International Journal of Advanced Research in Biological Sciences
ISSN : 2348-8069
www.ijarbs.com

Research Article

Identification of Compounds Present in *Schefflera racemosa* (Wight) Harms

B. Malar Selvi[1], J. Brijitta[2*], S. Padmavathy[1]

[1]Department of Botany, Nirmala College, Coimbatore- 641018, Tamilnadu, India
[2]Centre for Nanoscience & Nanotechnology, Sathyabama University, Chennai-600119, Tamilnadu,
*Corresponding author: *brijitta@live.com*

Abstract

From pre-historic to modern age plants have been used as a curative for many diseases and infections. This curative property in plants is imparted by the secondary metabolites present in them which are excellent pharmacological agents. The current study is designed to identify the secondary metabolites present in the leaves of Schefflera racemosa (Wight) Harms a plant endemic to the Western Ghats. Phytochemical analysis of the plant extract in various solvents was carried out to determine the various secondary metabolites such as alkaloids, tannins, saponin, etc. Most of the secondary metabolites were extracted in methanol and hence methanolic extract of the plant leaves have been used for the entire studies. Techniques like HPTLC, HPLC and GC-MS have been used to identify the number of secondary metabolites present in the methanolic plant extract. Some of the secondary metabolites identified using GC-MS are known to exhibit antimicrobial, anti-inflammatory, antifungal and antioxidant properties. The results of the current study indicate that the methanolic extract of the leaves of Schefflera racemosa contains effective compounds exhibiting therapeutic potential.

Keywords: GC-MS, HPLC, Plant Extract

Introduction

The use of plants, plant extracts and plant-derived chemicals for the treatment of a number diseases has its roots from pre-historic times. From ancient to modern times majority of the world's population relies on traditional medicines for primary health care, home remedies and most of which involve the use of plants. Plants synthesize two types of organic metabolites, viz., primary and secondary. Primary metabolites are involved in growth and development, respiration, photosynthesis, etc. Some of the primary metabolites are chlorophyll, carbohydrates, proteins, nucleic acids, lipids, etc. Secondary metabolites present in plants include alkaloids, steroids, tannins, and phenol compounds, flavonoids, steroids, resins fatty acids gums [Joshi et al, 2009; Balandrin et al, 1985; Cragg et al, 2005; Velanganni et al, 2011; Ramachandra Rao et al, 2002]. It is the combination of secondary metabolites present in the plants which imparts medicinal properties to the plant material and is capable of producing definite physiological action in the body. Some of the compounds extracted from different parts of the plants can be used to cure diarrhea, dysentery, cough, cold, cholera, fever bronchitis, etc. Apart from documenting, scientific validation of the pharmacological properties of the medicinal plants can lead to the formulation of cost-effective and efficient drugs. Validation of the plants medicinal property is performed either by isolating the secondary metabolites or by performing in-vitro or in-vivo experiments. Hence a detailed and systematic study on plants for their medicinal value can result in novel curative pharmacological formulations for the treatment of many diseases. Many of the drugs marketed now-a-days have been derived from plants and were discovered through their traditional use in curing diseases. Podophyllotoxin- antitumor, anticancer agent from *Podophyllum peltatum*, vincristine and vinblastin - antitumor, antileukemic

agent from *Catharanthus roseus*, camptothecin - anticancerous agent from *Camptotheca acuminate*, taxol- antitumor agent from *Taxus brevifolia*, Anisodamine – anticholinergic from, ephedrine – sympathomimetic and antihistamine from *Ephedra sinica*, quinine- antimalarial and antipyretic from *Cinchona ledgeriana*, reserpin- antihypertensive, tranquilizer from *Rauvolfia serpentina* and digoxin – Cardiotonic from *Digitalis purpurea* are well known examples of such drugs [Balandrin et al, 1993; Pandey et al, 2011; Chinou, 2008]. Phytochemical compounds exhibiting cytotoxic, anti-tumor, anti-oxidant, anti-inflammatory activity could lead to the production of new drugs for the treatment of cancer [Gullett et al, 2010; Menichin et al, 2009]. Phytochemical constituents present in the plant plays a vital role in the identification of the crude drugs. Hence, phytochemical screening is an important analysis in identifying new sources of therapeutically and industrially important compounds. There are several reports [Thomas et al, 2002; Dantas et al, 2006; Sukhramani et al, 2013; Patil et al, 2009] of plants belonging to the *racemosa* family such as *Barringtonia racemosa, Symplocos racemosa, Cayaponia racemosa*, *Ficus racemosa,* etc., exhibiting anti-cancerous, antimicrobial, antioxidant, anti-inflammatory, analgesic, anti-hyperglycemic activities. In the current study, the secondary metabolites present in *Schefflera racemosa (S. racemosa)* a plant endemic to the Western Ghats is determined using various characterization techniques such as phytochemical screening, HPTLC, HPLC and GC-MS. The results of the study are reported.

Materials and Methods

Sample preparation for phytochemical screening

Approximately 50 gram of the powdered leaves was weighed and moistened with ammonia and evaporated to dryness. The dried powder was extracted with chloroform and filtered. The chloroform layer was extracted with 10% sulfuric acid using a separating funnel. The pH of the aqueous layer was adjusted to 8 using 28% ammonium hydroxide solution. This solution was extracted with chloroform wherein the organic extract so obtained was concentrated by evaporating the chloroform at room temperature. The aqueous extract was dried by heating and a semi solid mass was obtained. The dried extract was used for phytochemical analysis.

Phytochemical screening

Phytochemical analysis of the extracts after evaporating the solvents was performed as per the standard microbiological assays and tests [Indian Pharmacopoeia, 1996]. Chemical tests were carried out using aqueous extract to identify various constitutes using standard methods of [Sofowara, 1993; Trease and Evans, 1989; Harborne, 1973].

Test for Alkaloid

Mayer and Wagner's reagent was added to a mixture of 3 ml of aqueous extract and 3 ml of 1% HCl in a steam bath. Turbidity of the resulting precipitate was taken as an evidence for the presence of alkaloid [Ameyaw etal, 2012].

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 [Ameyaw etal, 2012].

Test for Tannins

About 1 ml of the aqueous extract was stirred with 2 ml of ultrapure water and added a few drops of $FeCl_3$ solution. Formation of green precipitate indicated the presence of tannins [Ameyaw etal, 2012].

Tests for steroids

(a) Salkowski Test- To 2 ml of chlroformic extract, a few drops of concentrated sulfuric acid was added. Formation of a red coloured ring at the surface of the solution indicated the presence of steroids [Singh et al, 2013]. (b) Lieberman Burchardt test- Formation of reddish ring at the junction of 2 layers obtained by mixing Chloroform solution of the extract with a few drops of acetic anhydride and 1 ml of concentrated sulfuric acid indicates the presence of steroids [Nath et al, 1946]

Test for Flavonoids

Alcoholic solution of the extract was mixed with a few drops of 10 % lead acetate. Formation of a yellow

precipitate was taken as a positive test for flavonoids [Sofowara, 1993; Harbrone, 1973].

Test for Terpenoids

2 ml of the organic extract was dissolved in 2 ml of chloroform and evaporated to dryness. 2 ml of concentrated sulphuric acid was then added and heated for about 2 min. Formation of greyish colour indicated the presence of terpenoids [Singh et al, 2013] .

Test for Phlobatannins

About 2 ml of aqueous extract was added to 2 ml of 1% HCl and the mixture was boiled. Deposition of a red precipitate was taken as an evidence for the presence of phlobatannins [Ajayi et al, 2011].

Test for Coumarin

Evaporated 5 ml of the ethanolic plant extract and dissolved the residue in 2 ml of hot distilled water. To one half of this solution added 0.5 ml of 10% NH_4OH and the other half was kept as the control. Observation of intense fluorescence upon showing the solution to UV light indicated the presence of coumarins [Harborne, 1973].

Test for Cardiac Glycosides

About 100 mg of the extract was treated with 1 ml of glacial acetic acid containing 100µL of 3% $FeCl_3$. This was carefully underlayed with 1ml of concentrated sulphuric acid. The formation of brown ring at the interface indicated the presence of deoxy sugar characteristic of cardenolides [Ajayi et al, 2011].
Test for Quinone: To about 1 ml of the plant extract 1 ml of con. H_2SO_4 was added, formation of red colour indicated the presence of quinines [Ayoola et al, 2008].

Test for Total Phenolic Content

About 100 mg of the aqueous and methanolic plant extract was weighed separately and dissolved in 1 ml of 1% hydrochloric acid in methanol solvent (v/v). The extracts were centrifuged at 6000 rpm for 60 mins. To 100 µl of the supernatants 750 µl of Folin-Ciocalteau reagent was added and allowed to stand at room temperature. To this solution 750 µl of sodium bicarbonate was added and left to react in the dark for 90 minutes. Ultrapure water was used as a blank in the analysis. The absorbance was determined using UV-visible spectrophotometer (Jasco V650) at λ_{max} of 765 nm. The TPC was calculated by comparing the absorbance with the tannic acid calibration curve according to the formula:

$$TPC\left(\text{«}g/g\right) = C \times V/g \qquad (1)$$

In the above equation C is the concentration of the tannic acid equivalent from standard curve (*µg/ml*), V is the volume of the extract used in millilitres and g is the weight of extract in grams. Standard tannic acid solution [Humadi and Istudor, 2009] was prepared by dissolving 10 mg of tannic acid in 10 mL of ultrapure water; this solution was further diluted to a total volume of 100 mL. The calibration curve was obtained by measuring the absorbance for various concentrations of tannic acid. The total phenolic content is expressed as tannic acid equivalent (*µg TAE/g*).

Preparation of Methanolic Plant Extract

Healthy, disease free, mature leaves of *S. Racemosa* were collected from Kodaikkanal. The extraction was carried out using routine protocol [Abhilash et al, 2011] .The leaves were thoroughly washed, shade dried and ground to fine powder with the help of a blender. 25 gram of the powdered leaves was extracted with 250 mL of methanol for 15 days in a shaker at 150 RPM. The process of extraction was repeated three times. The crude extract so obtained was concentrated to 5 mL by evaporating the methanol using rotary flash evaporator. The concentrated extract was filtered using a 0.1µm Millex filter and stored at 20 ^{o}C in an airtight bottle until further use.

High Performance Thin Layer Chromatography (HPTLC)

An HPTLC plate with aluminum support silica gel was cut with ordinary household scissors. The markings on the plate were made with soft pencil. The gel plates of silica were impregnated with 4 % solution of sodium acetate in methanol – water 3:2 for about 10seconds. The gel plate was dried at room temperature. CAMAG Linomat IV spotter on

automated instrument has been used for the measurements. The gel plate was eluted with a mixture of Toluene: Methanol: Ethyl acetate: Formic acid (3.5:1:5.5:0.1) as the mobile phase. After eluting the plates were dried and densitometrically scanned using the CAMAG TLC SCANNER II and the peak area was recorded.

High Performance Liquid Chromatography (HPLC) Analysis

The column used was Phenomenex Luna– 5u (Gemini) C18 column, 4.6 × 250mm. The mobile phase consisted of Acetonitrile: Water (60:40) mixture. The flow rate was 0.5 mL/min, and the injection volume was 20 µL. The monitoring wavelength was 272 nm. The identification of each compound was based on a combination of retention time and spectral matching. A Shimadzu SPD-20A analytical HPLC system was employed for the analysis.

Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis

GC-MS is a technique where compounds can be identified by their mass spectral patterns and GC retention indices. The methanolic plant extract was analysed using a gas chromatograph, equipped with a glass column packed with 2% SP-2100 on 100/120 Supelcoport. The helium flow rate was maintained at 20 ml min^{-1} and the column temperature was programmed from 170 to 280 ^{o}C at 10 C min^{-1}. The GC was connected to a Hewlett Packard 5985 mass spectrometer by a jet separator and mass spectra was collected every 4.5 s. The ionizing potential was fixed at 70 ev.

Results and Discussion

Phytochemical Screening

Figure 1. shows the standard calibration curve obtained for tannic acid. The total phenolic compounds expressed as tannin equivalent in 25 grams of the dried plant leaves was found to be 1.36 grams.

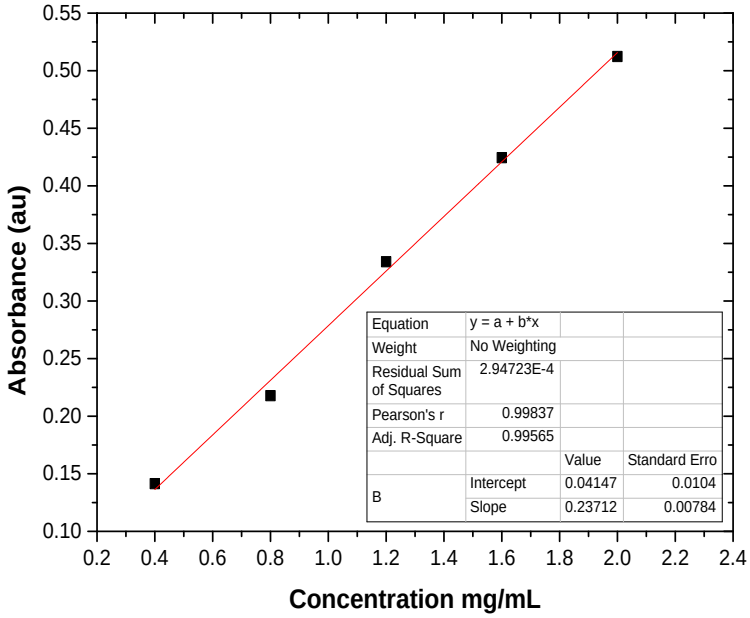

Fig. 1. Calibration curve for tannic acid

Table 1 show the phytochemical analysis carried out using various solvents. Compounds like alkaloids, Saponins, tannins, steroids, flavinoid, terpenoid, pholotannins, coumarin, cardio, phenols and quinine were found to be present in the plant extract. Steroidal compound was extracted in all the solvents. Since almost all the compounds were extracted in Methanol, we have used Methanol as the extraction medium for all other tests reported in this paper. Further phytochemical analysis of methanol and water extract revealed the presence of phenolic compounds, this indicates that the plant possess antibacterial activity [Puupponen-Pimiä, et al, 2001] .

Table 1. Phytochemical analysis of various solvent extracts

	Alkal oid	Sapo	Tanni	Steroi	Flava noid	Terpi	Pholo	Coum arin	Cardi	Total pheno l	Quino
Petroleum ether	-	-	-	+	-	-	-	-	-	-	-
Chloroform	-	-	-	+	+	-	-	-	-	-	-
Ethyl Acetate	-	-	-	+	+	+	-	-	-	-	-
Methanol	+	+	+	+	+	+	-	+	_	+	+
Water	-	-	+	+	+	+	-	+	+	+	-
Hexane	-	-	-	+	+	-	-	-	-	-	-

HPTLC

Figure 2 show the bands formed in TLC plates exposed to various UV wavelengths for the crude extract of *S. Racemosa*. Most of the compounds were eluted at 254 nm, *i.e.*, more bands in this wavelength and hence 254 nm is set for detecting the number of compounds present. About 15 peaks were obtained in the chromatogram, which means that 15 non-volatile compounds are present in the crude plant extract. Table 2 shows the number of compounds, their retention time and detection wavelength for the various compounds present.

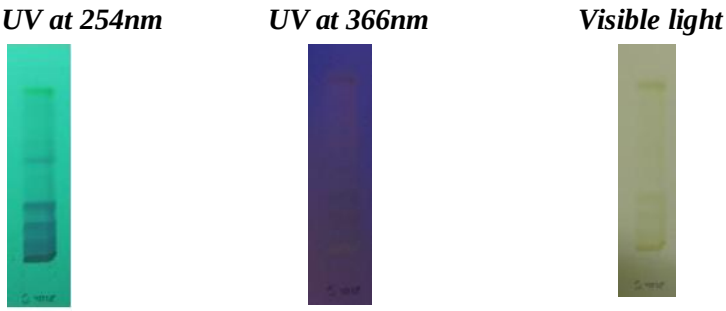

Fig 2: Bands formed in TLC plates exposed to various UV wavelengths

Table 2. Number of compounds, their retention time, and area and height and detection wavelength for the various compounds

Peak No	Rf	Height	Area	Lambda Max
1	0.05	61.4	1400.7	332
2	0.10	54.0	2249.3	327
3	0.20	50.8	2151.1	325
4	0.29	48.6	1994.6	328
5	0.35	16.2	415.5	323
6	0.39	14.9	206.5	315
7	0.42	15.2	403.0	321
8	0.52	28.4	1321.1	296
9	0.60	16.6	530.0	312
10	0.66	13.4	300.4	208
11	0.70	14.6	382.6	235
12	0.75	15.2	352.8	288
13	0.79	15.3	386.5	400
14	0.85	16.4	661.8	400
15	0.98	3.6	50.9	206

HPLC

Results of HPLC analysis of the methanolic extract, at 272 nm, showed the presence of 8 non-volatile compounds. The retention times for the two major volatile compounds were found to occur at 5.783 and 7.383. The HPLC spectrum is shown in Fig. 3 and the number of compounds and their retention times in the HPLC chromatogram is shown in Table 3

.

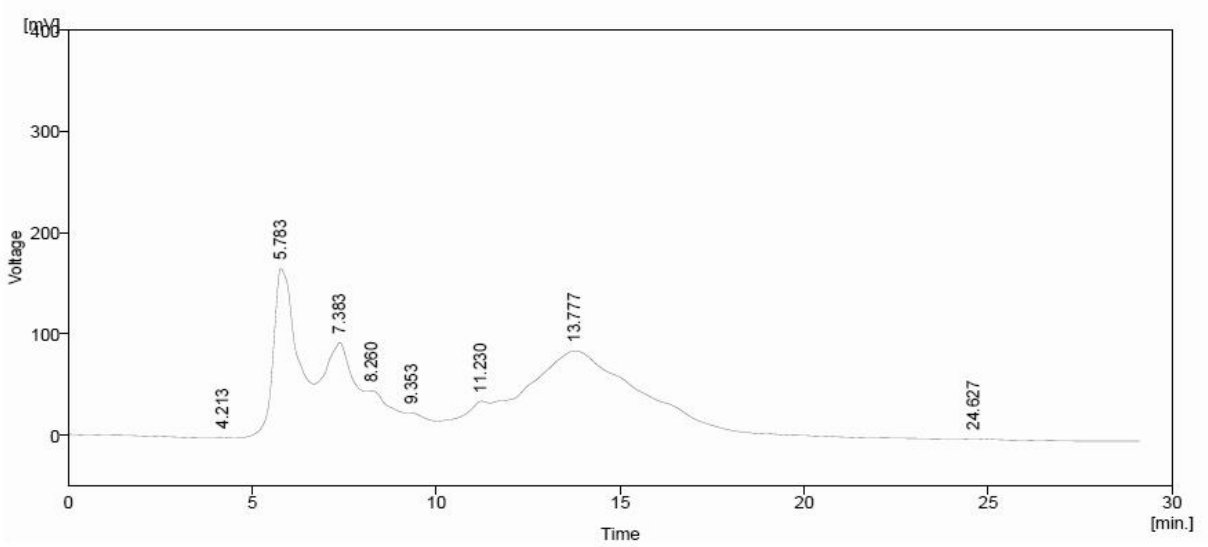

Fig 3. HPLC chromatogram for *S. Racemosa recorded* at 272 nm.

Table 3. Number of compounds, peak area and height and the retention time for various compounds seen in the HPLC chromatogram

S. No	Retention Time (min)	Area (mV.s)	Height (mV)	Area (%)	Height (%)	W05 (min)
1	4.213	13.470	0.847	0.1	0.3	0.27
2	5.783	4878.828	141.537	31.6	55.5	0.54
3	7.383	1508.710	44.782	9.8	17.6	0.55
4	8.260	105.940	4.593	0.7	1.8	0.30
5	9.353	33.259	1.561	0.2	0.6	0.22
6	11.230	127.594	5.713	0.8	2.2	0.37
7	13.777	8727.876	55.003	56.6	21.6	2.30
8	24.627	31.976	0.766	0.2	0.3	0.83
	Total	**15427.652**	**254.803**	**100.0**	**100.0**	

GC-MS

Interpretation of the spectrum of GC-MS was carried out using the database of National Institute Standard and Technology (NIST) MS database libraries. The mass spectrum of the crude extract was compared with the spectrum of the known components from the NIST library. The GC-MS spectra (Fig. 4) showed 21 peaks indicating the presence of twenty one phytochemical constituents. The various compounds which are identified and their retention times are shown in Table 4.

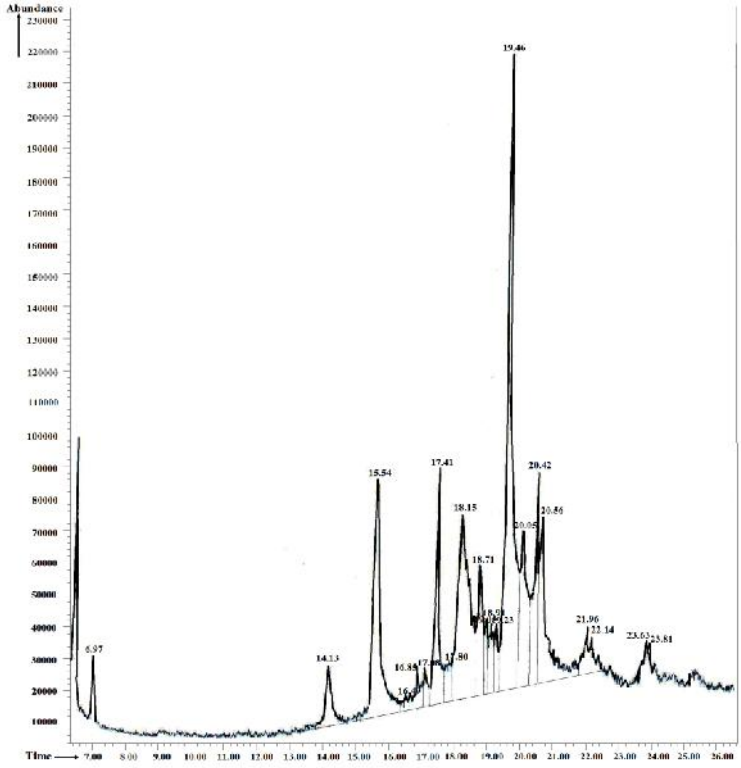

Fig. 4. GC-MS Spectrum for the methanolic extract of *Schefflera racemosa* leaves

Most of the compounds analyzed in GC-MS exhibit medicinal properties. The compound 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one is a flavanoid and it can exhibit antimicrobial and anti-inflammatory activity. n-Hexadecanoic acid and tetradecanoic acid are fatty acids which have potential antibacterial and antifungal activity. 5-hydroxymethyl-2-furaldehyde exhibits antibacterial activity. 2,3-Dihydrobenzofuran and methyl salicylate exhibits anti-oxidant properties. The GC-MS results have been compared to that of the photochemical analysis. The results were found to be closely matching.

Table 4. Compounds identified using GC-MS

S. No	Retention Time	Name of Compound	Area Percentage
1	14.13	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one	2.98
2	15.54	1,1-diethyl-4,4-dimethyl-2Tetrazene	11.53
3	16.32	1-O-Methyl-3,6-anhydro-α-D-mannopyranose	0.38
4	16.49	N-[2-[4-[5-(3,3-dimethyloxiran-2-yl)-4-hydroxy-3-methyl-pent-2-enoxy]phenyl]ethyl] Benzamide	0.45
5	16.85	Methyl Salicylate	1.59
6	17.08	2-Methyl-2-heptenal	1.02
7	17.41	n-Hexadecanoic acid	6.5
8	17.80	Tetradecanoic acid	1.48
9	18.15	2,3-Dihydrobenzofuran	15.57
10	18.71	N-benzylmethylamine catecholborane	4.67
11	18.91	Tris(dimethylamino)methane	1.36
12	19.05	Tetraacetyl-d-xylonic nitrile	1.90

13	19.23	2H-Pyrano[3,2-b]pyridine	1.56
14	19.46	3-Furanmenthonal	23.89
15	20.05	3-Bromopropyne	7.73
16	20.42	5-(hydroxymethyl)-2-furaldehyde	5.46
17	20.56	Thiocyanic acid,2-propynyl ester	7.99
18	21.96	Pentadecylamine	1.44
19	22.14	Dimethylhydrozone Propanal	1.08
20	23.63	3,3'- Iminobispropylamine	0.22
21	23.81	Benzeneethanol. 4-(2-hydroxyethyl)phenol	0.90

Conclusion

The present study has been found useful in the identification of several constituents present in the methanolic extract of the leaves of *S. racemosa* by phytochemical, HPTLC, HPLC and GC-MS analysis. The presence of various bioactive compounds justifies the use of the whole plant for various ailments by traditional practitioners. However isolation of individual phytochemical constituents and subjecting it to biological activity will definitely give fruitful results.

References

Abhilash, P. A., P. Nisha, A. Prathapan, Suresh V. Nampoothiri, O. Lijo Cherian, T. K. Sunitha, and K. G. Raghu. "Cardioprotective effects of aqueous extract of Oxalis corniculata in experimental myocardial infarction." Experimental and toxicologic pathology 63, no. 6 (2011): 535-540.

Ajayi, I. A., O. Ajibade, and R. A. Oderinde. "Preliminary phytochemical analysis of some plant seeds." *Res. J. Chem. Sci* 1, no. 3 (2011): 58-62.

Ameyaw, Y., V. Y. A. Barku, J. Ayivor, and A. Forson. "Phytochemical screening of some indigenous medicinal plant species used in the management of diabetes mellitus in Ghana." *Journal of Medicinal Plants Research* 6, no. 30 (2012): 4573-4581

Ayoola, G. A., H. A. Coker, S. A. Adesegun, A. A. Adepoju-Bello, K. Obaweya, E. C. Ezennia, and T. O. Atangbayila. "Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria." *Tropical Journal of Pharmaceutical Research*7, no. 3 (2008): 1019-1024.

Balandrin, M. F., Kinghorn, A. D., & Farnsworth, N. R. (1993). Plant-derived natural products in drug discovery and development. *Human Medicinal Agents from Plants. American Chemical Society, Washington, DC*.

Balandrin, M. F., Klocke, J. A., Wurtele, E. S., & Bollinger, W. H. (1985). Natural plant chemicals: sources of industrial and medicinal materials. *Science*, *228*(4704), 1154-1160.

Chinou, I. (2008). Primary and secondary metabolites and their biological activity. Thin Layer Chromatography in Phytochemistry, Waksmundzka-Hajnos, M., J. Sherma and T. Kowalska,(Eds.). CRC Press, Boca Raton.

Cragg, G. M., & Newman, D. J. (2005). Biodiversity: A continuing source of novel drug leads. *Pure and applied chemistry*, *77*(1), 7-24.

Dantas, I. N. F., G. C. M. Gadelha, D. C. Chaves, F. J. Q. Monte, C. Pessoa, M. O. de Moraes, and L. V. Costa-Lotufo. "Studies on the cytotoxicity of cucurbitacins isolated from *Cayaponia racemosa* (Cucurbitaceae)."*Zeitschrift Fur Naturforschung, C* 61, no. 9/10 (2006): 643

Gullett, Norleena P., A. R. M. Ruhul Amin, Soley Bayraktar, John M. Pezzuto, Dong M. Shin, Fadlo R. Khuri, Bharat B. Aggarwal, Young-Joon Surh, and Omer Kucuk. "Cancer prevention with natural compounds." In *Seminars in oncology*, vol. 37, no. 3, pp. 258-281. WB Saunders, 2010

Harborne JB. Phytochemical methods, London, Chapman and Hall Ltd., 1973, pp.49-188

Indian Pharmacopoeia, Government of Indian, Ministry of health and human welfare, Controller of publications, New Delhi (India); 1996; 2:A53-A54

Humadi, Suhad S., and Viorica Istudor. "*Lythrum salicaria* (purple loosestrife). Medicinal use, extraction and identification of its total phenolic compounds."*Farmacia* 57 (2009): 192-200.

Joshi, B., Lekhak, S., & Sharma, A. (2009). Antibacterial property of different medicinal plants: *Ocimum sanctum, Cinnamomum zeylanicum, Xanthoxylum armatum* and *Origanum majorana*. *Kathmandu university journal of science, engineering and technology*,*5*(1), 143-150.

Menichini, Federica, Filomena Conforti, Daniela Rigano, Carmen Formisano, Franco Piozzi, and Felice Senatore. "Phytochemical composition, anti-inflammatory and antitumour activities of four *Teucrium* essential oils from Greece." Food Chemistry 115, no. 2 (2009): 679-686.

Nath, M. C., M. K. Chakravorty, and S. R. Chowdhury. "Liebermann-Burchard reaction for steroids." *Nature* 157.10 (1946).].

Pandey, M., Debnath, M., Gupta, S., & Chikara, S. K. (2011). Phytomedicine: An ancient approach turning into future potential source of therapeutics. *J Pharmacognosy and Phytotherapy*,*3*, 27-37.

Patil, V. V., R. B. Pimprikar, N. G. Sutar, A. L. Barhate, L. S. Patil, A. P. Patil, R. Y. Chaudhari, and V. R. Patil. "Anti-hyperglycemic activity of *Ficus racemosa* Linn leaves." *Journal of Pharmacy Research* 2, no. 1 (2009): 54-57.

Puupponen-Pimiä, R., L. Nohynek, C. Meier, M. Kähkönen, M. Heinonen, A. Hopia, and K-M. Oksman-Caldentey. "Antimicrobial properties of phenolic compounds from berries." *Journal of applied microbiology* 90, no. 4 (2001): 494-507.

Ramachandra Rao, S., & Ravishankar, G. A. (2002). Plant cell cultures: chemical factories of secondary metabolites. *Biotechnology advances*, *20*(2), 101-153.

Singh, Bhulan Kumar, Krishna Kolappa Pillai, Kanchan Kohli, and Syed Ehtaishamul Haque. "Effect of *Cissampelos pareira* root extract on isoproterenol-induced cardiac dysfunction." *Journal of natural medicines* 67, no. 1 (2013): 51-60.

Sofowara A (1993). Medicinal plants and Traditional medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria. p. 289.

Sukhramani, Prakash S., G. Vidyasagar, and Piyush M. Patel. "In-vitro screening of *Ficus racemosa* for Anticancer activity." *Research Journal of Pharmacognosy and Phytochemistry* 5.3 (2013): 119-122.

Thomas, T. Jose, Beena Panikkar, A. Subramoniam, M. Krishnan Nair, and K. R. Panikkar. "Antitumour property and toxicity of *Barringtonia racemosa* Roxb seed extract in mice." *Journal of Ethnopharmacology* 82, no. 2 (2002): 223-227

Trease G.E. and Evans W.C., Pharmacognosy, 11th edn., Bailliere Tindall, London, 45-50 (1989)

Velanganni, J., Kadamban, D., & Tangavelou, A. C. (2011). Phytochemical screening and antimicrobial activity of the stem of *Mallotus philippensis* (lam.) Muell. Arg. Var. Philippensis (Euphorbiaceae). *International Journal of Pharmacy & Pharmaceutical Sciences*, *3*.