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Studies on phytochemical analysis and screening for active compounds in some ferns of Ranchi and Latehar districts

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Abstract
The principle objective was to evaluate chemical constituents of some locally and wildly grown fern plants of Ranchi and Latehar districts. Fern plants are growing under different environmental conditions. The aim of the present study was to evaluate the chemical composition of essential oils from two chemically unexplored fern ses of Ranchi and latehar district. So, the content of different bio-macromolecules and secondary metabolites are different in these plants. Ferns are least exploited group of plants in India, when compared to other countries of the world. Phytochemical studies of the two plants under investigation are important while evaluating plant wealth of the region under study.

Lygodium flexuosum was growing in areas under comparatively less water availability whereas *Ampelopteris prolifera* was growing under high water and moisture content. *Lygodium flexuosum* and *Ampelopteris prolifera* contains lots of pigments, carbohydrates, amino acids, proteins, lipids and several secondary metabolites. The present study was made to find out the chemical composition (in case of rhizome, fronds, and petiole of the two ferns) and their relationship with antimicrobial activity of these two plant species. Methanol extract showed maximum numbers (eight) of the compounds. Reducing sugars are present in petroleum ether extract and methanol extract of *Lygodium flexuosum* and in *Ampelopteris prolifera* it is present in the methanol extract only. Alkaloids are present in the benzene extract and methanol extract of *L.* flexuosum and in *A. prolifera* these compounds are present in methanol extract only. *Lygodium flexuosum* rhizome extract possessed more anti bacterial principles (than *Ampelopteris prolifera*, when compared), soluble in methanol and acetone which suppressed the growth and multiplication of the tested bacterial species than leaves and petiole. The presence of phenolics, flavonoids and triterpenoids in acetone extract might be responsible for its maximum anti bacterial activity in *Lygodium flexuosum* and little in *Ampelopteris* prolifera. Petroleum ether extract did not show the presence of chemicals tested in *Ampelopteris prolifera*. Methanol extract showed the presence of flavonoids and phenolics. The anti bacterial activity of different plant parts of these two ferns and their active constituents would be helpful in treating various kinds of diseases. *Lygodium flexuosum* is potential anti bacterial agent for the bacteria causing eczema, dysmenorrhoea and spermetorrohea and leaf and petiole extract is effective in jaundice and gastro intestinal ailments. The results confirm the presence of constituents which are known to exhibit medicinal as well as physiological activities.

Keywords: flavonoids, rhizome, petiole, methanol, benzene and phytochemical, extract, *Lygodium flexuosum, Ampelopteris prolifera*

Introduction

Like angiospermous plants, phytochemical studies of fern plants do not worked out extensively. From phytochemical analyses, It has been observed that fern plants contain higher levels of carbohydrate, amino acids, protein, lipid and secondary metabolites and these are economically useful product of any region (Britto *et al*.1992, Kale 2007, Kaur *et al*. 1986, Kale & Upadhye, 2005, Gopalakrishnan *et al*. 1993, Jesudass *et al*. 1993, Rathore & Sharma, 1990, Vyas & Sharma, 1988, Vyas *et al*. 1989, 1995) [8, 19, 20, 21, 7, 13, 18, 38]. Phytochemical characterization of plant material is important as it relates to the therapeutic actions. It is perhaps obvious that different species of plants would have different chemical constituents.

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Phytochemicals are organic, non-nutritive, naturally occurring chemicals found in plant foods They are nonessential nutrients, meaning that they are not required by the human body for sustaining life. It is well-known that plant produces these chemicals to protect them but recent research demonstrates that they can also protect humans against diseases.

It has already been stated (Arts & Hollman, 2005) [3] that phytochemicals are a large group of plant-derived compounds hypothesized to be responsible for much of the disease protection conferred from diets high in fruits, vegetables, beans, cereals, and plant-based beverages such as tea and wine. There are more than thousand known phytochemicals.

Further the importance of Secondary metabolites like phenolic compounds and alkaloids as medicinal value has been highlighted. Later on many studies (Gehlot *et al*. 1995; Manickam *et al*. 2005; Parihar & Bohra, 2001, 2002, Parihar & Bohra, 2003, 2004, Parihar *et al*. 2004, 2007) [12, 27, 29, 30, 31, 32, 33, 34, 35, 36, 37] were made to evaluate the importance of ferns from chemical and pharmacological aspects. Humans use secondary metabolites as medicines, flavorings, and recreational drugs.

A Detailed studies on phytochemical research of the fern

plants and anti microbial activity of the plant extract reveal the quantitative biochemical analysis are available (Britto *et al*. 1992, 1993, 1994, Gopalakrishnan *et al*. 1993, Jesudass *et al*. 1993, Kaur *et al*. 1986, Kale & Upadhye, 2005, Vyas & Sharma, 1988) [8, 20, 21].

Recent studies on the fern plants which are growing under different environmental conditions, so, the content of different bio-macromolecules and secondary metabolites are different in these plants and it has been reported (Mohammadkhani & Heidari, 2008) [28] that Proline is unique amino acid and its accumulation is a wide spread plant response to environmental stress, including low water potential, Phenolic compounds (Bhattacharyya (Goswami) & Halder, 2009, Kukuc & Kivanc, 2003, Maher *et al*. 1994, Manickam *et al*. 2005, Parihar *et al*. 2004, Taiz & Zeiger, 2003) [27, 37, 5, 25] act as defensive mechanisms and it accumulates in mature organ of the plants.

Like angiospermous plants, phytochemical studies of fern plants do not worked out extensively. Fern plants are growing under different habitat and especially under stress conditions. These plants have the potentiality as a source of economically important products used mainly in folk-medicine.

Phytochemical analysis of these plans shows the presence of some specific chemicals having Anti microbial properties which would definitely help in developing the pesticides which are eco friendly.

An attempt has also been made to find out the relationships among the above said characteristic morphology and habitat. Secondary metabolites like phenolic compounds and alkaloids have great importance for medicinal value. Nowadays many workers are interested (Gehlot *et al*. 1995, Parihar & Bohra, 2001, 2002, 2002a, 2002b, 2003, 2004.) [12, 29, 30, 31, 32, 33, 34, 35, 36, 37] to evaluate the importance of ferns from chemical and pharmacological aspects.

Fern plants are growing under different environmental conditions. So, the content of different bio-macromolecules and secondary metabolites are different in these plants. Phytochemical studies of the two plants under investigation are important while evaluating plant wealth of the region under study. It is also not possible to determine the source of any economically useful materials without any phytochemical study. *Ampelopteris prolifera* (figure-1.) and *Lygodium flexuosum* (Figure-2) contains lots of pigments, carbohydrates, amino-acids, proteins, lipids and several sec. metabolites. The present study was made to find out the chemical composition of these two plant species of the study area of Jharkhand.

Lygodium flexuosum (L) Sw. (Figure-2.) is an annual fern and exists in hilly areas and forests. The plant has been described as an expectorant and its root extract in mustard oil is is considered an effective remedy for the treatment of wounds and eczema, leaf paste of *Lygodium flexuosum* (L) Sw. when applied over the body for seven days cures jaundice. Roots of the plant have also been employed against dysmenorrhoea and cure spermetorrohea. The leaf paste is applied over the skin disease treatment and also given orally for the same purpose. The present investigation was done to evaluate the different parts of the plant for its potential as anti-bacterial agent.

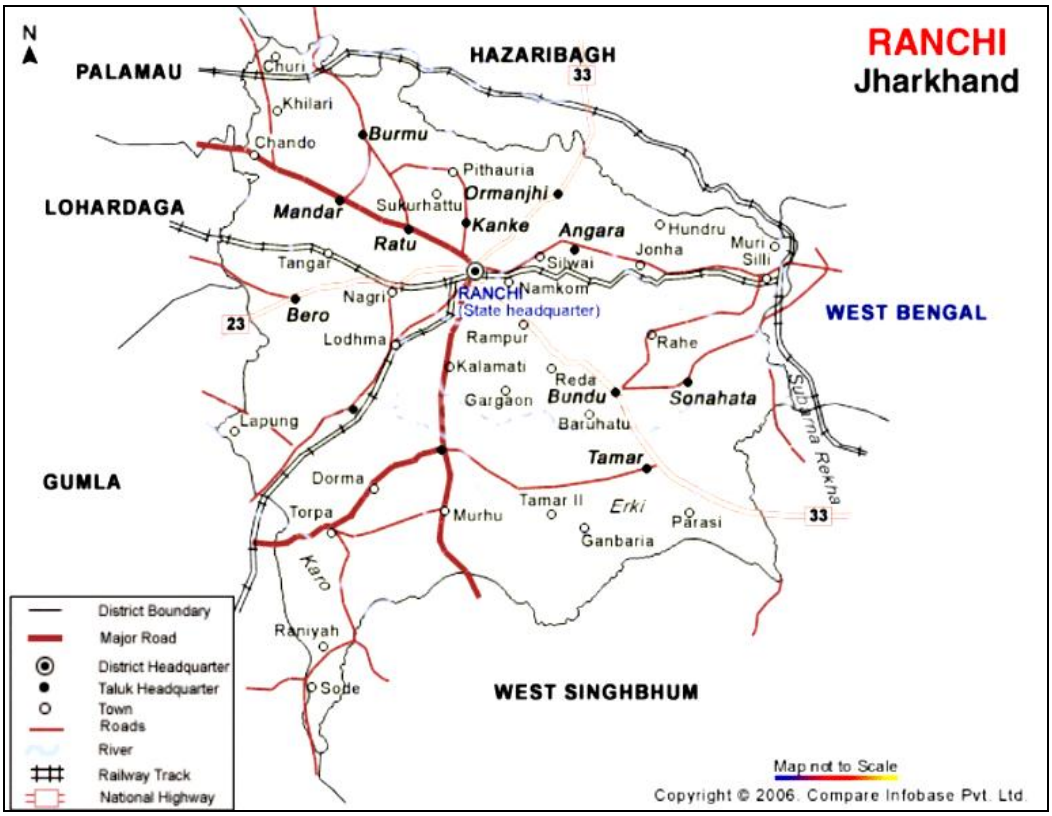
The phytochemical evaluation of both plants showed that the phenolics, triterpenoids and flavonoids were present in active acetone extract. Petroleum ether extract did not show the presence of chemicals tested. Methanol extract showed the presence of flavonoids and phenolics. None of the extract showed the occurrence of alkaloids. The presence of phenolics, flavonoids and triterpenoids in acetone extract (Table 3) might be responsible for its maximum anti bacterial activity in *Lygodium flexuosum* and little in *Ampelopteris prolifera* (Figure-1.) when compared. Now a day's many workers (Aguinaldo *et al*. 2005, Halder *et al*. 2008, Harborne, 1973, Kirk & Allen, 1965, Manickam *et al*. 2005, Trease & Evans, 1989, Parihar & Bohra, 2001,2002, 2002a, 2002b, 2003, 2004; Vyas *et al*. 1995) [29, 30, 31, 32, 33, 34, 35, 36, 37, 6, 27, 16, 41] are interested to evaluate the importance of ferns from chemical and pharmacological aspects.

Site description

Physical characteristics of Ranchi and Latehar district of Jharkhand state are very special as it is situated in East Deccan zone of India. The area is undulating with elevation from 152 m to 1200 m or above. The important river Swarnarekha originated from Ranchi district while life line of Jharkhand Damodar River, South Koel and North Koel originate from Latehar district. Ranchi district cover an area of 7698 km2, out of which 1,732 km. are under forest cover. The elevation ranges from 457 m to 700m. Latehar district has an area of 3637 km2. Out of which 2660 km2 is under forest. The elevation has sharp decline up to 400 m in north where as it is higher (1200 m) in south covering Netarhat and Mahuadar area. The area of study lies between 220 5‘- 230 6‘ N Lat. to 840 9‘ to 850 9‘ E L and 230 – 230 4 N Lat. To 840 – 850 E L. The climate of these two districts is sub-tropical with average rain fall of 1270 mm. On the northern edge of higher rain fall of 2032 mm has been recorded. In Ranchi district rain fall varies from 1272 mm to 1527 mm. The soil also play important role in determining the vegetation of the area. It is agreed that type of soil in this ultimate stage of evolution is determine by the climate that provide in that region (Wolfanger and Louis, 1929, Bharti and Pravesh, 2010). The variation of rocks caused wide difference in overlying soil consistency (Wadia, 1935). Maturity is reached only in residual soil which remains undisturbed during the period required for soil evolution under the given geographical condition. The soil of these two districts is red soil while black soil is restricted to part of North Koel. The lateritic soil which is a mixture of hydrated oxide of Aluminum, Iron with small amount of manganese oxide. Lateritic soil is found above altitude of 900 m. and above. This has maximum number of fern biodiversity at Netarhat and Mahuadar of Latehar district. The laterite is converted into red soil by biological action. Due to wide variations in altitude of Latehar and Ranchi district the vegetation is both dry deciduous and moist deciduous type.

The topography of Ranchi district is very undulating having small and high tops. The entire district covers a plateau area with 457 to 750 meter high tops, steep valleys with many perennial and annual streams & rainfalls of 1272-1527 mm. On the northern edge of higher rain fall has been recorded. Climate of district is sub-tropical. Ranchi district covers an area of 7698 km^2. Total 1732 km.(22.5%) are under forest cover. The tropic of cancer at 23 ½ ^{0} North passes through Ranchi District (at Ormanjhi). A few pockets like Jonha fall, Hundru fall, Seeta fall, Taimara ghati, Chuttu palu, Ormanjhi, Panchghag and Bandhgaon form ideal place for luxuriant

growth of pteridophytes especially *Lygodium flexuosum* and *Ampeloteris prolifera*, the two plants investigated. The physical characteristics of Ranchi district of Jharkhand state are very special as it is situated in East Deccan zone of India.



Map 1: Ranchi District of Jharkhand

Materials

Ampelopteris prolifera (Retz.) Copel. (Figure-1) and *Lygodium flexu*osum (L.) Sw. (Figure-2) growing in different climatic conditions of the Ranchi district was collected by the author. For this work author made an extensive tour of the different areas of the Ranchi district and its surrounding areas to collect both the fern species studied (Map No. 1 showing surveyed areas). The best season for the collection of fern has been the months of July to November. However, at higher altitudes and in more shaded and damp places of forest areas, these plants were found growing till the months of December to January in case of *Lygodium flexu*osum (L.) Sw. whereas *Ampelopteris prolifera* (Retz.) Copel. was found growing throughout the year. *Lygodium flexu*osum has been found growing in both dry deciduous as well as moist deciduous forests. The fern species *Lygodium flexu*osum have been found growing luxuriantly at an altitude of about 600 meters or more. It is widely distributed climbing fern of Ranchi district which was collected from Jonha, Seeta Fall, Ormanjhi, Hundru Fall, Panch Ghagh, Ranchi hills, Taimara Ghati (600m to 700 m). Whereas *Ampelopteris prolifera* (Retz.) Copel. was collected from Taimara Ghati, Dasam Fall and from Bandgaon (600 m) a border area of Ranchi and West Singhbhum district. It is very restricted in distribution in Ranchi district. The sporulation period of both the species were noted carefully. Collected specimens of both the fern species were housed in the department of Botany, Doranda College, Ranchi.

Methods

Preperation of plant extract

Healthy and mature plants for the present investigation were collected from nature at different locations during growing period. Specimens were documented at the department of Botany. The specimens were shade dried at room temperature for a period of three 3 weeks depending on the water content. The completely dried materials were separated in to fronds, roots, rhizomes and powdered by blender and used for various estimations.

Methods for the study adopted as that of Harborne, 1973 [16], Sofowara, 1993 [39], Trease & Evans, 1989 [41]. The collected plant samples were thoroughly washed, shade dried and then powdered with the help of a blender. 50 g of the powder was extracted using standard methods (Sofowara, 1993, Harborne, 1973, Trease & Evans, 1989) [39, 16, 41] successively with 250 ml of Benzene, Petroleum ether, Chloroform, Methanol, Acetone and Distilled Water using a Soxhlet extractor for 8 hrs. at a temperature of 50-60^{o}C (not exceeding the boiling point of the solvent). The flasks were plugged with cotton wool, wrapped in aluminum foil, shaken vigorously and allowed to stand in the refrigerator for 24 hrs. The extract obtained were evaporated to dryness using a rotary evaporator and stored in refrigerator in reagent bottles. All the extracts were concentrated and preserved in airtight bottle until further use.

Each extract was tested for growth /contamination by plating them on nutrient agar at 37^{0} C for 24 hrs. No growth was

observed visually and the extract was subsequently used to assay for antimicrobial activity using the agar diffusion method. The percentage yield of the extract was determined using the expressions. Then the extracts were used for preliminary phytochemical screening by different chemical tests viz., Molisch's, Fehling's, Benedicts's and Barfoed's tests for carbohydrates; Biuret and Millon's tests for proteins; Ninhydrins tests for Amino acids; Salkowski and Lieberman Burchard's reactions for steroids; Borntrager's test for glycosides; Mayer's, Hager's and Wagner's test for alkaloids; and Ferric chloride, Lead acetate, Potassium dichromate and dilute tests for tannins and phenolics.

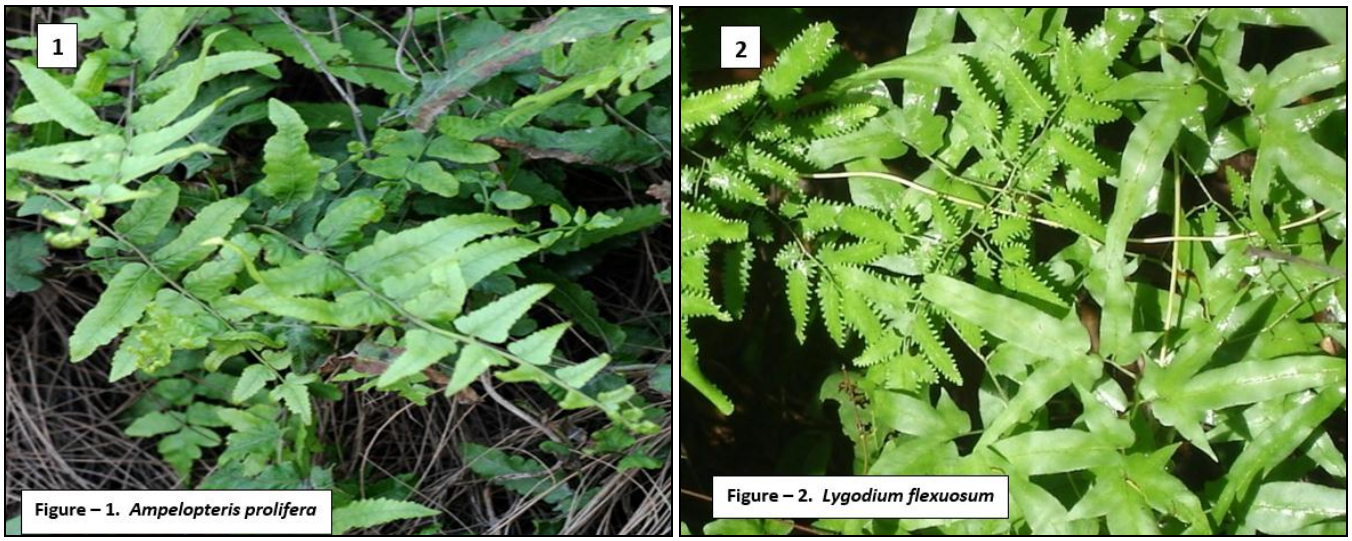


Plate 1.

Preliminary phytochemical screening for inorganic and organic elements were completed by using standard procedures. The results of organic and inorganic tests are given in table no- 01 to 07 respectively. From this analysis methanolic plant extract was found to have more active constituents than other extracts.

Phytochemical screening

Chemical tests were carried out using extract to identify various constitutes using standard methods (Sofowara, 1993 Harborne, 1973, Trease & Evans, 1989) [39, 16, 41] applied in previous studies.

Tests for organic constituents

1. Test for flavonoids: Three methods (Sofowara, 1993, Harborne, 1973) [39, 16] **were** used to determine the presence of flavonoids in the plant sample. 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H_2S0_4. A yellow coloration observed in each extract indicated the presence of flavonoids. The yellow coloration disappeared on standing. Few drops of 1% aluminium solution were added to a portion of each filtrate. A yellow coloration was observed indicating the presence of flavonoids. A portion of the powdered plant sample was in each case heated with 10 ml. of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml. of the filtrate was shaken with 1 ml. of dilute ammonia solution. A yellow coloration was observed indicating a positive test for flavonoids.

2. Tests for glycosides: Liebermann's test: 2 ml of the organic extract was dissolved in 2 ml of chloroform and then 2 ml of acetic acid was added in it. The solution was cooled well in ice. Sulphuric acid was then added carefully. A color change from violet to blue to green indicates the presence of a steroidal nucleus (that is, a glycone portion of glycoside).

Test for Glycosides:- **(Borntrager's test)**

Coarsely powdered plant material(1g.) was mixed with H_2SO_4 (5ml.), then it was heated for 3 minutes, thereafter it was filtered, after that filtrate was mixed with NaOH (0.5ml.) and allowed to stand for 3 minutes. A Reddish brown ppt. obtained which shows Glycosides present.

3. Test for Alkaloids: **(Dragendorffs reagent test)**

Two sets of Plant extract (0.5g.) was mixed with 1% HCL and stirred on steam bath, then solution was filtered. After that in 1 ml. of filtrate, 2 drop of Mayer's reagent was added. Two solutions mixed and made 100 ml. by distilled water.

A turbidity of extract filtrate appears which shows presence of Alkaloids in the extract.

Test for Alkaloid: 3 ml aqueous extract was stirred with 3 ml. of 1% HCl on steam bath. Mayer and Wagner's reagent was then added to mixture. Turbidity of the resulting precipitate was taken as an evidence for the presence of alkaloid.

4. Test for Saponins: 5 ml. of aqueous extract was shaken vigorously with 5 ml. of distilled water in a test tube and warmed. The formation of stable foam was taken as an indication of the presence of saponins.

Persistence of foaming which gives evidence for the presence of saponins.

5. Test for Tannins: (Ferric chloride test):- About 2 ml. of the aqueous extract was stirred with 2 ml. of distilled water and few drops of $FeCl_3$ solution were added. Formation of

green precipitate was indication of presence of tannins.

Test for tannins: About 0.5 g. of the plant extract samples was boiled in 20 ml. of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

6. **Test for Phenols**

Procedure (for water-soluble phenols)

The $FeCl_3$ (iron (III) chloride) test for phenols is not completely reliable for acidic phenols, but can be administered by dissolving 15 mg. of the unknown compound in 0.5 ml. of water or water-alcohol mixture and add 1 to 2 drops of 1% aqueous iron (III) chloride solution.

A red, blue, green, or purple color is a positive test.

Tests for inorganic constituents

1. Test for Chloride: To the 10 ml. of extract 05 ml. of dil. HNO_3 was added to neutralize its alkalinity, then few drops of $AgNO_3$ was added to it. Later on conc. HNO_3 was added to one part of it and dil. NH_4OH to another part. Formation of white precipitate which was insoluble in conc. HNO_3, but soluble in NH_4OH. The white precipitate of silver chloride, soluble in dilute NH_4OH, confirms the presence of chloride salt in the soil sample.

2. Test for sulphate: Dil. HCl was added to 10 ml. of extract followed by a few drops of $BaCl_2$ solution. Later on conc. HCl was added to it when precipitate was formed. Development of white precipitate observed which was soluble in conc. HCl. This indicates the presence of sulphate salt in the plant extract.

3. Test for phosphate: To the 05 ml. of the extract few drops of conc. HNO_3 was added and boiled for few seconds. After cooling down of solution, 2-3 drops of ammonium molybdate solution was added to it. Development of canary yellow precipitate was obtained which confirms the presence of phosphate salt in the extract.

4. Test for nitrate: 1-2 ml. of 0.5 % diphenylamine and conc. H_2SO_4 was added to 10 ml. of extract. A blue precipitate was formed, which indicates the presence of nitrate salt in the extract.

5. Test for carbonate: 10 ml. of extract was taken and 4-5 drops of dil. H_2SO_4 was added to it then, solution was heated for some time. Effervescence with the evolution of an odorless and colorless gas was observed. The gas evolved was CO_2, thus indicating the presence of carbonate salt in the plant extract.

6. Test for Iron: Few drops of potassium ferrocyanide were added to 10 ml. of extract. Appearance of a blue color was observed. This blue color was due to the formation of a ferro-ferricyanide complex, thus indicating the presence of iron in the extract.

7. Test for calcium: To the 10 ml. of extract of plant 2 ml. of glacial acetic acid was added. Then, 2-3 drops of methyl orange indicator was added to the above solution. The solution turns pink. Few drops of NH_4OH were added to it which caused disappearance of pink color. Later on 2-3 ml. of ammonium oxalate was added to it. A white precipitate of calcium oxalate was formed which confirms the presence of calcium salt in the extract.

Results

The result shows that amount of soluble carbohydrates are higher in young fronds than mature fronds (with sorus) of these two plants. Further, higher level of soluble carbohydrates is noticed in all the parts of *Ampelopteris prolifera* compared to *Lygodium flexuosum* (Table-6, 7). Whereas, insoluble carbohydrate levels are more in rhizome (Bhattacharyya (Goswami) & Halder, 2008) [5] parts. The levels of insoluble carbohydrates of rhizome of *Lygodium flexuosum* are significantly more than *Ampelopteris prolifera*.

A comparative phytochemical analysis is made among *Lygodium flexuosum* (L.) Sw. and *Ampelopteris prolifera* (Retz.) Copel. Among the ten extracts of the selected ferns, each extract contains minimum two compounds. Methanol extracts showed maximum numbers (eight) of the compounds (Table- 1 to 7). Reducing sugars and sugars are present in the petroleum ether and methanol extracts of *L. flexuosum* and in *A. prolifera* it is present only in methanol extract. Alkaloids are present in the benzene extract and methanol extract of *L. flexuosam* and in *A. prolifera* these compounds are present in methanol extract only. Phenolic compounds are present only in the methanol extracts of *L. flexuosum.* Flavonoids are present in petroleum ether, benzene, chloroform and methanol extracts of *L. flexuosum* whereas it is present in benzene and methanol extracts of *A. prolifera*. Saponins and tannins are present in the methanol and chloroform extracts of *L. flexuosum* and *A. prolifera*. Saponine is also present in water extract in both the cases. Glycosides are present in methanol extract in both the ferns.

Lygodium flexuosum is a seasonal plant but *Ampelopteris prolifera* is perennial growing throughout the year. That's why insoluble carbohydrate level in rhizome is high. It has been reported (Henry *et al*. 2003) [17] that higher amount of starch is found in rhizome. Levels of Amino acid are more in rhizome of the two plants but *L. flexuosum* has comparatively higher levels of amino acid. But Amino acid contents are more in young frond (Bhattacharyya (Goswami) & Halder, 2009) [9] than mature frond in both the species. Earlier worker (Guha *et al*. 2004) also showed similar result in *Adiantum capillaries-veneris*. The amount of protein is more or less higher in *Ampelopteris prolifera*. Among these two plants, *Ampelopteris prolifera* is growing under more moisturous condition.

Inorganic constituents were also tested in both the fern species and it was found that iron, potassium, phosphate, sulphur, calcium and chloride were present in both the cases whereas magnesium and sodium were found absent in both the cases (Table- 2 and 5).

The results confirm the presence of constituents which are known to exhibit medicinal as well as physiological activities. The phytochemical characteristics of the leaf extract of *Lygodium flexuosum* and *Ampelopteris prolifera* investigated are summarized in table-1-6. The results reveal the presence of medicinally active constituents like tannins, alkaloid, flavonoids, phenols, glycosides and saponins in the leaves of

L. flexuosum. While phenolics are absent in *Ampelopteris prolifera* but other organic compounds are present. The alkaloids contained in plants are used in medicine as anesthetic agents.

The presence of saponins in plants has been reported to be responsible for the tonic and stimulating activities observed in Chinese and Japanese medical herbs. The results obtained in this study thus suggest that the identified phytochemical compounds may be the bioactive constituents responsible for the efficacy of the leaves of the plants studied. The presences of some of these compounds have also been confirmed to have antimicrobial activity. Hence it could be inferred that the plant extracts could be a source for the industrial manufacture of drugs useful in the chemotherapy of some microbial infection.

Table 1: Phytochemical screening of rhizome and frond *Lygodium flexuosum*

S.No	Tests	Benzene	Petrolium Ether	Chloroform	Alcohol (Methanol)	Water
1	Carbohydrates	-	+	-	+	-
2	Proteins & Aminoacids	-	-	-	-	-
3	Fixed Oil & Fats	-	+	+	+	-
4	Alkaloids	+	-	-	+	-
5	Glycoside	-	-	-	+	-
6	Saponine	-	-	+	+	+
7	Flavanoids	+	+	+	+	-
8	Phenolics	-	-	-	+	-

Table 2: Analysis of inorganic constituents in the rhizome and frond of *Lygodium flexuosum*

S. No.	Elements	Observations
1	Sulphate	+
2	Phosphate	+
3	Potassium	+
4	Iron	+
5	Magnesium	-
6	Sodium	-
7	Chloride	+
8	Carbonate	-
9	Nitrate	-
10	Calcium	+

Table 3: Phytochemicals detected in various extracts of *Lygodium flexuosum*

Extract Used	Flavonoids	Alkaloids	Triterpenoids	Phenolics
Petroleum ether	-	-	-	-
Acetone	+	-	+	+
Methanol	+	-	+	+
Water	-	-	-	+

Table 4: Phytochemical screening of rhizome and frond *Ampelopteris prolifera*

S. No	Tests	Benzene	Petrolium Ether	Chloro-Form	Alcohol (Methanol)	Water
1	Carbohydrates	_	-	-	+	-
2	Proteins & Aminoacids	_	-	-	-	-
3	Fixed Oil & Fats	_	+	+	+	-
4	Alkaloids	-	-	-	+	-
5	Glycoside	-	-	-	+	-
6	Saponine	-	-	-	+	+
7	Flavanoids	+	-	-	+	-
8	Phenolics	-	-	-	-	-
9	Tanins	-	-	+	+	-

Table 5: Analysis of inorganic constituents in the rhizome and frond of *Ampelopteris prolifera*

S. No.	Elements	Observations
1	Sulphate	+
2	Phosphate	+
3	Potassium	+
4	Iron	+
5	Magnesium	-

6	Sodium	-
7	Chloride	+
8	Carbonate	-
9	Nitrate	-
10	Calcium	+

Table 6: Comparative analysis of organic constituents in the root powder of *Lygodium flexuosum* and *Ampelopteris prolifera*

		Lygodium flexuosum		*Ampelopteris prolifera*	
S. No	**Tests**	**Alcohol (Methanol)**	**Water**	**Alcohol (Methanol)**	**Water**
1	Carbohydrates	+	-	+	-
2	Proteins & Aminoacids	-	-	-	-
3	Fixed Oil & Fats	+	-	+	-
4	Alkaloids	+	-	+	-
5	Glycoside	+	-	+	-
6	Saponine	+	+	+	+
7	Flavanoids	+	-	+	-
8	Phenolics	+	-	-	-

Table 7: Comparative analysis of inorganic constituents in the root powder of *Lygodium flexuosum* and *Ampelopteris prolifera*

		Lygodium flexuosum	*Ampelopteris polifera*
S. No.	**Elements**	**Observations**	**Observations**
1	Sulphate	+	+
2	Phosphate	+	+
3	Potassium	+	+
4	Iron	+	+
5	Magnesium	-	-
6	Sodium	-	-
7	Chloride	+	+
8	Carbonate	-	-
9	Nitrate	-	-
10	Calcium	+	+

Discussion

The aim of this study was to determine the chemical properties of both the fern species studied and characterization of the various active substances. Phytochemical componds which are found in these species are known to have beneficial importance in industrial and medical sciences. Pteridophytes show medicinal utility and many of them are being used medicinally from ancient time. The tribal communities, ethnic groups and folklore throughout the world are utilizing plant parts like rhizome, stem, fronds, pinnae and spores in various ways for the treatment of various ailments (Kumar & Kaushik, 1999) [24] since ancient time. The medicinal values are caused by presence of chemical compounds in the ferns. Hence this study is focused to analyze the compounds which are present in some Indian medicinal ferns with five solvent extracts. Maximum numbers (nine) of the compounds are screened in methanol extracts of both the ferns. The numbers of contribution about the taxonomy, ecology and distribution of Pteridophytes have been published (Dixit 1975, Dixit & Bhatt, 1975) [10, 11] from time to time but enough attention have not been paid towards their medicinal useful aspects. The alkaloids and flavonoids are present in all the extracts of the selected ferns except water extract. Alkaloids and flavonoids are the source of antimicrobial activities, flavnoids have been referred as natures biological response modifiers; they show anti allergic, anti-inflammatory, antimicrobial and anticancer activity. Cardiac glycosides are known to work by inhibiting the Na+/K+ pump (Aiyelaagbe and Paul 2009) [9]. Tannins may have the potential values as cytotoxic (Harborne, 1973) [16] agents. Tannins are present in the methanol extracts of both the selected ferns. Hence the selected ferns may be used as anti-cancer agents. Phenolic compounds are the important source for antimicrobial and insecticidal activities. The methanol extracts of the two ferns showed the presence of phenolic compounds. Saponins have been implicated (Mandal *et al*. 2005) [26] as bioactive antibacterial agents. Saponins are present in the methanol extracts of both the selected ferns. Finally the result of the present study clearly shows that, due to the presence of maximum numbers of the compounds, the selected ferns may be used in anti-microbial and anti-cancer agents.

This study also leads to the further research in the way of isolation and identification of the active compound from the selected ferns. It will help to produce new medicines with less side effect, less costly affordable and more effective in the treatment of various infectious diseases in future. These preliminary chemical analysis of the two fern species investigated reveal the medicinal potentiality. It possesses various useful active compounds that benefit for mankind and could be harnessed for industrial and medicinal sciences. A detail study on qualitative and quantitative aspects of the phytochemicals present in the two fern species studied are required in future.

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