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PRELIMINARY PHYTOCHEMICAL SCREENING AND GC-MS PROFILING OF AN ENDANGERED MEDICINAL PLANT *BRYONIA LACINIOSA* L

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Abstract:

Petroleum ether and benzene leaf extracts of an endangered medicinal plant *Bryonia laciniosa* L was screened for primary and secondary metabolites and GC-MS profiling. Phytochemical screening of benzene and petroleum ether solvent extracts of *Bryonia* leaf showed the presence of Carbohydrates, proteins and amino acids, alkaloids, anthraquinones, flavonoids, glycosides, phenols, tannins, and triterpenoids. Both the extracts showed negative response to saponin and volatile oil. The petroleum extract showed Positive response to steroids and sterols and benzene extract did not respond to steroids and sterols. GC- MS analysis identified 25 biologically active constituents present in the ethanolic extract of leaves of *Bryonia laciniosa*.

Key Words: *Bryonia*, Cucurbitaceae, Phytochemical & GC-MS

Introduction:

Ethnomedicinal practices are preferred largely because medicinal plants are less expensive, readily available and reliable and they are considered to have fewer side effects than modern medicines. Medicinal plants are the wealthy bio-resources of drugs of traditional medicinal systems, modern medicines, nutraceuticals, food supplements and folk medicines, pharmaceuticals, intermediate and chemical entitled for synthetic drugs (Abraham,1981).Plants are the traditional source for many of the chemicals used as pharmaceuticals, biochemical, fragrance, food colours and flavours. Most valuable phytochemicals are the products of secondary metabolism and possess sufficient chemical or structural complexity.

Many plants synthesize substances that are useful to the maintenance of health in humans and other animals. These substances especially alkaloids serve as plant defense mechanism against predation by micro organisms, insects and herbivores. Traditional healers use the leaves and the seeds of this plant for treatment of fevers. It is also taken in impotency and used as a tonic. Whole plant is used to treat ague, bronchitis, carbuncles, cholera, colic, consumption, convulsions, cough, delirium, fertility, megalopleny, paralysis and snake bite. The chloroform extract of *Bryonia laciniosa* has exhibited a significant antiinflammatory activity (Gupta *et al*., 2003). Analgesic and antipyretic activity of methanol extract of *Bryonia laciniosa* also has been shown in standard animal models (Sivakumar *et al*., 2004).

Experimental Plant *Bryonia Laciniosa:*

Materials and Method:

Collection and Identification of Plant:

From the survey of Kundri hill, Thukkanaikkan Palayam range of Sathyamangalam reserve forest an endangered medicinal plant *Bryonia laciniosa* L. belongs to the family Cucurbitaceae It is commonly known as lollipop climber and it is also known as "Shivlingi" in India. It was selected to analyse the phytochemicals and GC-MS profiling. Identification was done by using Flora of the Presidency of Madras, Gamble and Fischer (1915-1936) and The Flora of the Tamil Nadu Carnatic, Matthew (1983 and 1988). The plant was authenticated by BSI, Coimbatore, India and herbarium was prepared and deposited in the Botany department, VCW.

Preparation of Plant Material:

Fresh leaves of the plant *Bryonia laciniosa* were collected and washed thoroughly under running tap water. Then the leaves were cut into small pieces and shade dried. The dried leaves were then pulverized to powder using a mechanical grinder. And the powder was preserved in air sealed polythene cover.

Qualitative Phytochemical Screening:

Plants are the traditional source for many of the chemicals used as pharmaceuticals, biochemicals, fragrance, food colours and flavours. Most valuable phytochemicals are the products of secondary metabolism and possess sufficient chemical or structural complexity. Many plants synthesize substances that are useful to the maintenance of health in humans and other animals.

Phytochemical screening of benzene and petroleum ether solvent extracts of *Bryonia* leaf was carried out following the methods of Harborne (1984); Kokate *et al*. (1995) and Prabhakaran (1996). Carbohydrates, proteins and amino acids, alkaloids, anthraquinones, flavonoids, glycosides, phenols and tannins, saponins, steroids and sterols, triterpenoids and volatile oil were qualitatively analyzed.

Tests for Carbohydrate Fehling's Test:

5 ml of Fehling's solution was added to 2 ml of extract and boiled in a water bath. The formation of yellow or red precipitate indicates the presence of reducing sugars.

Iodine Test:

2 ml of dilute iodine solution was added to the extract. The appearance of blue colour indicates the presence of starch.

Tests for Proteins and Amino Acids Biuret Test:

To 1 ml of extract, equal volume of 40% sodium hydroxide solution and 2 drops of 1% copper sulphate were added. The appearance of violet colour indicates the presence of proteins.

Ninhydrin Test:

To the extract, 2 drops of freshly prepared 0.2% ninhydrin reagent was added and heated. The appearance of pink or purple colour indicates the presence of proteins, peptides or amino acids.

Tests for Alkaloids Dragendorff Reagent:

To 1 ml of the extract, 1 ml of Dragendorff's reagent was added. The appearance of orange red precipitate indicates the presence of alkaloids.

Borntragers Test for Anthraquinones

5 ml of extract was added with 10 ml of benzene. The mixture was shaken and the appearance of a pink, red or violet colour in the lower phase indicates the presence of free anthraquinones.

Tests for Flavonoids Shinoda Test:

To 1 ml of the extract, magnesium turnings and 1-2 drops of concentrated hydrochloric acid were added. Formation of pink colour indicates the presence of flavonoids.

Tests for Glycosides Legal Test:

The extract was dissolved in pyridine and freshly prepared sodium nitro prusside solution was added. The formation of pink to red colour indicates the presence of glycosides.

Tests for Tannins and Phenolic Compounds:

- ✓ To 1 ml of the extract, few ml of 5% neutral ferric chloride was added. The development of a dark bluish black colour indicates the presence of tannins.
- ✓ To 1 ml of the extract, few ml of gelatin solution was added. The formation of a white precipitate reveals the presence of tannins and phenolic compounds.

Test for Saponins (Foam Test).

5 ml of the extract was taken in a test tube and few drops of 5% sodium bicarbonate solution were added. The mixture was shaked vigorously and kept for 3 min. Formation of honey comb like froth shows the presence of saponins.

Tests for Steroids and Sterols:

Salkowski's Test:

The extract was dissolved in 2 ml of chloroform and equal volume of concentrated sulphuric acid was added along the sides of the test tube. The upper layer - turns red and lower layer turns yellow with green fluorescence, indicating the presence of the steroids and sterol compounds, in the extract.

Tests for Triterpenoids:

Liebermann - Burchard's Test:

The extracts were dissolved in 2 ml of chloroform and 10 drops of acetic anhydride and 5 drops of concentrated sulphuric acid were added. Appearance of red to violet colour indicates the presence of triterpenoids.

Tests for Volatile Oil:

2 ml aliquot of extract was evaporated on a porcelain crucible. If the residue has an aromatic smell it indicates the presence of volatile oil.

GC –MS Analysis:

Preparation of Extract:

2 µl of the ethanol extract of *Bryonia laciniosa* leaf was employed for GC- MS analysis.

Instruments and Chromatographic Conditions:

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (30 × 0.25 mm ID ×1EM df, composed of 100% Dimethyl poly siloxane), operatin4.3g in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 0.5 EI was employed (split ratio of 10:1) injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C/min, then 5°C/min to 280°C/min, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 550 Da.

Results and Discussion

Phytochemical Screening:

The results on the phytochemical screening of petroleum ether and benzene leaf extract of *Bryonia laciniosa* Presented in Table-1. The presence of carbohydrates, proteins and amino acids, alkaloids, flavonoids, glycosides, phenols and tannins, anthraquinones and triterpenoids are revealed by both extracts. Both extracts showed negative response to saponin and volatile. Petroleum ether revealed positive response to steroids and sterols. This is in consonance with the findings of Caroline and Mallaiah (2011) who reported the presence of saponin, triterpenoid and glycosides in *Bryonia*.

Qualitative Phytochemical Screening of Leaf Sample of *Bryonia laciniosa:*

S.No	Constituents	Petroleum Ether	Benzene
1	Carbohydrates	+	+
2	Proteins & Amino acids	+	+
3	Alkaloids	+	+
4	Flavonoids	+	+
5	Glycosides	+	+
6	Phenol &Tannins	+	+
7	Saponins	-	-
8	Steroids & Sterols	+	-
9	Anthraquinones	+	+
10	Triterpenoids	+	+
11	Volatile oil	-	-

The phytocomponents like alkaloids, saponins and glycosides were reported to have various biological functions which include anticancer, antiinflammatory and antimicrobial activities. Phenolic compounds which are commonly found in both edible and inedible plants are reported to have multiple biological effects, including antioxidant activity and promotion of health benefits. This is in line with the report of Ryan *et al*. (2002). The petroleum ether showed negative response to volatile oil and saponin and benzene showed negative response to saponins, steroids and sterols and volatile oil. This may be due to the solubility nature of different organic solvents. Tannins are astringent, bitter plant polyphenols that either bind and precipitate or shrink proteins and they have traditionally been considered antinutritional but it may be employed medicinally in anti diarrheal, hemostatic and antihemorrhoidal compounds. Its presence in the plants suggests it to be of medicinal value because tannins have shown potential antiviral effects as reported by Lin *et al. (*2004). Eseyin *et al*. (2014) reported that natural products of plant origin have been found to exhibit strong anti oxidant activity due to the presence of phenols, flavonoids, carotenoids and lycopene. In the present study, phenols and flavonoids are present in the leaves which make the plant medicinally useful. Consumption of the plant has the potential of protecting the consumer from oxidative stress and its attendant health implications.

GC-MS Analysis:

Phytocomponents identified in the ethanol leaf extract of *Bryonia laciniosa* by GC-MS method were twenty five. The major components are1-Monolinoleoylglycerol trimethylsilyl ether, Phytol, Ethyl tetracosanoate, Tetracosanoic acid, methyl ester (CAS), 9,12-Octadecadienoyl chloride, (Z,Z), Undecane (CAS), 9,10,12,13-Tetrabromooctadecanoic acid, Dodecane (CAS) and Nonacosane (CAS) with area as 13.06%, 9.35%, 8.95%, 5.81%, 5.86%, 4.63%, 3.85% and 3.56% respectively.

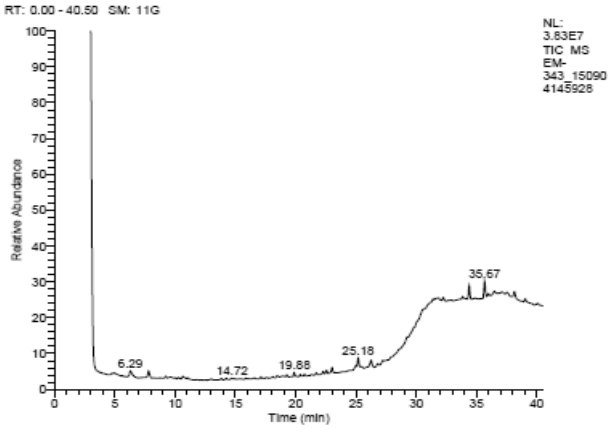
The retention time varies between 4.97 and 39.04. The least area percentage components are pentadecanoic acid (0.80%), carbonic acid (0.85%), Methyl1,3-dihydro-2H- isobenzofuran – 4 – carboxylate (1.01%), Quercetin 7,3',4' – Trimethoxy (1.08%), 9,12-Octadecadienoic acid (Z,Z) (1.30%), Chlorpyrifos (1.38%), Quinoline, 6-methyl-(CAS) (1.44%) and Tetratetracontane (CAS) (1.64%), Lucenin 2 (1.81%), Neophytadiene (1.85%) and 2-Hexadecen-1-ol (1.85%).The molecular weight ranges from 143 to 618. The highest molecular weight compounds recorded are Tetratetracontane (CAS) (618) and Lucenin 2 (610) and the less molecular weight compound is Quinoline, 6-methyl- (CAS) (143). All these phytocomponents have been found to show various biological activities.

Table 2: Phytocomponents identified in the ethanol leaf extract of *Bryonia laciniosa* by GC-MS

S.No	R.T	Compound Name	Molecular Formula	Molecular Weight	Area %
1	4.97	Bicyclo[3.1.0]hexan-2-ol	C10H18O	154	2.42
2	6.29	Undecane (CAS)	C11H24	156	4.63
3	7.80	Dodecane (CAS)	C12H26	170	3.82
4	9.63	Quinoline, 6-methyl- (CAS)	C10H9N	143	1.44
5	10.63	Lucenin 2	C27H30O16	610	1.81
6	18.45	Carbonic acid	C21H42O3	342	0.85
7	19.88	Neophytadiene	C20H38	278	1.85
8	19.88	2-Hexadecen-1-ol	C20H40O	296	1.85
9	21.72	Pentadecanoicacid	C17H34O2	270	0.80
10	22.29	Methyl 1,3-dihydro-2H-isobenzofuran-4-carboxylate	C10H10O3	178	1.01
11	22.55	Chlorpyrifos	C9H11Cl3N	349	1.38
12	23.02	Hexadecanoic acid	C18H36O2	284	2.56
13	25.18	Phytol	C20H40O	296	9.35
14	26.26	9,12-Octadecadienoyl chloride, (Z,Z)-	C18H31ClO	298	5.81
15	26.81	Quercetin 7,3',4'-Trimethoxy	C18H16O7	344	1.08
16	29.22	9,12-Octadecadienoic acid (Z,Z)-, 2,3-bis[(trimethylsilyl)oxy]propyl ester (CAS)	C32H58OSi	498	1.30
17	30.53	Silane, trimethyl[[(3á)-stigmast-5-en-3-yl]oxy]-(CAS)	C32H58OSi	486	3.30
18	31.38	1-Monolinoleoylglycerol trimethylsilyl ether	C27H54O4Si2	498	13.06
19	33.85	Tetratetracontane (CAS)	C44H90	618	1.64
20	34.40	Tetracosanoic acid, methyl ester (CAS)	C25H50O2	382	5.86
21	35.67	Ethyl tetracosanoate	C26H52O2	396	8.95
22	36.49	9,10,12,13-Tetrabromooctadecanoic acid	C18H32Br4O2	596	3.85
23	37.55	Corynan-17-ol, 18,19-didehydro-10-methoxy-(CAS)	C20H26N2O2	326	3.50
24	38.16	Nonacosane (CAS)	C29H60	408	3.56
25	39.04	Octasiloxane	C16H50O7Si8	578	2.52

The phytocomponents identified in the leaf sample of *Bryonia laciniosa* belong to alcohol, terpenoid, alkane, azanapthalene, glucoside, ester, acid, organic phosphate, flavonoid and silicon compound groups. 1-Monolinoleoylglycerol trimethylsilyl ether with higher area percentage (13.06%) showed the predicted antimicrobial, antioxidant, antiinflammatory, antiarthritic, antiasthmatic and antidiuretic activities. The next higher compound phytol with area percentage (9.35%) showed the antifungal, wound healing, antiviral, antidiabetic, anticancer and antiinflammatory activities. It is used commonly as an aperient medicine and tonic and is globally distributed in the paleotropics. *Bryonia* plants are used to treat adenopathy, asthma, headache, phthisis, tuberculosis etc. The bioactive molecule goniothalamin isolated from this plant showed potent cytotoxicity, weak antibacterial and significant antifungal activity against a wide range of gram positive and gram negative bacteria and fungi.

Figure 1: GC-MS Chromatogram of Ethanol Extract of *Bryonia Laciniosa* Leaf



GC- MS analysis identified 25 biologically active constituents present in the ethanolic extract of leaves of *Bryonia laciniosa* (Table-2, Fig-1). The mass spectra of the six major constituents were obtained. The major bioactive constituents are 1-Monolinoleoylglycerol trimethylsilyl ether (13.06%), Phytol (9.35%), Ethyl tetracosanoate (8.95%), Tetracosanoic acid, methyl ester (CAS) (5.86%), 9, 12-Octadecadienoyl chloride, (Z,Z)(5.81%) and Undecane (CAS)(4.63%). Out of these bioactive constituents 1-Monolinoleoylglycerol trimethylsilyl ether is present in high quantity. These compounds are reported to possess antimicrobial, wound healing, antidiabetic, anticancer and anti inflammatory activities. Similar findings were reported by Senthilkumar and Kamaraj (2010) in *Cucumis anguri*.

Thus, this type of GC-MS analysis is the first step towards understanding the nature of active principles in this medicinal plant and this type of study will be helpful for isolation of phytochemicals. In conclusion *Bryonia laciniosa* contains biologically active compounds that may serve as candidate for the discovery of new drugs in the treatment of many disorders.

References:

1. Abraham, Z. 1981. Glimpses of Indian Ethnobotany. Oxford and IBH Publishing co., NewDelhi.
2. Anjanadevi, N. 2 016. Phytochemicals and GC-MS Profilling in the bulb of *Scilla indica* Baker. Journal of Global biosciences, 5(3):3769-3775.
3. Caroline,V.J.E. and Mallaiah,B. 2011. In vitro mutagenesis in endangered medicinal Cucurbit *Bryonopsis laciniosa* (L.) Naud. Int.J. Genetic Eng& Biotech., 2(1): 67-75.
4. Eseyin,O.A., Sattar,M.A. and Rathore,H.A. 2014.A review of the pharmacological and biological activities of the aerial parts of *Telfairia occidentalis* Hook.f. (Cucurbitaceae). Tropic.Journ.Pharm.Res., 13(10): 1761-1769.
5. Gamble,J.S. and Fischer,C.E.C.1915- 1936. The Flora of the Presidency of Madras. Vol-I, II and III., Adlard& Son Ltd, London.
6. Gupta,M., Sivakumar,T., Mazumdar,U.K., Vamsi,M.L., Karki,S.S., Sampathkumar,R. and Manikandan,L. 2003. Evaluation of anti-inflammatory activity of chloroform extract of *Bryonia laciniosa* in experimental animal models. Biol.Pharm.Bull., 29:449-632.
7. Harborne,J.B. 1984. Phytochemical methods, Chapman and Hall, London (2^{nd} Ed.)
8. Kokate,C.K., Khandelwal,K.R., Pawer,A.P. and Gohaiz,S.B. 1995. Practical Pharmacognosy, VallabhPrakasham, New Delhi, 4^{th} Ed. P-107.
9. Lin,L.U., Shu-wen,L.,Shi-bo,J. and Shu-guang,W. 2004. Tannin inhibits HIV- 1entry by targeting gp41. Acta. Pharmacol.Sin., 25(2):213-218.
10. Matthew,K.M. 1983.The Flora of the Tamil Nadu Carnatic, Vol- III.Part- I, II & III. The Rapinet herbarium, ST.Joseph's College, Thiruchirapalli, India.
11. Matthew,K.M. 1988. Further Illustrations on The Flora of the Tamil Nadu Carnatic, Vol-IV.
12. Prabhakaran,P. 1996. Chemical investigation of finding medicinal plants and reated synthetic studies. Ph.D. Thesis, M.K.U. Madurai, Tamil Nadu, India.
13. Ryan,D., Antolovich,M., Prenzler,P and Robards Kand Lavee,S.2002. Bio transformations of phenolic compounds in OleaeuropeaL. Scientist Horticulturae, 92: 147-176.
14. Senthilkumar,S. and Kamaraj,M. 2010. Analysis of phytochemical constituents and antimicrobial activities of *Cucumis anguri* L. against clinical pathogens. American- Eurasian.Journ. Agricul& Environ.Sci., 7(2): 176-178.
15. Sivakumar,T., Perumal,P., Kumar,R.S., Vamsi,M.L., Gomathi,P., Mazumder,U.K. and Gupta,M. 2004. Evaluation of analgesic, antipyretic activity and toxicity study of *Bryonia laciniosa* in mice and rats. Am.J.Chin.Med., 32(4):531-539.