International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (1): 889-892
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | Open Access | MCI Approved
UGC Approved Journal

Phytochemical Analysis of *Ruellia tuberosa* Tuber Ethanolic Extract Using UV-VIS, FTIR and GC-MS Techniques

M.N.L.C. Harika[1] and P. Radhika[2*]
Department of Biochemistry, Andhra University, Visakhapatnam-530003, Andhra Pradesh, India.

Received: 12 Oct 2018 / Accepted: 14 Nov 2018 / Published online: 1 Jan 2019
Corresponding Author Email: parvataneni_radhika@yahoo.com

Abstract
Aim: The aim of this study was to evaluate various bioactive compounds present in the roots of *Ruellia tuberosa* collected from the biodiversity park located at Visakhapatnam. The study of ethanolic isolates based on characterization using gas chromatography- Mass spectroscopy. **Materials and Methods:** Preliminary phytochemical analysis showed the presence of alkaloids, phenolics, flavonoids, steroids, terpenoids, coumarins, tannins, cardiac glycosides, carbohydrates and amino acids. Further investigation was done using Ultraviolet-Visible spectroscopy, Fourier transform infrared spectroscopy (FTIR) and GC-MS analysis. **Results:** UV-VIS and IR spectral analysis showed the peaks of corresponding functional groups present in the phytochemical constituents. GC-MS analysis of *R. tuberosa* tuber shows total sixteen compounds from its ethanolic extract. Out of these compounds, the highest abundance of them found to be Flavone (50%), E, E, Z-1, 3, 12-nonadecatriene 5, 14-diol (41.8%), Phytol (41.4%) and Methyl-6-octadecenoate (40.2%) present, based on the highest percentage of peak area. **Conclusion:** The present study demonstrated that *Ruellia tuberosa* tuber has rich source of secondary metabolites.

Keywords
FTIR, GCMS analysis, *Ruellia tuberosa,* UV-VIS spectroscopy.

*****

INTRODUCTION
Plants have been used for medicinal purposes long before historic period. Now a day's plants and plant-based medicines are the basis of many of the modern pharmaceuticals [1]. Plants contain various secondary metabolites naturally occurring in the leaves, roots, fruits and flowers having tremendous medicinal values. The extraction and characterization of biologically active components from medicinal plants have resulted in the introduction of new drugs with high medicinal value. In the past few years, a remarkable effort has been deployed leading to the isolation of many bioactive drugs from plants.

Ruellia tuberosa (Menow weed) commonly known as cracker plant [2]. It is an erect perennial herb, belongs to the family of Acanthaceae. It is having tuberous fusiform roots and frequently found in gardens and waste lands. *R.tuberosa* is an ornamental plant has emetic properties and used for

treatment for stones in bladder. Decoction of leaves is used for chronic bronchitis [3]. In Siddha system of medicine, leaves are given with liquid copal as remedy for gonorrhea and ear diseases [4]. In folk medicine, it has been used as diuretic, antipyretic, antidiabetic, analgesic and having anticancer activities [5, 6]. The roots having antioxidant, anti-spermatogenic, anti-inflammatory, antibacterial, antifungal and anti-insecticidal activities [7-10]. The root extracts showed effect on pancreatic diabetics and antidiabetic activity in rats [11-12]. From previous literature various phytochemicals were identified in the tuber ethanolic extract of *R.tuberosa* [13]. Gas chromatography–Mass spectroscopy is a reliable and reproducible analytical protocol for the identification, characterization and quantitation of bioactive principles from herbal extracts. In this present investigation we study the phytochemical analysis of *R.tuberosa* tubers collected from Visakhapatnam area, Andhra Pradesh.

MATERIALS AND METHODS

Collection of Plant Material

Fresh tubers of *Ruellia tuberosa* were collected from the Biodiversity Park, Visakhapatnam, Andhra Pradesh. The plant was identified at the Taxonomy section of Department of Botany and authenticated with voucher specimen number 22235 by Prof. S. B. Padal, Andhra University, Visakhapatnam, Andhra Pradesh, India.

Preparation of Plant Extract

Ruellia tuberosa fresh tubers were collected, washed thrice, dried in a shady place and coarsely powdered. Extraction was done using soxhlet method, approximately 25 g of the powdered plant material was introduced into the extraction chamber of the soxhlet extractor, using ethanol as solvent. At the end of the extraction, the extracts were concentrated using rotary evaporator and concentrated extract was carefully stored for further analysis.

Phytochemical analysis

Tuber ethanolic extract was used to investigate for the presence of various phytochemical constituents like alkaloids, phenolics, flavonoids, steroids, terpenoids, saponins, coumarins, tannins, carbohydrates, cardiac glycosides and proteins using standard methods [14-15] (Table 1).

UV-VIS Spectral analysis

UV-VIS spectral analysis was used to investigate the functional groups of organic compounds present in from the tuber extract. SHIMADJU UV-1800 spectrophotometer was used for analysis, in the range of 200-400nm wavelength.

FTIR Analysis

FTIR technique measures the absorption of infrared radiation by the sample material versus wavelength. The infrared absorption peaks identify molecular functional groups and structures. IR analysis of ethanolic tuber extract used to know the functional groups present in metabolites or phytochemical constituents present in the extract in the range of 400-4000cm^{-1} using BRUCKER FTIR spectrophotometer.

GC-MS analysis of *Ruellia tuberosa*

The phytochemical investigation of ethanol extract of *R.tuberosa* was performed on a GC-MS equipment (Thermo Scientific Co.) Thermo GC-TRACE ultra ver.:2.2, Thermo TSQ QUANTUM XLS Experimental conditions of GC-MS system were maintained as follows: DB 5-MS capillary standard non-polar column, dimension: 30Mts, ID: 0.25 mm, Film thickness: 0.25µm. Flow rate of mobile phase was set at 1.0 ml/min (carrier gas: He). In the gas chromatography part, oven temperature was 40 °C raised to 290 °C at 5 °C/min and injection volume was 1.0 µl. A range of 40-600 m/z maintained with a scan interval of 0.5 seconds. Total GC running time was 35min. The identification of compounds was based on comparison of their mass spectra with those of WILEY and NIST Libraries.

Identification of Phytocomponents in *Ruellia tuberosa*

The retention indices, peak area percentage and mass spectra fragmentation pattern of GC-MS chromatogram of ethanol extract of *Ruellia tuberosa* was compared with the database of National Institute of Standards and Technology (NIST), NIST08.LIB, WILEY8.LIB and with published literature data. The name, molecular weight, formula, structure and nature of the compounds were ascertained.

RESULTS

Phytochemical analysis

Phytochemical analysis of tuber ethanolic extract revealed the presence of alkaloids, phenolics, flavonoids, steroids, terpenoids, coumarins, tannins, cardiac glycosides, carbohydrates and amino acids.

UV-VIS Spectral analysis

The UV-VIS profile showed different peaks ranging from 200-400nm with different absorption respectively. The UV spectroscopic analysis of tuber extract showed the presence main UV absorption peaks at 318, 307, 295, 285, 234, 228, 224, 220 and 218nm. (Fig. 1).

FTIR analysis

The FTIR spectroscopic studies Revealed different characteristic peak values with various functional compounds in the extract (fig. 2). The peaks were identified at 3329.48, 2922.90, 2852.35, 1708.96, 1624.33, 1519.14, 1406.64, 1342.10, 1103.33, 1035.23, 989.08 and 924.71 cm^{-1}. Band at 3329.48cm^{-1} corresponds to O-H groups of tannins, flavonoids (phenolic compounds), glucose and –NH stretching of the proteins. Band at 2922.90 cm^{-1} corresponds to C-H asymmetric stretching of alkanes [16]. Band at 2852.35 cm^{-1} corresponds to C-H stretching of alcohols. Band at 1708.96 cm^{-1} indicates the C=O stretching of carbonyl group. Peak at 1624.33 cm^{-1} corresponds the C=C stretching. Bands at 1519.14, 1406.64, 1342.10cm^{-1}corresponds to C-H stretching [17]. Bands at 1103.33, 1035.23cm^{-1} indicates O-H stretch of carboxylic acids and C=O group. Peaks at 924, 873, 825, 769, 713, 684cm^{-1} indicates the C-H stretching of alkenes. These groups can majorly contributed by alkaloids, flavonoids, tannins, phenolic compounds and carbohydrates present in ethanolic tuber extract.

GCMS analysis

From GCMS analysis of extract total 16 compounds were identified which were presented in Table- 2. Those compounds were identified from NIST library and literature data. Structures of some compounds having similar basic ring structure and differs in presence of functional groups. The compounds were identified depending on retention time, structure, percentage of peak area. The GC-MS spectrum was presented in fig. 3. The major compounds present in ethanolic extract of tubers were Flavone [18], E, E, Z-1, 3, 12-nonadecatriene 5, 14-diol, Phytol and Methyl-6-octadecenoate [19]. The retention time (RT) of the compounds were 17, 19.52, 18.68 and 18.9 min respectively and the relative peak areas were 50%, 41.8%, 41.4% and 40.2% respectively.

The other compounds identified were Cyclohexane 3-(1-methyl ethyl)- (11.2%), 3-cyclohexane -1-ol,4-methyl1-(1-methylethyl)- (9.5%), α-Pinene (8.8%) [20], Phenol 2,4-bis (1,1-dimethyl ethyl)- (8.4%), n-Hexadecanoic acid (24.2%), Coumarin,3-[2-[1-methyl-2-imidazolylthio]-1-oxoethyl]- (24.3%), Oleic acid (25.2%), Morin (16.4%) [21], (E)-13-docosenoic acid (20.9%), 1H-Pyrolo[2,3-b] quinoxalin-2-imine, 2,3,3a,4,9,9a-Hexahydro-1,N-diphenyl-(11.3%), Phenol,2,2-methylene bis 6-(1,1-dimethyl ethyl)-4-ethyl (6.5%) [22], 4-Piperidine acetic acid,1-acetyl-5-ethyl-2-[3-[2-hydroxyethyl]-1 H-indol-2-yl]-a-methyl-,methyl ester(5.1%) [18].

This investigation concluded that the ethanolic extraction of *Ruellia tuberosa* tubers have been produced number of active phytochemical constituents responsible for many biological activities. These phytoconstituents might be utilized for the development of traditional medicines, which may create a new way to treat many incurable diseases.

DISCUSSION

A number of studies have been made on plant *R. tuberosa* and proved that the plant had number of medicinal properties. The present study was carried out to characterize the bioactive constituents present in tuber extract of *R. tuberosa* using UV-VIS, FTIR and GC/MS techniques. UV-VIS spectrophotometer was performed for identification of phytoconstituents present in ethanolic extract of tuber. The plant extract was scanned in the wavelength ranging from 200-400nm by using UV-VIS spectrophotometer. In the UV-VIS spectra the identification of peaks in the region from 200-400nm showed the presence of unsaturated groups and heteroatoms. The FTIR spectrum confirmed the presence of alcohols, phenols, alkanes, alkenes, carbonyl, carboxylic acids and aromatic compounds in extract [23].

The GC-MS analysis supports the presence of important bioactive compounds. The height of the peak corresponds to the relative concentration of compound. The compounds which were eluted at different timings through gas chromatogram are picked up by the mass analyzer and produced particular fragmentation pattern. This fragmentation pattern was compared to the compounds present in the reference library (NIST) in which the structure of compounds was determined. The results of the GC/MS analysis of ethanolic tuber extract provide different peaks determining the presence of 16 phytochemical compounds. The major phytoconstituents were Flavone (50%), E, E, Z-1, 3, 12-nonadecatriene 5, 14-diol (41.8%), Phytol (41.4%) and Methyl-6-octadecenoate (40.2%) showed the highest percentage of peak area [19] showed in fig. 4. The results showed that important bioactive compounds were present in tuber extract and were responsible for various pharmacological activities.

CONCLUSION

The present study was carried out to characterize the bioactive constituents present in tuber extract of *R.tuberosa* using UV-VIS, FTIR and GC/MS techniques. The plant extract was scanned in the wavelength ranging from 200-400nm by using UV-VIS

spectrophotometer and the characteristic peaks were detected. The FTIR spectrum confirmed the presence of alcohols, phenols, alkanes, alkenes, carbonyl, carboxylic acids and aromatic compounds in extract. The results of the GC/MS analysis of ethanolic tuber extract provide different peaks determining the presence of 16 phytochemical compounds. The major phytoconstituents were Flavone (50%), E, E, Z-1, 3, 12-nonadecatriene 5, 14-diol (41.8%), Phytol (41.4%) and Methyl-6-octadecenoate (40.2%) showed the highest percentage of peak area. The results showed that important bioactive compounds were present in tuber extract and were responsible for various pharmacological activities.

AKNOWLEDGEMENT

The author gratefully acknowledges and thankful to SAIF, IIT, Madras for providing GC-MS facilities and Department of Pharmacy, Andhra University, Visakhapatnam for providing FTIR facilities.

REFERENCES

1. Newman D. J., Cragg G. M., Snader K. M., The influence of natural products upon drug discovery. Nat Prod Rep,17 (3): 215-34, (2000).
2. Pandey C. N., Medicinal plants of Gujrat, Gujrat Ecological Education and Research Foundation. Gujrat, India 2005.
3. The Wealth of India, A Dictionary of Indian Raw material and Industrial product, Publication and Information Directorate, Council of Scientific and Industrial Research, New Delhi, India 1972.
4. Rehman R., Ibrar M., Hameed., Hussain F., Short Communication: Pharmacognostic and Pharmacological evaluation of *Ruellia tuberosa*. Pak J Pharm Sci, 29 (6): 2099-2102, (2016)
5. Chiu N.Y., Chang K.H., The illustrated medicinal plants of Taiwan. Mingtong Med J, 226 1, (1995)
6. Nagarjuna Reddy V., Nagarathna P.K.M., Divya M., Evaluation of anticancer activity of *Ruellia tuberosa* on EAC induced mammary tumor. Int J Pharmacol Toxicol, 1(2): 36-42, (2013)
7. Rajendrakumar N., Vasantha K., Murugan M., Mohan V.R., Antioxidant Activity of Tuber of *Ruellia tuberosa* L.(Acanthaceae). Int J Pharmacogn Phytochem Res, 6(1): 97-103, (2014)
8. Bhogaonkar P.Y., Kanerkar U.R., Indurwade N.H., Chondekar R.P., Antispermatogenic effect of the aqueous root extract of *Ruellia tuberosa* L. on albino rats. Trends in life sci, 1 (1): 1-4, (2012)
9. Ashraful Alam M., Nusrat Subhan., Abdul Awal M., Shohidul Alam M., Mokaddez Sarder Lutfun Nahar *et al.*, Antinociceptive and anti-inflammatory properties of Ruellia *tuberosa*. Pharm Bio, 47(3): 209-214, (2009)
10. Abdul Kader Md., Shumaia P., Aktar uzzaman Chowduri Md., Ekramul Haque Md., Antibacterial, antifungal and anti-insecticidal activities of *Ruellia tuberosa* (L.) root extract. J bio-sci, 20: 91-97, (2012)
11. Asadullah M., Aulanni'am Aulanni'am., Arie Srihardyastutie., Effects of *Ruellia tuberosa* L. root extracts on the Pancreatics of Diabetic Rats. J Pure App Chem Res, 7 (2): 116-121, (2018)
12. Kurniawati., Aulanni'am A.N.L., Arie Srihardyastutie., Anna Safitri., The Effects of root extract *Ruellia tuberosa* L on Histopathology and Malondialdehyde Levels on the Liver of Diabetic Rats. IOP Conf. Series: Mat Sci Eng (2018) 299:012022. doi:10.1088/1757-899X/299/1/012022.
13. Rajendra kumar N., Vasantha K., Mohan V.R., GC-MS analysis of bioactive components of tubers of *Ruellia tuberosa* L. (Acanthaceae). Am J Phytomed Clin Ther, 2 (2): 209-216, (2014)
14. Trease E.G., Evans W.C., Text book of Pharmacognosy.14th ed. WB Saunders Company, UK, 119, 1997.
15. Harborne J.B., Phytochemical Methods, 2nd ed. London, UK, Champman and Hall Ltd 1976.
16. Ghaidaa Jihadi Md., Aseel Mohammad Omran., Haider M.H., Antibacterial and Phytochemical analysis of *Piper nigrum* using Gas Chromatography-Mass spectrum and Fourier transform infrared chromatography. Int J Pharmacogn Phytochem Res, 8(6): 977-996, (2016)
17. Mahesha M.P., Kanivebagilu A.V., Airody V.A., Extraction, characterization and biological studies of phytochemicals from *Mammea suriga*. J Pharm Analysis, 5(3): 182-189, (2015)
18. Saahithya S., Sridhar R., Sriram P., Gas chromatography and Mass spectroscopy analysis (GC-MS) of the phytochemical constituents of *Momordica charantia* (Indian variety Co.1). The Pharma Innovation J,7(5): 29-32, (2018)
19. Srinivasan K., Sivasubramanian S., Kumaravel S., Phytochemical profiling and GCMS study of *Adhathoda vasica* leaves. Int J Pharm Bio Sci, 5(1): 714-720, (2014)
20. Ajay kumar., Chemical composition of essential oil isolated from the Rhizomes of *Kaempferia galanga* L. Int J Pharm Bio Sci, 5(1): 225-231, (2014)
21. Arul priya R., Saravanan K., Phytochemical and GC-MS studies of traditional herbaceous plant pumpkin (*Cucurbita pepo*) L. J Cell Tissue Res, 17(1): 6035-6041, (2017)
22. Karthikeyan V., Baskaran A., Sebastian Rajasekaran C., Gas Chromatography-Mass spectroscopy (GC-MS) analysis of ethanolic extracts of *Barleria acuminata* Nees. Int J Pharmacol Res, 6(2): 55-61, (2016)
23. Jain P.K., Anjali Soni., Preeti Jain., Jeetendra B., Phytochemical analysis of *Mentha spicata* plant extract using UV-VIS, FTIR and GC/MS technique. J Che Pharm Res, 8(12):1-6, (2016)