ANALYSIS OF PHYTOCOMPONENTS IN THE METHANOLIC EXTRACT OF *Justicia gendarussa* Burm.f.

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ABSTRACT

Justicia gendarussa Burm.f. belongs to the family Acanthaceae. In local population of West Papua, it is used as male contracetive. The objective of this study is to identify the present in the methanolic extract of Justicia gendarussa Burm.f. by GC-MS analysis. Twenty grams of the powdered plant materials are subjected to three methanol washes followed by filtration of the combined filtrate through the filter paper, and evaporate to give a final yield of 7% extract. The extract is subjected to GC-MS analysis. The GC-MS analysis of the sample is performed using an Agilent 6980N Network GC System with autosample. Data handling is done using GC-MS solution software. The identification of compounds is based on comparison of their mass spectra with those of WILEY version 8.0 Libraries. Twenty three compounds are identified. The major constituents are 2-ethylidene-1-methyl-3-phenylimidazolidine, 9,12-Octadecadienoic acid (Z,Z), and 5,6,8,9-tetramethoxy-2-methylpepero (3,4,5-jk)-9,10-dihydrophenanthracene.

Keywords: *GC-MS, Justicia gendarussa Burm.f., Acanthaceae, Phytocomponents*

INTRODUCTION

Justicia gendarussa Burm.f. (Indonesian Plant Medicine), the local name is Gandarusa, belongs to *Acanthaceae* and common in forests of West Papua, Indonesia. Traditionally, the extract of this plant is consumed by the West Papua community as a male contraceptive (Soerjowinoto and Poejoarinto, 1985).

In Ayurveda, the plant is useful for treatment of inflammation, bronchitis, vaginal discharges, eye diseases, dyspepsia, and fever. The decoction of the leaves and tender shoots are diaphoretic and they are given in chronic rheumatism. Oil prepared from the leaves is useful in eczema and the mixture of leaves is given internally for hemiplegia, cephalalgia, facial paralysis (Kavitha et al., 2014). In Indonesia, this plant is clinically used for male contraceptive drug (Prajogo et al., 2007).

Gendarussa is a native plant from South Asia and South East Asia. Gendarussa is erect undershrub, 0.6-1.2m in height with subterete branches. This plant grows wildly in the forest, river embankment, curbs, and shrubs, ranging from lowland to the altitude of 1,500 m asl. This crop is planted as a living fence and preserved as a

medicinal plant. In Java, willow grows at an altitude of 1-500 m asl (Syamsuhidayat and Hutapea, 2000).

Plants of different habitats will produce different profiles of secondary metabolites as well. *Piper crocatum* leaf extract, acollection of nursery Palembang contain essential oils such as sesquisa-binene hydrate (22.83%), β-bisabolo (17,24%), γ=curcumene (11.16%), anymol (3.9%), and trans-caryophyllane (2.37%) (Adnan et al., 2011). While GC-MS analysis of *P. crocatum* leave extract from Magelang Central Java contains 16 components. The main component consist of sabinen (44.91%) and β=mirsen (18.8%) (Marliyana et al., 2013).

The present work was carried out to identify some of the phytocomponents present in the methanolic extract of the leave of *J. gendarussa* Burm.f. by GC-MS technique, to ascertain the medicinal properties of the plant.

MATERIALS AND METHODS

Collection of the plant materials

Justicia gendarussa Burm.f. plant was collected from the Taman Husada Graha Famili, Surabaya, Indonesia. *Justicia gendarussa* Burm.f. was identified and authenticated at Department of Biology, Faculty of Science and Technology, Airlangga University, Surabaya, Indonesia.

Preparation of the extracts

Justicia gendarussa Burm.f. leaf was shade dried, crushed by hand, and ground into coarse powder using a mortar. Twenty grams of the powdered plant materials were subjected to three methanol washes followed by filtration of the combined filtrate through the filter paper, and evaporated to give a final yield of 7% extract. The extract was subjected to GC-MS analysis.

GC-MS analysis

The GC-MS analysis of the sample was performed using an Agilent 6980N Network GC System with autosampler, Detector Agilent 5973 inert MSD, fitted with J&W Scientific, HP-5 5% phenylmethylsiloxane capillary column (30m X0.32 mm, with 0,2um film thickness). The oven temperature was programmed from 50^0C to 280^0 C at 100^0C/min and a hold for 10 min. Helium was used as the carrier gas at flow 1.3 mL/min. The injector temperature was 280^0C, injection size 1µL, with split ratio 1:10. The interface and MS ion source were maintained at 230^0C and 150^0C, respectively, the mass spectra were taken at 70eV with a mass scan range of 200-700 amu. Data handling was done using GCMS solution software. The identification of compounds was based on comparison of their mass spectra with those of WILEY version 8.0 Libraries.

RESULTS AND DISCUSSION

The GC-MS chromatogram of *Justicia gendarussa* Burm.f. methanolic extract showed 23 peaks (Figure 1) and have been identified after comparison of the mass

spectra with WILEY version 8.0 (Table 1), indicating the presence of 23 phytocomponents.

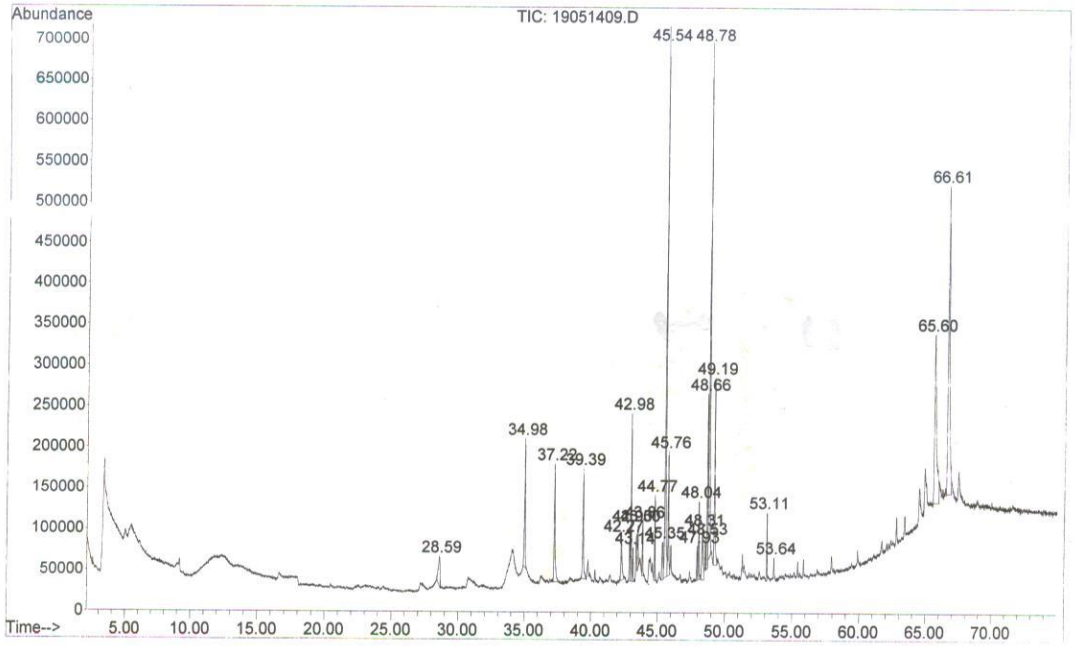

Figure 1. *GC-MS chromatogram of the methanolic extract of Justicia gendarussa Burm.f. leaf*

From the results, it was observed that tweenty three phytocomonent was identified, 2-ethylidene-1-methyl-3-phenylimidazolidine (14.351%), 9,12 Octadecadienoic acid (Z,Z) (13.731) and 5,6,8,9-tetramethoxy-2-methylpepero (3,4,5-jk)-9,10-dihydrophenanthracene (11.440) were the major components in the extract (Table 1).

The quantitative estimation of phytoconstituents of the leaves of *Justicia gendarussa* were collected from Anand farm and nursery Gandhinagar, Gujarat contained carotenoids (7.88 ±0.394 %), alkaloids (1.62 ±0.081 3%), phenolics (2.21 ±0.11 %), flavonoids (2.03 ±0.105 %), triterpenic acids (0.199 ±0.009 %), sugar (8.74 ±0.435%), and starch (5.85 ±0.292%) (Sonala et al., 2011). *Justicia gendarussa* was collected from Botanical Garden, Forest Research Institute, New Forest, Dehradun, contains β-sitosterol, b-Sitosterol-b-D-glycoside and aromadendrin (Bachheti et al., 2011). Samples of *Justicia gendarussa* were collected from Kishoreganj, Bangladesh containedthree compounds, stigmasterol, lupeol, 16-hydroxylupeol (Uddin et al., 2011).

The methanolic extract of *Justicia wynaadensis* from Irpu Hills, Western Ghats, Karnataka by GC-MS analysis to ascertain it's usage by the local community as a plant possessing medicinal properties. Twenty four compounds were identified. The major constituents are Dihydrocoumarin, Phytol and Palmitic acid. Significant quantities of Linoleic acid, Stearic acid, Squalene and phytosterols such as Campesterol and Stigmasterol were also present (Ponnamma and Manjunath, 2012). In this study Phytol was also identified (Table 1).

Table 1. *Phytocomponents identified in the methanolic extracts of Justicia gendarussa Burm.f. leaves by GC-MS*

Number	Name of Compund	Retention Time (min)	Average % Age of Compounds in Leaf
1	Dodecamethyl cyclohexasiloxane	28.587	1.672
2	Tetradecamethyl cycloheptasiloxane	34.983	3.647
3	4-oxo-4H-pyrido [1,2-a] pyrimidine-3-carbonitrile	37.222	4.008
4	Hexadecamethyl cycloheptasiloxane	39.385	2.968
5	1-methyl-5,7-indoline dicarboxaldehyde	42.274	1.717
6	4-(3,4-Dimethoxybenzylidene)-1-(4-nitrophenyl)-3-phenyl-2-pyrazolin-5-one	42.900	1.254
7	Neophytadiene	42.984	3.152
8	[R- [R@, R@-(E)]]-3, 7, 11, 15-tetramethyl-2-Hexadecene	43.137	0.710
9	Ethyl 5,6,7,8-tetrahydroquinoline-3-carboxylate	43.504	0.991
10	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	43.856	1.032
11	Methyl Palmitat	44.765	1.640
12	2-ethylidene-1-methyl-3-phenyl imidazolidine	45.537	14.351
13	Hexadecanoic acid (CAS)	45.759	3.245
14	O,O-diethyl ester Phosphorochloridothioic acid	47.929	0.667
15	methyl ester-9,12-Octadecadienoic acid (Z,Z)	48.043	1.603
16	Methyl Linolenat	48.311	2.273
17	Phytol	48.533	0.563
18	Methyl ester-16-methyl-Heptadecanoic acid	48.662	4.283
19	9,12-Octadecadienoic acid (Z,Z)	48.785	13.731
20	9,12,15-Octadecatrien-1-ol, (Z,Z,Z)	49.190	4.759
21	Octadecanoic acid	53.110	1.358
22	Bis(2-ethylhexyl) ester-hexanedioic acid,	53.637	0.446
23	5,6,8,9-tetramethoxy-2-methylpepero (3,4,5-jk)-9,10-dihydrophenanthracene	65.605	11.440

Gendarussa leaves obtained from Pacet, Indonesia contained 6,8-di-C-α-L-arabinosyl-apigenin, 6-C-α-L-arabinosyl-8-C-β-D-xylosyl-apigenin (Prajogo, 2002), and justidrusamides A-D (Kiren et al., 2014). Whereas the species collected from India contained β-sitosterol, friedelin, lupenol (Chakravarty et al., 1982), and O-disubstituted aromatic amines (2-amino-O-methyl-benzyl alcohol, 2-(2'-amino-benzyl-amino)-O-methyl-benzyl alcohol, 2-amino-benzyl alcohol, 2-(2'-amino-benzylamino)-benzyl alcohol) (Kim et al., 2011).

Environmental factors, such as the site of cultivation, altitude, temperature, sun exposure time, rainfall, climate, and soil can influence the primary and secondary metabolites of plants. These factors may affect secondary metabolites

qualitatively and quantitatively, so their bioactivities could be varied (Nivas and Gaikwad, 2014).

According to the references, there is some activity of the phytocomponent indentified (Table 2).

Table 2. *Activity of some of the phytocomponents identified in the methanolic extracts of Justicia gendarussa Burm. f by GC-MS*

Number	Name of Compund	Activity
1	Dodecamethyl cyclohexasiloxane	Antimicrobial (Mahmoud et al., 2013)
2	Tetradecamethyl cycloheptasiloxane	Antimicrobial, antifouling imunomodulatory and antitumor activities (Arun, and Varsha, 2014; Sheeba and Viswanathan, 2014; Thangavel et al., 2014)
3	Octadecanoic acid (stearic acid)	Antioxidant (Sonala et al., 2011)
4	Neophytadiene	Anti-bacteria (Aparna et al., 2012)
5	3, 7, 11, 15-tetramethyl-, [R- [R@, R@-(E)]]-2-Hexadecene,	Anti-bacteria (Aparna et al., 2012)
6	Hexadecanoic acid (CAS)	Anti-inflammation (Natarajan and Dhas, 2013), anti-diabetes (Choi et al., 2013), anti-bacteria (Lucie et al., 2013), Antioxidant (Sonala et al., 2011)
7	Methyl ester-16-methyl-Heptadecanoic acid	Insecticidal activity (Diezel et al.,, 1993), Antioxidant (Sonala et al., 2011)
8	Methyl ester-9,12-Octadecadienoic acid (Z,Z)	anti-inflammatory, acnereductive, and moisture retaining properties (Letawe et al., 1998; Darmstadt et al., 2002; Yang et al., 2014), Antioxidant (Sonala et al., 2011).
9	Methyl ester-9,12,15-Octadecatrien-1-ol, (Z,Z,Z)	Anti-thrombotic effects (Hansen and Harris, 2007), Anti-inflammation (Hansen and Harris, 2007; Harris et al., 2008; Mozaffarian, 2005), anti-bacteria (Mozaffarian, 2005)
10	Methyl Palmitat	Ascaricidal Activity (Wang et al., 2009). Antifungal Activity (Lima et al., 2011), antioxidant (Choi, 2016) nematicide (Munakata, 1983)
11	Phytol	Cancer preventive (Sonala et al., 2011)

Octadecanoic acid or stearic acid is used in the manufacture of pharmaceutical products. Recently, it has been used in the development of a drug delivery system, because it is considered to be inert, inexpensive, and biocompatible, as well as of a low toxicity (Killen and Corrigan, 2001). In addition, stearic acid has been used for masking the bitter taste of pharmaceutical compounds (Robson et al., 1999).

In medicine industry, Bis(2-ethylhexyl) ester hexanedioic acid, (or Adipic Acid) has been incorporated into controlled-release formulation matrix tablets to obtain pH-independent release for both weakly basic and weakly acidic drugs. It has also been incorporated into the polymeric coating of hydrophilic monolithic

systems to modulate the intragel pH, resulting in zero-order release of a hydrophilic drug. The disintegration at intestinal pH of the enteric polymer shellac has been reported to improve when adipic acid was used as a pore-forming agent without affecting release in the acidic media. Other controlled-release formulations have included adipic acid with the intention of obtaining a late-burst release profile (Rowe et al., 2009).

CONCLUSION

The methanol extract of gendarussa leaves contained 23 compounds and the major components in the extract are 2-ethylidene-1-methyl-3-phenylimidazolidine (14.351%), 9,12-Octadecadienoic acid (Z,Z) (13.731%), and 5,6,8,9-tetramethoxy-2-methylpepero (3,4,5-jk)-9,10-dihydrophenanthracene (11.440%).

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