Research Article

Screening of Phytochemistry and Secondary Metabolites: A Case Study on *Nyctanthes arboritis*

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ABSTRACT

Medicinal plants are sources of important therapeutic aids for alleviating human ailments. In a survey to see the scientific understanding behind its medicinal value, an attempt is made in this study, to analyze major bioactive compounds present in the ethanol extract from Nyctanthes arbors by GC-MS. The phytochemical constituent of plant leaf ethanolic extract contains steroids, phenol, alkaloids, saponin, tannin and flavonoids. GC-MS analysis were reveals the presence of 5 compounds such as Ar-turmerone, Curlone, Dibutyl phthalate, Hexadecanoic acid, ethyl ester, 9-Octadecenioc acid, ethyl ester and 1, 2-Benzenedicarboxylic acid, disooctyl ester. The ethanolic extract of *Nyctanthes arboritis* possess various potent bioactive compounds and is recommended as a plant of phyto-pharmaceutical importance.

Keywords: *Nyctanthes arboritis*, GC-MS, phytochemical constituents, ethanol extract

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INTRODUCTION

Natural remedies from medicinal plants are ground to be safe and efficacious. Many flora species have been used in folkloric medicine to care for several complaints. Granting to the World Health Organization (WHO) in 2008, more than 80% of the world's population rely on traditional medical specialty for their primary health maintenance needs. Higher plants as sources of bioactive compounds continue to take on a predominant part in the maintenance of human wellness. Accounts available on green plants represent a reservoir of effective chemotherapeutants; these are non-phytotoxic, more systemic and easily biodegradable. A cognition of the chemical elements of plants is suitable not only for the discovery of curative factors, but as well because such information may be of great value in revealing new sources of economic phytocompounds for the synthesis of complex chemical substances and for identifying the genuine significance of folkloric remedies. *Nyctanthes arboritis linn. (Division: Magnoliophyta; Class: Magndiopsida; Order: Lamiales; Family: Oleaceae) is normally known as night jasmine and is a common plant in India.* It is distributed wild in sub-Himalayan region and also found in Indian gardens as an ornamental plant. It is employed in traditional medicine as Stomachic, carminative, piles various skin diseases, hair tonic and used for treatment of cancer [1].

Several pharmacopoeia containing monographs of the plant materials describes only the physicochemical parameters. GC-MS is the best technique to identify the bioactive components of long chain hydrocarbons, alcohols, acid. Used in the analysis of the herbal medicines, there are at least two significant advantages for GC–MS, that is: (1) with the capillary column, GC– MS has in general very good separation ability, which can produce a chemical fingerprint of high quality; (2) with the coupled mass spectroscopy and the corresponding mass spectral database, the qualitative/quantitative composition information of the herb investigated could

be provided by GC–MS, which will be extremely useful for the further research for elucidating the relationship between chemical constituents in herbal medicine and its pharmacology in further research [2]. Thus, GC–MS should be the most preferable tool for the analysis of the volatile chemical compounds in herbal medicines. Therefore, the present study was carried out to determine the phytochemical constituents from *Nyctanthes arboritis* leaves by GC-MS by using ethanolic extract.

MATERIALS AND METHODS

Plant material

The plant materials (*Nyctanthes arboritis*) were collected from Karur district of Tamil Nadu in India during the period of October – December 2013. The plant samples were pre-cleaned by double distilled water to remove solid and dust particles.

Plant sample extraction

Five grams of the powdered plant sample were soaked in 20 ml of absolute methanol overnight and then filtered through Whitman No. 1 filter paper along with 2 mg sodium sulfate to take out the sediments and traces of pee in the filtrate. The filtrate is then concentrated by bubbling nitrogen gas into the answer and was pre-concentrated to 2 milliliter of the final content. The selection contains both polar and nonpolar Phyto-components.

Qualitative/quantitative analysis of phytochemical constituents

The solvent extracts were subjected to routine qualitative secondary metabolite analysis to distinguish the nature of phytochemical constituents present in the sample [3] and the quantitative analysis of secondary metabolites by [4].

Steroids: Three ml of test solution and a minimum amount of chloroform was added with 3-4 drops of acetic anhydride and one drop of concentrated H2SO4. Purple color, thus formed changes into blue or green color showing the presence of steroids. Alkaloids: A 3 ml of test solution was taken with 2N HCl. Aqueous layer formed was decanted and then appended with one or a few drops of Mayer's reagent. Establishment of white precipitate or turbidity indicates the presence of alkaloids. Phenols: A 3 ml of test solution in alcohol was added with one drop of neutral ferric chloride (5%) solution. Establishment of an intense blue color shows the presence of phenols.ls. Flavonoids: A 3 ml of test solution in alcohol was added with a bit of magnesium and one (or) two drops of concentrated HCl and heated. Formation of red or orange color shows the presence of flavonoids. Saponins: A 3 ml of test solution was added to water and shacked. Formation of foamy lather indicates the presence of Saponins. Tannins: A 3 ml of test solution was added with water and lead acetate. Formation of white precipitate indicates the presence of tannins.

GC-MS analysis:

GC-MS analysis was carried out on a GC Clarus 500 Perkin Elmer System and Gas Chromatography interfaced to a Mass Spectrometry (GC-MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (30mm X 0.25mm IDx1 (micron) Mdf, composed of 100% Dimethyl poly Siloxane), Operating in electron impact mode at 70 e' V; Helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection Volume of 2 µl was employed (split ratio of 10:1); Injector temperature 250^0 C; Ion-source temperature 280^0 C. The oven temperature was programmed from 110^0 C (isothermal for 2min), with an increase of 10^0 C /min, 200^0 C, then 5^0 C/min to 280^0 C, ending with a 9 min isothermal at 280^0 C. Mass Spectra were taken at 70 eV., a scan fragments from 45 to 450 Da. Total GC running time was 36 min.

RESULTS AND DISCUSSION

Qualitative secondary metabolites screening

The ethanolic extract of *Nyctanthes arbors* leaves contain some groups of secondary metabolites such as steroids, phenol, alkaloids, saponin, tannin and flavonoids are presented in (**Table 1**). Flavonoids have been demonstrated to have antibacterial, anti-inflammatory, anti-allergic, anti-viral activity [5]. Alkaloids often have pharmacological effects and are used as medication and recreational drugs [6]. The average value of steroids, phenol, alkaloids, saponin, tannin and flavonoids were 0.53, 0.36, 0.24, 0.34, 0.20 and 0.42, respectively are presented in (**Table 2**). Saponins have been covered to show

hypercholesterolemia and tumor inhibiting activity in experimental animals [7]. Tannins and phenols, which together constitute the polyphenolic group, are known to have antioxidant, anticancer and antimicrobial activities [8].

Apart from that, the result of the ethanol extract of *Nyctanthes arboritis* were identified by GC-MS analysis (**Fig. 1**). Gas Chromatography-Mass Spectrometry (GC-MS) is a valuable for tool for reliable identification of Phyto compounds [9]. Therefore this type of GC-MS analyses are the initiatory step towards realizing the nature of active principles in this medicinal plant and this character of survey will be helpful for further detailed work. Further investigation into the pharmacology of Nyctanthes arbors and their variety and detailed Phytochemistry may add new knowledge to the information in the traditional medical systems. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration % in the ethanol extract of whole plant of *Nyctanthes arboritis* are presented in (**Table 3**). In the present survey, 5 compounds have been distinguished from the ethanol extract of the whole plant of Nyctanthes arbors by Gas Chromatography-Mass Spectrometry analysis. The five compounds were Ar-tumerone, Curlone, Dibutylphthalate, Hexadelanoic acid – Ethyl ester, 1, 2-Benzene dicarboxylic acid-Diisooctyl ester.

Table 1: Qualitative Phytochemical Constituent of *Nyctanthes arboritis*

Phytochemical Constituents	Results
Phenol	+++
Flavonoids	+++
Alkaloids	++
Steroids	+++
Saponin	++
Tannins	++

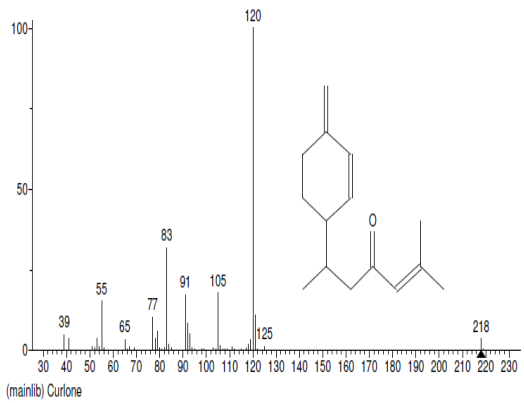
Table 2: Quantitative Phytochemical Constituent of *Nyctanthes arboritis*

Phytochemical Study	Results (mg/g)
Phenol	0.36
Flavonoids	0.42
Alkaloids	0.24
Steroids	0.53
Saponin	0.34
Tannins	0.20

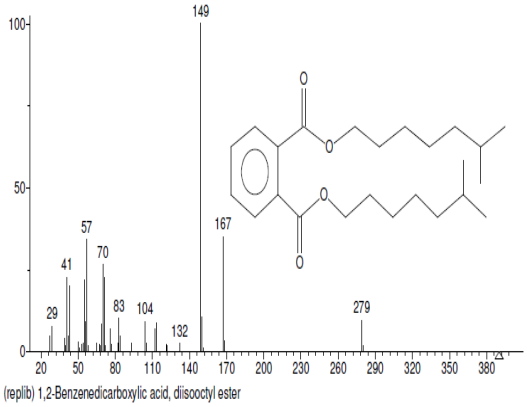
Table 3: Secondary Metabolites and their Applications from *Nyctanthes arboritis*

S. No	RT	Name of the compound	Molecular formula	Molecular Weight	Peak area %	Compound Nature	Applications
1	9.66	Ar-tumerone	$C_{15}H_{20}O$	216	8.42	Ketone compound	Antimicrobial / Anticancer insecticidal activity
2	10.08	Curlone	$C_{15}H_{22}O$	218	1.14	Ketone compound	Antimicrobial / Anticancer insecticidal activity
3	12.81	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	11.16	Plasticizer compound	Antimicrobial / Antifouling activity
4	13.14	Hexadecanoic acid, Ethyl ester	$C_{18}H_{36}O_2$	284	0.85	Palmitic acid ester	Antioxidant activity
5	20.53	1,2-Benzenedicarboxylic acid, Diisooctyl ester	$C_{24}H_{38}O_4$	390	76.73	Plasticizer compound	Antimicrobial / Antifouling activity

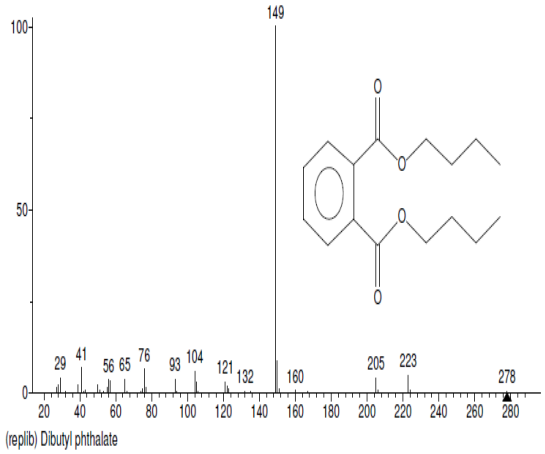
RT – Retention time



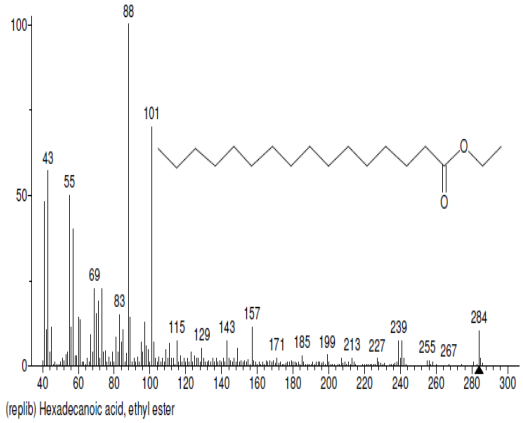
a. Curlone



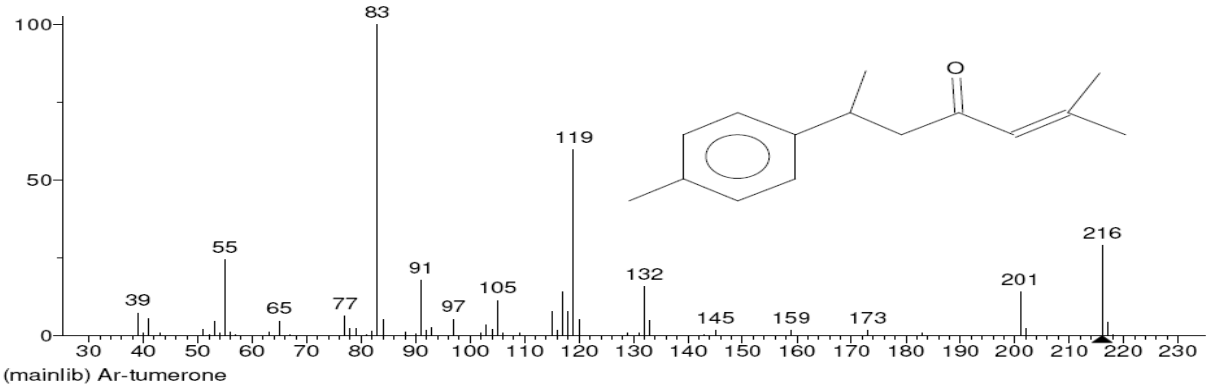
b. 1, 2-Benzene dicarboxylic acid, diisooctyl ester



c. Dibutyl phthalate



d. Hexadelanoic acid, ethyl ester



e. Ar-tumerone

Figure 1: Ethanol Extract of *Nyctanthes arboritis* were Identified by GC-MS Analysis

CONCLUSION

It was concluded that ethanolic extract of the leaves of *Nyctanthes arbors* possesses various potent bioactive compounds and is recommended as a plant of Phyto-pharmaceutical importance. Further subjects are required to explore the potential compounds responsible for the biological activity from Nyctanthes arbors for application in drug delivery, nutritional or pharmaceutical studies.

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