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A STUDY ON PHYTOCHEMICAL INVESTIGATION OF *DRYNARIA QUERCIFOLIA* LINN RHIZOME

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ABSTRACT

In the present research investigation we extracted the powdered rhizome of *Drynaria quercifolia* linn by Soxhletion method using different solvents. Then extracts were subjected to preliminary phyto-chemical investigation. The proximate analysis was carried out for the plant rhizome powder. The total ash value was 9.93%, Acid insoluble ash value was 4.49%, and Water-soluble ash value was 6.96% and extractive values of alcohol and water was found to be 9.87% and 13.94%. The materials were subjected to successive extraction with solvents. The solvents used were petroleum ether, chloroform, methanol and water in the ascending order of polarity. Pet ether extract was Light brown color, highly viscous and sticky and the yield was 3.12%, chloroform extract was brownish black color, viscous and sticky and the yield was 5.72%, methanol extract was brown color and sticky and yield was 19.67%, and the water extract was brown and sticky and yield was 16.33%. All the extract was subjected for qualitative chemical evaluation to detect the phyto-constituents present in them. Pet ether extract revealed the presence of phytosterols and fixed oils and fats. Chloroform extract revealed the presence of sterols, methanolic extract shows the presence of alkaloids, sterols and tannins and the water extract has shown the presence of tannins, proteins, amino acids, carbohydrates, gums and mucilages. To identify the constituents present in different extracts, the TLC was performed.

Keywords:

Drynaria quercifolia,
TLC,
Petroleum ether,
Chloroform,
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INTRODUCTION: Medicinal plant is any plant which in one or more of its organ contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs (According to WHO). Medicines are prepared from variety of plant materials like leaves, stems, root, barks and so on. They mainly contain biologically active ingredients and are used primarily for treating mild or chronic ailments. Herbs can be prepared at homes in many ways using either fresh or dried ingredient eg: roots, bark or other plant parts can be boiled into strong solutions called “Decoctions” then honey and sugar can be added to infusions and decoctions to make syrups. Now herbal medicines can also prepared in the form of pills, capsules or powders or in more concentrated liquid form called extracts and tinctures, they can also be applied topically in creams or ointments or applied directly to the skin as poultices.

The plant used in the present study belongs to Pteridophyta, and family Polypodiaceae. The plant is an epiphytic fern with a short thick, fleshy, creeping rhizome and densely clothed with reddish brown soft scales. The fronds are two types. The sterile fronds are small, somewhat concave and become brown on aging. The fertile fronds are long stalked, large pinnately lobed, leathery having network of small quadrangular areoles with or without free vein sori. They are small, numerous and two in each primary areoles. The plant part used in folk medicine was rhizome. Rhizome is used for bitter, anodyne, constipating, anti-inflammatory, and is used as tonic, which is useful in typhoid fever, phthiriasis, dyspepsia, cough, arthralgia, cephalalgia, diarrhea, foul ulcers and inflammation. It is very specifically used in the treatment of migraine¹.

Drynaria quercifolia FAM: Polypodiaceae (Asvakatri) is found throughout India, especially in

the plains or very low down in the mountains, on trees or rocks of South China, Malaysia and Tropical Australia. *Drynaria quercifolia* is an epiphytic fern with short thick fleshy creeping rhizome 2cm or thicker, the young parts are densely scaly, scales very dark brown, to about 2cm long narrowed gradually from the peltate base to the very narrow apes not stiff edges pale and closely and finely toothed. Nest leaves 40 cms long and 30 cm wide, lobed at depth of 2-5cm, lobes are broad and rounded. Stripes of foliage leaf about 30cm long; lamina to about 100cm long and 40cm wide, lobed to less than 1cm from the mid rib; oblique 25cm long and 4.5cms wide, rather shortly acuminate, separated by rather narrow sinuses, thin but stiffly leathery in texture.





FIG. 1: PLANT, RHIZOMES AND LEAVES OF DRYNARIA QUERCIFOLIA

The plant is known to have therapeutic uses in tuberculosis, fever, dyspepsia and cough. The fronds have astringent properties. The fronds are pounded and used as a poultice for swelling because of its antibacterial and astringent properties.⁵ Rhizome and roots is used as tonic in typhoid fever and dyspepsia². Traditional use of this drug is in diarrhea, typhoid, cholera, jaundice, fever, headache, skin diseases and syphilis³. It single is found to strengthen and promote the repair of sinews, muscles and bones. They are effective for lower back and ligament injuries⁴. In another combination of drug, Drynaria is used for expelling rheumatism⁵. Drynaria rhizome is used topically in traditional Chinese medicine to stimulate hair growth and to treat baldness. In the treatment of hyperthyroidism, Drynaria along with other drugs are used. In these conditions drynaria is used externally as well as internally⁵. *D. quercifolia* along with other combination of herbs is used in pain from traumatic injury, such as sprains and contusions with bruising and swelling⁵.

MATERIALS AND METHODS:

Collection of Plant Material: The rhizome of *Drynaria quercifolia* linn (Fam: Polypodiaceae)

were collected from Updi District in the month of April 2008 and authenticated by Dr. Gopalkrishna Bhatt, Professor, Sri Poornaprajna College, Udupi, Mangalore, and Karnataka, India. The plant material was dried, powdered and stored in airtight containers until further studies.

Proximate Analysis:

Determination of total ash value: Accurately weighed 5gms of powdered rhizome of *Drynaria quercifolia* Linn (Polypodiaceae) was taken in a dried silica crucible. It was incinerated at temperature 450°C, until free from carbon and then cooled. The weight of total ash was taken and the percentage of it was calculated with reference to the air-dried sample.

Determination of acid insoluble ash value: The total ash obtained was boiled for 5 min with 25 ml of 2N HCl, filtered and the insoluble matter was collected on ash less filter paper. Then it was washed with hot water, ignited in tarred crucible cooled and the residue obtained was weighed. Finally the percentage of acid insoluble ash was calculated with reference to the air-dried drug (table 1).

TABLE 1: ASH VALUE OF DRYNARIA QUERCIFOLIA RHIZOME LINN

Name of plant	Ash Value		
	Total ash	Acid insoluble ash	Water soluble ash
<i>Drynaria quercifolia</i> L	9.93%	4.49%	6.96%

Determination of water-soluble ash value: The total ash obtained was boiled with 25 ml of water for few minutes. The insoluble matter was collected on ash less filter paper, washed with hot water and ignited for 15 mins at temperature not exceeding 450°C. The difference in weight represents the water-soluble ash. The percentage of water-soluble ash was calculated with reference to the air-dried drug.

Determination of alcohol and water-soluble extractive value: 20 gms of air dried, coarsely powdered rhizome of *Drynaria quercifolia* linn powdered was macerated with 100 ml of alcohol (90%) in a closed flask for 24 hrs, shaking frequently during the first 6hrs and was allowed to stand for 18 hrs Then it was filtered rapidly and precautions were taken against loss of alcohol. 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105°C and weighed. The percentages of alcohol soluble extracts were calculated with reference to the air-dried drug. The procedure followed as above using chloroform water instead of alcohol.

TABLE 2: EXTRACTIVE VALUE OF DRYNARIA QUERCIFOLIA RHIZOME

Name of plant	Extractive values (Percentage w/w)	
	Alcohol soluble extractive value	Water soluble extractive value
<i>Drynaria quercifolia</i>	9.87%	13.94%

Determination of Moisture Content: Accurately weighed 5gms of powdered rhizome of *Drynaria quercifolia* Linn. was taken in a china dish. It was kept for 30 mins in a hot air oven at 105 - 110°C. The percentage of moisture content was then calculated with reference to the air-dried drug at different times.

TABLE 3: MOISTURE CONTENT OF DRYNARIA QUERCIFOLIA RHIZOME

Time (mins)	Moisture content (%)
30	12.82
45	10.94
60	7.84
75	5.92
90	5.53

Sequential Extraction of the Drug *Drynaria quercifolia* rhizome: The method is based on the extraction of active constituents present in the drug using various solvents ranging from non-polar to polar. The solvents used are petroleum ether, chloroform, methanol and water. The

successive solvent extraction procedure was adopted for the preparation of various extracts of *Drynaria quercifolia rhizome*. The materials were subjected to successive extraction with solvents in their ascending order of polarity in this process the substance, which is soluble in a solvent with particular range of polarity was extracted in the solvent and remaining marc further extracted with next solvent.

The 2 kg powdered drug was taken and subjected for successive solvent extraction The extraction was carried out for 18 hrs with the following solvents in the increasing order of the polarity i.e. Petroleum ether, chloroform, methanol and distilled water.

TABLE 4: PHYSICAL APPEARANCE AND YIELD OF DIFFERENT EXTRACTS OF POWDERED DRYNARIA QUERCIFOLIA RHIZOME

Solvent used	Color and consistency	Percentage yield
Pet ether	Light brown color, viscous and sticky	3.12%
Chloroform	Brownish black color and sticky	5.72%
Methanol	Brown color and sticky	19.67%
Distilled water	Dark brown color and non sticky	16.33%

Preparations of Petroleum Ether Extract: About 2kg of dried rhizome powder of *Drynaria quercifolia* was extracted with 600 ml of petroleum ether using Soxhlet apparatus for 18 hrs at 60-80°C. The extract was concentrated to 1/4 of its original volume by distillation as it was adopted to recover the solvent, which could be used again for extractions.

Preparation of Chloroform Extract: After pet ether extraction, the remaining dried marc was extracted with chloroform to get chloroform extract.

Preparation of Methanol Extract: After chloroform extraction, the remaining dried marc was extracted with methanol to get methanol extract.

Preparation of Aqueous Extract: After methanol extraction, the remaining dried marc was extracted with water to get water extract. For the preparation of aqueous extract, the above dried marc was macerated for 3 days with distilled water and the residue was removed by filtration and filtrate was concentrated to obtain aqueous extract. All the extracts were concentrated by distillation of the solvent and evaporating them to dryness at low temperature. They were then weighed and the percentage of different extractive values was calculated in terms of air-dried weight of the plant material.

Preliminary Phytochemical Screening: The powdered rhizome was subjected to systematic phytochemical screening by successively extracting them in different solvents and testing for the presence of chemical constituents.

Qualitative Chemical Examination of Extracts:

Detection of alkaloids: Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrates were tested carefully with alkaloid reagents.

Mayer's Test: Filtrates were treated with Mayer's reagent (potassium mercuric iodide). The formation of a yellow cream precipitate indicated the presence of alkaloids.

Wagner's Test: Filtrates were treated with Wagner's reagent (iodine in potassium iodide) and observed. Formation of brown or reddish brown precipitate indicated the presence of alkaloids.

Detection of Flavonoids:

Lead Acetate Test: The extracts were treated with few drops of 10% lead acetate solution. The formation of yellow precipitate confirmed the presence of flavonoids.

Detection of Proteins and Amino Acids:

Millons Test: The extracts were treated with 2 ml of Millons reagent. The formation of white precipitate, which turned to red upon heating, indicated the presence of proteins and amino acids.

Biuret Test: The extract: were treated with 1ml of 10% sodium hydroxide solution and heated. A drop of 0.7% copper sulphate solution to the above mixtures was added. The formation, of purplish violet color indicated the presence of proteins.

Detection of Glycosides:

Modified Borntrager's Test: The extracts were treated with ferric chloride solution and heated on a boiling water bath for about 5 mins. The mixture was cooled and shaken with equal volume of benzene. The benzene layer was separated and treated with half of its volume of ammonia solution. The formation of rose pink or cherry red color in the ammonical layer indicated the presence of anthranol glycoside.

Legal's Test: The extracts were treated with sodium nitroprusside in pyridine and methanolic alkali. The formation of pink to red color indicated the presence of cardiac glycosides.

Baljet Test: The extract of drug was treated with sodium picrate and the formation of a yellowish orange color confirmed the presence of cardiac glycosides.

Liebermann-Burchard's Test: The extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was added through the sides of the test tube. The formation of brown or pink colored rings at the junction confirmed the presence of steroidal or triterpenoid saponin glycosides respectively.

Keller Killani Test: 0.5g of dried extract was dissolved in 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was then under laid with 1 ml of concentrated H_2SO_4 . A brown ring obtained at the junction of two liquids indicates the presence of a deoxysugar.

Detection of saponins:

Froth's Test: The extracts (alcoholic and aqueous) were diluted with 20 ml of distilled water separately and further shaken for 15 min in a graduated cylinder. A layer of foam measuring about 1 cm was formed which indicated the presence of saponins.

Detection of phytosterols:

Liebermann-Burchard's Test: The extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was added through the sides of the test tube. The formation of a brown colored ring at the junction of two liquids confirmed the presence of steroids.

Detection of phenolic compounds and tannins:

Ferric Chloride Test: The extract was treated with few drops of neutral ferric chloride solution (5%). The formation of bluish black color indicated the presence of phenolic compounds.

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. The

formation of white precipitate indicated the presence of tannins.

Lead acetate Test: The extracts were treated with few drops of 10% lead acetate solution. The formation of yellow precipitate confirmed the presence of flavonoids.

Alkaline reagent Test: The extract was treated with few drops of sodium hydroxide separately. Formation of intense yellow color, which turned colorless on addition of few drops of dilute acid, indicated the presence of flavonoids.

Shinoda Test: The extracts were treated with few fragments of magnesium metal separately followed by drop wise addition of concentrated hydrochloric acid. The formation of magenta color indicated the presence of flavonoid.

Vanillin in Hydrochloric Acid Test: The extracts were treated with few drops of vanillin hydrochloride reagent. The formation of pinkish red color indicated the presence of tannins.

Detection of Proteins and Amino Acids:

Millons Test: The extracts were treated with 2 ml of Millons reagent. The formation of white precipitate, which turned to red upon heating, indicated the presence of proteins and amino acids.

Biuret Test: The extract: were treated with 1ml of 10% sodium hydroxide solution and heated. A drop of 0.7% copper sulphate solution to the above mixtures was added. The formation of purplish violet color indicated the presence of proteins.

Ninhydrin Test: To the extracts, 0.25% ninhydrin reagent was added and boiled for few minutes. Formation of blue color indicated presence of amino acid.

Detection of fixed oils and fats:

Stain Test: Small quantity of extracts was pressed between two filter papers separately. An oily stain on filter paper indicated the presence of fixed oil.

Soap Test: The extracts were heated on water bath with 0.5 N alcoholic potassium hydroxide solutions. Formation of soap indicated the presence of fixed oils and fats.

Detection of Carbohydrates: Extracts were dissolved individually in 5ml of distilled water and filtered. The filtrates were used to test the presence of carbohydrates.

Molisch's Test: Filtrates were treated with 2 drops of alcoholic α -naphthol solution in a test tube and 2 ml concentrated sulphuric acid was added carefully along the sides of the test tube. Formation of violet ring at the junction indicated the presence of carbohydrates.

Benedict's test: Filtrates were treated with Benedict's reagent and heated on water bath. Formation of an orange red precipitate indicated the presence of reducing sugars.

Fehling's Test: Filtrates were hydrolyzed with dilute hydrochloric acid, neutralized with alkali and heated with Fehling's A and B solutions. A red precipitate was formed which indicated the presence of carbohydrates.

Barfoed's Test: Filtrates were treated with Barfoed's reagent and heated on Water bath. Formation of an orange red precipitate indicated the presence of reducing sugars.

Detection of Gums and Mucilage:

Alcohol Precipitate Test: 10 ml of aqueous extract was slowly added to 25 ml of absolute alcohol with constant stirring. The precipitate was filtered

and dried in air. The precipitate was examined for its swelling properties.

Ruthenium Red Test: To the above precipitate, ruthenium red solution was added. The formation of red and pink color indicated the presence of gums and mucilage.

Detection of Glycosides: Extracts were hydrolyzed with dilute hydrochloric acid and the hydrolysate was subjected to glycosides tests.

Modified Borntrager's Test: The extracts were treated with ferric chloride solution and heated on boiling water bath for about 5 mins. The mixture was cooled and shaken with equal volume of benzene. The benzene layer was separated and treated with half of its volume of ammonia solution. The formation of rose pink or cherry red color in the ammonical layer indicated the presence of anthranol glycoside.

Legal's Test: The extracts were treated with sodium nitroprusside in pyridine and methanolic alkali. The formation of pink to red color indicated the presence of cardiac glycosides.

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under laid with 1 ml of concentrated H₂SO₄. A brown ring obtained at the junction of two liquids indicate the presence of a deoxysugars.

Detection of Saponins:

Froth's Test: The extracts (alcoholic and aqueous) were diluted with 20 ml of distilled water separately and further shaken for 15 mins in a graduated cylinder. A layer of foam measuring about 1 cm was formed which indicated the presence of saponins.

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Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. The formation of white precipitate indicated the presence of tannins.

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Alkaline reagent Test: The extract was treated with few drops of sodium hydroxide separately. Formation of intense yellow color, which turned

colorless on addition of few drops of dilute acid, indicated the presence of flavonoids.

Shinoda Test The extracts were treated with few fragments of magnesium metal separately followed by drop wise addition of concentrated hydrochloric acid. The formation of magenta color indicated the presence of flavonoid.

Vanillin in Hydrochloric Acid Test: The extracts were treated with few drops of vanillin hydrochloride reagent. The formation of pinkish red color indicated the presence of tannins.

Detection of Proteins and Amino Acids:

Millons Test: The e tracts were treated with 2 ml of Millons reagent. The formation of white precipitate, which turned to red upon heating, indicated the presence of proteins and amino acids.

Biuret Test: The extract: were treated with 1ml of 10% sodium hydroxide solution and heated. A drop of 0.7% copper sulphate solution to the above mixtures was added. The formation, of purplish violet color indicated the presence of proteins.

Ninhydrin Test: To the extracts, 0.25% ninhydrin reagent was added and boiled for few minutes. Formtion of blue color indicated presence of amino acid.

Detection of fixed oils and fats:

Stain Test: Small quantity of extracts was pressed between two filter papers separately. An oily stain on filter paper indicated the presence of fixed oil.

Soap Test: The extracts were heated on water bath with 0.5 N alcoholic potassium hydroxide solutions. Formation of soap indicated the presence of fixed oils and fats.

Detection of Carbohydrates: Extracts were dissolved individually in 5ml of distilled water and filtered. The filtrates were used to test the presence of carbohydrates.

Molisch's Test: Filtrates were treated with 2 drops of alcoholic α -naphthol solution in a test tube and 2 ml concentrated sulphuric acid was added carefully along the sides of the test tube. Formation of violet ring at the junction indicated the presence of carbohydrates.

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Fehling's Test Filtrates were hydrolyzed with dilute hydrochloric acid, neutralized with alkali and heated with Fehling's A and B solutions. A red

precipitate was formed which indicated the presence of carbohydrates.

Barfoed's Test Filtrates were treated with Barfoed's reagent and heated on Water bath. Formation of an orange red precipitate indicated the presence of reducing sugars.

Detection of Gums and Mucilage:

Alcohol Precipitate Test: 10 ml of aqueous extract was slowly added to 25 ml of absolute alcohol with constant stirring. The precipitate was filtered and dried in air. The precipitate was examined for its swelling properties.

Ruthenium Red Test: To the above precipitate, ruthenium red solution was added. The formation of red and pink color indicated the presence of gums and mucilage.

TABLE 5: QUALITATIVE CHEMICAL ANALYSIS OF DIFFERENT SOLVENT EXTRACTS OF *DRYNARIA QUERCIFOLIA*

Tests	Extracts			
	Petroleum ether	Chloroform	Methanol	Water
ALKALOIDS				
• Mayer's test	-	+	+	+
• Wagner's test	-	+	+	+
• Dragendorff's test	-	+	+	+
• Hager's test	-	+	+	+
GLYCOSIDES				
• Mod. Borntrager's test	-	-	-	-
• Legals test	-	-	-	-
• Libermann-Buchard's test	+	+	+	-
• Baljet test	-	-	-	-
SAPONINS				
• Forth test	-	-	-	-
PHYTOSTEROLS				
• Libermann-Buchard's test	+	+	+	-
PHENOLICS AND TANNINS				
• Ferric chloride test	-	-	+	+
• Gelatin test	-	-	-	-
• Lead acetate test	-	-	+	+
• Alkaline reagent test	-	-	-	-
• Shinoda test	-	-	-	-
• Vanillin Hcl test	-	-	-	-
PROTEINS AND AMINO ACIDS				
• Million's test	+	-	+	+
• Biuret test	-	-	-	-
• Ninhydrin test	+	-	+	+

FIXED OILS AND FATS				
• Stain test	+	-	-	-
• Soap test	+	-	-	-
CARBOHYDRATES				
• Molisch's test	-	-	+	+
• Benedict's test	-	-	+	+
• Barfoed's test	-	-	+	+
GUMS AND MUCILAGE				
• Alcohol ppt test	-	-	-	+
• Ruthenium test	-	-	-	+

Thin Layer Chromatography (TLC Studies): TLC studies were carried out for different extracts. The different solvent systems of different polarities were prepared and TLC studies were carried out to select the solvent system capable of showing better resolution.

Apparatus TLC plate, development chamber, capillary tube, micropipette and spraying apparatus.

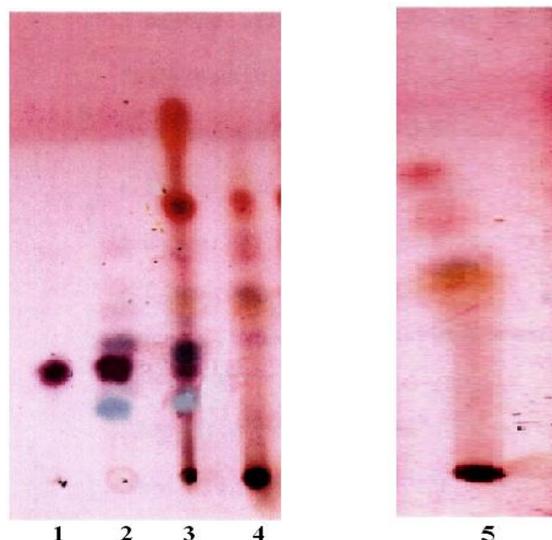
Adsorbent: Silica gel 60F (0.25 readymade aluminum sheets (Merck)).

Sample preparation: Small quantities of samples were taken and dissolved in their respective solvents.

Visualization: UV at 254 nm and 366 nm, Iodine chamber and spraying reagent.

Method: The above prepared sample solutions were applied on pre-coated TLC plates using capillary tubes and developed in a TLC chamber using suitable mobile phase. The developed TLC plates were air dried and observed under UV at both 254 nm and 366 nm. They were later sprayed with different spraying reagents and some were placed in hot air oven for 1min for the development of color in separated bands. The R_f values were calculated for different sample.

$$R_f = \frac{\text{Distance traveled by the solute}}{\text{Distance traveled by the solvent front}}$$



TLC PLATES

RESULTS AND DISCUSSION:

Proximate Analysis: The rhizome of *Drynaria quercifolia* Linn. was subjected to evaluate its total ash value, acid insoluble ash, water-soluble ash, water-soluble extractive value, alcohol soluble extractive value and moisture content.

Preliminary Phytochemical Screening: The successive solvent extraction procedure was adopted for the preparation of various extracts of *Drynaria quercifolia* rhizome and quantity taken was 100gms. The materials were subjected to successive extraction with solvents. The solvents used are petroleum ether, chloroform, ethanol and water in the ascending order of polarity. In this process the substance, which is soluble in a

solvent with particular range of polarity was extracted in the solvent and remaining marc further extracted with next solvent.

Qualitative chemical analysis of extracts: All the five extracts obtained from successive solvent extraction were subjected to qualitative chemical evaluation to detect the chemical constituents present in them. Petroleum ether extract revealed

the presence of phytosterols, fixed oils and fats. The chloroform extract shows the presence of sterols. The methanolic extract shows the presence of alkaloids, carbohydrates, glycosides, tannins, proteins and amino acids and the water extract has shown the presence of saponins, tannins, carbohydrates, proteins and amino acids (table 6).

TABLE 6: TLC ANALYSIS OF DIFFERENT SOLVENT EXTRACTS OF *DRYNARIA QUERCIFOLIA* RHIZOME

Name of the extract	Mobile phase	Detection agents	Number of spots	Rf values
Pet ether	Hexane: ethyl acetate (7:3)	Iodine chamber and anisaldehyde sulphuric acid spray	02	0.17
				0.21
Chloroform	Hexane: ethyl acetate (7:3)	Iodine chamber and anisaldehyde sulphuric acid spray	03	0.21
				0.27
				0.35
Methanol	Hexane: ethyl acetate (7:3)	Iodine chamber and anisaldehyde sulphuric acid spray.	05	0.08
				0.15
				0.17
				0.27
Water	Hexane: ethyl acetate (7:3)	Iodine chamber and anisaldehyde sulphuric acid spray	03	0.31
				0.18
				0.22
Methanol	Chloroform: Methanol (7:3)	Dragendorff's reagent	03	0.27
				0.19
				0.22
				0.25

CONCLUSION: The line of investigation can be concluded that rhizome of *Drynaria Quercifolia* Linn have contains Alkaloids, Glycosides, Saponins, Phytosterols, phenolics, Tannins, Proteins, Amino acids, Fixed oils, Fats, Carbohydrates, Gums and Mucilage's.

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