WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES

SJIF Impact Factor 7.421

Volume 8, Issue 2, 1357-1369 Research Article ISSN 2278 – 4357

PHYTOCHEMICAL SCREENING OF A MEDICINAL HERB *CLEOME MONOPHYLLA* L

P. Vijayashalini* and P. Abirami

PG and Research Department of Botany, Vellalar College for Women (Autonomous), Erode-638 012, Tamil Nadu, India.

Article Received on 23 December 2018,

Revised on 13 Jan. 2019,
Accepted on 03 Feb. 2019

DOI: 10.20959/wjpps20192-13196

***Corresponding Author**
P. Vijayashalini
PG and Research Department of Botany, Vellalar College for Women (Autonomous), Erode-638 012, Tamil Nadu, India.
vijayashalinip@gmail.com,

ABSTRACT

The present study deals with the phytochemical analysis of a medicinal herb *Cleome monophylla* L. Hexane, chloroform, ethanol and aqueous extracts of the whole plant have been screened for qualitative and quantitative determination, the result revealed the presence of primary and secondary metabolites such as flavonoids, carbohydrates, protein and amino acids, phenols and tannins, glycosides, anthraquinones, quinones terpenoids and coumarins. All the extracts showed negative response to fatty acids. The aqueous extract showed negative response to alkaloids and saponins.

KEYWORDS: *Cleome monophylla,* hexane, chloroform, alkaloids, phenols, tannins.

INTRODUCTION

Medicinal plant is an important element of indigenous medical systems in all over the world. About 80% of world populations still use the herbal drugs for treatment of various diseases. In India, it is reported that traditional healers use 2500 plant species and 100 species of plants serve as regular sources of medicine.[1] The ethno botany provides a rich resource for natural drug research and development.[2] The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents.[3] Phytochemicals are primary and secondary compounds, chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have alkaloids, terpenoids and phenolic compounds.[4] Phytochemicals are responsible for medicinal activity

of plants.[5] In plant the phyto chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack.[6,7]

The secondary metabolites are important for the human consumption as food and as medicinal compounds used in the pharmaceutical industry require special attention.[8] Over 50% of the modern clinical drugs are of natural origin and natural products play an important role in drug development program of the pharmaceutical industry.[9] Successful determination of bioactive compounds from plant material is largely dependent on the type of solvent used in the extraction procedure.[10] The present work deals with qualitative and quantitative phytochemical screening of *Cleome monophylla* L. and various phyto compounds detected in plant extracts are known to have health benefits, physiological activities and medicinal activity.

Cleome monophylla L. belongs to the family Capparidaceae (spindle flower family). The genus *Cleome* has been recorded approximately 150 species. In India it represented by 15 species. *Cleome monophylla* L. is an erect, annual herb upto 90 cm, branchlets glandular and pubescent and it is commonly grow in cultivated lands and road sides. *C.monophylla* is a single leaved species in the compound leaf genera. It is commonly called as spindle pod and Tamil vernacular name is Naaikadugu and Ellukku sakkalathi. The synonym is *Cleome cordata, C. subcordata* and *C. massae* (Plate-I). *C. monophylla* is commonly used in traditional and folklore medicines for treating various diseases such as ulcer, boils, wounds, cough, headache, swellings, hasten maturation, ear discharges,[11] fever,[12,13] headache,[14] and bile enlargement.[15] The leaf extract of *C. monophylla* had anti-HIV-1 reverse transcriptase activity.[16] The plant possesses anti inflammatory, anthelmintic and antidermatosis activity.[17]

MATERIALS AND METHODS

Collection, Identification and Authentication of Plant

From the survey of Burgur range of reserve forest, Erode District, Tamil Nadu, India, a medicinal plant *Cleome monophylla* L. were collected during August 2016. Identification was done by using Floras.[18,19] The plant was authenticated by BSI, Coimbatore, India. The Herbarium number in BSI is BSI/SRC/5/23/2016/Tech./1818.

Preparation of Plant Material

Fresh plant of *Cleome monophylla* L. were collected and washed thoroughly under running tap water. Then the plant parts were cut into small pieces and shade dried. The dried parts were then pulverized to powder using a mechanical grinder. The powder was preserved in air sealed glass bottles.

Preparation of extracts

The plant powder was extracted successively using Soxhlet apparatus with hexane, chloroform and ethanol, based on the Polarity. Each time before extracting with next solvent, powdered material were dried in an air-oven below 50°C. The extracts were dried over anhydrous sodium sulfate, stored in sealed vials in refrigerator (5-8°C) until analysis.

Finally the marc was macerated with chloroform water for 24 h to obtain the aqueous extract. The extract was concentrated by distilling off the solvent and then evaporating to dryness on a water bath.[20]

Qualitative Phytochemical Screening

Qualitative phytochemical analysis was carried out according to Harborne,[20] Kokate*et al*.[21] and Lowry *et al*.[22] Carbohydrates, proteins and amino acids, alkaloids, flavonoids, glycosides, saponins, phenols, tannins, anthraquinones, steroids quinones, terpenoids, fatty acids and coumarin were qualitatively analysed.

Tests for Alkaloids

The extracts were dissolved in dilute H_2So_4 and filtrate was treated with Dragendorff's, Hager's, Mayer's and Wagner's reagent separately. Appearance of orange brown, yellow cream, pink and reddish brown precipitates in response to the above reagents respectively indicates the presence of alkaloids.

Tests for Flavonoids (Shinoda Test)

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

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 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.

Test for quinones

To 2ml of extract, 1ml of concentrated H2So4 was added and shaken well for 5 minutes formation of red colour confirms the presence of quinone.

Tests for Carbohydrate

The extracts were treated with Benedict's and Fehling's reagents under suitable conditions. Appearance of brick red colour in response to the above reagents indicates the presence of carbohydrates.

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.

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.

Test for fatty acids

0.5 ml of extract was mixed with 5 ml of ether. These extract was allow it for evaporation on filter paper and dried the filter paper. The appearance of transparencies on filter paper indicates the presence of fatty acids.

Tests for Steroids (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.

Test for Anthraquinones (Borntragers)

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.

Test for terpenoids

Crude extract was dissolved in 2 ml of chloroform and evaporated to dryness. To this, 2ml of concentrated H_2SO_4 was added and heated for about 2 minutes. A grayish colour indicates the presence of terpenoids.

Test for coumarin

To 2 ml of extract 10% NaOH was added and shaken well for 5 minutes. Yellow colour indicates the presence of coumarin.

Quantitative Screening

Determination of Alkaloid

The sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added. Beaker was covered and allowed to stand for 4 h. Then it was filtered and the extract was concentrated on a water bath to one quarter of its original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to stand till its settlement. The precipitate was easily collected from the solution and was washed with dilute ammonium hydroxide and filtered. The residue was the alkaloid which was weighed after complete dryness and the percentage was calculated.[24]

Determination of total flavonoids

Aluminium chloride colorimetric method was used with some modifications to determine flavonoid content. 1 ml of sample plant extract was mixed with 3ml of methanol, 0.2 ml of 10% Aluminium chloride, 0.2 ml of 1 M Potassium acetate and 5.6 ml of distilled water and remains at room temperature for 30 minutes. The absorbance was measured at 420 nm. Quercetin was used as standard (1mg/ml). All the tests were performed in triplicates. Flavonoid contents were determined from the standard curve and were expressed as Quercetin Equivalent (mg/g of extracted compound).[25]

Determination of Saponin

20 g of each grounded sample was put into a conical flask and 100 cm3 of 20% aqueous ethanol was added. Then the flask was heated on a hot water bath for 4 h. with constant stirring at about 55°C. The mixture was then filtered and the residue was again extracted with another 200 ml 20% ethanol. The combined extract was reduced to 40 ml on a hot water bath at about 90°C. The concentrate was transferred into a 250 ml separator funnel, added 20 ml diethyl ether in it followed by vigorous shaking. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in oven, weighed and saponin content was calculated as percentage.[29]

Determination of Total Phenolic Content

The dilute extracts of different concentrations were taken in 10ml. glass tubes and total volume made to 3ml. with distilled water these are then mixed with 0.5ml. Folin – Ciocalteau reagent (1:1 with water) and 2 ml. Na2CO3 (20 %). A blue coloured complex, molybdenum blue developed in each tube, as the phenols undergo a complex redox reaction with phosphomolibdic acid in Folin – Ciocalteau reagent in alkaline medium. The tubes containing the blue solutions were warmed for 1 min., cooled and absorbance was measured at 650 nm against the reagent blank. The standard curve was prepared using known concentrations of catechol at 650 nm. The total phenol content in the test samples was calculated from the standard curve and expressed as mg. catechol equivalent of phenol/ g. sample.[28]

Determination of Tannins

Estimation of tannins was carried out by using Folin-Denis reagent Reagents 1. Folin-Denis reagent: To 750 ml of water, 100 gm of sodium tungstate was added. 20gm of phosphomolydic acid and 50ml of 85% phosphoric acid were also added. The whole mixture was refluxed for 2 hours. It was cooled and diluted to 1000ml. 2. Saturated sodium carbonate solution: 35gm of anhydrous sodium carbonate was dissolved in 10 ml of water at 70-80oC and cooled overnight. Clear liquid was decant and used.[27]

Determination of Glycosides

1g of the extract was extracted with 10ml 70% alcohol and the mixture filtered. From the filtrate, 8ml was transferred to a 100ml volumetric flask and the volume was completed to the mark with distilled water. 8ml of the mixture was added to8ml of 12.5% lead acetate (to

precipitate resins, tannins and pigments). The mixture was shaken well, completed to the volume (100ml) with distilled water and filtered. 50ml of the filtrate was pipette into another 100ml volumetric flask and 8ml of 4.7% disodium hydrogen phosphate (Na2HPO4) solution was added to precipitate excess lead. The mixture was made up to the volume with distilled water and mixed. This was filtered twice through filter paper. Baljets reagent (10ml) was added to 10ml of the purified filtrate. A blank sample of 10ml of distilled water was also added to 10ml baljets reagent. The two solutions were allowed to stand for one hour (time necessary for maximum colour development) a blank of 20ml distilled water was used. The intensity of the colour was read at 495nm using spectrophotometer. The colour was stable for several hours.

The percentage of glycosides = A×100 g % 17 Where A = the absorbance of the colour at 495nm.[26]

Determination of Steroids

A portion of 2 ml was taken from a solution of 2.5 g of powdered plant material prepared in 50 ml of distilled water after vigorous shaking for 1 hour. The extract solution was washed with 3 ml of 0.1 M NaOH (pH 9) and later mixed with 2 ml of chloroform and 3 ml of ice cold acetic anhydride followed by adding two drops of concentrated H_2SO_4 cautiously. The absorbance of both sample and blank were measured spectrophotometrically at 420nm.[30]

Determination of Anthraquinone

50 mg of the fine powder sample was soaked in 50 ml of distilled water for 16 hours. This suspension was heated in water bath at 70°C for one hour. After the suspension was cooled, 50ml of 50% methanol was added to it and then filtered. The clear solution was measured by spectrophotometer at a wavelength of 450nm and compared with a standard solution containing 1mg/100ml alizarin and 1mg/100ml purpurin with the absorption-maximum 450nm.[24]

Determination of total Terpenoides

10g of plant powder were taken separately and soaked in alcohol for 24 hours. Then filtered, the filtrate was extracted with petroleum ether; the ether extract was treated as total Terpenoids.[31]

Determination of Coumarins

500 ml of plant extract, 2 ml distilled water and 500 ml of lead acetate (5%, w/v) solution were added in a test tube. After shaking thoroughly, 7 ml of distilled water was added and mixing well, 2 ml of this solution was taken in another test tube and 8 ml of 0.1 M (v/v) hydrochloric acid solution was added. The solution was kept for 30 minutes at room temperature and absorbance was recorded at 320 nm using UV-Visible spectrophotometer. The total coumarin content was expressed as mg of coumarin equivalents per gm of sample extract (mg CE/g).[32]

RESULT AND DISCUSSION

The morphology of the *Cleome monophylla* is given in Plate-I. The qualitative phytochemical analysis of successive solvent extracts of *C.monophylla* is given in Table-I. The qualitative phytochemical result showed the presence of flavonoids, phenols and tannins, quinones, carbohydrates, proteins and amino acids, glycosides, anthraquinones, terpenoids and coumarin in all tested extracts, they were mentioned in (+) symbol. The remaining constituents namely fatty acids were absent in all tested extracts, they were expressed in (-) symbol. The alkaloids, saponins and steroids are absent only in aqueous extract but it shows positive result of remaining extracts. Related study was carried out by many authors,[33,34] alkaloids showed negative response in aqueous extract, similar findings were reported by,[35] in *C.viscosa* and *C. burmanni* but this result is disagrees with the work of Disticraj *et al*.,[36]

Quantitative determination phytoconstituents were carried out the powdered plant material by used various standard methods. The result obtained from this analysis showed the presence of phytochemicals from highest to least amount, it is given in the Table-II. The highest amount of alkaloids, flavonoids, glycosides, phenols, tannins, saponins, steroids and coumarins reported in ethanolic extract and n-hexane extract have greatest amount of terpenoids and quinone. The least amount of alkaloids, flavonoids, glycosides, steroids, terpenoids and coumarins represented in chloroform extract and phenols, tannins, saponin have lowest level in n-hexane extract. Antroquinones wer found only traces in all extracts. Similar findings were studied by[37] who reported anthraquinone present in least amount in methanol and aqueous extract of *C.gynandra.* In quantitative analysis, alkaloids, flavonoids and saponins were high in ethanloic extract of *Cleome rutidosperma* reported by Okonwn et al.,.[38]

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.[39] Tannins are astringent and bitter, 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.[40] Similar results were reported by Ryan.[41] The secondary metabolites were used to cure various diseases, Determinations of these metabolites are helpful to know the medicinal as well as food value of respective plants.[42]

Cleome monophylla **L.**

Table I: Qualitative phytochemical screening of successive solvent extracts of *Cleome monophylla* L.

S. No.	Name of the phytoconstituents	Name of the extracts			
		Hexane	Chloroform	Ethanol	Aqueous
1.	Alkaloids	+	+	+	_
2.	Flavonoids	+	+	+	+
3.	Saponins	+	+	+	_
4.	Phenols & Tannins	+	+	+	+
5.	Quinones	+	+	+	+
6.	Carbohydrates	+	+	+	+
7.	Proteins & Amino acids	+	+	+	+
8.	Glycosides	+	+	+	+

9.	Fatty Acids	_	_	_	_
10.	Steroids	+	+	+	_
11.	Anthraquinones	+	+	+	+
12.	Terpenoids	+	+	+	+
13.	Coumarin	+	+	+	+

('+') indicates presence; while ('–') stands for absence.

Table II: Quantitative Phytochemical Screening of *Cleome monophylla* L.

S. No.	Name of the Phytoconstituents	Name of the extracts			
		Hexane	Chloroform	Ethanol	Aqueous
1.	Alkaloids	2.35 ±0.01	0.98±0.05	3.25±0.02	Bdl
2.	Flavonoids	5.35±0.05	0.94±0.01	5.94±0.06	-
3.	Saponins	0.25±0.01	0.35±0.04	0.45±0.03	-
4.	Phenols	1.33±0.17	2.56±0.01	10.11±0.04	5.35±0.05
5.	Tannins	0.44 ±0.06	1.25±0.10	3.21±0.11	2.31±0.07
6.	Quinones	2.35±0.04	0.55±0.06	0.09 ±0.01	1.35±0.04
7.	Glycosides	2.11±0.12	0.36±0.06	14.5±0.08	10.33±0.08
8.	Steroids	1.83±0.01	1.44±0.01	4.03±0.04	-
9.	Anthraquinones	In traces	In traces	In traces	In traces
10.	Terpenoids	5.46±0.01	1.34 ±0.07	3.95±0.03	2.16±0.12
11.	Coumarin	0.95±0.05	0.36±0.04	0.834±1.02	0.05±0.07

CONCLUSION

The present study revealed the *C. monophylla* L. has more active principles like, alkaloids, flavonoids, phenol, tannin, Saponins, carbohydrates, proteins and amino acids, glycosides, quinones, steroids, anthraquinones, terpenoids and coumarins. These phytoconstituents seemed to have a potent drug for various diseases. This study would provide the preliminary scientific evidence for the ethnobotanical and traditional uses of *C.monophylla*.

REFERENCES

1. Pei S.J., Ethnobotanical approaches of traditional medicine studies: some experiences from *Asia. Pharmaceut. Bio.,* 2001; 39: 74-79.
2. Farnsworth N.R. The Role of Ethno Pharmacology in Drug Development. Ciba Foundation Symposium 154. Bioactive Compounds from Plants. John Wiley & Sons, Baffins Lane, Chichester (England), 1990; 2-21.
3. Nostro A., Germano M.P., Dangelo V., Marino A., Cannatelli M.A. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. *Lett Appl. Microbiol,* 2000; 30: 379-384.
4. Krishnaiah D., Sarbatly R., Bono A. Phytochemical antioxidants for health and medicine: A move towards nature. *Biotechnol. Mol. Biol. Rev.,* 2007; 1: 97-104.

5. Svithramma N., Venkateswarlu P., Suhrulatha D., Basha S.K.M. and Venkataramanadevi C.H. Studies of Boswellia ovalifoliolata Bal And Herny- An endemic and endangered medicinal plant. *Bio. Sci.,* 2010; 5: 359-362.
6. Gibson E.L., Wardel J., Watts C.J. Fruit and Vegetable Consumption, Nutritional Knowledge and Beliefs in Mothers and Children. *Appetite,* 1998; 31: 205-228.
7. Mathai K. Nutrition in the Adult Years. In Krause's Food, Nutrition, and Diet Therapy, 10th ed., ed.L.K. Mahan and S. Escott-Stump, 2000; 271: 274-275.
8. Hill A. F. Economic botany. A text book of useful plants and plant products. 2nd edn. *Mcgraw-Hill book company Inc.NY*, 1952.
9. Tresina P.S., Mohan, V.R. Preliminary phytochemical, FT-IR and antibacterial evaluation of leaf *Eugenia floccose* Bedd. (Myrtaceae), *World. J. Pharmaceut. Res*., 2014; 3(5): 714-725.
10. Tiwari P., Kumar B., Kaur M., Kaur G. and Kaur H. Phytochemical screening and extraction: a review. *Int Pharm. Sci.,* 2011; 1(1): 98-106.
11. Kalaichelvi K., Dhivya S.M. and Vijayashalini P. Indigenous knowledge on herbaceous medicinal plants among the local people of Mavanatham and Ittarai villages, Thalamalai hills, Sathyamangalam reserve forest range, Tamil Nadu, India. *Int. J. Plant. Animal & Environment. Sci.,* 2017; 7(3): 10-18.
12. Tamilselvi S.S., Venkatachalapathi A. and Paulsamy S. Ethnomedicinal plants used by irula tribes of Maruthamalai hills of Coimbatore District, Western Ghats, India. *Int. J. Pharm. Bio. Sci*., 2016; 7(3): (B) 533 – 553.
13. Tugume P., Kakudidi E.K., Buyinza M., Namaalwa J., Kamatenesi M., Mucunguzi P. and Kalema J. Ethnobotanical survey of medicinal plant species used by communities around Mabira central forest reserve. *Uganda. J. Ethnobiol & Ethnomed*, 2016; 12(5): 1-28.
14. Ssegawa P., Kasenene J.M. Medicinal plant diversity and uses in the Sango bay area, Southern Uganda. *J. Ethno. Pharmacol*, 2007; 113: 521-540.
15. Bandana K. And Sudhanshu K. A. checklist of some leafy vegetables used by tribals in and around Ranchi, Jharkhand. *Zoos' Print. J*., 2000; 16(3): 442-444.
16. Hurinanthan V. Anti-HIV activity of selected South African medicinal plants [Ph.D. dissertation], 2013.
17. Khare C.P. Indian Medicinal Plants–An Illustrated Dictionary. First Indian Reprint, *Springer (India) Pvt. Ltd.* New Delhi, 2007.

18. Gamble J.S. and Fischer, C.E.C. 1936. The Flora of the Presidency of Madras. Vol-I, II and III, *Adlard& Son Ltd, London*, 1915.
19. Matthew K.M. Materials for a Flora of the Tamil Nadu Carnatic, 1981; I: II,III,IV.
20. Anonymous. The Indian Pharmacopoeia. *Govt. of India publication, New Delhi,* 1966; 947-950.
21. Harbone J.B. Phytochemical methods, *Chapman and Hall, London* (*2nd Ed.),* 1984.
22. Kokate C.K., Khandelwal, K.R., Pawer, A.P. and Gohaiz, S.B. Practical Pharmacognosy, *Vallabh Prakasham, New Delhi, 4th Ed*, 1995; 107.
23. Lowry O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. J.Biol.Chem, 1951; 193: 265. (The original method).
24. Harborne J. B. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, *Chapman and Hall, London, UK*, 1973.
25. Aiyegoro O.A. and Okoh A.I. Preliminary pytochemical screening and in vitro antioxidant activities of the aqueous extract of Helichrysum longifolium DC. BMC Complement *Altern. Med*, 2010; 10: 21.
26. Evans W. C. Trease and Evans Pharmacognosy 14th Edition. *W. B. Saunders Company Limited, New York*, 1999; 1-340.
27. Anonymous. Firewood crops. National Academy of sciences, *Washington D.C*., 1980; 114-117.
28. Malik E.P., Singh M.B., Plant Enzymology and Hittoenzymology (1st Edn.) *Kalyani Publishers: New Delhi*, 1980; 286.
29. Obadoni B.O., Ochuko P.O. Phytochemical studies and comparative efficacy of the crude extract of some homeostatic plants in Edo and Delta states of Nigeria. *Global J. Pure Appl. Sci*., 2001; 8: 203- 208.
30. Trease G.E. and Evans W.C. 1989. Pharmacognosy. 13th (ed). *ELBS/Bailliere Tindall, London.* Pp. 345-6, 535-6, 772-3.
31. Ferguson N. M. A Text book of Pharmacognosy. *Mac Milan Company, New Delhi,* 1956; 191.
32. Osorio A.C. and Martins J.L.S. Determination of coumarin in fluid extract and guaco dye by spectrophotometry derived from first order. *Rev Bras Cienc Farm,* 2004; 40: 481-486.
33. Manikandan V. and Prabhakaran J. Qualitative and GC-MS analysis of phytochemical constituents of Tick weed (*Cleome viscosa* L.). *Int. J. Curr. Biotech*, 2014; 2(2): 25-30.

34. Vidhya R. and Umavandhana R. Phytochemical screening and GC-MS analysis of methanolic leaf extract of *Cleome viscosa* L. *Indo-Asian. J. Multidisp. Res,* 2016; 2(6): 863-868.
35. Pillai L.S. and Nair B.R. Pharmacognostical standardization and phytochemical in *Cleome viscosa* L. and *Cleome burmanni* W. & A. (Cleomaceae). *J. Pharma. Res*., 2011; 5(2): 1231-1235.
36. Disticraj S. and Jayaraman P. Pharmacognostical and phytochemical analysis of *Asparagus racemosus* Willd, *Anisomeles malabarica* (L.) R.Br. *Cleome monophylla* L. and *Coleus forskohlii* L. *Int. J. Pharma & Biol. Sci*., 2015; 5(4): 61-66.
37. Sango C., Marufu L. and Zimudzi C. Phytochemical, anti-nutrients and Toxicity Evaluation of *Cleome gynandra* and *Solanum nigrum*: common indigenous vegetables in Zimbabwe. *British. Biotech. J.,* 2016; 13(3): 1-11.
38. Okonwn K., Ekeke C. and Mensah S.I. Micromorphological and phytochemical studies on *Cleome rutidosperma* L. *J. Adv.Bio & Biotech*, 2017; 11(3): 1-8.
39. Ryan D., Antolovich M., Prenzler P and Robards K. and Lavee S. Biotransformations of phenolic compounds in *Oleaeuropea* L. *Scientist Horticulturae*, 2002; 92: 147-176.
40. Lin L.U., Shu-wen L., Shi-bo J. and Shu-guang W. Tannin inhibits HIV- 1entry by targeting gp41. *Acta. Pharmacol. Sin*, 2004; 25(2): 213-218.
41. Eseyin O.A., Sattar M.A. and Rathore H.A. A review of the pharmacologicaland biological activities of the aerial parts of *Telfairia occidentalis*Hook.f. (Cucurbitaceae). *Tropic. Journ. Pharm. Res*, 2014; 13(10): 1761-1769.
42. Ashish C., M. Goyal K. and Chauhan P. GC-MS Techniques and its analytical applications in science and technology, *Nat. Insti. Pharmaceut. Education & Res,* 2014; 9(4): 325 - 342.