



RESEARCH ARTICLE

PHYTOCHEMICAL PROFILING OF ETHANOLIC BULB EXTRACT OF BELLICORYNE
PLUMBAGINIFOLIA

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ABSTRACT

Plants are a rich source of secondary metabolites with interesting biological activities. Natural product with antioxidant properties could trigger this goal. To quantify the major secondary metabolites and the antioxidant potential of ethanolic bulb extract of *Bellicoryne plumbaginifolia*. In the present study ethanolic bulb extract of *B. plumbaginifolia* was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS) and the compound structures were identified with help of National Institute of Standards and Technology (NIST) library. GC-MS analysis of test plant revealed the presence of 20 bioactive compounds. Among them 3 The prevailing compounds were 2-Pentadecanone, 6,10,14-trimethyl- Hexahydrofarnesyl acetone Hexahydrofarnesyl acetone 6,10,14-Trimethyl-2-pentadecanone (6.87%), n-Hexadecanoic acid Hexadecanoic acid n-Hexadecanoic acid Palmitic acid Pentadecanecarboxylic acid (25.75%) are important bioactive compounds which act as essential drugs for dangerous diseases and disorders and other compounds are used in antimicrobial, anti-inflammatory, antioxidant, cytotoxicity and cancer preventive activities.

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INTRODUCTION

A great proportion of the natural products used as drugs are derived from plants. The poisonous or healing properties of plants were discovered by man in his search for food. Some plants were found to have very dramatic effect on the human body and some were found to cure certain diseases. The knowledge of these plants was passed on through the generations and thus man gathered considerable experience of drugs which could be obtained from the plants in his surroundings. It became the task of the medicine man to maintain this knowledge and pass it on to his successor. The medicine men were often also priests and thus the actual knowledge became enmeshed in a veil of myth and magic. This process can still be observed in the developing countries; consequently the study of drugs used by traditional healers is an important object of pharmacognostical research. Over 50% of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs in the pharmaceutical industry¹.

Medicinal plants are the source of many potent and powerful drugs. The plant derived drugs are healthier and safer alternate to the synthetic drugs². Different parts of medicinal plants like root, stem, flower, fruit, seed etc. are used to obtain pharmacologically active constituents. Medicinal activities of

plants can be attributed to the secondary metabolites such as alkaloids, flavonoids, glycosides, tannins, terpenoids and essential amino acids present in these plants. These active principles are isolated for direct use as drugs, lead compounds and or pharmacological agents³. Even today compounds from plants continue to play a major role in primary health care as therapeutic remedies in many developing countries⁴. Standardization of plant materials is the need of the day. Several pharmacopoeia containing monographs of the plant materials describe only the physicochemical parameters. Hence the modern methods describing the identification and quantification of active constituents in the plant material can be useful for proper standardization of herbals and its formulations. Also the WHO has emphasized the need to ensure the quality of medicinal plants products using modern controlled technique and applying suitable standards⁵. Nowadays there were a number of dramatic advances in analytical techniques including TLC, UV, NMR and GC-MS that were powerful tools for separation identification and structure determination of Phytochemicals. In GC-MS used to identify the bioactive constituents of long chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino and nitro compounds etc.,

Bellicoryne plumbaginifolia (Liliaceae) is extensively used in most of the Indian herbal pharmaceuticals and nutraceuticals⁶. Juice of pounded bulb is given every half an hour duration

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(depends on the severity of bites) for snake bite. Leaf paste is applied on the spider bitten area. Paste of bulb is applied over the head for mental disorder. Keeping this in view, the present study has been undertaken to investigate the phytoconstituents present in ethanolic extract of *B. plumbaginifolia*. Hence the present study focused on Phytochemical profiling of ethanolic bulb extract of *B. plumbaginifolia* using Gas chromatography and mass spectrometry.

MATERIALS AND METHODS

Collection of plant samples

The bulb of the medicinal plants was used for the present study, the plants of *Bellicoryne plumbaginifolia* was collected from Kannikars of Kanyakumari District, Tamil Nadu, India. The plant parts were identified taxonomically and authenticated according to various literatures, Flora of Madras Presidency and Wealth of India including other pertinent taxonomic literature.

Phytochemical Analysis

The collected bulbs samples were washed thoroughly two times with running tap water and once with sterile water, air-dried, powdered using a pulverizer and used for extraction. About 50 grams of air-dried and coarsely powdered plant material was extracted successively with 250 ml of methanol using a Soxhlet extractor for at least 15 refluxs. After complete extraction, the extract in the round bottom flask were removed and condensed using rotary evaporator. After solvent evaporation, extracts were weighed for the percentage yield calculation. The thick syrup plant extract were labeled and stored at 5°C in sterile screw-capped vials for further use.

Preliminary phytochemical screening of methanol extract of *B. plumbaginifolia* Bulbs was carried out to detect the phyto-constituents using standard conventional protocols⁷⁻⁹. Alkaloids, carbohydrates, tannins and phenols, flavonoides, gums and mucilage, fixed oils and fats and saponins were qualitatively analyzed.

Gas Chromatography-Mass spectrometry (GC-MS) analysis:

The GC-MS analysis was carried out using a Clarus 500 Perkin- Elmer (Auto System XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold - Perkin Elmer Turbomas 5.2 spectrometer with an Elite-1 (100% Dimethyl ply siloxane), 300 m x 0.25 mm x 1 µm capillary column. The instrument was set to an initial temperature of 110°C, and maintained at this temperature for 2 min. At the end of this period, the oven temperature was raised upto 280°C, at the rate of an increase of 5°C/min, and maintained for 9 min. Injection port temperature was ensured as 250°C and Helium flow rate as 1 ml/min. The ionization voltage was 70 eV. The samples were injected in split mode as 10:1. Mass Spectral scan range was set at 45-450 (mhz). The chemical constituents were identified by GC-MS. The fragmentation patterns of mass spectra were compared with those stored in the spectrometer database using National Institute of Standards and Technology Mass Spectral database (NIST-MS). The percentage of each component was calculated from relative peak area of each component in the chromatogram.

Identification of Compounds: The identity of the components in the extract was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures. National Institute of Standards and Technology library sources were also used for matching the identified components from the plant material.

RESULT AND DISCUSSION

Qualitative phytochemical analyses for alkaloids, carbohydrates, tannins, phenols, gums and mucilage, fixed oils and fats, saponins, proteins, volatile oils, flavonoids and steroids were screened in ethanolic extracts of *Bellicoryne plumbaginifolia* Bulbs. The screening of the extract indicated the presence of alkaloids, tannins and saponin in the ethanolic extracts of bulb (Table 1). The plant extract yield percentage on the usage of methanol agreed with the earlier reported¹⁰ obtained in *Hypochaeris radicata* L. The plant extract obtained using soxhlet is varied among the herbal plants to plant. In a plant, different parts having differently yielded¹¹. The plant extract yield percentage on the usage of methanol agreed with the earlier reported¹² obtained in *Brassica oleracea*.

Table 1 Phytochemical screening of ethanolic extract of *Bellicoryne plumbaginifolia*

Phyto-constituents	Observation
Alkaloids	+
Flavonoids	+
Terpenoids	+
Phenolic Compounds	+
Saponins	+
Tannins	+
Glycosides	+
Cardiac Glycosides	+
Coumarins	-

+: presence; -: absence

Preliminary quantities of phytochemical screening of ethanolic extract of *B. plumbaginifolia* revealed the presence of alkaloids, flavonoids, terpenoids and phenolic compounds which are essential to prevent diseases and to maintain a state of well being. Recent studies have been focused on finding the natural substance of medicinal plant that decrease the inflammation and reduce oxidative stress and there by counteracting the macromolecular damage. It is well known that reactive oxygen species interact with key bimolecular such as proteins and enzymes which regulate major metabolic path way and decrease their functional efficiency. Table 2 shows that *B. plumbaginifolia* contains rich amount of bioactive compounds which exhibit antioxidant property the quantitative analysis revealed that *B.plumbaginifolia* contain rich amount of phenolic compounds and flavonoids.

Table 2 Quantitative Phytochemical Analysis

S. No.	Phytochemicals	Quantity mg/gm of dry material
1.	Alkaloids	1.55
2.	Terpenoids	0.79
3.	Total phenols	4.21
4.	Gallic acid	3.84
5.	Cinnamic acid	0.45
6.	Flavonoids	2.15
7.	Rutin	1.24
8.	Quercetin	1.86

It is well known that plant flavonoids and phenols in general are highly effective in scavenging free radical and providing antioxidant defense in living cells. Polyphenols and flavonoids isolated from medicinal plants have been used for the prevention and cure of various diseases which are mainly associated with free radicals¹³.

Twenty compounds were identified in *B. plumbaginifolia* Bulbs extract by GC-MS analysis. The chromatogram is obtained by fraction of *B. plumbaginifolia* Bulbs. The active principle, area of the peak, height (%), Retention Time (RT), Molecular formula and Molecular weight were presented in Table 3. The prevailing compounds were 2-Pentadecanone, 6,10,14-trimethyl- Hexahydrofarnesyl acetone Hex 6,10,14-Trimethyl-2-pentadecanone (6.87%), n-Hexadecanoic acid Hexadecanoic acid n-Hexadecanoic acid Palmitic acid Pentadecanecarboxylic acid (25.75%), 9-Octadecen-1-ol, (Z)- cis-9-Octadecen-1-ol cis-9-Octadecenyl Alcohol Adol 320 (5.42%), Z,Z-2,13-Octadecadien-1-ol (2Z,13Z)-2,13-Octadecadien-1-ol (5.95%), 2-Hexadecen-1-ol, 3,7,11,15-Tetramethyl- (12.20%), 9,12-Octadecadienoic acid (Z,Z)- cis-9,cis-12-Octadecadienoic acid (13.48%), (Z)6-Pentadecen-1-ol (6Z)-6-Pentadecen-1-ol (4.92%) etc. Figure 1 shows the chromatogram with retention time, molecular weight, height, area% of the standards.

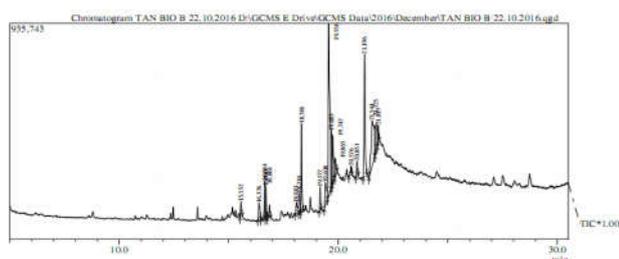


Fig 1 GC-MS Chromatogram of *B. Plumbaginifolia* BULBS

The differences between the compounds that we have found in the roots, stems and leaves of *Aristolochia clematidis* were studied by GC-FID. This study was performed on the alcoholic extracts of the three parts of the plant. From this study we have concluded that the compounds found in the root and stem are very similar. The aristolochic acid derivatives are present in both extracts, but in the leaves these derivatives are in very low concentration¹⁴. Ramamurthy¹⁵ reported that the roots and leaves of *H. indicum* were studied by GC-MS. This study was performed on the alcoholic extracts of the two parts of the plant. From this study we have concluded that the compounds found in the root and leaves are very dissimilar. The organic acid derivatives are present in both extracts, but in the leaves these derivatives are in very high concentration.

Plants are integral part of human civilization. Medicinal plants are also been relied upon by over 80% of the world population for their basic health care needs. Drugs based on the Plants are of prime importance for several remedies in traditional and conventional medicine throughout the world and serves as a substitute for drug supply in modern medicine¹⁶. Medicinal plants with therapeutic properties are used for the treatment of many infectious diseases of humans as they contain many bioactive phytochemical constituents which are of curative effects. By consuming medicinal plants, can boost the immune system and increase antioxidant activity in humans. The high level of use as a medicinal plant due to easily available, cheap and relatively no side effects¹⁷. Tetracosane, also called tetracosane, is an alkane hydrocarbon. Tetracosane showed some cytotoxic activity against AGS, MDA-MB-231, HT-29 and NIH 3T3 cells¹⁸. It also used as a good antibacterial activity.

The analytical methods GC/MS is suitable for medicinal herbs organic compounds determination. The sample preparation method is rapid and precise.

Table 3 GC-MS Profile of the Phytochemical of *B. Plumbaginifolia* Bulbs

Peak #	RT	Name of the Compound	Molecular Formula	M.W	Area%
1	15.550	Diethyl Phthalate \$ \$ Diethylphthalat	C ₁₂ H ₁₄ O ₄	222	1.23
2	16.375	Pentadecanal- \$ \$ 1-Pentadecanal \$ \$ n-Pentadecanal \$ \$	C ₁₅ H ₃₀ O	226	2.05
3	16.642	Oxirane, tetradecyl- \$ \$ Hexadecane, 1,2-epoxy- \$ \$ Hexadecylene oxide \$ \$ 1,2-Epoxyhexadecane \$ \$ 1,2-Hexadecane oxide \$ \$ 1,2-Hexadecene	C ₁₆ H ₃₂ O	240	3.17
4	16.683	(Z)6-Pentadecen-1-ol \$ \$ (6Z)-6-Pentadecen-1-ol # \$ \$	C ₁₅ H ₃₀ O	226	2.30
5	16.858	Tetradecanal \$ \$ Myristaldehyde \$ \$ 1-Tetradecanal \$ \$ 1-Tetradecyl Aldehyde \$ \$ -	C ₁₄ H ₂₈ O	212	1.36
6	18.083	Decanoic Acid \$ \$ Capric Acid \$ \$ Sodium Caprate Sodium Decanoate1-	C ₁₀ H ₂₀ O ₂	172	1.86
7	18.233	2,6,10-Trimethyl,14-Ethylene-14-Pentadecne \$ \$ Neophytadiene	C ₂₀ H ₃₈	278	1.93
8	18.317	2-Pentadecanone, 6,10,14-trimethyl- \$ \$ Hexahydrofarnesyl acetone \$ \$ 6,10,14-Trimethyl-2-pentadecanone	C ₁₈ H ₃₆ O	268	6.87
9	19.175	Isoborneol \$ \$ Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, exo- \$ \$ exo-2-Hydroxy-1,7,7-trimethylnorbornane	C ₁₀ H ₁₈ O	154	1.56
10	19.408	cis-9-Hexadecenal \$ \$ 9-Hexadecenal, (Z)- \$ \$ (Z)-9-Hexadecenal \$ \$ Z-9-Hexadecenal \$ \$ (9Z)-9-Hexadecenal	C ₁₆ H ₃₀ O	238	3.71
11	19.558	n-Hexadecanoic acid \$ \$ Hexadecanoic acid \$ \$ n-Hexadecanoic acid \$ \$ Palmitic acid \$ \$ Pentadecanecarboxylic acid	C ₁₆ H ₃₂ O ₂	256	25.75
12	19.683	9-Octadecen-1-ol, (Z)- \$ \$ cis-9-Octadecen-1-ol \$ \$ cis-9-Octadecenyl Alcohol \$ \$ Adol 320	C ₁₈ H ₃₆ O	268	5.42
13	19.742	Z,Z-2,13-Octadecadien-1-ol \$ \$ (2Z,13Z)-2,13-Octadecadien-1-ol	C ₁₈ H ₃₄ O	266	5.95
14	19.865	Ethyl Pentadecanoate \$ \$ Einecs 255-223-8 \$ \$ N-Pentadecanoic Acid Ethyl Ester	C ₁₇ H ₃₄ O ₂	270	2.04
15	20.575	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-	C ₁₅ H ₂₄	204	1.07
16	20.850	1-Hexadecanol \$ \$ n-Cetyl alcohol \$ \$ n-Hexadecan-1-ol	C ₁₆ H ₃₄ O	242	1.31
17	21.192	2-Hexadecen-1-ol, 3,7,11,15-Tetramethyl-	C ₂₀ H ₄₀ O	296	12.20
18	21.542	9,12-Octadecadienoic acid (Z,Z)- \$ \$ cis-9,cis-12-Octadecadienoic acid	C ₁₈ H ₃₂ O ₂	280	13.48
19	21.725	(Z)6-Pentadecen-1-ol \$ \$ (6Z)-6-Pentadecen-1-ol	C ₁₅ H ₃₀ O	226	4.92
20	21.817	Linolenic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C ₂₁ H ₃₆ O ₄	352	1.81

Source: - Dr. Duke's Phytochemical and Ethno botanical Databases

There is a difference between the compounds extracted from herbs by infusion and tincture but the important thing is that the organic acid and fatty acids derivatives are present in both of them. In the flower extracts organic acid derivatives and vitamins (polyunsaturated fatty acids) are present in very high amount. In conclusion flavonoids, terpenic compounds, fatty acids, phytol, alkaloids and especially organic acid derivatives are responsible for the therapeutic activity of this plant.

The analytical methods used GC/MS is suitable for medicinal herbs organic compounds determination. The sample preparation method is rapid and precise. There is a difference between the compounds extracted from herb by infusion and tincture but the important thing is that the organic acid and fatty acids derivatives are present in both of them. On the other side the study shows that their concentration is higher in the roots and stems. The present study focused on identification of several constituents present in the ethanolic leaves extract of *B. plumbaginifolia* Bulbs. This type of GC-MS analysis is the first step towards understanding nature of active compounds in this medicinal plant and helpful for the further detailed study. In this plant contains various bioactive compounds justifies the use of the whole plant for various ailments by traditional practitioners.

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