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# GC-MS ANALYSIS AND IDENTIFICATION OF PHYTOCONSTITUENTS FROM HEXANE EXTRACTS OF *ADHATODA VASICA* NEES AND *ADHATODA BEDDOMEI* CB CLARKE LEAVES

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**Keywords:**

*Adhatoda vasica*, *Adhatoda beddomei*, GC-MS, Vasicolinone and methyl commate

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**ABSTRACT:** The medicinal plants, *Adhatoda vasica*, and *Adhatoda beddomei* are commonly used in the treatment of several illnesses and respiratory disorders *viz*., cold, severe cough, chronic bronchitis, asthma and tuberculosis. The present study aimed to identify the phytochemical constituents and quantification of bioactive contents of hexane extracts of these plants, followed by metabolic fingerprinting, which was carried out by GC-MS analysis. Assessment of phytochemical screening showed the presence of various phytochemicals like glycosides, phytosterols, flavonoids, terpenoids, and phenols in hexane extract of *A. vasica* and *A. beddomei.* A rapid method has been used for the comparative study of *A. vasica* and its related plant species *A. beddomei* using gas chromatography-mass spectrometry (GC-MS). The phytocompounds comparison in two plants by GC-MS was similar in 12 compositions. Squalene, β-sitosterol, tetratetracontane, and lupeol were the major constituents identified in the hexane extract of *A. vasica* and *A. beddomei*. The extracts showed the presence of several phytoconstituents, including bioactive components, and provide reference data for future research of its active constituents.

**INTRODUCTION:** Plants are a beneficial source in the discovery of novel herbal drugs or medicine [1]. Herbal drugs have become very popular for the past few decades for its potency and powerful pharmacological activities such as antioxidants, enzyme inhibitors, immuno-suppressive, hypocholesterolemic, antiasthmatic, anticancer [2], *etc*.

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Phytochemistry is the study of chemical or phytoconstituents which are derived from plants. These compounds are known as secondary metabolites, which are having various therapeutic properties and benefiting human health [1]. Crude plants generally consist of mixtures of various chemical constituents. Amongst, secondary metabolites are extensively used to prepare the herbal formulations and their derived product [2]. Phytochemical investigation plays a major role in the detection of bioactive phytocompounds [3].

Chromatographic techniques mainly contribute to novel drug discovery from herbal drugs, particularly in the area of identification and isolation of phytoconstituents and its characterization [4].

Plant extract screening can be performed by chromatographic techniques such as gas chromatography (GC) hyphenated with mass spectrometric (MS) detection method. GC is one of the important chromatographic techniques used for the fast identification of phytochemicals since it allows the profiling of plant materials complex structures and precisely identify the compounds. Therefore, GC-MS offers a new methodology for finding the unknown components in the plant materials over effective separation abilities of GC and specific structural elucidation by MS.

*Adhatoda vasica* Nees (Acanthaceae) is widespread throughout the temperate regions of South Asia, used for more than 3000 years in Indian traditional medicine for the prevention, management, and treatment of several illnesses and respiratory disorders *viz*., cold, severe cough, chronic bronchitis, asthma and tuberculosis [5,] *etc*. It is commonly known as vasaka or malabar nut tree. *Adhatoda beddomei* CB Clarke is one of the species of *Adhatoda* called vasa or adalodakam in Tamil, rarely distributed in Western Ghats of Kerala and Tamil Nadu and used in Ayurveda, the ancient Indian System of Medicine (ISM) [6]. It has been the choice for the treatment and management of several diseases such as fever, cough, asthma, bronchitis, leprosy, blood disorders, heart troubles, inflammation, jaundice, tumors, and tuberculosis [7, 8]. The scientific literature revealed that plants in the genus *Adhatoda* mainly comprised of chemicals belong to such as alkaloids, tannins, saponins, phenolics, flavonoids and fatty acids that are beneficial to human health [9, 10].

However, both plants are closely related to each other and have similar morphological characteristics. In order to intensively elucidate the major difference in the chemical constituents between two species and control the quality of medicinal plants. In our approach, we developed a method to compare the chemical profiles of phytoconstituents established on gas chromato-graphy-mass spectrometry (GC-MS) study to demonstrate the difference in the chemical composition of two species.

## EXPERIMENTAL:

**Plant Material:** *A. vasica* (AV) and *A. beddomei* (AB) leaves were collected from Gingee Hills, Villupuram (Dt) and Chengalpattu, in the month of October 2017. The plants were authenticated by Prof. P. Jayaraman (Botanist), Plant Anatomy Research Centre (PARC), Tambaram, Chennai, Tamil Nadu, India, with a reference number of *A. vasica* and *A. beddomei* was PARC/2018/3653 and PARC/2018/3654 respectively. The plant specimens were preserved in the Herbarium at Interdisciplinary Institute of Indian System of Medicine (IIISM), SRM Institute of Science and Technology, Kattankulathur, Kancheepuram, Tamil Nadu, India. Collected leaves were cleaned with running tap water, dried under shade and powdered using cutter mill, sieved through 60 mesh sieve and stored in an airtight container at room temperature.

**Preparation of Hexane Extracts:** The powdered leaves of each plant (100 g) were extracted in 250 mL of hexane for 72 h at room temperature and repeat the process to complete the extraction. Further, the extracts were filtered, evaporated to dryness under reduced pressure using a rotary vacuum evaporator, and the samples were analyzed by GC-MS. The percentage yield for hexane extracts obtained from the *A. beddomei* and *A. vasica* was 0.42% w/w and 0.56% w/w, respectively.

**Preliminary Phytochemical Evaluation:** Prepared hexane extracts were analyzed for the presence of phenols, flavonoids, tannins, alkaloids, saponins, amino acids, and reducing sugars, using the standard procedure [11, 12].

**Determinations of Bioactive Contents:** Determinations of total phenol [13] flavonoid [14] alkaloids [15, 16] glycosides [17] steroids [18] saponins [19] and terpenoid contents [20] were carried out using a standard procedure.

**Analysis and Identification of Compounds by GC-MS:** The analysis was done using Shimadzu GCMS-QP2010 Plus (Shimadzu, Europe) equipped with capillary column Elite-1 fused silica capillary column (30mm × 0.25mm i.d., 0.25µm thickness, composed of 100% Dimethylpolysiloxane). 99.999% of helium was used as carrier gas with 1.00 mL/min flow rate. The column temperature was set at 110-280 °C (110 ºC isothermal for 2 min with an increase of 10 °C/min, to 200 °C, then 5 ºC/min to 280 °C, ending with 9 min isothermal at

280 °C). The electron-impact ionization was applied for the mass spectrum. The ion source was maintained at the temperature of 280 °C, and the ionization voltage was set at 70 eV with a scan interval of 0.50 sec. The total running time of GC was 50 min.

**Identification of Compounds:** Interpretation of GC-MS spectrum was achieved by NIST (National Institute Standard and Technology, Gaithersburg, MD, USA) database 2005. The spectra of unknown compounds were correlated with that of known compounds reserved in the NIST library.

**RESULTS AND DISCUSSION:** *Adhatoda vasica* and *Adhato dabeddomei* are popular Indigenous System of Medicine (ISM) in India and popular drugs in Ayurveda and Unani belong to the family Acanthaceae [21, 8]. The therapeutic properties of the plants attribute to their bioactive compounds like alkaloids *viz*., vasicine, deoxyvasicine and vasicinone [22] glycosides, flavones, triterpenes, polysaccharides, vitamin C, polyphenols, proteins, quinines, coumarins and essential oils [23]. Vasicine is a major principle constituent of *Adhatoda* species.

After successful extraction of the plant materials in the investigation, the preliminary phytochemical screening revealed that the hexane extracts of *A. vasica* and *A. beddomei* contains glycosides, phytosterols, flavonoids, terpenoids, and phenols. Alkaloids and saponins were absent in hexane extract of *A. beddomei,* whereas the results were positive in *A. vasica*, as summarized in **Table 1**.

**TABLE 1: PRELIMINARY PHYTOCHEMICAL SCREENING OF HEXANE EXTRACTS OF *A. VASICA* AND *A. BEDDOMEI***

| Phytochemical Tests | Hexane Extracts | |
|---|---|---|
| | *A. vasica* | *A. beddomei* |
| Alkaloids | + | - |
| Glycosides | + | + |
| Saponins | + | - |
| Phytosterols | +++ | +++ |
| Flavonoids | + | + |
| Terpenoids | ++ | ++ |
| Phenols | + | + |
| Proteins | - | - |
| Amino acids | - | - |
| Carbohydrates | - | - |
| Fixed oils | - | - |
| Gums and Mucilage | + | + |

(+)- Low; (++)- Medium; (+++)- Strong; (-)- Absence

The bioactive content of total phenolic, flavonoid, alkaloid, glycoside, sterol, saponin, and terpenoids were determined in the hexane extracts of *A. vasica* and *A. beddomei* and the results were depicted in **Table 2** and **Fig. 1**. The content of total phenols in the extract expressed as gallic acid equivalents varied between *A. vasica* and *A. beddomei* (6.15 mg/g and 4.73 mg/g, respectively) of dry extract. As shown in the table, phenol content was high in the hexane extract of *A. vasica* than *A. beddomei*.

**TABLE 2: DETERMINATIONS OF TOTAL PHENOLIC, FLAVONOID, ALKALOID, GLYCOSIDE, STEROID, SAPONIN AND TERPENOID CONTENTS (mg/g) IN *A. VASICA* AND *A. BEDDOMEI***

| Quantitative Parameters | Hexane Extracts | |
|---|---|---|
| | *A. vasica* (mg/g) | *A. beddomei* (mg/g) |
| Phenolics | 6.15 ± 0.0002 | 4.73 ± 0.0004 |
| Flavonoids | 13.12 ± 0.0003 | 6.98 ± 0.0004 |
| Alkaloids | 3.48 ± 0.002 | ND |
| Glycosides | 7.74 ± 0.004 | 15.34 ± 0.02 |
| Sterols | 51.98 ± 0.0004 | 27.96 ± 0.0004 |
| Saponins | 0.26 ± 0.001 | ND |
| Terpenoids | 4.85 ± 0.005 | 10.83 ± 0.01 |

Values are in mean ± standard deviation, n=3
ND- Not Detected
Phenolics equivalent to Gallic acid, flavonoids equivalent to Quercetin, alkaloids equivalent to Caffeine, glycosides equivalent to Sennoside, sterols, and saponins equivalent to Diosgenin and terpenoids equivalent to Ursolic acid.

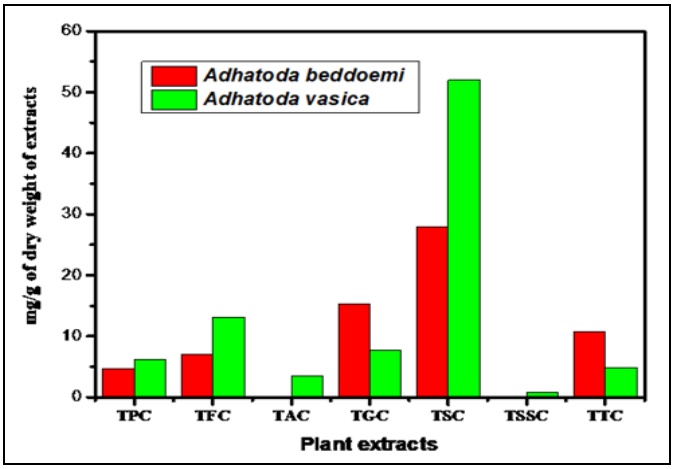


**FIG. 1: QUANTIFICATION OF BIOACTIVE CONTENTS IN THE HEXANE EXTRACTS OF *A. VASICA* AND *A. BEDDOMEI*.** (TPC-Total Phenolic Content; TFC-Total Flavonoid Content; TAC- Total Alkaloidal Content; TGC- Total Glycoside Content; TSC-Total Sterol Content; TSSC-Total Steroidalsaponin Content; TTC-Total Terpenoid Content); ND-Not detected (Below Detectable Range)

The pattern of variation in flavonoid (quercetin equivalent) and sterol (diosgenin equivalents) were similar with phenol content, with the highest content in *A. vasica* and lowest in *A. beddomei*

whereas glycoside content (equivalent to sennoside) were high in the hexane extract of *A. beddomei* (15.34 mg/g) and *A. vasica* (7.7 4 mg/g). Terpenoid content (ursolic acid equivalent) was high in the hexane extract of *A. beddomei* (10.83 mg/g) than *A. vasica* (4.85 mg/g). The alkaloid and saponin contents (equivalent to caffeine and diosgenin, respectively), were high abundance in the hexane extract of *A. vasica* whereas absent in *A. beddomei*.

**GC-MS Analysis:** A mass spectrum of compounds obtained in the hexane extracts of *A. vasica* and *A. beddomei* were compared with NIST library. The major compounds in thehexane extract of *A. vasica* are diethyl phthalate, methyl 12-methyltetra-decanoate, phytol, *2'-deoxy*-5-(hydroxymethyl) *cytidine*, 9, 12, 15-octadecatrienal, 4-methyl-1-undecene, di-n-octyl phthalate, vasicolinone, nonadecane, squalene, (6E, 10E, 14E, 18E)-2, 6, 10, 15, 19, 23-Hexamethyl-1, 6, 10, 14, 18, 22-tetracosahexaen-3-ol, β-tocopherol, tetratetra-contane, α-tocopherol, oxalic acid, decyl propyl ester, stigmasta-5, 22-dien-3-ol, ergost-5-en-3-ol, 3, 5, 24-trimethyltetracontane, β-sitosterol, vitamin K1, olean-12-en-3-one, norandrostane, lupeol, simiarenol, cycloartenyl acetate, dodecahydro-1H-fluorene, (+)- methoprene, 4- tetradecanol, amorphane-B, 2, 6, 10-trimethyl, 14-ethylene-14-pentadecne, neolyratol, 1, 2-epoxyoctadecane, 2, 5-methano-1H-inden-7(4H)-one, tetrahydro the identified compounds were listed in **Table 3**. The GC-MS chromatogram of *A. vasica* were presented in **Fig. 2**.

**TABLE 3: MAJOR COMPOUNDS IDENTIFIED IN THE HEXANE EXTRACT OF *A. VASICA* LEAVES**

| S. no. | Compound name | Retention time, $R_t$ (min) | Molecular formula | Molecular weight |
|---|---|---|---|---|
| 1 | Diethyl phthalate | 17.043 | $C_{12}H_{14}O_4$ | 222 |
| 2 | Methyl 12-methyltetradecanoate | 21.851 | $C_{16}H_{32}O_2$ | 256 |
| 3 | Phytol | 24.584 | $C_{20}H_{40}O$ | 296 |
| 4 | *2'-Deoxy-5*-(hydroxymethyl)*cytidine* | 27.904 | $C_9H_{13}N_3O_4$ | 227 |
| 5 | 9,12,15-octadecatrienal | 28.221 | $C_{18}H_{30}O$ | 262 |
| 6 | 4-methyl-1-undecene | 30.291 | $C_{12}H_{24}$ | 168 |
| 7 | Di-n-octyl phthalate | 30.782 | $C_{24}H_{38}O_4$ | 390 |
| 8 | Vasicolinone | 33.909 | $C_{19}H_{19}N_3O$ | 305 |
| 9 | Nonadecane | 34.577 | $C_{19}H_{40}$ | 268 |
| 10 | Squalene | 34.778 | $C_{30}H_{50}$ | 410 |
| 11 | (6E,10E,14E,18E)-2,6,10,15,19,23-Hexamethyl-1,6,10,14,18,22-tetracosahexaen-3-ol | 37.234 | $C_{30}H_{50}O$ | 426 |
| 12 | β-tocopherol | 37.797 | $C_{28}H_{48}O_2$ | 416 |
| 13 | Tetratetracontane | 38.484 | $C_{44}H_{90}$ | 618 |
| 14 | α-tocopherol | 38.797 | $C_{29}H_{50}O_2$ | 430 |
| 15 | Oxalic acid, decyl propyl ester | 39.720 | $C_{15}H_{28}O_4$ | 272 |
| 16 | Ergost-5-en-3-ol | 40.006 | $C_{28}H_{48}O$ | 400 |
| 17 | Stigmasta-5,22-dien-3-ol | 40.369 | $C_{29}H_{48}O$ | 412 |
| 18 | 3,5,24-trimethyltetracontane | 40.926 | $C_{43}H_{88}$ | 604 |
| 19 | β-sitosterol | 41.141 | $C_{29}H_{50}O$ | 414 |
| 20 | Vitamin K1 | 41.414 | $C_{31}H_{46}O_2$ | 450 |
| 21 | Olean-12-en-3-one | 41.560 | $C_{30}H_{48}O$ | 424 |
| 22 | Norandrostane | 41.789 | $C_{18}H_{30}$ | 246 |
| 23 | Lupeol | 42.209 | $C_{30}H_{50}O$ | 426 |
| 24 | Simiarenol | 42.362 | $C_{30}H_{50}O$ | 426 |
| 25 | Cycloartenyl acetate | 42.879 | $C_{32}H_{52}O_2$ | 468 |
| 26 | Dodecahydro-1H-fluorene | 43.142 | $C_{13}H_{22}$ | 178 |
| 27 | (+)-Methoprene | 43.242 | $C_{19}H_{34}O_3$ | 310 |
| 28 | 4-tetradecanol | 43.625 | $C_{14}H_{30}O$ | 214 |
| 29 | Amorphane-B | 44.280 | $C_{15}H_{28}$ | 208 |
| 30 | 2,6,10-trimethyl,14-ethylene-14-pentadecne | 44.458 | $C_{20}H_{38}$ | 278 |
| 31 | Neolyratol | 45.568 | $C_{10}H_{16}O$ | 152 |
| 32 | 1,2-epoxyoctadecane | 48.099 | $C_{18}H_{36}O$ | 268 |
| 33 | 2,5-methano-1H-inden-7(4H)-one, tetrahydro- | 48.386 | $C_{10}H_{14}O$ | 150 |

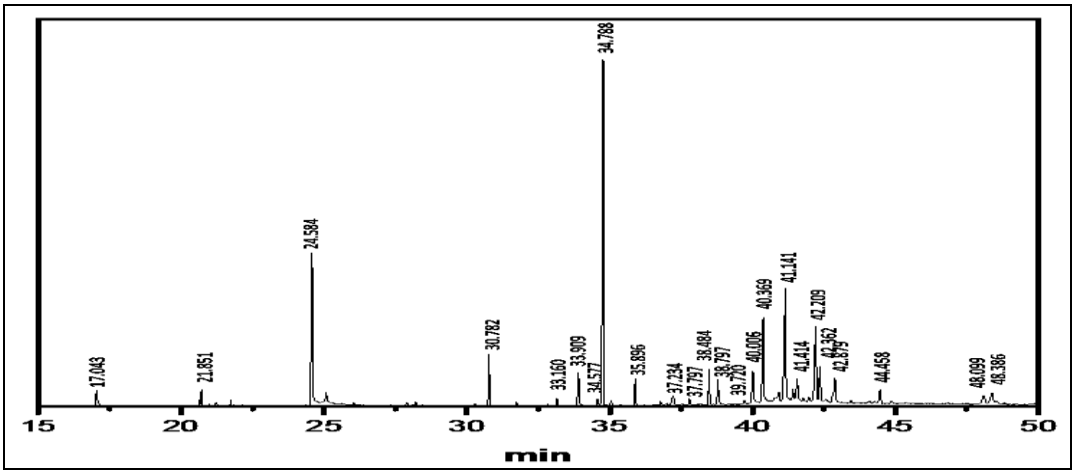


**FIG. 2: GC-MS CHROMATOGRAM OF HEXANE EXTRACT OF *A. VASICA***

The major components in the hexane extract of *A. beddomei* are diethyl phthalate, citronellyl propionate, phytol, di-n-octyl phthalate, docosane, tetracosane, octadecane, squalene, geranyl linalool, octacosane, β-tocopherol, tetratetracontane, α-tocopherol, 5-ethyl-5-methyl-2-phenyl-2-oxazoline, campesterol, stigmasterol, 3-chloropropanoic acid, 1-cyclopentylethyl ester, β-sitosterol, 5-phenyl-sulfonylgeranyl geraniol, vitamin K1, methyl commate D, 4,22-stigmastadiene-3-one, *urs-12-ene*, simiarenol, 1-heptatriacontanol, 4-sitosterol-3-one, cycloeucalenol acetate, 2, 6, 10-trimethyl, 14-ethylene-14-pentadecne, 1, 2-epoxyhexadecane. The identified principles with retention time ($R_t$), molecular formula, and molecular weight were illustrated in **Table 4**. The GC-MS chromatogram of *A. beddomei* hexane extract with different retention times, as depicted in **Fig. 3**.

**TABLE 4: MAJOR COMPOUNDS DETECTED IN THE HEXANE EXTRACT OF *A. BEDDOMEI* LEAVES**

| S. no. | Compound name | Retention time, $R_t$ (min) | Molecular formula | Molecular weight |
|---|---|---|---|---|
| 1 | Diethyl phthalate | 17.046 | $C_{12}H_4O_4$ | 222 |
| 2 | Citronellyl propionate | 21.231 | $C_{13}H_{24}O_2$ | 212 |
| 3 | Phytol | 24.546 | $C_{20}H_{40}O$ | 296 |
| 4 | Di-n-octyl phthalate | 30.781 | $C_{24}H_{38}O_4$ | 390 |
| 5 | Docosane | 31.737 | $C_{22}H_{46}$ | 310 |
| 6 | Tetracosane | 33.160 | $C_{24}H_{50}$ | 338 |
| 7 | Octadecane | 34.567 | $C_{18}H_{38}$ | 254 |
| 8 | Squalene | 34.766 | $C_{30}H_{50}$ | 410 |
| 9 | Geranyl linalool | 35.058 | $C_{20}H_{34}O$ | 290 |
| 10 | Octacosane | 35.903 | $C_{28}H_{58}$ | 394 |
| 11 | β-tocopherol | 37.798 | $C_{28}H_{48}O_2$ | 416 |
| 12 | Tetratetracontane | 38.510 | $C_{44}H_{90}$ | 618 |
| 13 | α-tocopherol | 38.807 | $C_{29}H_{50}O_2$ | 430 |
| 14 | 5-Ethyl-5-methyl-2-phenyl-2-oxazoline | 39.150 | $C_{12}H_{15}NO$ | 189 |
| 15 | Campesterol | 39.989 | $C_{28}H_{48}O$ | 400 |
| 16 | stigmasterol | 40.347 | $C_{29}H_{48}O$ | 412 |
| 17 | 3-Chloropropanoic acid, 1-cyclopentylethyl ester | 40.575 | $C_{10}H_{17}ClO_2$ | 204 |
| 18 | β-sitosterol | 41.095 | $C_{29}H_{50}O$ | 414 |
| 19 | 5-Phenylsulfonylgeranyl geraniol | 41.317 | $C_{26}H_{38}O_3S$ | 430 |
| 20 | Vitamin K1 | 41.392 | $C_{31}H_{46}O_2$ | 450 |
| 21 | Methyl commate D | 41.756 | $C_{31}H_{50}O_4$ | 486 |
| 22 | 4,22-Stigmastadiene-3-one | 41.936 | $C_{29}H_{46}O$ | 410 |
| 23 | *Urs-12-ene* | 42.174 | $C_{30}H_{50}$ | 410 |
| 24 | Simiarenol | 42.328 | $C_{30}H_{50}O$ | 426 |
| 25 | 1-heptatriacontanol | 42.570 | $C_{37}H_{76}O$ | 536 |
| 26 | 4-sitosterol-3-one | 42.808 | $C_{29}H_{48}O$ | 412 |
| 27 | Cycloeucalenol acetate | 42.866 | $C_{32}H_{52}O_2$ | 468 |
| 28 | 2,6,10-trimethyl,14-ethylene-14-pentadecne | 44.457 | $C_{20}H_{38}$ | 278 |
| 29 | 1,2-epoxyhexadecane | 48.069 | $C_{16}H_{32}O$ | 240 |

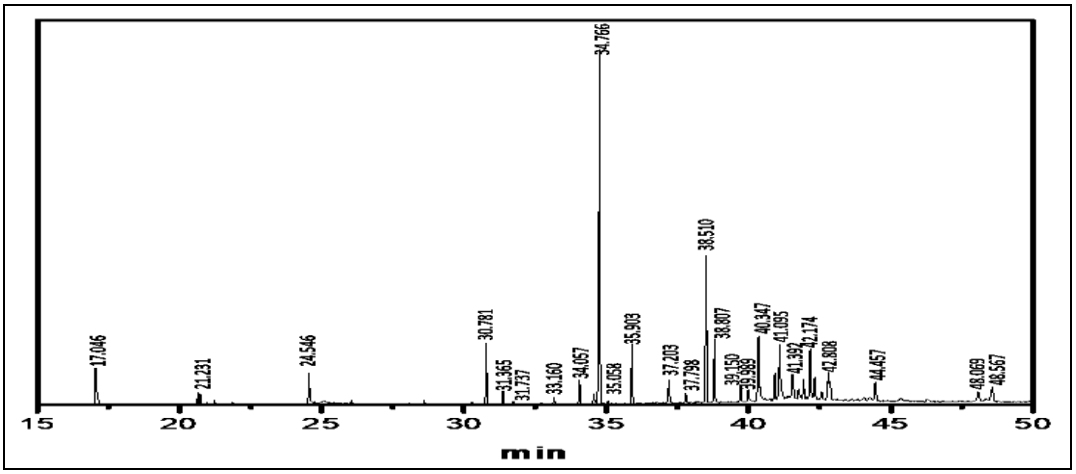


**FIG. 3: GC-MS CHROMATOGRAM OF HEXANE EXTRACT OF *A. BEDDOMEI***

The comparison of the constituents obtained from these plants by GC-MS was similar in 12 compositions, as illustrated in **Fig. 4**. However, squalene, β-sitosterol, tetratetracontane and lupeol were the predominant in the constituents of *Adhatoda* species. Two compounds, campesterol and methyl commate D were observed in *A. beddomei* extracts which possess various therapeutic properties *viz*., antioxidant [24] and antifungal and larvicidal activity [25, 26] whereas vasicolinone and amorphane-B, were detected only in *A. vasica* extract, amongst vasicolinone reported to have antitubercular activity [27] and amorphane-B reported to possesses antibacterial activity [28]. The structure of the compounds which were present in *A. vasica* and *A. beddomei* were displayed in **Fig. 5** and **Fig. 6**, respectively. *A. vasica* showed the highest distribution of the secondary metabolites when compared to *A. beddomei*. Based on the presence of the detected compounds, the hexane extracts of *A. vasica* and *A. beddomei* may find potential applications in the herbal and pharmaceutical industries.

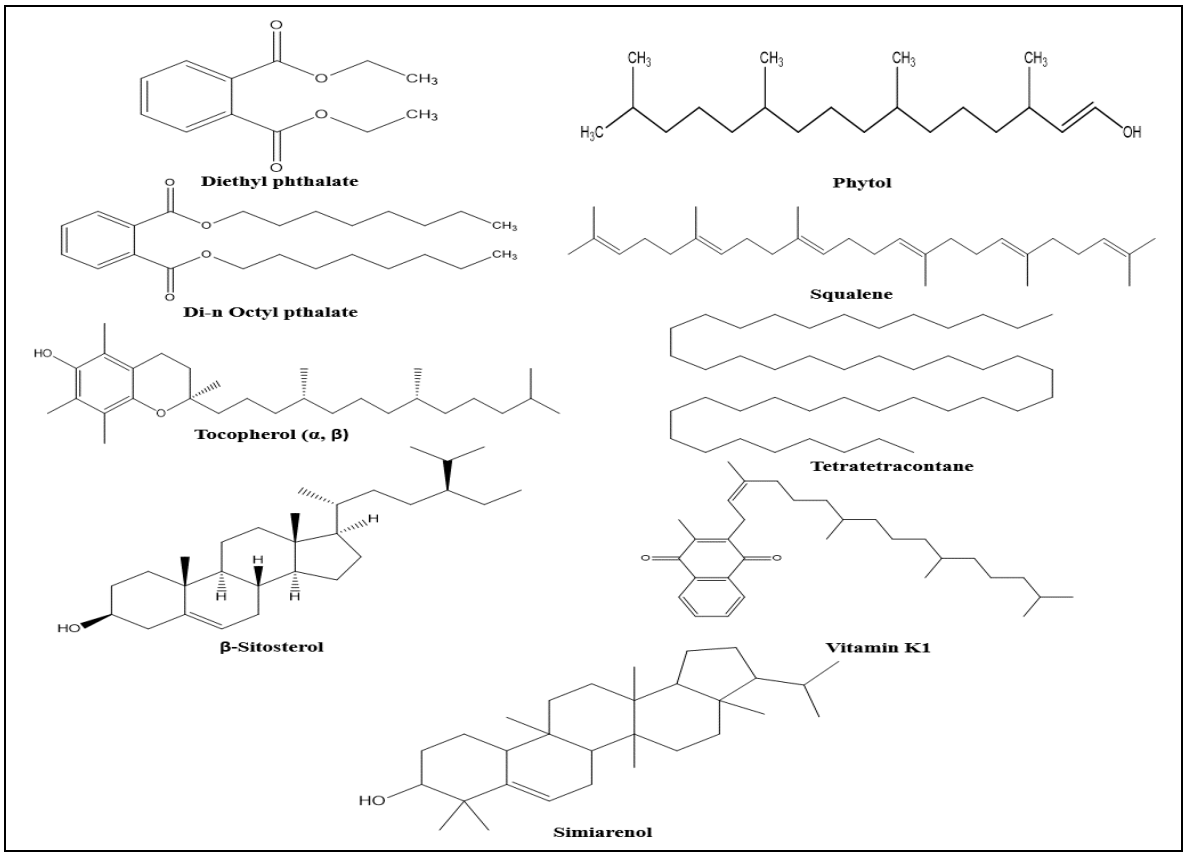


**FIG. 4: COMPOUNDS PRESENT IN THE HEXANE EXTRACT OF *A. VASICA* AND *A. BEDDOMEI***

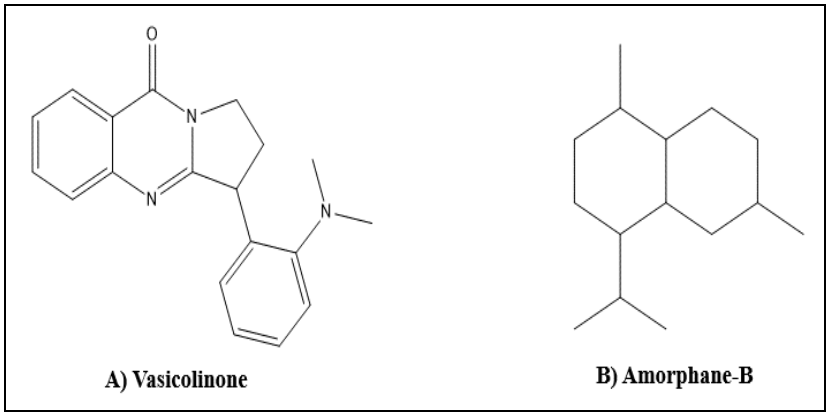


**FIG. 5: COMPOUNDS PRESENT IN HEXANE EXTRACT OF *A. VASICA* A) VASICOLINONE; B) AMORPHANE-B**

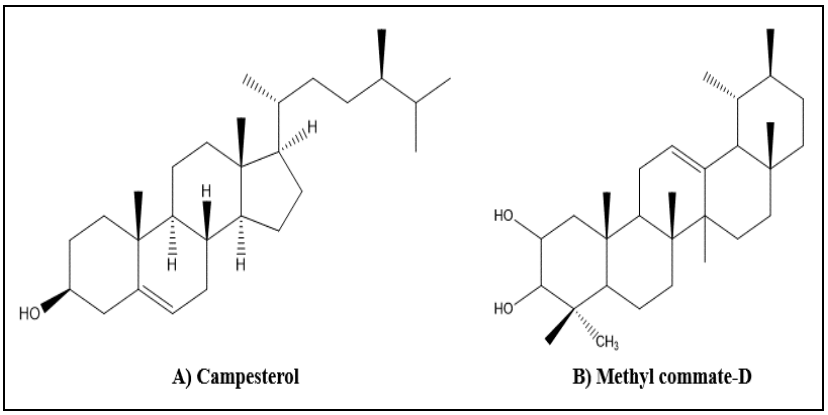


**FIG. 6: COMPOUNDS PRESENT IN HEXANE EXTRACT OF *A. BEDDOMEI* A) CAMPESTEROL; B) METHYL COMMATE-D**

**CONCLUSION:** To the best of our knowledge, this present study provides the first comprehensive approach to reveal the phytochemical compositional differences between hexane extracts of *A. vasica* and *A. beddomei* leaves. The combined information from phytochemical investigation and GC-MS techniques had been successfully differentiated through qualitative and chemical differences in chemical profiles of *A. vasica* and *A. beddomei* leaves.

Further investigation may lead to the isolation, characterization of bioactive compounds, and their pharmacological screening will be helpful for drug development.

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**CONFLICTS OF INTEREST:** The authors declare no conflict of interest.

## REFERENCES:

1. Boligon AA and Athayde ML: Importance of HPLC in analysis of plants extracts. Austin Chromatography 2014; 1(3): 1-2.
2. Yang M, Sun J, Lu Z, Chen G, Guan S, Liu X, Jiang B, Ye M and Guo DA: Phytochemical analysis of traditional Chinese medicine using liquid chromatography coupled with mass spectrometry. Journal of Chromatography A 2009; 1216(11): 2045-62.
3. Wolfender JL, Rodriguez S and Hostettmann K: Liquid chromatography coupled to mass spectrometry and nuclear magnetic resonance spectroscopy for the screening of plant constituents. Journal of Chromatography A 1998; 794(1-2): 299-16.
4. Costa DC, Costa HS, Albuquerque TG, Ramos F, Castilho MC and Sanches-Silva A: Advances in phenolic compounds analysis of aromatic plants and their potential applications. Trends in Food Science and Technology 2015; 45(2): 336-54.
5. Rao KV, Munjal M, Patnayak A, Karthik L and Kumar G: Phytochemical composition, antioxidant, antimicrobial and cytotoxic potential of methanolic extracts of *Adhatoda vasica* (Acanthaceae). Research Journal of Pharmacy and Technology 2013; 6(9): 1004-09.
6. Abhyankar G and Reddy VD: Rapid micropropagation via axillary bud proliferation of *Adhatoda vasica* Nees from nodal segments. Indian Journal of Experimental Biology 2007; 45: 268-71.
7. Dhuley JN: Antitussive effect of *Adhatoda vasica* extract on mechanical or chemical stimulation-induced coughing in animals. Journal of Ethnopharmacology 1999; 67(3): 361-65.

8. Srinivasan M, Padmaja B and Nair S: GC-MS profiling and *in-vitro* radical scavenging effect of *Adhatoda beddomei*. Journal of Pharmacognosy and Phytochemistry 2014b; 2: 55-59.
9. Kirtikar KR and Basu BD: Indian Medicinal Plants, International Book Distributors, Book seller and Publisher. 2005; 3: 1899.
10. Shinawie A: Wonder drugs of medicinal plants. Ethnobotany. Molecular and Cellular Biochemistry 2002; 213(1-2): 99-109.
11. Ishtiaq S, Ahmad M, Hanif U, Akbar S and Kamran SH: Phytochemical and *in-vitro* antioxidant evaluation of different fractions of *Amaranthus graecizans* subsp. Silvestris (Vill.) Brenan. Asian Pacific Journal of Tropical Medicine 2014; 7: S342-S347.
12. Najafi S and Deokule SS: Pharmacognostic study of *Tylophora dalzellii* Hook. f. Journal of Medicinal Plants Research 2010; 4(5): 403-06.
13. McDonald S, Prenzler PD, Antolovich M and Robards K: Phenolic content and antioxidant activity of olive extracts. Food Chemistry 2001; 73(1): 73-84.
14. Chang CC, Yang MH, Wen HM and Chern JC: Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis 2002; 10(3): 178-82.
15. Shamsa F, Monsef H, Ghamooshi R and Verdian-rizi M: Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. Thai Journal of Pharmaceutical Sciences 2008; 32: 17-20.
16. Rao TM, Rao BG and Rao YV: Antioxidant activity of *Spilanthes acmella* extracts. International Journal of Phytopharmacy 2012; 3(2): 216-20.
17. Solich P, Sedliakova V and Karlíček R: Spectrophotometric determination of cardiac glycosides by flow-injection analysis. Analytica Chimica Acta. 1992; 269(2): 199-03.
18. Kim E and Goldberg M: Serum cholesterol assay using a stable Liebermann-Burchard reagent. Clinical Chemistry 1969; 15(12): 1171-79.
19. Xu B and Chang SK: Phytochemical profiles and health-promoting effects of cool-season food legumes as influenced by thermal processing. Journal of Agricultural and Food Chemistry 2009; 57(22): 10718-31.
20. Ni Q, Xu G, Wang Z, Gao Q, Wang S and Zhang Y: Seasonal variations of the antioxidant composition in ground bamboo *Sasaargentea striatus* leaves. International Journal of Molcular Sciences 2012; 13(2): 2249-62.
21. Srinivasan K and Sivasubramanian S: Influence of drying temperature on quality parameters of *Adhatoda vasica* leaves. International Journal of Life Sciences Biotechnology and Pharma Research 2014a; 2: 280-85.
22. Kumar KS, Debjit B, Pankaj T and Rakesh K: Indian traditional herbs *Adhatoda vasica* and its medicinal application. Journal of Chemical and Pharmaceutical research 2010; 2(1): 240-45.
23. Karthikeyan A, Shanthi V and Nagasathaya A: Preliminary phytochemical and antibacterial screening of crude extract of the leaf of *Adhatoda vasica*. International Journal of Green Pharmacy 2009; 3: 78-80.
24. Kmiecik D, Korczak J, Rudzińska M, Kobus-Cisowska J, Gramza-Michałowska A and Hęs M: β-sitosterol and campesterol stabilisation by natural and synthetic antioxidants during heating. Food Chemistry 2011; 128(4): 937-42.
25. Motlhanka D, Houghton P, Miljkovic-Brake A and Habtemariam S: A novel pentacyclic triterpene glycoside from a resin of *Commiphora glandulosa* from Botswana. African Journal of Pharmacy and Pharmacology 2010; 4(8): 549-54.
26. Kumar VA, Ammani K, Siddhardha B, Sreedhar U and Kumar GA: Differential biological activities of the solvent extracts of *Ceriops decandra* (Griff.) and their phytochemical investigations. Journal of Pharmacy Research 2013; 7(7): 654-60.
27. Jha DK, Panda L, Lavanya P, Ramaiah S and Anbarasu A: Detection and confirmation of alkaloids in leaves of *Justicia adhatoda* and bioinformatics approach to elicit its anti-tuberculosis activity. Applied Biochemistry and Biotechnology 2012; 168(5): 980-90.
28. Wu SJ, Fotso S, Li F, Qin S and Laatsch H: Amorphane Sesquiterpenes from a Marine Streptomyces sp. Journal of Natural Products 2007; 70(2): 304-06.


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