Ma et al., 2015).

Research examining various species within the genus *Selaginella* has demonstrated that phenolics, flavonoids, and saponins are the predominant phytochemicals. This means that different species have similar patterns of phytochemicals (Ma et al., 2015). This adds to the credibility of the existing outcomes. Statistics show that phytochemicals have a regular distribution and association. This shows that they may be generated through metabolic pathways that are connected to each other. It is known that these kinds of interactions between phytochemicals make medicinal plants work better as a whole (Wink, 2010). This research corroborates and enhances prior studies regarding the phytochemical composition of *Selaginella repanda*. Research plant could be a good source of natural antibacterial and antioxidant chemicals, as research reveals.

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