Pharmacogn. Res., 2024; 16(1):51-58.
https://www.phcogres.com

Original Article

Analysis of Phytoconstituents, Antioxidant and Anti-bacterial Properties of an Ethnobotanical Herb *Cotula anthemoides* L. Occurring in Purulia District of West Bengal, India

Ghanashyam Mahato[1,*], Rajani Kanta Mahato[1], Bangamoti Hansda[2]

[1]Department of Botany, Achhruram Memorial College, Jhalda, West Bengal, INDIA.
[2]Department of Botany, Narazola Raj College, Midnapore, West Bengal, INDIA.

ABSTRACT

Background: Since antiquity, traditional healers use herbs for the treatment of various ailments. *Cotula anthemoides* L., an ethnobotanical herb, widely used by traditional healers of Purulia district, West Bengal, India for the treatment of skin ailments. Plants contain broad range of bioactive constituents which is mainly responsible for its versatile properties. **Objectives:** The objectives of the present study are to analyze the phytochemicals and evaluation of antioxidant and antibacterial activities of the Aerial Part (AP) and underground Root Part (RT) of *C. anthemoides*. **Materials and Methods:** Total Phenol (TPC) and Flavonoid Contents (TFC) were estimated against Gallic acid and Quercetin. Antioxidant properties were evaluated by using 2, 2-diphenyl-1- picrylhydrazyl (DPPH). Antibacterial properties were examined by well-diffusion method. Secondary metabolites and fatty acids were analyzed by High Performance Liquid Chromatography (HPLC), Gas Chromatography Mass Spectrometry (GCMS), respectively. **Results:** Both aerial and root part exhibited effective zone of inhibition against gram +ve *Staphylococcus aureus* and gram –ve *Escherichia coli* bacteria. There is a positive correlation between total phenol and flavonoid content with free radical scavenging properties. Aerial part contains comparatively higher concentration of phenolic compounds which is verified by assessment of DPPH antioxidant properties. HPLC and LC-MS study revealed the presence of phenolic acids, phenol alcohols and flavonoids. The FA analysis identified the presence of saturated FAs in the range of C12 to C26 and unsaturated FAs of C16, C18, C20 and C22. Conclusion: Definite range of phenolic compounds beside with other bio active constituents in the herb keep up the ethnobotanical status done by local healers.

Keywords: *Cotula anthemoides*, Antioxidant, Anti-bacterial, Secondary metabolites, Fatty acids.

Correspondence:
Dr. Ghanashyam Mahato
Assistant Professor, Department of Botany, Achhruram Memorial College, Jhalda, West Bengal, INDIA.
Email: ghana.mahato@gmail.com

Received: 18-03-2023;
Revised: 22-08-2023;
Accepted: 22-09-2023.

INTRODUCTION

Human beings have been searching for new plant-based drugs with better therapeutic potentials from ancient period. Traditional medicines can provide the right way to the researcher to discover new plant based medicinal products against some specific ailments.[1] Herbal products have gained much importance in recent years because of their efficacy and cost effectiveness.[2] Botanicals have been used to treat human diseases because they have a wide variety of bioactive molecules such as alkaloids, terpenoids, tannins, and phenolic compounds, those have a definite physiological action on the human body.[3] Exploration of new antibacterial and free radical scavenging agents from natural sources is increasing due to the limitations of presently accessible therapy and the appearance of antibiotic resistance strains.[4] In purulia, "West Bengal, India" has a wide diversity of ethnobotanicals, not yet been investigated to establish the state made by local traditional healer.

An ethnobotanical perennial herb *Cotula anthemoides* L. belonged to the family Asteraceae, is commonly known as 'Dingla'. These plants are grown in large quantities in rocky areas of Purulia, West Bengal. The whole plant has been used by the tribal people of Purulia district for the treatment of skin diseases such as carbuncle, boil, eczema and wound from time immemorial. This plant in different name as "guertoufa", is also used in North African countries as folk medicine.[5] Several studies have reported the presence of phytoconstituents in *Cotula anthemoides*, including flavonoids, alkaloids, tannins, and saponins.[5] These compounds have been linked to different

DOI: 10.5530/pres.16.1.7

pharmacological activities, such as antioxidant, anti-bacterial, anti-inflammatory, and anti-cancer effects.[6]

Antioxidants are compounds that can neutralize harmful molecules called free radicals, which can damage cells and contribute to various diseases, including cancer, cardiovascular disease, and neurodegenerative disorders. Several studies have shown that *Cotula anthemoides* has high antioxidant activity due to the presence of flavonoids and other compounds.[5,6] Antibacterial activity is another important pharmacological property of *Cotula anthemoides.* Several studies have reported the antibacterial effects of the plant against different strains of bacteria, including *Staphylococcus aureus, Escherichia coli,* and *Salmonella typhi*. These effects have been attributed to the presence of tannins, saponins, and other compounds that can disrupt bacterial cell membranes and inhibit their growth.[5,6]

Forty-one compounds have been identified from the essential oil extracted from aerial part of different species of *Cotula corronnopifolia* L., *C. cinerea* L., including *C. anthemoides*.[5,6]

The study was selected based on the widespread use of *C. anthemoides* in ethnobotany and its availability in the district. However, no such detailed explanatory note on overall chemical features of secondary metabolities of the underground Root Part (RT) as well as Aerial Part (AP) is available. This study aims to analyze the phytoconstituents present in *Cotula anthemoides*, evaluate its antioxidant activity using different assays, and investigate its antibacterial effects against selected bacterial strains from the aerial and underground plant parts.

MATERIALS AND METHODS

Collection and authentication of plant material

Plant material (the whole plant of *C. anthemoides*) was collected from the surrounding area of Bandwan (22°52'33.6"N; 86°30'25.2"E), West Bengal, India, in July, 2018 during its flowering stage (Jul-Sep). Morphology of the collected plant was studied and was identified and authenticated by Botanical Survey of India, Kolkata and preserved as voucher number GM-08.

Extraction and Assay for Antibacterial activity

AP and RT were processed separately and thoroughly washed with running tap water and chopped into diminutive pieces and shade dried for 14 days. After drying, AP and RT were grinded individually to powder by grinding machine. Powder of AP and RT was used separately for diverse qualitative and quantitative analyses.

Preliminary qualitative test for antibacterial property were done by taking about 10 g of each AP and RT powder separately in soxhlet apparatus and was extracted using 100 mL of 9:1 aqueous ethanolic solvent at 60°C for 18 hr.

Evaluation of antibacterial activity was carried out by agar well diffusion method from the aqueous ethanolic extract of both AR and RT. Fresh bacterial cultures of 100 µL *Staphyllococcus aureus* (MTCC 3160) and *Escherichia coli* (MTCC 443) were spread on agar plates and under aseptic condition wells of 6mm diameter was punched off with sterile cork-borer followed by filled with 50 µL of above AP and RT extracts in each. The Petri-plates were then set aside under refrigeration for 15 min for pre-diffusion and then incubated at 37°C for 24 hr in inverted position. The antibacterial screening was evaluated by measuring the diameter of zone of inhibition. The experiment was done in triplicate and supported by control experiment.

Evaluation of Total Phenolic Content (TPC), Total Flavonoid Content (TFC), Antioxidant Potentialities (AOP) and Total Protein (TP)

AP and RT powder were mixed with methanolic water (9:1 v/v) and kept overnight under refrigeration. After separation of the supernatant the methanolic extract was subjected for Total Phenolic Content (TPC) analysis for both of AP and RT by Folin-Ciocalteu method. OD value was measured by spectrophotometer at 765 nm (Jasco V-630, USA) and expressed as gm of gallic acid (Sigma, USA) Equivalent (GAE) per 100 gm dry weight.[7]

Similarly, Total Flavonid Content (TFC) of the methanolic extract was measured by adding 10% aluminium chloride, 5% sodium nitrite and water in a ratio of 1:1:1:7.[8] After 25 min incubation Optical Density was measured spectrophotometrically at 510 nm (Jasco V-630, USA) and the result was expressed as gm of Quercetin (Chroma Dex, USA) Equivalent (QE) per 100 gm dry weight of Plant parts.

Antioxidant Potentialities (AOP) of methanolic extract of both the samples i.e., AP and RT were measured by the radical scavenging property of 2, 2-diphenyl-1-picrylhydrazyl (DPPH).[9] 0.16 mM aqueous methanolic (3:7 v/v) DPPH$^{\cdot}$ was mixed with the extract in 3:1 ratio and the degree of decolourisation was measured spectrophotometrically after 30 min incubation at 517 nm in a UV-spectrophotometer (Jasco V-630, USA). The IC_{50} value of the sample was calculated and expressed as the inverse of the IC_{50} value. The results were repeated thrice in all cases.

For analysis of total protein, AP and RT after soaking with 1:2:1 chloroform, methanol and water for 72 hr, chilled acetone was added to methanol phase in 4:1 (v/v) ratio for precipitation of protein. Solution was incubated at -20°C for 1 hr and then centrifuged three times at 12,000 rpm at 4°C for 15 min. The solution was concentrated by speed vac (Thermo SPD 2010) and subjected for TP analysis by using Bradford reagent and estimated against Bovine Serum Albumin (BSA) (Sigma, USA).

Analysis of secondary metabolites

AP and RT were treated with chloroform, methanol and water (1:2:1) and kept for 72 hr. Under refrigeration for lipid removal, following which the chloroform phase was discarded. Chilled acetone was mixed to methanolic phase in 4:1 (v/v) ratio for protein elimination. Then the Solution was incubated for 1 hr at -20°C and then centrifuged thrice at 12,000 rpm at 4°C for 15 min. Supernatant was subjected to High Pressure Liquid Chromatography (HPLC, Agilent, USA) for the analysis of phenolics. HPLC was attached with Zorbax SB-C_{18} column (4.6x150 mm, 3.5 micron, Agilent USA) and equipped with Photo Diod Array Detector. Gradient of two mobile phases were: methanol (A) and water with 0.02% aqueous H_3PO_4 (B) were set at: 25% A + 75% B for 5 min > 30% A + 70% B for 10 min > 45% A + 55% B for 30 min > and 80% A + 20% B for 60 min. The injection volume was 20 µL. The flow rate was kept at 0.4 mL/min and analytes were scanned at 280 nm wave length. The peaks were identified by comparing the relative retention time with proper peak integration, co-chromatography with standard and calibration against absorption spectra obtained from the authentic compounds (Sigma, USA; Chroma Dex, USA). Analytes were estimated using external method following standard validation guideline and finally the amounts of the analytes were expressed as µg/g of dry weight of sample.

Analysis of Fatty Acid Methyl Ester (FAME)

The total lipid was extracted from AP and RT following the modified method of Bligh and Dyer's.[10] The total Fatty Acids (FA) was derivatized into Fatty Acid Methyl Ester (FAME), extracted by n-hexane (HPLC grade, E. Merck, India), and analyzed by GCMS (Agilent Technologies, USA; 7890A GC system with 5975C triple axis detector MS) attached with HP5-MS (30 m x 0.25 mm x 0.25 µm) + 10m Duraguard capillary column. The MS was conditioned with ion trap at 200°C, transfer line 280°C at vacuum pressure of 2.21e-0.5 torr. The column temperature was programmed initially at 70°C with 1 min hold and ramping at a rate of 8°C/min up to 210°C with 5 min hold, and subsequent ramping of 1°C/min up to 230°C, then final ramping of 2°C/min up to 260°C. We employed He as carrier gas for GCMS, with the flow being kept at a rate of 1 mL/min. Injection temperature maintained at 250°C.

Identification of FAME was made by calculating Relative Retention Time (RRT) comparing with the authentic mixture of 37 FAME (Supelco, Lot No: LB80556, USA) as standard, and by plotting log RRT against carbon chain length. Identification was confirmed by comparing mass fragmentation pattern of the compounds from NIST (2011) data source. The amounts of the FAs were calculated as relative percentage of total FAME.

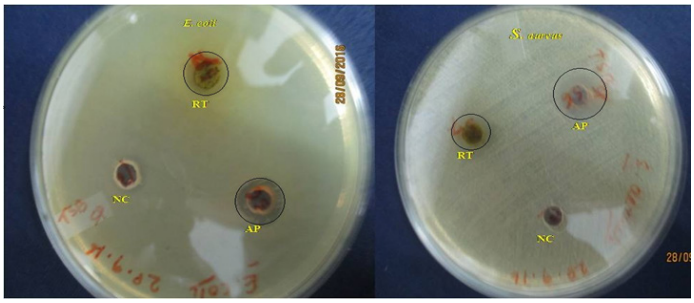

Figure 1: Plant extract showing inhibition zone against *E. coli* and *S. aureus* (RT= Root; AP= Aerial Part; NC= Negative Control).

RESULTS

Antibacterial assay and Chemical analysis of RT and AP

The zone of inhibition from the AP and RT extract was measured and expressed in Figure 1. *E. coli,* gram negative strain responded more efficiently than gm positive *S. aureus.* Total phenolic content is higher in AP (10.88 g of GA equivalent per 100 g of sample ± 0.35) than RT (3.28 g of GA equivalent per 100 g of sample± 0.19). AP (4.022 g of quercetin equivalent per 100 g ± 0.27) contains more flavonoid than RT (0.181 g of quercetin equivalent per 100 g ± 0.01). Antioxidant potential was estimated in terms of IC_{50} and expressed in mg/ml which was 0.376 ± 0.03 mg/mL in AP and 0.546 ± 0.04 mg/mL in RT. Protein content is also higher in AP (82.43 ± 0.98) µg/mL than RT (14.93±0.73 µg/mL).

Analysis of secondary metabolites

The secondary metabolite profile of CA Aerial Part (AP) revealed the presence of two phenolic acid (p-coumaric acid and ferulic acid), and one phenol alcohol (p-Cresol) whereas Root Part (RT) showed the presence gallic acid, 2,4-dihydroxybenzoic acid, p-cresol and a flavonoid quercetin as shown in Figures 2 and 3 respectively. The overall profile study thus indicated a predominance of phenolic acids over phenol alcohols and flavonoids in the sample.

Analysis of Fatty Acid Methyl Ester (FAME)

Fatty acid profile of studied plant showed variation on the presence of Fatty Acid Methyl Esters (FAMEs) and its concentrations (Tables 1 and 2). The identified FAMEs with their respective Retention time (Rt), relative area percentage and relative mole percentage were presented in Tables 1 to 2. Relative abundance of the identified fatty acids with respect to their retention time was shown in Figures 4 and 5. Fatty acid profile of *C. anthemoides* root and aerial parts reported the presence of saturated fatty acids in the range of C12 to C26 and unsaturation's of C16, C18 and C20. The concentration of C18 Poly unsaturated Fatty Acids (PUFA) were distinguishably more than that of the other unsaturated in both the plant parts. This may be as it is the precursor of many metabolic pathways of secondary metabolites for combating stress mechanisms of plants. Amongst the SFAs concentration

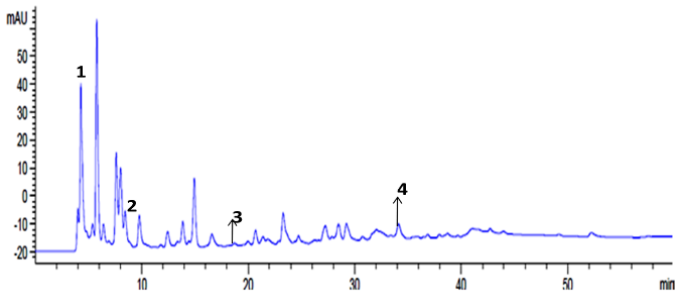

Figure 2: HPLC chromatogram of CA aerial part (AP) showing 1- p-Coumaric acid (Rt =5.801) and 2- Ferulic acid (Rt= 14.890), 3-p-Cresol (Rt= 18.443).

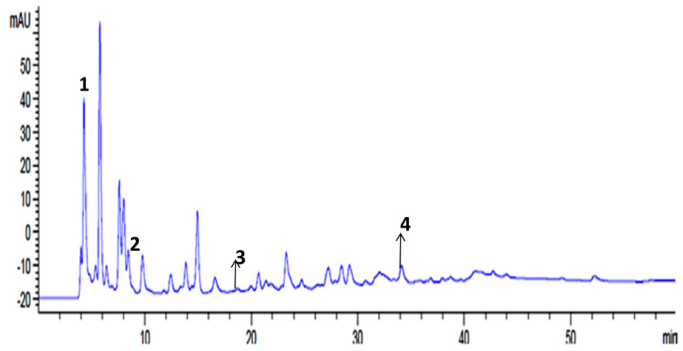

Figure 3: HPLC chromatogram of CA root (RT) showing 1-Gallic acid (Rt= 4.296), 2-2,4-dihydroxybenzoic acid (Rt= 8.101), 3- p –Cresol (Rt= 18.885), 4-Quercetin (Rt= 34.158).

C16 present in highest concentration in comparison to others (Tables 1 to 2).

DISCUSSION

Plants under the family Asteraceae are rich sources of secondary metabolites like alkaloids, phenolics, saponin, tannin, flavonoids, and lignans.[11] Due to the presence of these pytochemicals some plants of this family such as *Ageratum conyzoides, Anaphalis neegerriana* and *Blumea lacera* shows antioxidant, anti-bacterial, antimalarial, antiparasitic, anti-inflammatory and antiviral properties.[12,13]

Phytochemical analysis of roots and aerial parts give a piece of information that plant contains important secondary metabolites and exhibits anti-bacterial property against *S. aureus* and *E. coli*. AP contain higher amount of total flavonoid and phenolic contents in comparison to RT and also similarly shows higher free radical scavenging property in AP. These studies revealed the positive correlation between total phenol and flavonoid contents with antioxidant properties and corroborate the finding of some previous authors.[14] AP contains higher concentration of protein in comparison to RT.

On the basis of several reports, it has been found that phenolic compounds are known to be useful for its free radical scavenging properties due to the presence of anion radicals.[15,16] Plant extract showed free radical scavenging potentials are related to their

Table 1: Identified FAMEs from CA Aerial Part (AP) with Relative Area (RA %) and mole percentage (RMOL %).

Sl. No.	Rt	Name of the compounds	RA%	RMOL%
1	17.862	Dodecanoic acid, methyl ester (C12:0)	0.25	0.32
2	22.366	Methyl tetradecanoate (C14:0)	2.12	2.51
3	24.467	Pentadecanoic acid, methyl ester (C15:0)	0.89	0.79
4	26.044	9-Hexadecenoic acid, methyl ester, (Z) (C16:1n9)	0.24	0.23
5	26.813	Hexadecanoic acid, methyl ester (C16:0)	23.94	29.06
6	27.927	Cis-10-Heptadecenoic acid, methyl ester (C17:1n10)	0.4	0.43
7	28.408	Heptadecanoic acid, methyl ester (C17:0)	6.09	1.18
8	29.797	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (C18: 2n9)	35.88	54.21
9	29.910	9-Octadecenoic acid, methyl ester, (Z) (C18:1n9)	12.18	7.61
10	30.472	Octadecanoic acid, methyl ester (C18:0)	5.75	1.21
11	33.175	cis-11,14-Eicosadienoic acid, methyl ester (C20:2n11)	0.16	0.12
12	33.262	Cis-13-Eicosenoic acid, methyl ester (C20:1n13)	0.6	0.38
13	35.295	Heneicosanoic acid, methyl ester (C21:0)	0.46	0.26
14	37.009	Docosanoic acid, methyl ester (C22:0)	9.29	1.1
15	38.342	Tricosanoic acid, methyl ester (C23:0)	0.33	0.23
16	39.905	Tetracosanoic acid, methyl ester (C24:0)	1.68	0.55
17	43.790	Hexacosanoic acid, methyl ester (C26:0)	0.13	0.09

therapeutic potentials.[17] The presence of phenolic compounds in the extracts exhibits antioxidant properties through donation of hydrogen, forming the reduced form of DPPH. It is also found that Phenol or carbolic acid is bacteriostatic at concentrations of 0.1%-1% and bactericidal/ fungicidal at 1%-2%.[18] The results of antioxidant properties shows that plant was potently active and had properties to donate hydrogen to a free radical in order to remove odd electrons. Flavonoids also showed antioxidant activity due to the presence of their redox properties. Some flavonoids have a significant inhibitory potential against a wide array of enzymes such as phosphodiesterase, phospholipase A_2, protein tyrosine kinases and others.[19]

The fact that the methanol extracts of *C. anthemoides* exhibit bactericidal properties against *S. aureus* and *E. coli*, is a partial justification of the ethnobotanical uses of the plant against skin disease.

In DPPH assay antioxidant potentiality was measured simply by evaluating the ability of the antioxidants present in the plant extract to reduce purple coloured 1, 2- diphenyl 2-picryl hydrazyl (DPPH) radical to yellow coloured diphenyl picryl hydrazine. A higher free radical scavenging potentiality is associated with a lower IC_{50} value.[20,21] Potent antioxidant activity was observed during DPPH assay. Polyphenols are major antioxidant components present in most plant extracts.[22,23] The studied plant

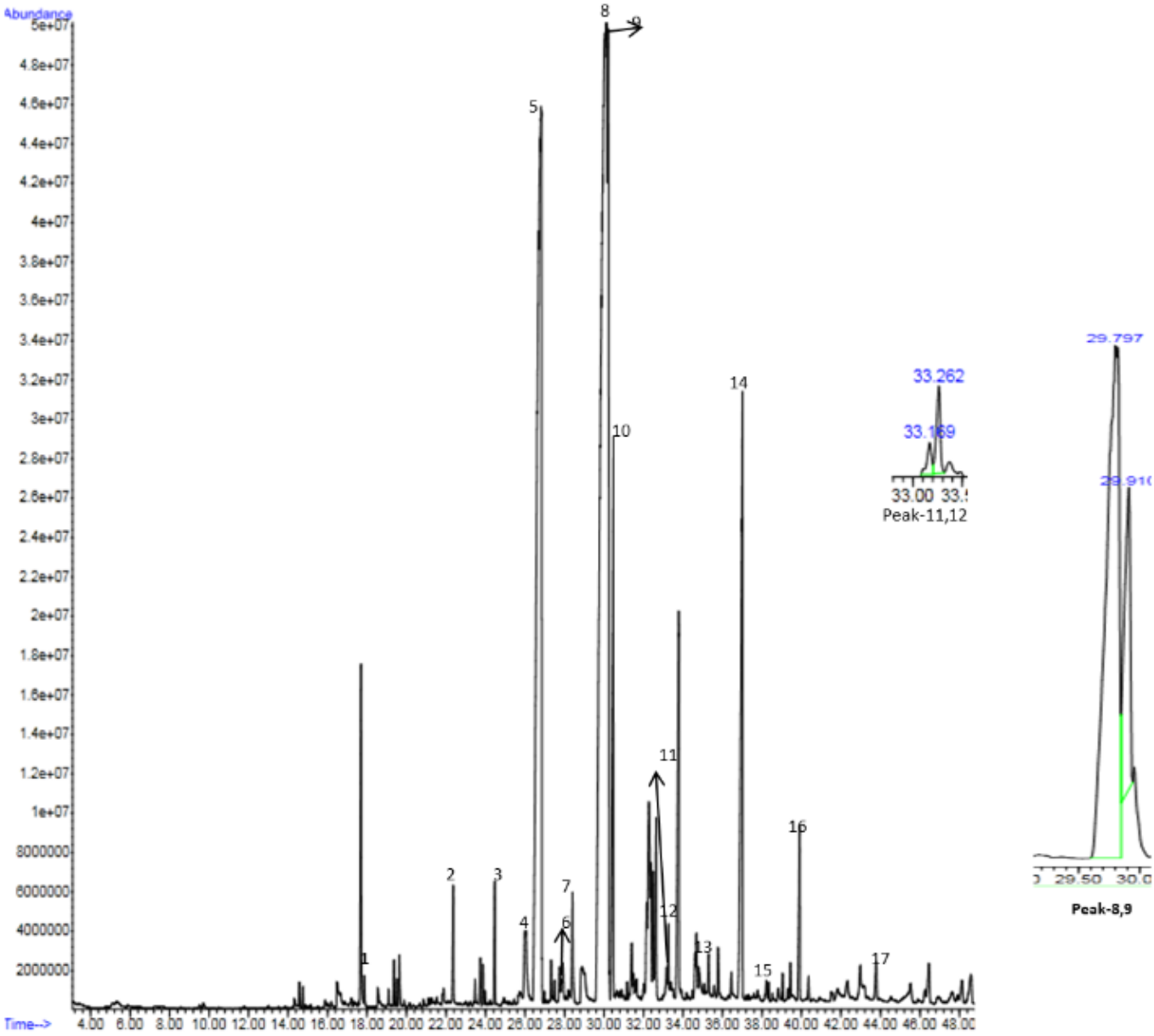

Figure 4: GC chromatogram of *C. anthemoides* Aerial Part (AP) showing all the identified fatty acid methyl esters. 1. C12:0, 2. C14:0, 3. C15:0, 4. C16:1n9, 5. C16:0, 6. C17:1n10, 7. C17:0, 8. C18: 2n9, 9. C18:1n9, 10. C18:0, 11. C20:2n11, 12. C20:1n13, 13. C21:0, 14. C22:0, 15. C23:0, 16. C24:0, 17. C26:0.

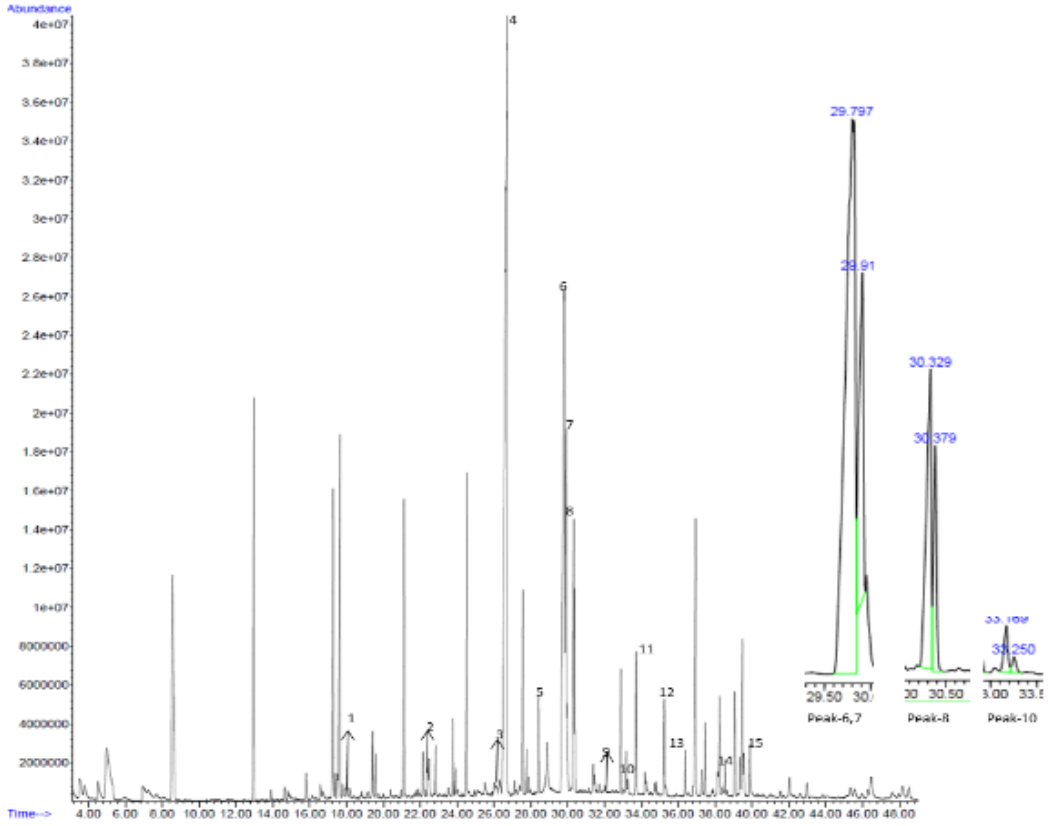

Figure 5: GC chromatogram of *C. anthemoides* Root Part (RT) showing all the identified fatty acid methyl esters. 1. C12:0, 2. C14:0, 3. C16:1n9, 4. C16:0, 5. C17:0, 6. C18: 2n9, 7. C18:1n9, 8. C18:0, 9. Octadecanoic acid, 11-methyl, methyl ester, 10. C20:1n11, 11. C20:0, 12. C21:0, 13. C22:0, 14. Methyl 21-methyldocosanoate, 15. C24:0.

Table 2: Identified FAMEs from CA Root Part (RT) with Relative Area (RA %) and mole percentage (RMOL %).

Sl. No.	Rt	Name of the compounds	RA%	RMOL%
1	17.931	Dodecanoic acid, methyl ester (C12:0)	0.44	1.11
2	22.403	Methyl tetradecanoate (C14:0)	0.5	6.94
3	26.294	9-Hexadecenoic acid, methyl ester (Z) (C16:1n9)	0.6	0.17
4	26.713	Hexadecanoic acid, methyl ester (C16:0)	45.06	53.58
5	28.421	Heptadecanoic acid, methyl ester (C17:0)	1.73	0.44
6	29.797	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (C18: 2n9)	23.42	18.68
7	29.910	9-Octadecenoic acid (Z)-, methyl ester (C18:1n9)	9.76	9.37
8	30.329	Methyl stearate (C18:0)	6.74	6.67
9	32.018	Octadecanoic acid, 11-methyl, methyl ester	0.14	0.47
10	33.256	cis-11-Eicosenoic acid, methyl ester (C20:1n11)	0.28	0.56
11	33.707	Eicosanoic acid, methyl ester (C20:0)	2.75	0.64
12	35.314	Heneicosanoic acid, methyl ester (C21:0)	0.25	0.06
13	36.934	Docosanoic acid, methyl ester (C22:0)	6.41	1.11
14	38.404	Methyl 21-methyldocosanoate	0.33	0.03
15	39.887	Tetracosanoic acid, methyl ester 9 C24:0)	1.43	0.17

extracts contain polyphenols which might be responsible for the antioxidant properties of the plant.

HPLC study tentatively identified some important phenlic acids such as p-coumaric acid, ferulic acid, gallic acid and 2,4-dihydroxybenzoic acid derivatives of some phenolic acids. These phenolic acids possess antibacterial properties against some pathogenic bacteria including *S. aureus* and *E. coli*.[24,25]

GC MS is most widely used technique for the analysis of fatty acids. Its main advantages are selectivity, sensibility and efficiency.[26] The human body can synthesize many fatty acids, except some essential Polyunsaturated Fatty Acids (PUFAs) such as the linoleic acid (C18:2n9; LA) and the α-linolenic acid (C18:3n9; ALA). C18:1n9, C18:2n9 and C18:3n9 are three predominate unsaturated fatty acids found in plants.[27] Traditional Mediterranean people showed lower rates of CVD because of their diet rich in MUFAs.[28] It has been speculated that MUFAs are cardioprotective. MUFAs may also have other health benefits. It reduces the risk of ovarian cancer by 30%.[28] PUFA in the diet contribute to reducing plasma cholesterol. Diet with enriched PUFA reduces blood pressure. The linoleic acid is the most abundant poly unsaturated fatty acid in nature, and it is the precursor of other omega-6 fatty acids. The omega-3 fatty acids are synthesized from α-linolenic acid. A balance of omega-3 and omega-6 fatty acid is essential for a proper health.[29] Free Fatty Acids (FFA) have antibacterial activity against a range of Gram-positive bacteria. Dodecanoic acid showed considerable inhibitory effect on *Staphylococcus aureus*.[30]

CONCLUSION

Studied plant shown to have evident inhibitory effect against the gram-positive *S. aureus*. In fact, the plant is active against the gram-negative facultative anaerobe, *E. coli* as well, which might point towards a broad-spectrum antibacterial activity of the plant. The studied plant extracts may contain polyphenols which might be responsible for the antioxidant properties of the plant. HPLC and GCMS identified some important phenolic compounds and fatty acids. All of these compounds might be synergistically imparting medicinal value to the plant samples.

ACKNOWLEDGEMENT

Author is greatly acknowledging University Grant Commission (ERO), Govt. of India for providing financial assistance from Project no. F.PSW-204/15-16 (ERO). Author is thankful to the Department of Botany, University of Calcutta for providing GCMS and HPLC instrumental facilities funded by Dept. of Science and Technology (FIST programme:SR/FST/LSI-459/2010 dated 10.03.2011, Govt. of India).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

RT: Root Tuber; **AP:** Aerial Part; **TPC:** Total Phenol Content; **TFC:** Total Flavonoid content; **FA:** Fatty Acid; **HPLC:** High Performance Liquid Chromatography; **GCMS:** Gas Chromatography Mass Spectrometry; **ZOI:** Zone of Inhibition; **GA:** Gallic Acid; **QE:** Quercetin; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **FAME:** Fatty Acid Methyl Ester; **RRT:** Relative Retention Time.

SUMMARY

It can be summarized that the synergistic effect of all these chemical compounds adds therapeutic property to the selected plant which is used by the ethnic community of Purulia region from time immemorial for curing carbuncle.

REFERENCES

1. Owolabi OO, James DB, Sani I, Andongma BT, Fasanya OO, Kure B. Phytochemical analysis, antioxidant and anti-inflammatory potential of *Feretia apodanthera* root bark extracts. BMC Complement Altern Med. 2018;18(1):12. doi: 10.1186/s12906-017-2070-z, PMID 29329564.
2. Peacock M, Badea M, Bruno F, Timotijevic L, Laccisaglia M, Hodgkins C, *et al.* Herbal supplements in the print media: communicating benefits and risks. BMC Complement Altern Med. 2019;19(1):196. doi: 10.1186/s12906-019-2602-9, PMID 31375101.
3. Noumedem JA, Tamokou JD, Teke GN, Momo RC, Kuete V, Kuiate JR. Phytochemical analysis, antimicrobial and radical-scavenging properties of *Acalypha manniana* leaves. Springer Plus. 2013;2:1-6.
4. Mondal M, Hossain MS, Das N, Khalipha AB, Sarkar AP, Islam MT, *et al.* Phytochemical screening and evaluation of pharmacological activity of leaf methanolic extract of *Colocasia affinis* Schott. Clin Phytosci. 2019;5(1):1-.
5. Tadrent W, Kabouche A, Touzani R, Kabouche Z. Chemotypes investigation of essential oils of "Guertoufa" herbs. J Mater Environ Sci. 2014;5(4):1200-5.
6. Djellouli M, Benmehdi H, Mammeri S, Moussaoui A, Ziane L, Hamidi N. Chemical constituents in the essential oil of the endemic plant *Cotula cinerea* (Del.) from the southwest of Algeria. Asian Pac J Trop Biomed. 2015;5(10):870-3. doi: 10.1016/j.apjtb.2015.06.007.
7. Harborne AJ. Phytochemical methods a guide to modern techniques of plant analysis. Springer Science+Business Media; 1998.
8. Goufo P, Trindade H. Rice antioxidants: phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols, γ-oryzanol, and phytic acid. Food Sci Nutr. 2014;2(2):75-104. doi: 10.1002/fsn3.86, PMID 24804068.
9. Brand-Williams W, Cuvelier ME, Berset CL. Use of a free radical method to evaluate antioxidant activity. LWT Food Sci Technol. 1995;28(1):25-30. doi: 10.1016/S0023-6438(95)80008-5.
10. Eg B. A rapid method of total lipid extraction and purification. Can J Biochem Physiol. 1959;39:911-7.
11. Patel Y, Dabgar YB, Joshi PN. Distribution and diversity of grass species in Banni grassland, Kachchh District, Gujarat, India. Int J Sci Res Rev. 2012;1(1):43-56.
12. Vijayalakshmi K, Alladi PA, Sathyaprabha TN, Subramaniam JR, Nalini A, Raju TR. Cerebrospinal fluid from sporadic amyotrophic lateral sclerosis patients induces degeneration of a cultured motor neuron cell line. Brain Res. 2009; 1263:122-33. doi: 10.1016/j.brainres.2009.01.041, PMID 19368830.
13. Ahmed FA, Rahman A, Mubassara S. Phytochemical composition, antioxidant activity and cytotoxicity of *Blumea lacera* Linn. from two different habitats. Jahangirnagar Univ J Biol Sci. 2014;3(1):37-45. doi: 10.3329/jujbs.v3i1.28276.
14. Aiyegoro OA, Okoh AI. Preliminary phytochemical screening and *in vitro* antioxidant activities of the aqueous extract of *Helichrysum longifolium* DC. BMC Complement Altern Med. 2010;10(1):21. doi: 10.1186/1472-6882-10-21, PMID 20470421.
15. Khanam Z, Wen CS, Bhat IUH. Phytochemical screening and antimicrobial activity of root and stem extracts of wild *Eurycoma longifolia* Jack (Tongkat Ali). J King Saud Univ Sci. 2015;27(1):23-30. doi: 10.1016/j.jksus.2014.04.006.
16. Pandey S. Preliminary phytochemical screening and *in vitro* antibacterial activity of *Bauhinia variegata* Linn. against human pathogens. Asian Pac J Trop Dis. 2015;5(2):123-9. doi: 10.1016/S2222-1808(14)60639-3.
17. De O. Phytochemical and vitamin content of indigenous spices of South Eastern Nigeria. J Sustain Agric Environ. 2004;6:30-4.
18. Al-Jadidi HS, Hossain MA. Studies on total phenolics, total flavonoids and antimicrobial activity from the leaves crude extracts of neem traditionally used for the treatment of cough and nausea. Beni Suef Univ J Basic Appl Sci. 2015;4(2):93-8.

19. Xie JH, Dong CJ, Nie SP, Li F, Wang ZJ, Shen MY, *et al.* Extraction, chemical composition and antioxidant activity of flavonoids from *Cyclocarya Paliurus* (Batal.) Iljinskaja leaves. Food Chem. 2015;186:97-105. doi: 10.1016/j.foodchem.2014.06.106, PMID 25976797.
20. Abid M, Yaich H, Cheikhrouhou S, Khemakhem I, Bouaziz M, Attia H, *et al.* Antioxidant properties and phenolic profile characterization by LC–MS/MS of selected Tunisian pomegranate peels. J Food Sci Technol. 2017;54(9):2890-901. doi: 10.1007/s13197-017-2727-0, PMID 28928529.
21. López-Laredo AR, Gómez-Aguirre YA, Medina-Pérez V, Salcedo-Morales G, Sepúlveda-Jiménez G, Trejo-Tapia G. Variation in antioxidant properties and phenolics concentration in different organs of wild growing and greenhouse cultivated *Castilleja tenuiflora* Benth. Acta Physiol Plant. 2012;34(6):2435-42. doi: 10.1007/s11738-012-1025-8.
22. Stuper-Szablewska K, Kurasiak-Popowska D, Nawracała J, Perkowski J. Quantitative profile of phenolic acids and antioxidant activity of wheat grain exposed to stress. Eur Food Res Technol. 2019;245(8):1595-603. doi: 10.1007/s00217-019-03262-8.
23. Mehmood A, Murtaza G. Phenolic contents, antimicrobial and antioxidant activity of *Olea ferruginea* Royle (Oleaceae). BMC Complement Altern Med. 2018;18(1):173. doi: 10.1186/s12906-018-2239-0, PMID 29866091.
24. Taviano MF, Filocamo A, Ragusa S, Cacciola F, Dugo P, Mondello L, *et al.* Phenolic profile, antioxidant and cytotoxic properties of polar extracts from leaves and flowers of *Isatis tinctoria* L. (Brassicaceae) growing in Sicily. Plant Biosyst An Int J Dealing Aspects Plant Biol. 2018;152(4):795-803. doi: 10.1080/11263504.2017.1338629.
25. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev. 1999;12(4):564-82. doi: 10.1128/CMR.12.4.564, PMID 10515903.
26. Chiu HH, Kuo CH. Gas chromatography-mass spectrometry-based analytical strategies for fatty acid analysis in biological samples. J Food Drug Anal. 2020;28(1):60-73. doi: 10.1016/j.jfda.2019.10.003, PMID 31883609.
27. Orsavova J, Misurcova L, Ambrozova JV, Vicha R, Mlcek J. Fatty acids composition of vegetable oils and its contribution to dietary energy intake and dependence of cardiovascular mortality on dietary intake of fatty acids. Int J Mol Sci. 2015;16(6):12871-90. doi: 10.3390/ijms160612871, PMID 26057750.
28. D'Alessandro A, De Pergola G. Mediterranean diet and cardiovascular disease: a critical evaluation of a priori dietary indexes. Nutrients. 2015;7(9):7863-88. doi: 10.3390/nu7095367, PMID 26389950.
29. Sokoła-Wysoczańska E, Wysoczański T, Wagner J, Czyż K, Bodkowski R, Lochyński S, *et al.* Polyunsaturated fatty acids and their potential therapeutic role in cardiovascular system disorders—a review. Nutrients. 2018;10(10):1561. doi: 10.3390/nu10101561, PMID 30347877.
30. Anzaku AA, Akyala JI, Juliet A, Obianuju EC. Antibacterial activity of lauric acid on some selected clinical isolates. Ann Clin Lab Res. 2017;5(2):1-5. doi: 10.21767/2386-5180.1000170.

Cite this article: Mahato G, Mahato RK, Hansda B. Analysis of Phytoconstituents, Antioxidant and Antibacterial Properties of an Ethnobotanical Herb *Cotula anthemoides* L. Occurring in Purulia District of West Bengal, India. Pharmacog Res. 2024;16(1):51-8.