IJPSR (2016), Vol. 7, Issue 4 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 06 November, 2015; received in revised form, 29 December, 2015; accepted, 26 January, 2016; published 01 April, 2016

IDENTIFICATION AND QUANTIFICATION OF PHENOLIC COMPOUNDS FROM *OPERCULINA TURPETHUM* (L.) SILVA MANSO LEAF BY HPLC METHOD

Anju Vijayakumari Jalaj* and Puthuparampil Madhavan Radhamany

Department of Botany, University of Kerala, Thiruvananthapuram- 695581, Kerala, India.

Key words:

Operculina turpethum, HPLC, salicylic acid, coumarin

Correspondence to Author:
Anju V. Jalaj

KSCSTE Senior Research Fellow, Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram- 695581, Kerala, India.

E- mail: anjustein@gmail.com

ABSTRACT: Plants served as potent sources of bioactive compounds since ages. The understanding of phytochemical constitution of each and every plant on earth is the preliminary and the most important step in drug discovery. As *Operculina turpethum*(L.) Silva Manso is traditionally used for various ailments, the understanding of phytochemical composition of the plant is very essential. The present study was an attempt to identify the phenolic compounds present in the leaf of the plant *O. turpethum*. Total phenol and flavonoid content was determined from the methanol extract of the leaf and the extract was subjected to HPLC analysis with seven standard compounds. A gradient mobile phase comprising acetonitrile and 0.1% phosphoric acid was used to get the phenolic profile. The quantity of each compound present in the extract was calculated from the peak area and expressed in mg/ ml of sample injected. The first mobile phase revealed the presence of compounds viz., coumarin, ferulic acid, catechin, salicylic acid, rutin, vanillin and gallic acid. Among the seven identified phenolics, coumarin was present in highest concentration (0.34mg/ml of sample injected) and vaniilin was present in least amount (0.002mg/ml of sample injected). All the compounds identified are reported to have pharmacological activities. This report is the first report on the identification of phytochemicals from the *O. turpethum* leaf.

INTRODUCTION: Plants produce amazing diversity of low molecular weight compounds through various secondary biochemical pathways, often in response to specific environmental stimuli such as herbivore-induced damage, pathogen Attacks or nutrient depravation [1, 2]. These secondary metabolites can be unique to specific species or genera and do not play any role in the plant's primary metabolic requirements [3]. They are structurally diverse and show differential occurrence among plant species. So they are used as tools in chemotaxonomic studies.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.7(4).1656-61

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.7 (4).1656-61

Based on their biosynthetic origin, plant secondary metabolites are classified into three major groups- phenolics, terpenoids and alkaloids [4]. Phenolic compounds are widely distributed groups of natural products in the plant kingdom. They range from simple, low molecular weight, single aromatic- ringed compounds to large and complex tannins and polyphenols. Based on the number of carbon atoms in the basic skeleton, they are classified as benzoquinones, naphthoquinones, stilbenes, flavonoids, norlignans, lignans and condensed tannins.

The role of phenolic compounds in plants include provide defence against a range of attacks from browsing animals, bacteria, fungi and insects, inhibit growth of competing plants, provide protection against the damaging effects of UV radiation, promote the growth of pollen tubes in the style of flowers and impart colour to the stem, leaves, flowers and fruits. The plant phenolics are viewed as potential sources of new natural drugs, antibiotics, insecticides and herbicides [5]. Phenolic

compounds act as antiviral, antitumor, biocidal and bioactive agents [6]. Qualitative and quantitative analysis of phenolic compounds found in any part of the plant is an essential prerequisite for testing efficacy of the particular plant part in treating various ailments.

Operculina turpethum (L.) is a large perennial twiner of the family Convolvulaceae. It is a rare and endangered medicinal plant of peninsular India, threatened by overexploitation [7]. It is also known as Indian Jalap and occasionally cultivated in botanical gardens as an ornamental plant. The Sanskrit name Trivrit and the Malayalam name Triputa is assigned to the plant due to the presence of three layered bark [8]. The leaf of the plant possesses antimicrobial properties [9] and fresh juice of the leaf is used for treating corneal opacity and conjunctivitis [10]. The ancient literature provides many clinical properties of the plant but they were not yet fully scientifically evaluated. As the phytochemical composition of a plant reflects biological use of the plant, understanding of the chemical profile of the plant is the very first step in any drug discovery process.

As per early reports, the leaf of the plant is rich in secondary metabolites such as flavonoids, cardiac glycosides and terpenoids [11]. But there is no report regarding the specific phenolic, terpenoid or glycosidic compounds present in the leaf. So the present work is designed to identify and quantify phenolic compounds present in leaf methanolic extract of *O. turpethum* by High Performance Liquid Chromatography (HPLC).

MATERIALS AND METHODS:

Procurement of plant material:

Operculina turpethum was collected from Pangappara, a place in Thiruvananthapuram district of Kerala, India. The material was verified and authenticated by Dr. G. Valsala Devi, Curator, Department of Botany, University of Kerala, Kariavattom and a Voucher specimen was deposited in the Department herbarium (KUBH 6987).

Preparation of leaf extract and standard solutions:

For extract preparation, leaves of the plant were collected, cleaned and shade dried for one month. The dried leaves were powdered using a blender and fine powder was used for extraction. Accurately weighed (10g) powder was continuously extracted with methanol in a soxhlet extractor for 48 hours. After extraction, the extract was concentrated on a rotary evaporator and dried to a constant weight. The weight of extract was measured and kept at 5^0C until use.

The seven standards used for the study include catechin, gallic acid, rutin, coumarin, ferulic acid, salicylic acid and vanillin. All the standard compounds were dissolved in one millilitre of HPLC grade methanol and this was passed through Whatman Nylon Membrane Filter before injecting it in the column.

Spectroscopic quantification of phenols and flavonoids:

Total phenol and flavonoid contents were estimated from the methanol extract according standard procedures [12, 13].

HPLC analysis:

The methanolic Extract and seven phenolic standards were subjected to High Performance Liquid Chromatography using 600 series HPLC pump and 2487 dual wavelength UV detector-254 and 360 nm of Waters company, USA, having Reprobond C18 column-4.6x250mm and 7725 Rheodyne injector. Sample volume of 20 microlitre each was injected in all cases and flow rate of 1.0 ml/min was maintained. The data analysis was done using Empower 2 software. The compounds were eluted by employing the following method.

Gradient elution of two solvents such as acetonitrile (solvent A) and 0.1% phosphoric acid in water (solvent B) were used for the detection of catechin, gallic acid, rutin, coumarin, Ferulic acid, salicylic acid and vanillin [14]. The total run time of the program was 30 minutes. The gradient program was begun with 85% B and was held at this concentration for the first 12 minutes. This was followed by 75% eluent B for the next 10 minutes with a linear gradient and after which its concentration was again increased to 85% B for the next 8 minutes.

RESULTS AND DISCUSSION: The total phenol and flavonoid content in the methanol extract was

determined and result is presented in **Table1**. Chromatograms of phenolic standards catechin, gallic acid, rutin, coumarin, Ferulic acid, salicylic acid and vanillin having retention times 6.873, 3.571, 20.498, 23.497, 15.787, 25.080 and 11.161 respectively were obtained using mobile gradient phase of acetonitrile and 0.1% phosphoric acid in water for 30 minutes run time when each chemical was individually analyzed. The result obtained is shown in **Fig. (1 – 7**). The chromatogram of methanol extract (**Fig.8**) showed the presence of tested standards. The quantity of each identified compound in the extract and other characters of them are presented in **Table 2.** The predominant unidentified peaks in the chromatogram of the extract need to be identified by the use of other advanced methods.

Natural phenolic compounds play an important role in cancer prevention and treatment**.** They are reported to possess antioxidant, anticarcinogenic, or antimutagenic and anti-inflammatory effects[15]. Flavonoids are known to inhibit production of heat shock proteins in several malignant cell lines, including breast cancer, leukemia and colon cancer[16].

The identified phenolic compounds were reported to possess several biological activities. Salicylic acid and its derivatives showed analgesic, antipyretic, cytotoxic and anti-inflammatory activities [17]. They are used extensively for the relief of headache, inflammation, arthritis pain, and some are employed in the treatment of heart attacks and strokes in the elderly [18]. Gallic acid and its derivatives also showed numerous pharmacological activities such as antioxidant, apoptotic and antimicrobial effects [19]. Coumarins, commonly known as benzopyrones act as antimicrobial, anti-tumoural, anti-HIV agents, CNS activators and enzyme inhibitors [20]. Ferulic acids are proved as platelet aggregation (PA)-inhibitory, tyrosinase-inhibitory, angiotensin converting enzyme (ACE)-inhibitory compounds and they exhibited superoxide dismutase (SOD)-like activities [21].

The flavonoid compound rutin is reported to possess hepatoprotective activities [22]. In addition, these compounds possess antimicrobial and antioxidant properties [23]. Catechin possess antiviral properties [24, 25]. So the present study revealed the presence of potent medicinal principles in *O. turpethum* and adds a data source for utilizing real healing potentials of the plant.

The identified phenolic compounds were reported to possess several biological activities. There are more phenolic compounds to be identified in the plant. The wide range of bioactivities reported for the identified compounds make the plant a potent source of antioxidant, antimicrobial, anticancer and hepatoprotective drugs.

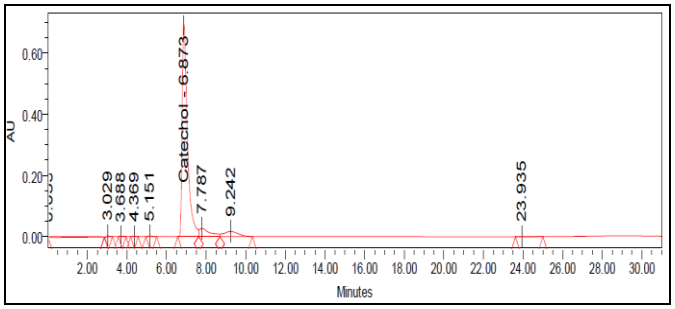

FIG.1: HPLC CHROMATOGRAM OF STANDARD CATECHIN

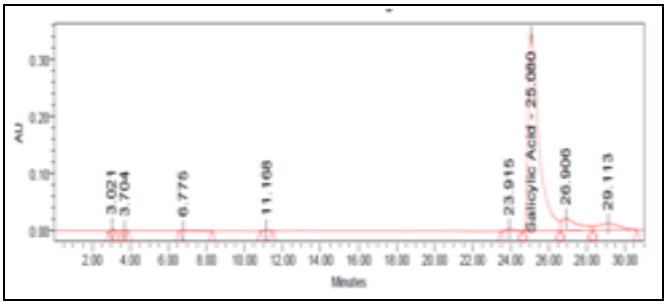

FIG. 2: HPLC CHROMATOGRAM OF STANDARD SALICYLIC ACID

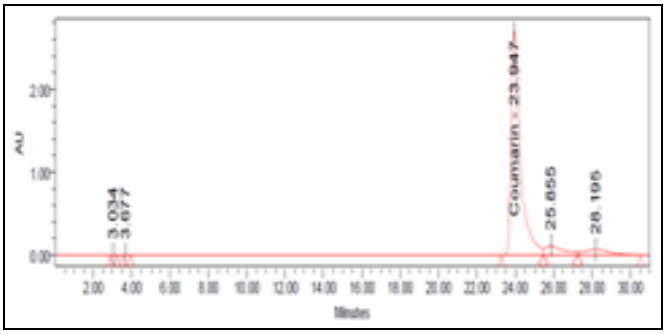

FIG.3: HPLC CHROMATOGRAM OF STANDARD COUMARIN

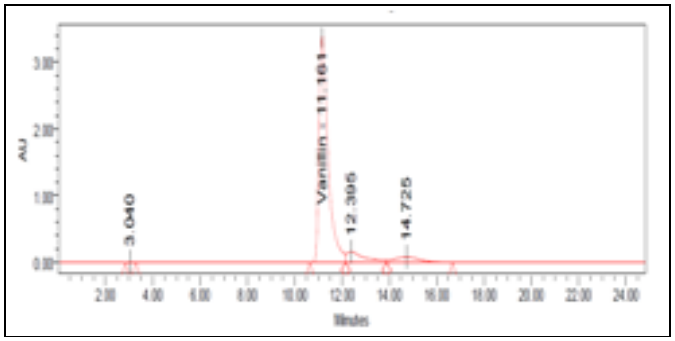

FIG.4: HPLC CHROMATOGRAM OF STANDARD VANILLIN

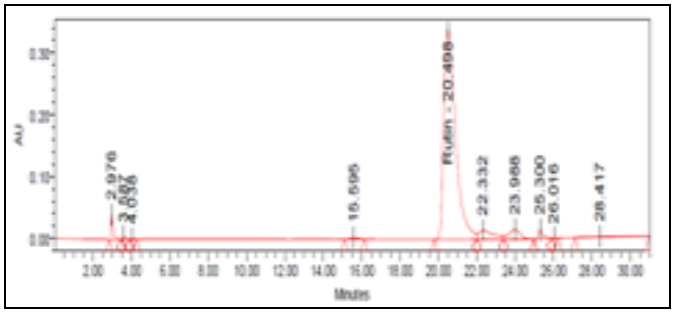

FIG. 5: HPLC CHROMATOGRAM OF STANDARD RUTIN

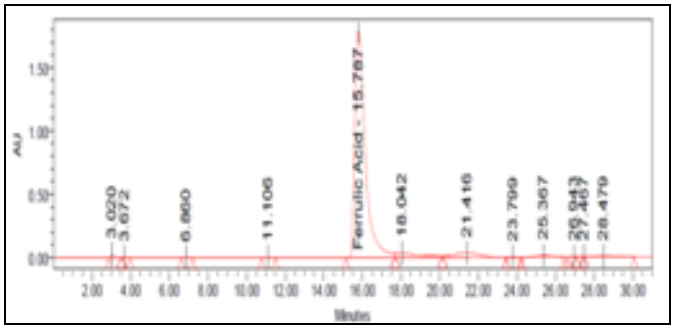

FIG.6: HPLC CHROMATOGRAM OF STANDARD FERULIC ACID

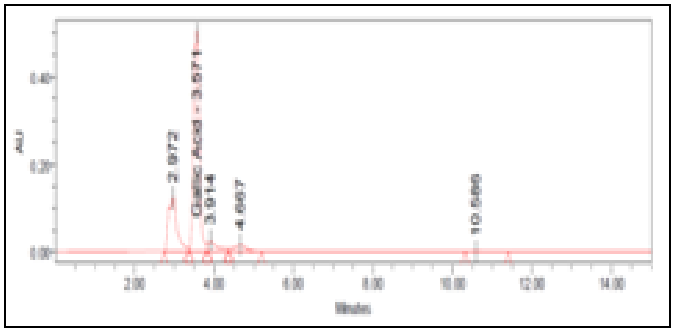

FIG.7: HPLC CHROMATOGRAM OF STANDARD GALLIC ACID

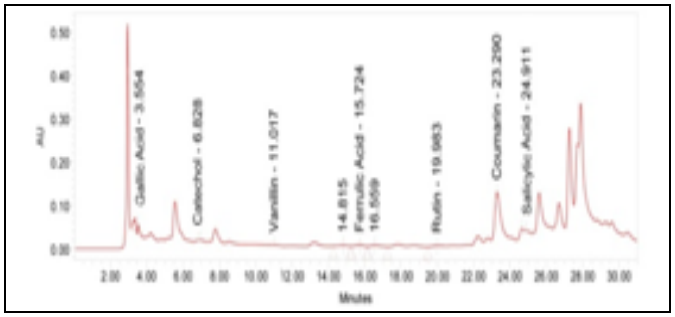

FIG. 8: HPLC CHROMATOGRAM OF METHANOL EXTRACT

TABLE 1: TOTAL PHENOL AND FLAVONOID CONTENT

Test	Value (mg/ml equivalents of standards)
Total phenol content	1.89 ± 0.01
Total flavonoid content	1.0 ± 0.00

TABLE 2: COMPOUNDS IDENTIFIED IN HPLC

Compound	Classification	RT (Sample)	Quantity (mg/ml)	Molecular formula	Molecular weight (g/mol)
Gallic Acid	Trihydroxy benzoic acid	3.554	0.15	$C_7H_6O_5$	170.12
Catechin	Flavan 3-ol	6.828	0.08	$C_{15}H_{14}O_6$	290.26
Vanillin	Phenolic aldehyde	11.017	0.002	$C_8H_8O_3$	152.15
Ferulic Acid	Hydroxy cinnamic acid	15.724	0.01	$C_{10}H_{10}O_4$	194.18
Rutin	Flavonoid glycoside	19.983	0.06	$C_{27}H_{30}O_{16}$	610.52
Salicylic acid	Monohydroxy benzoic acid	24.911	0.28	$C_7H_6O_3$	138.121
Coumarin	Phenylpropanoid	23.29	0.34	$C_9H_6O_2$	146.145

ACKNOWLEDGEMENTS: We are thankful to Kerala State Council for Science, Technology and Environment for providing fund and Bio-Medical Technology wing of Sree Chithra Thirunal Institute for Medical Science and Technology for providing lab facilities.

REFERENCES:

1. Reymond P, Weber H, Damond M and Farmer EE: Differential gene expression in response to mechanical wounding and insect feeding in Arabidopsis. Plant Cell 2000; 12: 707–719.
2. Hermsmeier D, Schittko U and Baldin IT: Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuate*, Large-scale changes in the accumulation of growth and defense-related plant mRNAs. Plant Physiology 2001; 125: 683–700.
3. Harborne JB: Introduction to ecological biochemistry. Elsevier, Fourth edition 1993.
4. Alan C, Cifford MN, Ashihara H: Plant secondary metabolites – Occurrence, structure and role in the human diet. Blackwell publishing, 2014:1-2.
5. A.Cozier, M.N. Cifford, H. Ashihara: Plant secondary metabolites – Occurrence, structure and role in the human diet. Blackwell publishing, 2014: 209.
6. Foundation for revitalization of local health and tradition, web.nchu.edu.
7. Sebastinraj J, Britto SJ and Kumar SSR: Micropropagation of *Operculina turpethum* (Linn.) Silva Manso Using Cotyledonary Node Explants. Academic Journal of Plant Sciences 2013; 6: 77-81.
8. Sivarajan VV and Balachandran I: Ayurvedic Drugs and their Plasnt Sources. Oxford and IBH publishing Co. Pvt. Ltd., 1994.
9. Alam MJ, Alam I, Sharmin SA, Rahman MM, Anisuzzaman and Alam MF: Micropropagation and antimicrobial activity of *Operculina turpethum* (syn. *Ipomoea turpethum*), an endangered medicinal plant. Plant Omics Journal 2010; 3: 40-46.
10. Balpal V: Nighantu Aadarsha. Chaukhamba Bharti Publishers, 2005.
11. Jalaj AV and Madhavan RP: Pharmacognostic Studies on Leaf of *Operculina turpethum* (L.) Silva Manso. International Journal of Advanced Research 2014; 2 (12): 585-590.
12. Malik CP and Singh MB: Plant Enzymology and Histo-Enzymology. Kalyani Publishers, 1980: 286.
13. Mervat MM, El Far, Hanan AA and Taie: Antioxidant Activities, Total Anthocyanins, Phenolics and Flavonoids Contents of Some Sweetpotato Genotypes under Stress of Different Concentrations of Sucrose and Sorbitol. Australian Journal of Basic and Applied Sciences 2009; Vol 3: 3609-3616.
14. Mradu G, Saumyakanti S, Sohini M and Arup M: HPLC Profiles of Standard Phenolic Compounds Present in Medicinal Plants. International Journal of Pharmacognosy and Phytochemical Research 2012; 4(3):162-167.
15. Huang WY, Cai YZ and Zhang Y: Natural phenolic compounds from medicinal herbs and dietary plants: Potential use for cancer prevention. Nutrition and Cancer 2010: 62: 1-20.
16. Davis WL and Matthew SB: Antioxidants and cancer III: quercetin. Alternative Medicine Review 2000; 5: 196–208.

17. Moncada S and Vane JR: Mode of action of aspirin-like drugs. Advanced Internal Medicine 1979; 1-22.
18. Rainsford KD: Aspirin and the Salicyclates. Butterworth, 1984.
19. Esteves M, Siquet C, Gaspar A, Rio V, Sousa JB and Reis S: Antioxidant Versus Cytotoxic Properties of Hydroxycinnamic Acid Derivatives - A New Paradigm in Phenolic Research. Arch. Pharm. Chem. Life. Sci 2008; 341:164–173.
20. Borges F, Roleira FMF, Santana L and Uriarte E: Simple Coumarins and Analogues in Medicinal Chemistry: Occurrence, Synthesis and Biological Activity. Current Medicinal Chemistry 2005; 12: 887-916.
21. Kayahara H, Miao Z and Fujiwara G: Synthesis and biological activities of ferulic acid derivatives. Anticancer Research 1999: 19: 3763-3768.
22. Tapas AR, Sakarkar DM and Kakde RB: Flavonoids as nutraceuticals: a review. Tropical Journal of Pharmaceutical Research 2008; 7: 1089–1099.
23. Rice ECA, Miller NJ and Paganga G: Structure-antioxidant relationships of flavonoids and phenolic acids. Free Radical Biology and Medicine 1996; 7: 933–956.
24. Cushnie TPT and Lamb AJ: Antimicrobial activity of flavonoids. International Journal of Antimicrobial Agents 2005; 26: 343–356.
25. Zandi K, Teoh BT, Sam SS, Wong, Mustafa MR and Abubakar S: Antiviral activity of four types of bioflavonoid against dengue virus type-2. Virology Journal 2011; 8: 560

How to cite this article:
Jalaj AV and Radhamany PM: Identification and Quantification of Phenolic Compounds from *Operculina Turpethum* (L.) Silva Manso Leaf by HPLC Method. Int J Pharm Sci Res 2016; 7(4): 1656-61.doi: 10.13040/IJPSR.0975-8232.7(4).1656-61.