

Development and Validation of an GC-MS Method to Quantify Phytoconstituents by Using *Entada pursaetha* DC

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Abstract Medicinal plants have rich source of secondary metabolites, which can be screened either qualitatively or quantitatively. It is an important procedure for qualifying the plant drug and their potentialities. The active phytoconstituents present in a plant reflect the therapeutic values of medicinal species. In order to discover a new bioactive compounds from plant resources which could become new leads or new drugs, plant extracts should be simultaneously evaluated by appropriate screening methods by either biological or pharmacological targets. For the extraction procedures, solvent extraction is commonly employed for extraction of most active principles from the plants. *Entada sp.* showed more than 27 different components of phytochemicals with a rich source of polysaccharides whereas polysaccharides only have major drug potential which was carried out by GC-MS analysis. In the recent studies, the bioactive components of seed of *Entada pursaetha* using GC-MS were investigated using Perkin-Elmer Gas Chromatography-Mass Spectrometry, while the Mass spectra of the compounds found in the extract was matched with previous literature.

Keywords Stem, Leaf, Compounds, Retention Time, Chemical Formula, GC-MS

1. Introduction

Medicinal plants are being the main resource of secondary metabolites which can be used as potent drugs. They have been an important source of medicine for curing innumerable ailments since thousands of years. Mainly old traditional remedies as world history of wisdom, they had been used as popular folk medicines (Sathyaprabha *et al.*, 2010). Based on the *in-vitro* screening of phytochemical

method has supported to conceive the preliminary knowledge to elect crude plant extracts with useful properties for future studies and analysis research towards secondary phytochemical and pharmacological investigations (Mathekaga and Meyer, 1998).

Kalimuthu and Prabakaran (2013) reported that the medicinal plants are increasingly valuable importance for extracting principal resources of nutraceuticals, traditional medicines and food supplements. The official values of medicinal plants are depending on their bioactive phytoconstituents. These phytoconstituents are being identified in medicinal plants can be used to cure many diseases and disorders and can be studied for their definite physiological activity in the human body. The isolation, identification and screening of chemical constituents present in herbal medicines have been of great interest to the scientists for discovering novel therapeutic drugs, and the effective in remedy for several diseases (Sylvestrea *et al.*, 2006).

Currently, Gas Chromatography-Mass Spectrometry (GC-MS) has become firmly established as a key technological platform for secondary metabolite profiling in both plant and non-plant species (Robertson, 2005). More recently, detailed studies on phytochemical constituents and their pharmacological properties of *E. africana* have been elucidated by Yusuf and Abdullahi (2019). *Entada phaseoloides* is an important medicinal species in China and Taiwan, which has been detected the two new derivatives of Dihydroxyphenylacetic acid (Chen *et al.*, 2013) and phenolic acid glucosides (Singh *et al.*, 2011). *Entada abyssinica* is also having rich sources of phenolic saponins and antioxidant glycosides which have been carried out antimicrobial and antioxidant properties (Teke *et al.*, 2011). The study revealed major bioactive compounds present in the plant part extract. Identification of these compounds in the plant serves as the basis in

determining the possible health benefits of the plant leading to further biologic and pharmacologic studies (Denwick, 2002).

2. Materials and Methods

2.1. Collection of Plant Material

The entire herb of *Entada pursaetha* DC plant was collected from the nearby area of Kollihills, Namakkal District fields (Tamil Nadu). The shade dried plant parts of *E. pursaetha* were pulverized to powder in a mechanical grinder. The required quantity of powder was weighed and extracted with a standard procedure.

2.2. Preparation of Extracts

A portion of dried aerial parts (100 g) of *Entada pursaetha* DC was placed in a soxhlet apparatus. Extraction was performed with 700 ml of methanol for 48 hours at a temperature not exceeding the boiling point of the solvent. The collected extract was filtered through a 45µm filter (Hsouna *et al.*, 2011). The filtered solute was concentrated in vacuum to dryness to give methanol extract. And then, the extract was stored in a refrigerator at 4 °C for further use and the sample extract was then subjected to GC-MS analysis.

2.3. GC-MS Analysis

Gas-Liquid Chromatography coupled to Mass Spectrometry (GC-MS) was performed to identify the chemical constituents of essential oils (Yang *et al.*, 2007). For that, the Clarus 680 GC was used in the analysis, a fused silica column packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane) (30 m x 25µm film thickness) and the individual component was separated by using Helium as carrier gas at a constant flow of 1 ml/min. The 1µL of essential oil injected into the instrument the oven temperature was as follows: Initial temperature 60 °C for 2 min, ramp 10 °C/min⁻¹ to 300 °C, hold 6 min, Injection auto temperature 260 °C; and Split injection flow ratio, 10:1. The mass detector Conditions were: transfer line temperature 240 °C and ion source temperature were 240 °C; and ionization mode electron impact at 70 eV, with a scan time 0.2 sec and scan interval of 0.1 sec the fragments from 40 to 600 Da. In this, one micro litre of the samples was loaded for the analysis. The sample was detected at different retention time by The South Indian Textile Research Association (Coimbatore, India). The retention factors in

the spectrum were then compared with the library.

2.4. Identification of Phytocomponents

Interpretation on mass-spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The name of phytocompounds, molecular weight, and percentage of distribution of the test materials were tabulated.

3. Results

Gas Chromatography-Mass Spectrometry (GC-MS) is a combined technique which is used for identifying different compounds or biochemical substances sample extracts. It works on the separation of the individual compound by gas chromatography (GC). According to their retention time (RT), the separated compounds were further analyzed at a molecular level by mass spectrometry (MS). The GC-MS analysis revealed the presence of phytochemical constituents from which some are higher hydrocarbon alkanes, ester, terpenes, flavonoids, organic compounds, steroids, and fatty acids.

The GC-MS Chromatogram (**Fig. 1**) showed 7 distinct peaks on the basis of RT and each peak depicted a phytocompound with specific molecular weight in leaf extract of *E. purseatha*. It can be identified along with their chemical structure MS spectrum. The name of phytochemical compounds, molecular formula, molecular weight, probability percentage at retention time (RT), and corresponding peak area % were listed in **Table 1**. The compounds are identified by the GC-MS analysis and their chemical formula viz, Triethoxysilanol (C₆H₁₆O₄Si), Benzoic acid, 2-hydroxy-, methyl ester (C₈H₈O₃), 2-nitro-1,3-propanediol (C₁₀H₁₆), 1,1-Difluoro-2-(2,4-difluorophenyl)-1-(2-quinolinyl)-3-(1,2,4-triazol-1-yl)-2-propanol (C₂₀H₁₄F₄N₄O) Hexadecanoic acid (CAS) (C₁₆H₃₂O₂), Octadecanoic acid (C₁₈H₃₆O₂), 2,5-bis(4'-Methoxynaphthyl)thiophene (C₂₆H₂₀O₂S) respectively. The major compounds observed namely Benzoic acid and 1,1-Difluoro-2-(2,4-difluorophenyl)-1-(2-quinolinyl)-3-(1,2,4-triazol-1-yl)-2-propanol. Other 5 compounds are trace in amount of distribution such as 2-hydroxy-, methyl ester (CAS), 2-nitro-1,3-propanediol, Hexadecanoic acid (CAS), Octadecanoic acid, and 2,5-bis(4'-Methoxynaphthyl) thiophene.

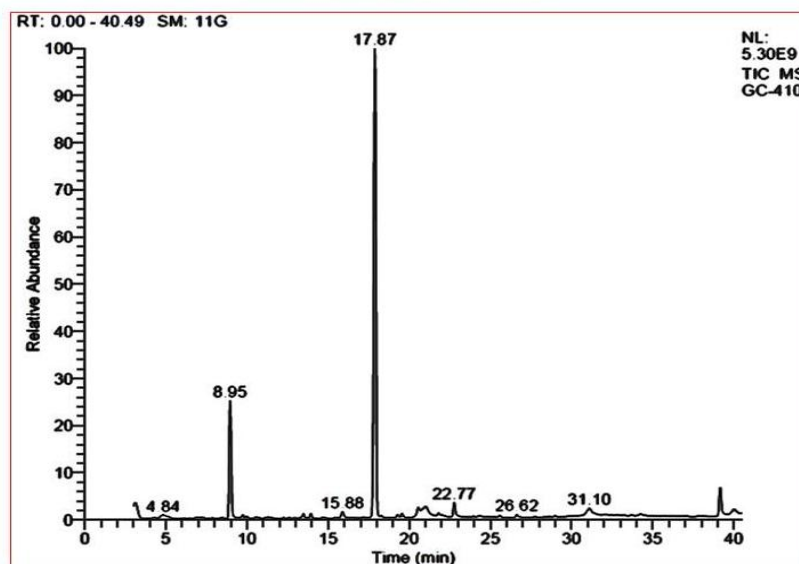


Figure 1. GC-MS Analysis of Ethanolic Leaf Extract of *Entada pursaetha*

Table 1. Phyto-Chemical Constituents from ethanolic extract of leaf and stem of *E. pursaetha*

S.No	RT	Name of Phyto-Chemical Constituents		Molecular Formula	Molecular Weight	Area%
1	4.84	Leaf Extracts	Triethoxysilanol	C ₆ H ₁₆ O ₄ Si	180	1.23
2	8.95		Benzoic acid,2-hydroxy-,methyl ester	C ₈ H ₈ O ₃	152	14.4
3	15.88		2-nitro-1,3-propanediol	C ₁₀ H ₁₆	121	0.23
4	17.87		1,1-Difluoro-2-(2,4-difluorophenyl)-1-(2-quinolinyl)-3-(1,2,4-triazol-1-yl)-2-propanol	C ₂₀ H ₁₄ F ₄ N ₄ O	402	62.8
5	22.77		Hexadecanoic acid (CAS)	C ₁₆ H ₃₂ O ₂	256	1.75
6	26.62		Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284	0.57
7	31.10		2,5-bis(4'-Methoxynaphthyl)thiophene	C ₂₆ H ₂₀ O ₂ S	396	1.78
8	4.84	Stem Extracts	(+)-trans-Ethoxy-2-(prop-1-ynyl) cyclohexane.	C ₁₁ H ₁₈ O	166	0.90
9	8.96		Benzoic acid, 2-hydroxy-methyl ester.	C ₈ H ₈ O ₃	152	16.40
10	15.95		6-Hepten-3-one, 4-methyl.	C ₈ H ₁₄ O	126	0.68
11	17.91		24-Oxalisol A.	C ₂₉ H ₄₈ O ₅	476	63.25
12	21.78		Thiophene, tetrahydro-2-methyl.	C ₅ H ₁₀ S	102	4.54
13	25.57		Eicosanebioic acid, dimethyl ester.	C ₂₂ H ₄₂ O ₄	370	0.38
14	32.80		Hexadecanoic acid, 2-hydroxy-1(hydroxymethyl) ethylester.	C ₁₉ H ₃₈ O ₄	330	0.23

Among all the extracts were identified only limited number of phytochemicals from GC-MS profiling. Whereas only one phytocompound such as Benzoic acid methyl ester exhibited the common occurrence in all selected plant part extracts and is also a major constituent of all samples. Other major compounds are diverse nature of nitrogenous compound, some monoterpenes and diterpenes in trace to considerable percentage. The overall GC-MS analysis did not show that much varied the metabolite composition in *E. pursaetha* which stored

unique metabolite which are responsible for their medicinal and pharmacological properties. The major compounds from *E. pursaetha* observed namely (+)-trans-Ethoxy-2-(prop-1-ynyl) cyclohexane (C₁₁H₁₈O), Benzoic acid, 2-hydroxy-methyl ester (C₈H₈O₃), 6-Hepten-3-one, 4-methyl (C₈H₁₄O), 24-Oxalisol A (C₂₉H₄₈O₅), Thiophene, tetrahydro-2-methyl (C₅H₁₀S), Eicosanebioic acid, dimethyl ester (C₂₂H₄₂O₄) and Hexadecanoic acid, 2-hydroxy-1(hydroxymethyl) ethylester (C₁₉H₃₈O₄) (Fig. 2 & Table 2).

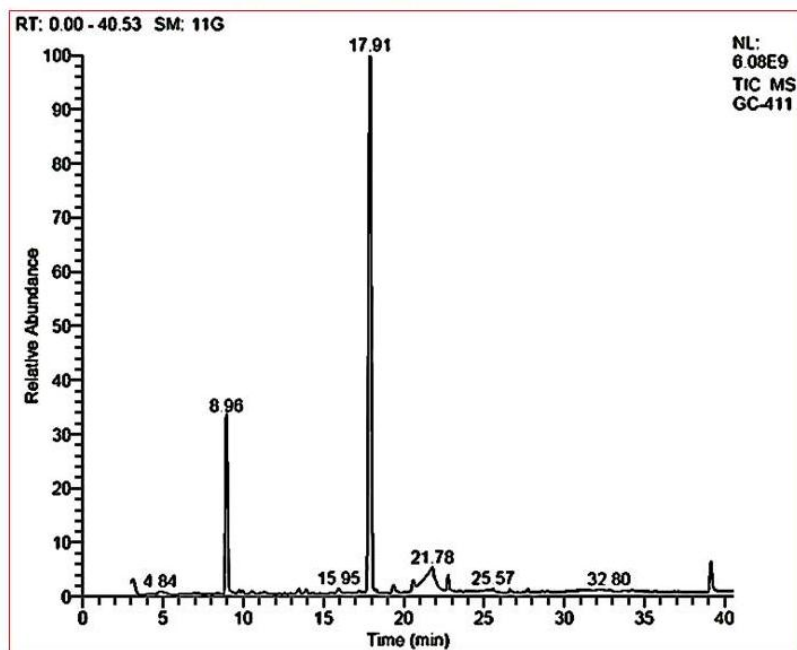


Figure 2. GC-MS Analysis of Ethanolic Stem Extract of *Entada pursaetha*

Table 2. Comparison of the Phyto-Chemical Constituents from ethanolic extract of leaf and stem of *E. pursaetha*

S.No	Name of Phyto-Chemical Constituents	Molecular Formula	References
1	Triethoxysilanol	C ₆ H ₁₆ O ₄ Si	Shalini <i>et al.</i> , 2016
2	Benzoic acid, 2-hydroxy-, methyl ester	C ₈ H ₈ O ₃	Kalpna <i>et al.</i> , 2012
3	2-nitro-1,3-propanediol	C ₁₀ H ₁₆	Björn and Alexander, 2011
4	1,1-Difluoro-2-(2,4-difluorophenyl)-1-(2-quinolinyl)-3-(1,2,4-triazol-1-yl)-2-propanol	C ₂₀ H ₁₄ F ₄ N ₄ O	-
5	Hexadecanoic acid (CAS)	C ₁₆ H ₃₂ O ₂	Radha <i>et al.</i> , 2016
6	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	Altameme <i>et al.</i> , 2015
7	2,5-bis(4'-Methoxynaphthyl)thiophene	C ₂₆ H ₂₀ O ₂ S	-
8	(+)-trans-Ethoxy-2-(prop-1-ynyl) cyclohexane.	C ₁₁ H ₁₈ O	Karthikeyan & Dhanapal, 2016
9	Benzoic acid, 2-hydroxy-methyl ester	C ₈ H ₈ O ₃	Zhang & Zhou, 2011
10	6-Hepten-3-one, 4-methyl	C ₈ H ₁₄ O	Schmeda Hirschmann, 2014
11	24-Oxoalisol A	C ₂₉ H ₄₈ O ₅	Wagner <i>et al.</i> , 2002
12	Thiophene, tetrahydro-2-methyl	C ₅ H ₁₀ S	Thamburaj <i>et al.</i> , 2013
13	Eicosanebioic acid, dimethyl ester	C ₂₂ H ₄₂ O ₄	Ponnaiah & Yokeswari, 2018
14	Hexadecanoic acid, 2-hydroxy-1(hydroxymethyl) ethylester	C ₁₉ H ₃₈ O ₄	Esen & Bilal, 2018

4. Discussion

Entada pursaetha is rich sources of phytochemical and it has been elucidated from the GC-MS analysis of the plant part extracts that enlisted about 20 different phytomolecules with varied proportions (Tables 1 – 2). The major compounds are observed two in each plant part extracts and similar observation was made from the same plant seed extract (Kalpna *et al.*, 2012; Bhogireddy *et al.*, 2015). Benzoic acid is major phytochemical reported in the

current study. Whereas in allied species of *E. africana* is also noted the same compound but due to geographical isolation and toptotype of the species may varied the percentage of proportions of active constituents (Dong *et al.*, 2012; Singh *et al.*, 2011). Various parts of the *E. africana* was reported different chemicals such as polysaccharides (Diallo *et al.*, 2001), betulin (Kwaji *et al.*, 2018), saponins (Yusuf *et al.*, 2019) and glycosides (Kwajiet *et al.*, 2017). However, in *E. pursaetha* needs further study for extraction, isolation and characterization of

tagged phytomolecules with specific geographical chemotype. *E. rheedii* is conspecific to *E. pursaetha* even though many researchers worked on that plant species and reported several chemical components which are active in antimicrobial, antioxidants, antiproliferative and other pharmacological properties (Nzowa *et al.*, 2009; Chen *et al.*, 2013).

Entadamide-A is a phenolic terpenoids isolated from the seeds of *E. rheedii* (Sufian *et al.*, 2015) which has been investigated for biological activities. The seed extract of present results showed the corresponding chemical moiety such as terpenoid derivatives obtained from GC-MS (Table 1). Many other studies are also noted the phytochemical profile of the *Entada* species and the phytochemicals are corresponding the pharmacological activities like antimicrobial and antioxidants (Rahman *et al.*, 2013; Nathan, 2017). Nzowa *et al.*, (2013) have been reported the tryptophan derivatives in seed kernel of *E. rheedii*. The present results are also on par with above investigation that has been enumerated many pyridine and purine compounds in *E. pursaetha* plant part extract (Tables 1-2).

The GC-MS results obtained from the study outlay on interesting with narrow array of phytochemicals. *Entada pursaetha* possess a good store of biochemical constituents which provides the basis of medicinal potential. The combined effects of those secondary compounds are responsible for the medicinal and therapeutic values. The prime phytochemical potential indulged in the *E. pursaetha* has been successfully explored as an outcome of this study.

5. Conclusions

The present findings were carried out to determine the possible bioactive components phytochemical of leaves and stem of *E. pursaetha* using GC-MS. However, isolation of individual phytochemical constituents of leaves and stem of *E. pursaetha* using GC-MS and subjecting it to the biological activity will be definitely giving fruitful results and will open a new area of investigation of individual components and their pharmacological potency. From these results, it could be concluded that *E. pursaetha* contains various bio-active compounds. Evaluation of pharmacological activity is under progress. The phytochemicals detected were the higher hydrocarbon alkanes, ester, terpenes, flavonoids, organic compounds, steroids, and fatty acids. A few numbers of fatty acids were polyunsaturated and saturated fatty acids such as Tetradecanoic acid, n-Hexadecanoic acid. Therefore, it is recommended as a plant of phytopharmaceutical importance.

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