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Quantitative Estimation of Minerals, Secondary Metabolites and GC-MS Profile of *Chomelia asiatica* Linn. Root

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Abstract

The ethanolic root extract of *Chomelia asiatica* was found to contain macroelements such as total nitrogen, phosphorus, potassium, calcium, sodium and magnesium contents. Total nitrogen content was recorded maximum. Microelements test revealed the presence of iron content only. Manganese, zinc, selenium, cobalt and boron were not recorded. Secondary metabolites such as total phenolic substance, tannin content and total flavonoid substance were recorded during quantitative estimation. The phenolic substance was found to be elevated. Flavonoid and tannin substances were less than the phenol compounds. Analysis through Gas Chromatograph-Mass Spectrometry identified 19 phytocomponents. 2',4',6'-Trideutero-2,4,6-trimethylbenzaldehyde was found to be maximum with 8.63 peak area percentage at 31.40 retention time.

Keywords: Chomelia, GC-MS Analysis, Minerals, Secondary Metabolites

1. Introduction

The quest for good health and immortality has been a continuous human endeavour since the beginning of civilization throughout the World. In all ages and civilizations, man's dependence on plants for food and medicine was well chronicled. Plants have persistent to participate a superseding role in preserving and safeguarding the human health since prehistoric times. Human beings are suffering from illness and diseases. The search for relief from ailments prompted them to explore their surroundings for a remedy. As a result they started to use various natural hidden agents of the plants, as they were being forefront. Majority of plants are collected from the forests that are the principal repositories of herbal plants. The herbal medicines are beginning to find their due place and recognition in society, which they rightly deserved due to the presence of array of chemical compound [1, 2]. Hence, the current research was taken up to estimate minerals, secondarily considered metabolic substances and GS-MS profiling in the root of a sporadic medicinal plant, *Chomelia asiatica* (L.) Okze, as it is traditionally used for skin diseases [3–5] by the folk healers of Kanjamalai.

2. Materials and Methods

2.1 Identification, Authentication and Seed Collection

A sporadic plant, *Chomelia asiatica* available at Kanjamalai, Salem district claimed to cure skin diseases was selected for the present investigation and it was identified using Floras and legitimated by Botanical Survey of India, Coimbatore. Matured seeds were collected and allowed to germinate and continued to grow in a pot up to one year. At the closing stages of the year roots were unruffled, shadow desiccated and powered and used for the analysis of macro and microelements, secondary metabolites and subjected to GC-MS analysis.

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2.2 Estimation of Minerals

2.2.1 Total Nitrogen

Total nitrogen content [11] was estimated using the microkjeldahl method that mentioned below.

Digestion Procedure- Fresh materials were oven dried and ground to 40-mesh size. The powdered substance was again at 70°C oven dried before digestion. 100mg of oven dried powdered material was taken in a microkjeldahl flask. To that 100mg of catalyst (copper sulphate:potassium sulphate:selenium dioxide 11:9:1), 4ml of Conc. H_2SO_4 and 4ml of hydrogen peroxide were added. Digestion was carried out by keeping the sample on a hot plate until a clear colourless solution obtained. After digestion, the clear colourless solution was prepared up to 25ml using distilled water.

Estimation- Aliquots of the digest in 5ml were taken into the distillation unit and 10ml. of 40% NaOH was added. Distillation was carried out for 20 minutes. 5ml of boric acid, indicator mixture, was taken in a conical flask and kept at the receiving end of the distillation unit. The ammonia liberated was absorbed by the boric acid indicator mixture kept in conical flask. The indicator solution was turned into pink in the beginning and became green at the end of complete distillation. The content (green in colour) was titrated against N/100 HCl, until pink colour reappeared. The quantity of nitrogen available in the test material was calculated considering that 1ml of N/100 HCl = 0.14 mg of nitrogen.

The other macro and micro nutrient analysis were carried out using Atomic Absorption Spectrophotometer (Model ECIL AAS 4127). The contents of Phosphorus (P), Potassium (K), Sodium (Na), Manganese (Mn), Magnesium (Mg), Iron (Fe), Zinc (Zn), Selenium (Se), Cobalt (Co) and Boron (B) Calcium (Ca), were analyzed with the respective cathode lamp [17].

2.2.2 Quantification of Phenolic Substance and Tannin Substance

Aliquots in ten microlitres (10 mg/2 ml) were taken in glass tubes. Using distilled water, the aliquots were prepared up to the quantity of 1 millilitre. Further added 0.5 millilitre of Folin-Ciocalteau phenol substance and sodium carbonate solution (20%) of 2.5 millilitre in each tube one after the other. The rejoinder potion was allowed to vortex. At once the glass tubes were positioned in gloomy place for 40 minutes and the absorbance was read at 725 nm against the reagent blank. The study was worked out in triplicate and the outcome was uttered as tannic acid equivalent [15].

The tannin substance was predictable after treating with the chemical polyvinyl polypyrrolidone [16] using the same extract. Weighed 100 milligram of Polyvinyl polypyrrolidone was taken in a 100 x 12 millimeter glass tube and supplemented with 1 millilitre distilled water and 1 ml of the test extract. The substance was vortexed and set aside incessant in the test tube at 4°C for 4 hrs. Then the test extract was subjected to centrifugal force (at 3000 rpm for ten minutes at laboratory warmth) and the top solution leaving the sediment was gathered. This top solution has only plain phenolics other than tannin substance (In the company of the chemical polyvinyl polypyrrolidone, the tannin substance would have been precipitated). The phenolic substance of the top solution was intended and uttered on a dry matter basis as the substance of tannin less phenolics. On getting above domino effect, the tannin substance of the test material was intended as below:

Tannin (%) = the full quantity phenolic content (%) – tannin less phenolic content (%)

2.2.3 Quantification of Flavonoids

Aliquot (appropriately 10 mg/2 ml) of diluted sample solution in 0.5 ml was assorted with two ml of purified water and consequently with 0.15 ml of five per cent sodium nitrite solution. After 6 minutes, 0.15 ml of ten per cent aluminium chloride solution was supplemented. This was allowed to set for 6 minutes and afterward 2 ml of four per cent sodium hydroxide solution was supplemented to the mixture. To fetch the final quantity to five ml, subsequently, glass distilled water was poured gently. After that the concoction was methodically assorted and allowed to position for an additional fifteen minutes. The blend was resoluted at 510 nanometer against water as blank. The outcomes were expressed as rutin equivalent [18].

2.3 Gas Chromatography/Mass Spectrometry Technique

C. asiatica root ethanol haul out at 2 µl was subjected to GC/MS technique.

2.3.1 Instrumentation Technique and Conditions of Chromatography

Using Gas chromatography clarus 500 Perkin Elmer system comprise a AOC-20i auto sampler, GC-MS investigation

was conceded. Gas Chromatograph interfaced to a Mass Spectrometer device is working under following environment: Column Elite-1 fused silica capillary column (30 × 0.25 mm ID ×1EM df, composed of 100% Dimethyl poly siloxane), functioning in electron force sort at 70 eV. Carrier gas Helium (99.999%) at a steady flow of 1ml/minute was used. An injection quantity of 0.5 EI was set off (10:1 split ratio. The temperature of the injector and ion-source was respectively at 250°C and at 280°C. The temperature of oven was considered from 110°C (isothermal for 2 minutes), with an enhance of 10°C/minute, up to 200°C/minute, then 5°C/minute up to 280°C/minute and concluding at 280°C with a 9 minutes isothermal conditions. At 70 eV, mass spectra were resolved with fragments from 40 to 550 Dalton and a scan gap of 0.5 second [12].

3. Experimental Results and Discussion

Macroelements contents such as total nitrogen, phosphorus, potassium, calcium, sodium and magnesium were found to be present in the root sample of *C. asiatica* (Table 1). The root sample analysis exhibited the presence of iron as an only microelement. Other minerals were found to be absent. Nitrogen is a fundamental component of protein and nucleic acids and many other organic molecules, which play an imperative role in plant life. Being essential for the construction of protoplasm, which is predominantly proteinaceous, the insufficiency of nitrogen inhibits cell division and cell magnification. In the present study, the nitrogen content was maximum in the root sample. Similar information was reported in *Faramea occidentalis* [14] and in *Pavetta indica* [13]. Phosphorus is an indispensable constituent of lipoprotein membranes of the cell nucleoproteins, organic molecules and many coenzymes. Calcium is crucial for neuromuscular mechanism [3]. Many enzymes would function efficiently in the presence of inorganic activators like Ca++, Mn, Mg and Cl, as they actively take part in the enzyme substrate complex [6].

Table 1. Analysis revealed the presence of macro and microelements in the root sample of *C. asiatica*

S. No.	Macroelements	mg/100gm root	Microelements	mg/100gm root
1	Total Nitrogen	1025.0	Iron	58.6
2	Phosphorus	408.0	Manganese	–
3	Potassium	134.0	Zinc	–
4	Calcium	159.0	Selenium	–
5	Sodium	28.9	Cobalt	–
6	Magnesium	56.7	Boron	–

The root sample contains phenolic substance, tannins and flavonoid substance (Table 2). Phenolic substance is of vast significance as cellular sustaining resources. They figure a central and vital part of cell wall structures. By means of this formulation, plants become modified to terrestrial life by edifying inflexible organs [7]. The occurrence and amount of phenolics in Rubiaceae plants was well documented by many scientists [8–10]. Tannins, the plant polyphenols are usually soluble in water and precipitate protein from aqueous solutions, located in vacuoles. This cluster of compound have established an enormous deal of concentration in current years, as it is optional that the utilization of tannin include beverages; particularly, green tea and red wines can alleviate otherwise thwart a diversity of illness. Flavonoids are well known phytochemicals having sound biological effects such as free radical scavenge activity, intonation of enzymatic activity and prospective utility as antibiotics and anti-inflammatory agents.

Table 2. Phytochemicals in ethanol root extract of *C. asiatica*

Test material	Total phenolic content (mg TAE/g root extract)#	Tannin content (mgTAE/g extract)#	Total flavonoid content (mg RE/g root extract)#
Root	75.3±3.1	55.2±2.4	09.1±0.1

Values are given in Mean±SE based on triplicates maintained in each test.

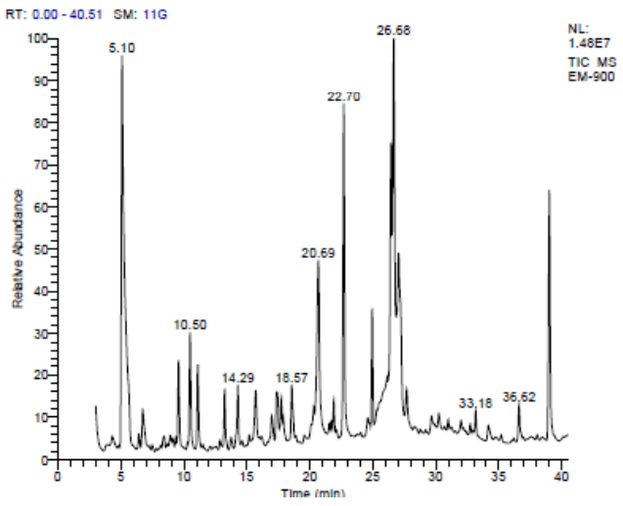

Figure 1. *Chomelia asiatica* root screened phytochemicals through GC/MS chromatogram.

Table 3. Phytochemicals documented from the root of *Chomelia asiatica* through GC-MS technique

Sl. No.	Retention time	Chemical compounds	Molecular Formula of Chemicals	Molecular Weight	Peak Area (%)
1	3.47	11-Hydroxyalliacolide	$C_{15}H_{22}O_5$	282	2.94
2	5.10	(R)-2-Amino-3-methoxypropan-1-ol hydrochloride dl-Serine (CAS)	$C_4H_{11}NO_2$	105	2.40
3	10.50	Hept-1-en-3-on	$C_7H_{12}O$	112	1.92
4	14.29	Tetracosamethyl cyclododecasiloxane	$C_{24}H_{72}O_{12}Si_{12}$	888	1.82
5	18.57	Tetradecanoic acid (CAS)	$C_{14}H_{28}O_2$	228	1.68
6	20.69	1-oxa-4-methoxy-5-hydroxy-5-(2-phenylethylcarbonyl)	$C_{14}H_{14}O_4$	245	1.95
7	22.70	1-(2-naphthyl)methylene-2-(phenoxycarbonyl)-Hydrazine,	$C_{18}H_{14}N_2O_2$	290	2.04
8	25.88	Arachidonoyl ethanolamide	$C_{22}H_{37}NO_2$	347	2.28
9	26.68	2-(dimethyl amino)methyl]aniline	$C_{23}H_{24}N_2S$	360	3.68
10	28.94	2-Methyl-2-phenylbutylamine	$C_{11}H_{17}N$	163	2.54
11	29.45	Hexanedinitrile, 2-methyl- (CAS)	$C_7H_{10}N_2$	122	3.13
12	29.81	Cyclopentene	$C_{24}H_{24}O_4$	376	1.92
13	30.98	2-Ethyl-1-dodecanol	$C_{14}H_{30}O$	214	3.60
14	31.40	2',4',6'-Trideutero-2,4,6-trimethylbenzaldehyde	$C_{10}H_9D_3O$	148	8.63
15	32.22	2-hexanoic acid, ethyl ester	$C_{19}H_{28}O_4Si$	348	4.10
16	33.16	(-)-2á-Hydroxy-9-oxoverrucosane	$C_{20}H_{32}O_2$	304	3.51
17	36.62	4,5-Dicyano-1-methyl 2-(3-nitrophenyl) imidazole	$C_{12}H_7N_5O_2$	253	2.75
18	37.19	à-d-Xylopyranoside	$C_{30}H_{51}B_3O_5$	524	3.88
19	37.39	Triphenyleno[2,3-b]thiophene-9,13-dione	$C_{20}H_{10}O_2S$	314	4.07

GC-MS Technique: The components recognized by this study in the root sample of *Chomelia asiatica* are accessible in the Table 3 with their molecular formula of chemicals, molecular weight of chemicals, retention time and percentage of area of peak. The chromatogram *C. asiatica* depicted 19 peaks representing the occurrence of 19 phytocomponents (Figure 1). 19 phytoconstituents were identified and deep rooted based on their characterization and assessment with the mass spectra of the chemical constituent listed in the National Institute Standard and Technology library. 2,4,6-trideutero,2,4,6-trimethylbenzaldehyde is the major component found in the root sample of *C.asiatica*. The compounds are grouped as aliphatic, aldehyde, sugars, alcohols, ketone, fatty acid, terpenoids, phenols, esters and tocopherol. The predicted activities of the components are wound healing, antimicrobial, antiinflammatory, antianginal and antiepileptic activities.

4. Conclusion

Estimation of minerals and secondary metabolites in the root samples of *C. asiatica* authenticated its therapeutic value. The secondary metabolites, microelements and macroelements of this sporadic medicinal plant are good candidates for drug formulation that may have more biological friendliness than synthetic drugs. The phytocomponents identified through GC-MS chromatogram have further strengthened this view, which may pave the way to integrate these phytocomponents in traditional as well as in modern system of medicines.

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