Research Article

Journal of Scientific Enquiry
Vol:3(1), Pages:50-53

Identification of secondary metabolites and fatty acids in *Curculigo orchioides* Gaertn., an important ethnomedicinal plant

Ghanashyam Mahato*, Rajani Kanta Mahato

Department of Botany, Achhruram Memorial College, Jhalda, Purulia, W.B.- 723202, India.

Received:13.05.2023 ; accepted: 15.06.2023; published online:30.06.2023

Abstract

Curculigo orchioides Gaertn, is a herb belonging to family Amaryllidaceae. The plant possess aromatic smell and used by ethnic community (Majhi, Munda, Santal, Birhor, Ho and Rajwar) from Purulia district, West Bengal, India as a whole either topically or orally in various monoherbal and polyherbal formulations for the treatment of various skin diseases such as carbuncle, acne, pimpleand ulcer. However, there is no scientific database on secondary metabolites and fatty acids of the plant yet. So, main emphasis of the present study is given on secondary metabolites and fatty acids of the plant.Secondary metabolites and fatty acids were analyses by HPLC (High-performance Liquid Chromatography) and GCMS (Gas Chromatography Mass Spectrometry). HPLC study revealed the presence of phenolic compounds and flavonoids. GCMS analyses identified the presence of saturated fatty acids in the range of C14 to C26 and C18 and C20 as unsaturated fatty acids. Assured levels of secondary metabolites along with fatty acids in the studied plant part support the ethnobotanical claim done by traditional healers.

Keywords: *Curculigo orchioides,* Ethnobotanical, Secondary metabolites, Fatty acids.

1. Introduction

Plants with ethnobotanical properties have been used as traditional medicines in various cultures since ancient times [1]. The presence of different phytochemicals in plants which are also known as secondary metabolites contributes to the medicinal properties of the plants and serves as potent drugs for human use. The secondary metabolites are diverse group of chemical compounds acts as beneficial agents in pharmaceutical production of novel drugs [2]. The development of plant based drugs starts with identification and authentication of bioactive components from the natural sources. Medicinal Plants are integral part of traditional societies' health care systems. The use of medicinal plants for different ailments is also very popular in certain parts of India. The primary health care of Purulia district of West Bengal, India is very much dependent on use medicinal plants available in their locality [3]. The people of this region prefer traditional medicine due to their belief to local healers and lack of modern medicinal facility in their area. Traditional healers use parts of different plants for the preparation of medicine for various diseases such as pox, asthama, bronchitis and cough.

Curculigo orchioides Gaertn., (family Amaryllidaceae) is one of the important medicinal plants and popularly known as "Kali musli" or Golden eye grass. It is a perennial herb available in the Purulia region of West Bengal and locally called as 'Talmuli'. Tuberous rhizome of *C. orchioides* Gaertn (Hypoxidance) is used as a medicine for the treatment of Carbuncle [4] and other diseases such as asthma, piles, urinary disorders, impotence, urinary disorders, and diarrhea [5]. The rhizome powder of *C. orchioides* is also known for its effect on skin health. The mixture of *C. orchioides* rhizome powder and honey is used to increase skin glow [6].

The medicinal properties of ethnobotnical plants are mainly due to the presence of secondary metabolites with several bio active properties such as antioxidant, anti-inflammatory, anti-diarrhea, and antimicrobial properties [7]. Traditional healers use medicinal plants for disease treatment on the basis of indigenous knowledge. The composition of medicinal plants in terms of phytochemicals and bioactive constituents are necessary to understand the mechanism of action on treating diseases. In lieu of the absence of available records on the secondary metabolites and fatty acids of an ethno medicinally important plant, the present study is expected to identify the secondary metabolites and fatty acids of *C. orchioides* to create a scientific database for ethnobotanical claim done by traditional healers from Purulia, West Bengal, India.

2. Materials and Methods

Collection and authentication of plant material

Plant material (the whole plant of *C. orchioides)* was collected from the surrounding area of Bandwan (22°52'33.6"N; 86°30'25.2"E), Purulia, West Bengal, India, in July, 2019 during its flowering stage (May-Sep). Plant was identified and authenticated by Botanical Survey of India, Kolkata and preserved as voucher number GM-06.

Extract preparation for secondary metabolites (HPLC analysis) and fatty acids (GC MS analysis)

#Electronic mail: ghana.mahato@gmail.com

 DOI: https://doi.org/10.54280/jse.233109

Briefly, 2.5 g plant powder was subjected to Bligh and Dyer's (1959) method to separate secondary metabolites, protein part from lipid contents using separating funnel. The upper methanolic fraction contains secondary metabolites and proteins mixed with chilled acetone 4:1 (v/v) and kept for 1 h at–20°C, then centrifuged twice for 10 mins. at 15,000 × *g* (4°C). The pellet containing protein was discarded and the supernatant was subjected to HPLC (Agilent, USA). The HPLC analysis was carried out in isocratic condition usingZorbax SB-C18 column (4.6 × 150 mm, 3.5 micron) and equipped with diodarray detector. HPLC grade methanol and water were used for resolution of compounds. The injection volume was 20 µl and the flow rate was kept at 0.4 ml/min in every case. Phenolic acids, flavonoids and some alcohols were taken as standards and compared with the peak area of the sample.

The chloroform phase separated out from the above procedure was trans esterified for the analysis of fatty acids. Methanol: Benzene: Conc.H_2SO_4 (5.3:0.5:0.8) was added and the mixture was put in water bath at 90°c for 8 hrs. Saponifiables were taken in hexane and subjected to GCMS (Agilent, USA) analysis. The GCMS analysis for fatty acids was done using Agilent Technologies 5975C GC-MS system (Agilent Technologies, USA) attached to HP-5 ms Capillary Column (30 m × 0.25 mm i.d. × 0.25 µm film thickness) and equipped with inert MSD triple axis mass detector conditioned at ion trap 200°C, transfer line 280°C, electron energy 70 eV (vacuum pressure- 2.21e-0.5 torr) was used for analysis. Pure helium gas (99.995%) was used as the carrier gas with flow rate of 1 ml/min. The initial temperature was set at 50 °C with increasing rate of 3 °C/min for 10 min and increased up to 300 °C. The compounds were identified on the basis of retention times and mass fragmentation patterns using Agilent Chem Station integrator and matching of the spectra with NIST (National Institute of Standards and Technology) library 2010.

3. Results and discussion

HPLC analysis of secondary metabolites

The secondary metabolite profile of CO (*Curculigo orchioides*) rhizome revealed the presence of three phenolic acids such as gallic acid, paracoumaric acid and 4- hydroxybenzoic acid and a flavonoid rutin(Fig. 1). The overall profile study indicated a predominance of phenolic acids over flavonoid in the sample. Amongst the identified phenolic acids paracoumaric acid found highest concentration in comparison to others (Table 1). These phytochemicals have several health benefits and cure various maladies. Inflammation, microbial, viral, parasitic infections, ulcers, psychotic diseases, ulcers, osteoporosis, cardiovascular issues, diabetes, neurogenerative disorders, diabetes, high blood pressure, and other diseases have all been reported to be successfully treated with the use of various bioactive compounds [8] and flavonoids that exist naturally have antibacterial properties [9,10]. The quantity and locations of methoxy and phenolic groups within the structures of flavonoids and phenolics affect their antibacterial action in different ways [11, 12]. The alkaloid extracts from medicinal plant species exhibit a variety of host-mediated biological activities, such as antibacterial, antimalarial, antihyperglycemic, and anti-inflammatory properties [13,14].

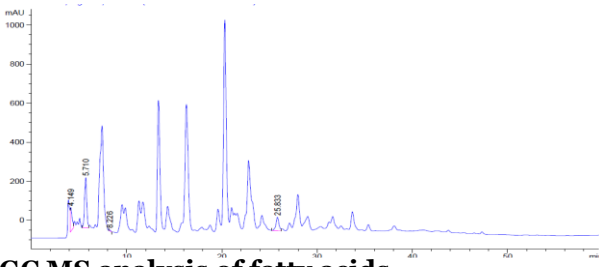
Comparatively to extracts from other solvents, the methanolic extract of the plant *C. orchioides* has more phenolic chemicals. Plants have antioxidant qualities because of the phenolic chemicals they contain, and methanolic extract has the greatest potential for scavenging free radicals.

Among the identified bioactive components, Para caumeric acid has more bioactive properties such as anti-oxidant, anti-inflammatory, anti-diabetic, anti-ulcer, anti-platelet, anti-cancer properties [15]. Gallic acid has antioxidant property [16]. 4-hydroxy butyric has anesthetic activity [17]. Rutin has antioxidant, anti-inflammatory, anti-microbial, cancer preventive, neuroprotective and cardioprotective activities [18].

Table 1. Name of the identified secondary metabolites

RT	NAME OF THE COMPOUNDS	Conc. (mg/gm)
4.149	Gallic acid (GA)	0.549±0.06
5.710	Para coumaric acid (PRCA)	0.671±0.02
8.226	4- hydroxy butyric acid (4HBA)	0.059±0.004
25.833	Rutin	0.348±0.06

Fig. 1. HPLC chromatogram of rhizome showing Rt in min along X-axis 4.149-Gallic acid; 5.710-Para coumaric acid; 8.226- 4-hydroxy butyric acid; 25.833- Rutin



GC MS analysis of fatty acids

Fatty acid profile of studied plant showed variation on the presence of fatty acid methyl esters (FAMEs) and its concentrations. The identified FAMEs with their respective retention time (Rt), molecular formula and molecular weight (Table 2). Relative abundance of the identified fatty acids with respect to their retention time was shown in Fig. 2. Fatty acid profile of *C. orchioides* rhizome reported the presence of saturated fatty acids (SFA) in the range

of C14 to C26 and poly unsaturated fatty acids (PUFA) of C18 and C20. There was dominance of SFAs than PUFAs. Methyl tetradecanoate has anti-cancer activity [19]. 6-Octadecenoic acid has antiplasmodial activity [20]. Eicosanoic acid has anti-inflammation, anti-fever, anti-allergy properties [21]. Docosanoic acid has reported to used as fungicide, insecticide and cosmetic additive [22].

Table 2. Name of the identified fatty acids

Sl. No.	RT	Name of identified compound	M F (MW)
1	23.147	Methyl tetradecanoate	$C_{15}H_{30}O_2$ (242)
2	26.226	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$ (270)
3	28.458	Heptadecanoic acid, methyl ester	$C_{18}H_{36}O_2$ (284)
4	29.205	6-Octadecenoic acid, methyl ester, (Z)-	$C_{19}H_{36}O_2$ (296)
5	30.420	5,8,11-Eicosatriynoic acid, methyl ester	$C_{21}H_{30}O_2$ (314)
6	33.338	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$ (326)
7	37.122	Docosanoic acid, methyl ester	$C_{23}H_{46}O_2$ (354)
8	39.190	Tetracosanoic acid, methyl ester	$C_{25}H_{50}O_2$ (382)
9	43.778	Hexacosanoic acid, methyl ester	$C_{27}H_{54}O_2$ (410)

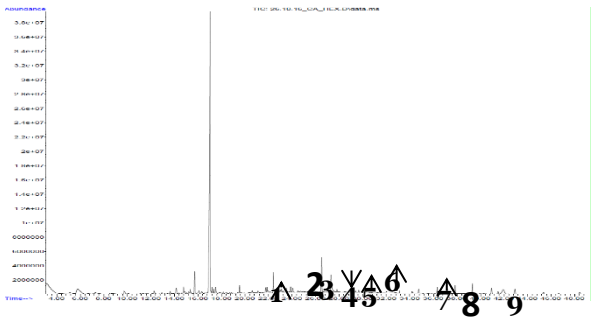


Fig. 2. Gas chromatogram of Fatty Acid Methyl Esters of *C. orchioides*showing Peak 1 – C14:0 (Rt =23.147), 2 - C16:0 (Rt =26.226), 3 - C17:0 (Rt =28.458), 4 - C18:1n9 (Rt =29.205), 5 - C20:3n9 (Rt =30.420), 06 - C20:0 (Rt =33.338), 7 - C22:0 (Rt =37.122), 8- C24:0 (Rt =39.190), 9- C26:0 (Rt =43.778). X-axis = Retention time in min. and Y-axis=Relative abundance.

4. Conclusion

Studied plant found phenolic acids, flavonoids and fatty acids which possess bioactive properties, so the results support the ethnobotanical claim done by traditional healers.

Acknowledgments

First author is greatly acknowledging University Grant Commission(ERO), Govt. of India for providing financial assistance from Project no. F.PSW-204/15-16(ERO). Authors are thankful to Dept. of Botany, University of Calcutta for providing GCMS & HPLC instrumenta lfacilities funded by Dept.of Science and Technology (FISTprogramme:SR/FST/LSI-459/2010 dated 10.03.2011, Govt. of India). We are indebted to about twenty traditional healers of Purulia district for giving us their valuable ethnobotanical information of this plant.

References

[1] R W Bussmann, and D Sharon, Traditional medicinal plant use in Northern Peru: tracking two thousand years of healing culture. Journal of Ethnobiology and Ethnomedicine, vol. 2, 47, (2006).

[2] Hussein, A Rehab, A Amira, and E Anssary, Plants secondary metabolites: the key drivers of the pharmacological actions of medicinal plants. Herbal medicine, 1, 3 (2019).

[3] S K Ghosh, N O Guria, A V Sarkar, and A N Ghosh, Traditional herbal remedies for various ailments within the rural communities in the district of Bankura and Purulia, West Bengal, India. Int. J. Pharm. Sci, 5, 195-198 (2013).

[4] Y Nie, X Dong, Y He, T Yuan, T Han, K Rahman, L Qin, and Q Zhang, Medicinal plants of genus Curculigo: traditional uses and a phytochemical and ethnopharmacological review. Journal of ethnopharmacology, 147(3), 547-563 (2013).

[5] K S Rao, and S H Mishra, Effect of rhizomes of *Curculigo orchioides* Gaertn. on drug induced hepatotoxicity. Indian Drugs, 33, 458–461 (1996).

[6] J Mehta, and K S Nama, A review on ethanomedicines of *Curculigo orchioides* Gaertn (Kali Musli): Black Gold. Int. J. Phar. & Biomedi. Res., 1(1), 12-16 (2014).

[7] A Marchica, R Ascrizzi, G Flamini, L Cotrozzi, MTonelli, G Lorenzini, C Nali, and E Pellegrini, Ozone as eustress for enhancing secondary metabolites and bioactive properties in Salvia officinalis. Industrial Crops and Products,170:113730 (2021).

[8] J C Casagrande, L F B Macorini, K A Antunes, U P D Santos, J F Campos, N M Dias-Junior, A Sangalli, C A Lima Cardoso, M do Carmo Vieira, L A Rabelo, and E J Paredes-Gamero, Antioxidant and cytotoxicactivity of hydroethanolic extract from Jacaranda decurrens leaves. PLoS One 9.11, e112748 (2014).

[9] T P T Cushnie, and A J Lamb, Antimicrobial activity of flavonoids. Inter. J. Antimi. Agents, 26(5), 343-356 (2005).

[10] B Havsteen, Flavonoids, a class of natural products of high pharmacological potency. Biochemical Pharmacology, 32(7), 1141-1148 (1983).

[11] T Wu, M He, and X Zang, A structure-activity relationship study of flavonoids as inhibitors of *E. coli* by membrane interaction effect. Biochimica et Biophysica Acta (BBA)– Biomembranes, 1828(11), 2751-2756 (2013).

[12] L E Alcaraz, S E Blanco, O N Puig, F Tomas, and F H Ferretti, Antibacterial activity of flavonoids against methicillin-resistant *Staphylococcus aureus* strains. Journal of Theoretical Biology, 205(2), 231-240 (2000).

[13] Boakye, and K Yiadom, Antimicrbial properties of cryptolepis. J. Pharm. Science, 68, 435-447 (1979).

[14] B Uday, D Dipak, and B Ranajit, Reactive oxygen species: oxidative damage and pathogenesis. Curr. Sci., 77(5), 658-665 (1999).

[15] A Venkatesan, J V R A Samy, K Balakrishnan, V Natesan, and S J Kim, In vitro antioxidant, anti-inflammatory, antimicrobial, and antidiabetic activities of synthesized chitosan-loaded p-coumaric acid nanoparticles. Curr. Pharm. Biotechnol., (2022). doi: 10.2174/1389201023666220822112923

[16] N Kahkeshani, F Farzaei, M Fotouhi, S S Alavi, R Bahramsoltani, R Naseri, S Momtaz, Z Abbasabadi, R Rahimi, M H Farzaei, and A Bishayee, Pharmacological effects of gallic acid in health and diseases: A mechanistic review. Iran J. Basic Med. Sci. 22(3), 225-237 (2019). doi: 10.22038/ijbms.2019.32806.7897.

[17] Y Godin, J Mark, and P Mandel, The effects of 4-hydroxybutyric acid on the biosysthesis of amino acids in the central nervous system. Journal of Neurochemistry, 15(10), 1085-1091 (1968).

[18] K S Sangeetha, S Umamaheswari, C U Reddy, and S N Kalkura, Flavonoids: Therapeutic potential of natural pharmacological agents. International Journal of pharmaceutical sciences and research,7(10), 3924 (2016).

[19] M R Mallu, S Vemula, and R K Kante, Molecular docking studies of bioactive compounds from Stevia rebaudiana for its anti-cancer activity. Journal of Pharmaceutical Sciences and Research, 1, 11(5), 2016-2018 (2019).

[20] D F Al-fekaiki, A K Niamah, and S T Al-Sahlany, Extraction and identification of essential oil from Cinnamomum zeylanicum barks and study the antibacterial activity. Journal of Microbiology, Biotechnology and Food Sciences, 6, 2021, 312-316 (2021).

[21] A L Gollo, V O Tanobe, G V de Melo Pereira, O Marin, S J Bonatto, S Silva, I R de Barros, and C R Soccol, Phytochemical analysis and biological activities of in vitro cultured Nidularium procerum, a bromeliad vulnerable to extinction. Scientific Reports, 24, 10(1), 1-3 (2020).

[22] Y Y Li, H Bi, D Zheng, Y Zhao, M A Ashraf, X Wang, and W X Peng, Pyrolysis Molecules of Chinese Buckeye Seed from Pharmacy Waste. Ekoloji, 26, 28(108), 99-103 (2019).