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Identification of Bioactive Compounds from Leaf Methanolic Extracts of *Adhatoda vasica* (L.) Nees and *Psidium guajava* L. by GC-MS Profiling

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Abstract

While *Adhatoda vasica* (Vasaka) is an important medicinal plant used as a remedy for treating bronchitis, tuberculosis and other lung disorders, *Psidium guajava* (Guava) is used against diabetes, diarrhea, dysentery, gastroenteritis and hypertension. We report here the methanolic extract and phytochemical analysis of both the plants from the leaves using gas-chromatography (GC) and mass spectrometry (MS). Analysis of vasaka revealed the presence of amrinone, a selective phosphodiesterase III inhibitor effective in the treatment of congestive heart failure; silicic acid associated with bone mineralization, collagen synthesis, skin, hair and nails health atherosclerosis, Alzheimer disease, immune booster; arsenous acid used for treating leukemia; methyltris (trimethylsiloxy) silane which is a potent antibacterial compound; tetrasiloxanedecamethyl- that exhibits antifungal activity. Analysis of guava revealed the presence of copaene (a tricyclic sesquiterpene) that has antioxidant activity, caryophyllene oxide which has relaxation effect, humulene that promotes appetite-suppressing effect and used for weight loss, γ-muurolene that is known for its antibacterial activity and also alloaromadendrene, a sesquiterpenoid with anticancer and antibacterial properties. Importantly, it also contains (±)-norephedrine, an appetite suppressant and used for nasal decongestion. Phenylephrine is used to relieve nasal discomfort caused by colds, allergies, and hay fever, and (-)-isolongifolol, an antioxidant. The data show that both the plants have important bioactive molecules that can be further exploited for treating human ailments.

Keywords: *Adhatoda vasica*, *Psidium guajava*, GC-MS analysis, phytochemicals

Introduction

Plants are a rich source of highly effective and safe medicinal compounds (1). Medicinal plants serve as complements or substitutes to the modern medical treatments and play pivotal roles in 75% of the rural areas of many countries (2,3). In other words, medicinal plants have been contributing to the health and security of rural folk. Such plants should be thoroughly investigated to find out their properties and efficacy (4). Plants biosynthesize organic compounds such as alkaloids, flavonoids, steroids, tannins, terpenoids and others (5,6). Phytochemicals are diverse with a wide range of functions. These phytochemicals derived from barks, flowers, leaves, roots and seeds are not only used in the human therapy, but also in agriculture and veterinary (7,8). Knowledge about the phytoconstituents of the plants is highly vital for the chemical synthesis of the complex molecules (9,10).

A. vasica also called as *Justicia adhatoda* or Malabar nut in English belongs to the family Acanthaceae and is a perennial shrub. It is native to Asia and the plant is used to treat asthma, chronic bronchitis, cold, cough, diarrhea, expectorant, fever, and rheumatic inflammatory swellings (11,12). *A. vasica* contains secondary metabolites like adhatodine, vasicine, vasicinone, vasicinolone, vasicol, vasicoline, and vasicolinone (13, 14, 15). *P. guajava* is a small tree, commonly called as guava, belongs to the family Myrtaceae, and native to Mexico (16). The plant is used for a number of diseases such as anti-inflammatory, for caries, diabetes, hypertension, pain relief, and wound healing (17). Secondary metabolites like carotenoids, flavonoids, phenolics, and triterpenes have been isolated from *P. guajava* and found to have biological activities (18,19). The fruit is a rich source of vitamin C. Though some secondary metabolites have been isolated (17,18,19), we report here new compounds like amrinone, silicic acid from vaska and copaene, caryophyllene oxide, γ-muurolene and -(-) norephedrine from gauva for the first time in the leaf methanolic extracts.

Material and Methods

Plant material

Leaves of *Adhatoda vasica* and *Psidium guajava* were collected from the Medicinal Plant Garden, Vignan's Foundation for Science, Technology and Research (Deemed to be University), Guntur. The plants were authenticated by Prof. V.S. Raju, Department of Botany, Kakatiya University, Warangal.

Methanolic extraction

Chemicals and solvents used in this study were of Analytical grade obtained from SD Fine Chemicals Pvt. Ltd., Mumbai. The leaves were washed in running tap water to remove the dust adhered and then shade dried for 4-5-days. The leaves were finely powdered and 200 grams of powder soaked in 600 ml of n-hexane until it was completely immersed for 48-hours. Thereafter, hexane was decanted and 600 ml of methanol was added until the powder was totally soaked. The plant extract was collected and filtered through Whatman Number 1 filter paper. The filtrates were concentrated at room temperature. The dried residue was dissolved in 1.0 ml of methanol and kept at 5 ^{o} C in a refrigerator until further use.

Gas chromatography-mass spectrometric (GC-MS) analysis

Methanolic extract was subjected to GC-MS for qualitative analysis of phytochemicals using standard procedure. The GC-MS instrument employed for the analysis of the phytochemicals was Agilent Technologies (Model: GC:7890B, MSD:5977A). The non-polar column used was HP-5-MS 30*o.25*0.2. The vehicle gas used was Helium with a flow rate of 1.0 ml/min. Oven and inject temperature are 50 ^{0} C; and 250 ^{0} C respectively. Mode of injection was split. The resultant m/z peaks were matched to the NIST11 library for compound search. The spectra obtained were recorded and shown. The spectra were analysed manually by referring to the available literature (20).

Results and Discussion

Present study was designed to identify the secondary metabolites in hexane and methanolic extract of *A. vasica* and *P. guajava* via GC-MS analysis. In all, 25 compounds have been detected in both the plants. The plants vasaca and guava are shown in the Figure 1a and 1b, and the list of compounds present is shown in the Tables 1 and 2 respectively. GC-MS spectral analysis of vasaka (Fig. 2a) and guava (Fig. 2b) are shown. Further, GC-MS spectra of amrinone (Fig. 3a) and silicic acid (Fig. 3b) from vasaka and copaene (Fig. 4a), caryophyllene oxide (Fig. 4b) from gauva are represented.


Fig. 1a. Twig of vasaka


Fig. 1b. Twig of guava

Though many compounds have been detected in the cold methanolic extract of *A. vasica*, and *P. guajava*, 25 and 21 important molecules have been listed in Tables 1 and 2 respectively. In vasaca leaf extract, amrinone (Fig. 3a), a pyridine phosphodiesterase-3-inhibitor has been detected (Table 1). It improves prognosis of patients with congestive heart failure. Eight molecules (bicyclo [2.2.1] heptan-2-one 4,7,7-trimethyl-, 3,5-dimethyl-4-benzylisoxazole, 9-borabicyclo [3.3.1] nonane, pyridazin-3(2H)-one, tris(tert-butyldimethylsilyloxy)arsane, 5-methyl-2-phenylindolizine, cyclotrisiloxane, and methyltris (trimethylsiloxy) silane have been identified with antimicrobial activity. Four compounds (3,5-dimethyl-4-benzylisoxazole, 9-borabicyclo [3.3.1] nonane, tris (tert-butyldimethylsilyloxy) arsane, and 5-methyl-2-phenylindolizine) also exhibit antioxidant properties (Table 1). Importantly, three compounds, namely, imidazole-4-carboxylic acid, pyridazin-3(2H)-one, and silicic acid (Fig. 3b) have been found associated in the treatment of neurological disorders such as Alzheimer, Huntington, depression and epilepsy. Also, another three compounds especially bicyclo[2.2.1] heptan-2-one, 4,7,7-trimethyl-, 3,5-dimethyl-4-benzylisoxazole, and pyridazin-3(2H)-one have been identified in this study with anticancer properties. Two compounds principally 3,5-dimethyl-4-benzylisoxazole and silicic acid are also allied with immune boosting. While 3,5-dimethyl-4-benzylisoxazole, and pyridazin-3(2H)-one have antiviral activities, tetrasiloxanedecamethyl- is known to act against a wide range of fungi. Similarly, silicic acid helps in collagen synthesis, skin, hair and nails health, and atherosclerosis, pyridazin-3(2H)-one displays antidiabetic activity (Table 1).

Table 1. List of secondary metabolites of *Adathoda vasica* as revealed by methanolic extract by GC-MS

S. No.	Name of the compound	Chemical formula	Medical use	Reference
1	2-Methoxydiethylamine	$(CH_3)2NH$	Unknown	-
2	Imidazole-4-carboxylic acid	$C_5H_6N_2O_2$	Acts against neurodegenerative disorders like Alzheimer's and Huntington's	21
3	4-[Ethylethanolamino]-1,2-naphthoquinone	$C_{10}H_7NO_2$ (naphthoquinone)	Unknown	-
4	Egtazic acid	$C_{14}H_{24}N_2O_{10}$	Chelating agent.Used for the treatment of animals with cerium poisoning	22
5	2-Cyclopenten-1-one, 5-hydroxy-2,3-dimethyl-	$C_7H_{10}O_2$	Unknown	-
6	3,4-Pentadienal	C_5H_6O	Unknown	-
7	1,2-Dimethyl cyclopropene	C_5H_{10}	Unknown	-
8	Cyclopropane carboxamide	C_4H_7NO	Its prodrugs act as β-blocking agents. It is a direct precursor of the phytohormone ethylene and also acts as a growth regulator	23 24

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9	3-Piperidinol, 1,4-dimethyl-	$C_7H_{15}NO$	Unknown	-
10	3-Picoline, 4-amino-, 1-oxide (Synonym 4-Amino-3-methylpyridin-1-ium-1-olate)	$C_5H_6N_2O$	Unknown	-
11	Bicyclo[2.2.1]heptan-2-one, 4,7,7-trimethyl-	$C_{10}H_{16}O$	Anti-inflammatory, antioxidant, antimicrobial, and anticancer effects	
12	Androst-4-en-3-one	$C_{19}H_{28}O$	Unknown	-
13	Mono-ethyl-malonate-mono-amide	$C_5H_8O_4$	Enzyme inhibitor	25
14	Amrinone	$C_{10}H_9N_3O$	Used in the treatment of acute heart failure, epilepsy, and for the synthesis of tumour necrosis factor (TNF-α)	26,27
15	3,5-Dimethyl-4-benzylisoxazole	$C_{19}H_{14}O_3$	Immunoregulatory function, antibacterial, antiviral, antiproliferative, anti-tubercular, antioxidant	28
16	9-Borabicyclo[3.3.1]nonane	$C_8H_{15}B$	Antiinflammatory, antioxidant and antimicrobial	29
17	Pyridazin-3(2H)-one	$C_4H_4N_2O$	Antibacterial, muscle relaxant, anti-depressant, antidiabetic, anti-hypertensive, analgesic, anti-tumor, antiviral, nephrotropic, antiinflammatory, anti-viral, anticancer, anti-aggregative, anti-epileptic	30
18	Arsenous acid	H_3AsO_3	Anticancer	31
19	Tris(tert-butyldimethylsilyloxy) arsane	$C_{18}H_{45}AsO_3Si_3$	Antioxidant and antibacterial	32
20	5-Methyl-2-phenylindolizine	$C_{15}H_{13}N$	Antimicrobial and antioxidant	33
21	Silicic acid	H_4SiO_4	Associated with bone mineralization, collagen synthesis, skin, hair and nails health atherosclerosis, Alzheimer disease, immune system enhancement	34
22	Cyclotrisiloxane	$H_6O_3Si_3$	Antimicrobial	35
23	Methyltris(trimethylsiloxy) silane	$C_{10}H_{30}O_3Si_4$	Antibacterial	36
24	Tetrasiloxane, decamethyl-	$C_{10}H_{30}O_3Si_4$	Antifungal	37
25	1,4-Bis(trimethylsilyl)benzene	$C_{12}H_{22}Si_2$	Anti-oxidative stress and anti-hyperlipidemia	38

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In *P. guajava*, new compounds associated with anticancer such as (1) copaene (Fig. 4a), (2) humulene, (3) γ-muurolene, and (4) β-caryophyllene oxide (Fig.4b) have been noticed (Table 2). Importantly, two compounds namely humulene and -(-)-norephedrine have been found implicated in appetite suppression and weight loss. Both phenylephrine and -(-)-norephedrine appear to help in nasal decongestion. On the other hand, analysis of gauva revealed the presence of copaene (a tricyclic sesquiterpene) that has antioxidant activity, caryophyllene oxide (Fig. 4b) which has relaxation effect, humulene that promotes appetite-suppressing effect and used for weight loss, γ-muurolene that is known for its antibacterial activity and also alloaromadendrene, a sesquiterpenoid with anticancer and antibacterial properties. Importantly, it also contains (±)-norephedrine, an appetite suppressant and used for nasal decongestion. γ-muurolene has good antibacterial activity. Further, carbromal is currently being used for treating mild insomnia (Table 2).

Table 2. List of secondary metabolites of *Psidium guajava* as revealed by methanolic extract by GC-MS

S. No.	Name of the compound	Chemical formula	Medical use	Reference
1	Aurin	$C_{19}H_{14}O_3$ (Dye)	Used as a dye intermediate	40
2	Copaene	$C_{15}H_{24}$ (Sesquiterpene)	Anticancer and antioxidant	41
3	β-Caryophyllene or Humulene, α-Humulene	$C_{15}H_{24}$ (Monocyclic sesquiterpene)	Anticancer and analgesic. Beneficial for colitis. Antibacterial, antibiofilm effects, Appetite suppressing effect, weight loss	42,43.44
4	γ-Muurolene	$C_{15}H_{24}$ (Terpene)	Antibacterial activity	45
5	(-)-Isolongifolol	$C_{15}H_{26}O$ (Tricyclic sesquiterpenol alcohol)	Sesquiterpene and antioxidant	39
6	4-isopropyl-1,6-dimethyl-1,2,3,4- tetrahydronaphthalene	$C_{15}H_{22}O$	Unknown	-
7	Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-	$C_{15}H_{24}$	Unknown	-
8	2-Propanone, 1-chloro-1-(phenylthio)-	$C_{10}H_{11}C$	Unknown	-
9	β-Caryophyllene oxide	$C_{15}H_{24}O$ (Sesquiterpene)	Anticancer and analgesic	43

10	Alloaromadendrene	$C_{15}H_{24}$	Unknown	-
11	Isoaromadendrene epoxide	$C_{15}H_{24}O$	Unknown	-
12	*cis*-Z-α-Bisabolene epoxide	$C_{15}H_{24}O$ (Sesquiterpene)	A component in the essential oils of plants with antimicrobial activity	46
13	N,N-dimethyl-urea	$C_3H_8N_2O$	Efficient ligand for the synthesis of pharma-relevant motifs through Chan-Lam cross-coupling strategy	47
14	Phenylephrine	$C_9H_{13}NO_2$	Relieves nasal discomfort caused by colds, allergies, and hay fever. An active vasopressor agent	48
15	4-Aminocinnamic acid	$C_9H_9NO_2$	Anion scavenging biopolyamide	49
16	Ethanedialdioxime	$C_2H_4N_2O_2$	Unknown	-
17	-(-)-Norephedrine	$C_9H_{13}NO$	Nasal decongestant and an appetite suppressant	50
18	N-(1-Bicyclo[2.2.1]hept-2-yl-ethyl)-butyramide	$C_9H_{14}O$	Not known	-
19	Benzaldehyde, 2-nitro-, diaminomethylidenhydrazone	$C_8H_9N_5O_2$	Cures infectious diseases	51
20	2-Butenediamide, (Z)-	$C_4H_{12}N_2$	Unknown	-
21	Carbromal	$C_7H_{13}BrN_2O_2$	For treating mild insomnia	52

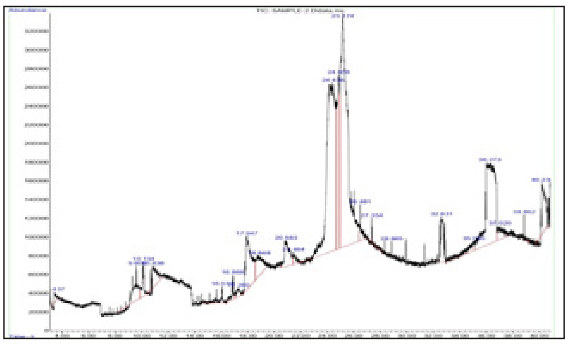

Fig. 2a. GC-MS profiling of *Adhatoda vasica*(L.) Nees leaf methanolic extract

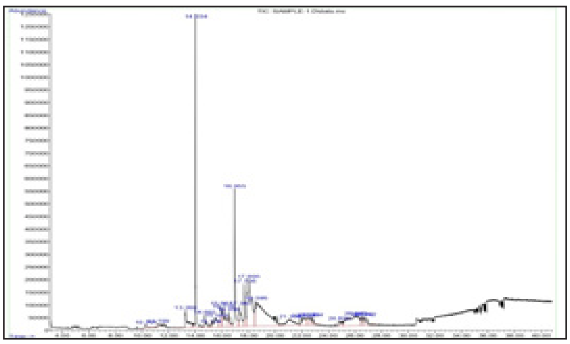

Fig. 2b. GC-MS profiling of *Psidium guajava* L. leaf methanolic extract

Identification of bioactive compounds from leaf methanolic extracts

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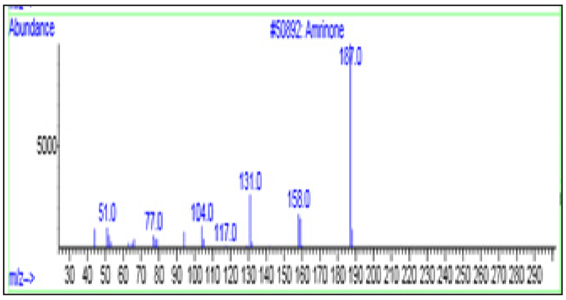

Fig. 3a. GC-MS spectrum of amrinone seen in vasaka extract

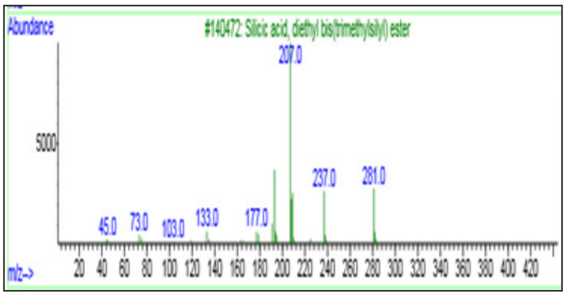

Fig. 3b. GC-MS spectrum of silicic acid observed in vasaka extract

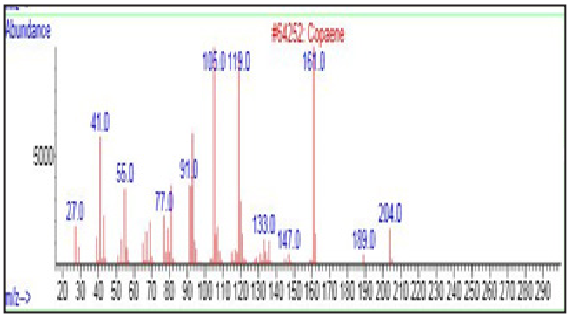

Fig. 4a. GC-MS spectrum of copaene noticed in guava

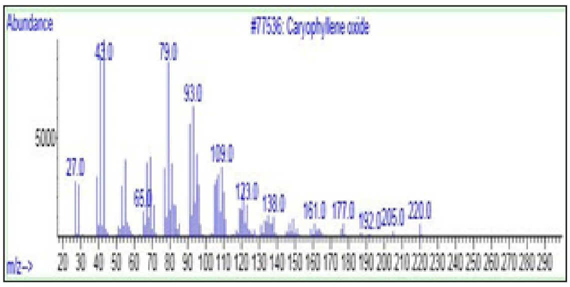

Fig. 4b. GC-MS spectrum of caryophyllene oxide detected in gauva

Discussion

Chemical profiling of medicinal plants is vital for the development and standardization of herbal formulations. Secondary metabolite analysis is also crucial for ensuring the safety of the herbal formulations. Further, such an analysis will also pave the way for the characterization and chemical synthesis of drug-like molecules.

A. vasica also called as Malabar nut is being used for respiratory disorders like asthma, chronic bronchitis, cold, and cough, (53). Alkaloids, fatty acids, flavonoids, saponins and tannins have been detected previously in the leaf methanolic extracts (54,55). Fatty acids and heterocyclic compounds with antibacterial, antifungal, anti-inflammatory, and antimycotic activity have been identified in the leaves, stems and flowers (56). 2-Acetyl benzyl amine, adhatodine, vasicine, vasicine acetate, vasicinone, vasicinolone, vasicol, vasicoline, vasicolinone have also been detected (57,58,59,60), and also their biological activities (61,62). Secondary metabolites such as 2-butylphenol, cyclobutylhexyl ester, 2,3-dihydro-3,5-dihydroxy6-methyl-4H-pyran-4-one, 3,4-dihydroxy-5-methyldihydro-furan-2-on, 2(OR) 3-(1,1-dimethylethyl)-4-methoxyphenol, hexadecanoic acid, 5-hydroxymethylfurfural, megastigmatrienone 3, tetradecanoic acid, oxalic acid, 1,3,5-triazine-2,4,6-triamine, and vomifoliol have been identified in the methanolic extracts of leaves (53). In the present study, new compounds have been detected in the cold methanolic extract. Amrinone, arsenous acid, silicic acid, methyltris(trimethylsiloxy)silane, tetrasiloxanedecamethyl- are being reported. All these new compounds have proven clinical activities (Table 1). These compounds if validated will be extremely useful for treating the diseases.

The fruit of guava is edible and rich in vitamin A and C (63). Many metabolites such as carotenoids, flavonoids, phenolics, terpenoids, triterpenes have been detected with useful biological activities (64,65,66). Methanolic

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and chloroform extracts with a number of secondary plant products have been isolated both from leaves and fruits (67). Its antimalarial (68), antihyperglycemic (69) and anticancer activities (70) have been known. In the present study, new compounds such as copaene, humulene, γ-muurolene; β-caryophyllene oxide, phenylephrine and -(-)-norephedrine are the few molecules which have pharmacological activity and used with health benefits. Such a variation in the detection of these compounds could be due to different solvents and also the diverse plant parts used. Diverse molecules detected in guava extracts have exhibited pharmacological activities thus far *in vitro* tests using animal models. But they need to be tested in clinical trials for the development of these molecules as drugs. This gap has to be bridged in order to exploit the medicinal use of this plant fully.

Conclusions

The results showed the presence of all together new compounds in both the plants. These compounds have not been reported previously. Importantly, these compounds appear to have pharmaceutical activity as revealed by literature search. Such compounds need to be isolated and purified for further characterization, and tested not only on animal models but also in clinical trials for their toxicological effects and also pharmacological efficacy.

Author contribution

Dr. S.Asha has designed the article structure. BM has carried out the phytochemical analysis from the two plants. All others have analyzed and interpreted the data. Dr. S. Asha has prepared the draft manuscript. All authors have refined the article. All authors have read and approved.

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Data availability

The data are available with Dr. S. Asha and with Bhargavi Maddineni.

Conflict of interest

Authors declare that they do not have any conflict of interest.

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