



## Metabolites Profiling and Biological Activities of Volatile Compounds of *Ruellia tuberosa* L. Leaves by GC-MS

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### ABSTRACT

*Ruellia tuberosa* L. is a common plant that traditionally has pharmacological potential. The leaves are believed to help accelerate wound healing. However, exploring the biochemical profile in it is still limited. This study aimed to establish phytoconstituent profiles on the leaves of *R. tuberosa* L. Therefore, a Gas Chromatography-Mass Spectrometer Analysis (GC-MS) was based. This study revealed 18 polar and non-polar compounds, with five compounds having known biological activity. Squalene dominates in the leaves of *R. tuberosa* L., with the highest content of 15.41%, a source of essential anti-oxidants. The study also revealed the presence of new compounds that have not been identified for activity, such as Salvialane, Tridecanoic acid, 12-oxo-, Phenazine, 2-methoxy-, Cyclododecanone, 2-methylene- and others. We conclude that *Ruellia tuberosa* L. is an essential herbal candidate that can be used for drug development.

**Keywords:** *bioactive compounds; GC-MS analysis; medicinal plants; Ruellia tuberosa* L.; *phytochemicals*

### INTRODUCTION

*Ruellia tuberosa* L., or "cracker plant," is a species of the Acanthaceae that grows a lot in hot and tropical to subtropical climates (1,2). *R. Tuberosa* L. is a shrub plant with a height of 30-45 cm (3). *R. Tuberosa* L. grows at altitudes above 150 meters above sea level, rapidly growing in moist and shady areas (4). This plant is native to Central America and is popular in India as an ornamental plant (5).

*R. tuberosa* L. has been reported to have benefits as a medicinal plant, and in pharmacology, it contains antimicrobials, anti-oxidants,

antipyretics, antidiabetics, and anti-cancer (6,7), anti-inflammatory (8), antinociceptive (9,10), antibacterial (11). The herbal decoction of *R. tuberosa* L. is also used as a substitute for ipecac in the medical world. It treats various diseases such as bladder, diabetes, diarrhea, typhoid, chronic jaundice, cholera, headache, and fever (8,12). *R. tuberosa* L. contains active phytochemicals such as flavonoids, phenolics, glycosides (13,14), steroids, triterpenoids, alkaloids (1), and tannins (15). Ethanol extract from *R. tuberosa* L. contains flavonoids that have the potential to be anti-inflammatory (16).

Previous research revealed the presence of flavonoids, glycosides, phenols, and saponins in *R. tuberosa* L plants with HPTLC (17). Furthermore, the LC-HRMS method can identify 12 compounds containing *R. tuberosa* L. (15). However, no work has been done on the phytoconstituents of this plant with GC-MS. Therefore, our goal is to perform quantitative and qualitative screening of phytoconstituents on the leaves of *R. tuberosa* L.

## MATERIAL AND METHOD

### *Plant material*

The naturally grown fresh plant *R. tuberosa* L. was collected from the Tapos region, Depok, Indonesia (6°24'53.8"S 106°53'16.6"E) in a plastic bag in mid-August 2022. It was identified by the Life Sciences Research Organization, BRIN (National Research and Innovation Agency) Indonesia. The identification results were deposited at the Herbarium Bogoriensis with specimen voucher No. LHU46085.

### *Extract preparation*

Healthy leaves are selected and washed with running tap water. The leaves are cut ( $\pm$  4 cm) and dried at 40 oC using the oven for 72 hours. Then, the dried leaves are mashed with an electric grinder until they become a fine powder. After the previous procedure, 50 g of leaf powder was transferred to 500 mL of ethanol to be macerated for five days in an impermeable room (18,19). Finally, the extract is filtered with Whatman No.1 paper and placed in a glass bottle for subsequent analysis.

### *GC-MS Analysis*

GC-MS analysis was performed using Gas Chromatography (Agilent 7890) equipped with a 5975 Mass Selective Detector and Chemstation data system (20,21). A total of 5  $\mu$ L of *R. Tuberosa* L. leaf extract was filtered by hammering a syringe filter and inserted into

column HP-5 by split method (8:1). The initial column temperature was set at 80 oC was raised every 3 oC/min to 150 oC and held for 1 minute, then increased by 20 oC/min to 180 oC and held for 26 minutes.

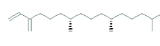
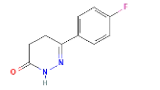

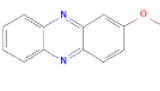
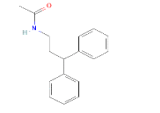
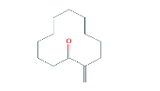
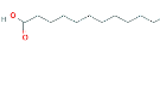
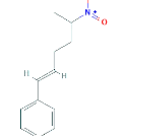
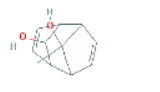
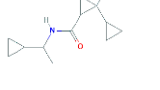
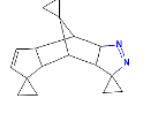
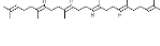
### *Data analysis*

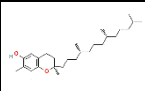
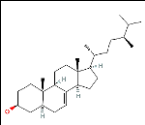
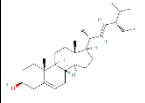
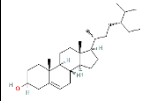
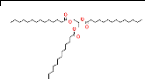
Qualitative data analysis was performed with Agilent MassHunter Qualitative Analysis Software. Identification of compounds in the spectrum is carried out based on retention time and the extent of peak integrals. Unknown mass fragments and mass spectra compared to NIST Chemistry WebBook (<https://webbook.nist.gov/chemistry/>) PubChem (<https://pubchem.ncbi.nlm.nih.gov/>), and SpectraBase (<https://spectrabase.com/>). The similarity of compounds matching > 70% is listed based on a literature search. After that, the name, molecular mass, and structure of the phytocomponent are determined to determine further their bioactivity potential based on relevant sources (22).

## RESULTS

Developing herbal formulations from natural ingredients has become an exciting research topic. The extraction process, instrument selection, and analysis are fundamental to the performance of potential compound discovery (23,24). This study was designed to identify the metabolites profiling in *R. Tuberosa* L. leaf by Gas Chromatography-Mass Spectroscopy (GC-MS). In this study, GC-MS could detect 18 phytoconstituents, consisting of pyridazinones, acyclic diterpenoids, phenazines and derivatives, diphenylmethanes, carbonyl, fatty acids, pyrrolopyrazines, tertiary alcohols, cyclopropane carboxylic acids and derivatives, triterpenoids, tocopherols, ergosterol and derivatives, stigmastanes and derivatives, triacylglycerols with details of each compound listed in (Table 1). Squalene is the compound with the most content of 15.41%.

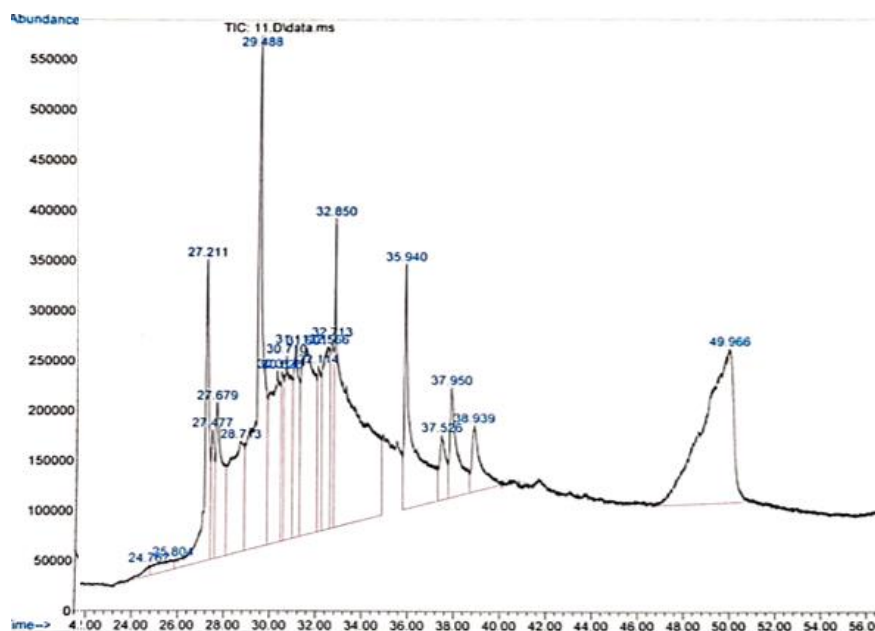
**TABLE 1:** Fitocompound present in leaves of *R. Tuberosa* L. using GS-MS analysis

No.	RT	Metabolites compound	% of area	M.F.	M.W. g/mol	Chemical structure	Library	Class of compound
1.	27.210	2,6,10-trimethyl, 14-ethylene-14-pentadecne	4,51	C20H38	278.516		PubChem	Unknown
2.	27.679	Salvialane	3,14	-	-	-	-	-
3.	28.713	Pyridazin-3 (2H)-one, 4,5-dihydro-6-(4-fluorophenyl) –	5,01	C10H9FN2O	192.19		PubChem	Pyridazinones
4.	29.486	(2e)-3,7,11,15-tetramethyl-2-hexadecen-1-ol	11,47	C20H40O	296,50		PubChem	Prenol Lipids: Acyclic diterpenoids
5.	30.313	Phenazine, 2-methoxy-	5,77	C13H10N2O	210.23		PubChem	Phenazines and derivatives
6.	30.527	Acetamide, n-(3,3-diphenylpropyl) –	1,28	C17H19NO	253.34		PubChem	Diphenylmethanes
7.	30.713	Cyclododecanone, 2-methylene-	4,01	C13H22O	194.31		PubChem	Organooxygen: Carbonyl
8.	31.113	Tridecanoic acid, 12-oxo-	3,44	C13H24O3	228.33		PubChem	Fatty Acyls: Fatty acids and conjugates
9.	31.603	(5-Nitrohex-1-enyl) benzene	8,16	C12H15NO2	205.25		PubChem	Pyrrrolopyrazines
10.	32.113	9-Methyltricyclo [4.2.1.1 (2,5) ] deca-3,7-diene-9, 10-diol	1,97	C11H14O2	178.23		PubChem	Tertiary alcohols
11.	32.568	Cyclopropane carboxamide, 2-cyclopropyl-2-methyl-N-(1-cyclopropylethyl) –	4,18	C13H21NO	207.31		PubChem	Cyclopropane carboxylic acids and derivatives
12.	32.713	Trispiro [ (3,4-diazatetracyclo [5.5.1.0 (2,6). 0 (8,12) ] trideca-3,10-diene)-5,9,13-tricyclopropane	1,86	C17H20N2	252.36		PubChem	-
13.	32.851	Squalene	15,41	C30H50	410.7		PubChem	Prenol lipids : Triterpenoids

14.	35.940	Vitamin E	6,13	C <sub>29</sub> H <sub>50</sub> O <sub>2</sub>	430.71		PubChem	Prenol lipids: Tocopherols
15.	37.526	7-Ergosterol	1,31	C <sub>30</sub> H <sub>50</sub> O <sub>2</sub>	442.7		PubChem	Steroids: Ergosterol and derivatives
16.	37.953	Stigmasterol	2,71	C <sub>29</sub> H <sub>48</sub> O	412.70		PubChem	Steroids: Stigmastanes and derivatives
17.	38.939	Stigmast-5-en-3-ol	1,97	C <sub>29</sub> H <sub>50</sub> O	414.71		PubChem	Steroids: Stigmastanes and derivatives
18.	49.965	2,3-bis (tetradecanoyloxy) propyl myristate	15,04	C <sub>45</sub> H <sub>86</sub> O <sub>6</sub>	723.2		PubChem	Glycerolipids: Triradylglycerols

Squalene is the most compound in the leaves of *R. Tuberosa* L., with as much content (15.41 %) coming from the class of prenyl lipids, with subclass triterpenoids. Squalene is composed of six isoprene units. Squalene includes compounds that are widely spread in various plants, such as apricot, broccoli, coconut, and corn. Squalene,

which belongs to the kingdom of organic compounds, is found in plants and deep-sea shark liver oil. In contrast to the least amount of compound named 2,6,10-trimethyl, 14-ethylene-14-pentadecne with as much content (1.22%) has never been reported. The identity of the compound is not yet known for sure.



**FIGURE 1:** Chromatogram of total ions GC-MS from the leaves of *R. Tuberosa* L.

## DISCUSSION

In this study, the dissolution used was ethanol (C<sub>2</sub>H<sub>6</sub>O) which can remove fat components (25). Ethanol belongs to the class of straight-chain primary alcohols (26), which are often used in various studies and are used in industries such as the manufacture of medicines, plastics, and cosmetics (27), food and beverage additives (28), and the chemical industry (29). Ethanol is also a major component in the fermentation of food,

beverages, and fruit products at large and small levels (30,31). Through ethanol extract, GCMS analysis found 18 compounds identified from the leaves of *R. Tuberosa* L, which belong to the class of unique compounds. Only five compounds whose biological activity has been reported in the study (Table 2), and 13 have not been reported (Table 3). This study also found a phytosterol group in the steroid class, a beneficial compound for living beings.

**TABLE 2:** Biological activity of the Active compound on the identified *R. Tuberosa* L.

No.	Metabolites of Compound	Biology Activity
1.	(2e)-3,7,11,15-tetramethyl-2-hexadecen-1-ol	Antimicrobial, antifungal, antibacterial, antiparasitic, antimutagenic (32), anti-oxidant (33), anti-inflammatory (34)
2.	Squalene	Anti-oxidant (35), anti-tumor, anti-cancer, hypolipidemic action in blood and liver (36), & reduces cardiovascular disease (37)
3.	Vitamin E	Anti-oxidant (38,39), Anti-inflammatory (40), antibacterial (41)
4.	Stigmasterol	Antiinflammatory (42,43), antidiabetic (44), antitumor (45), anticancer, & anti-allergic (46)
5.	Stigmast-5-en-3-ol	Anti-cancer (47), anti-diabetic, anti-hyperlipidemic and anti-tumor (48)

**TABLE 3:** The biological Activity of the active Compound on *R. Tuberosa* L. has not been identified.

No.	Metabolite of Compound	Biology Activity
1.	2,6,10-trimethyl, 14-ethylene-14-pentadecne	Unknown
2.	Salvialane	Unknown
3.	Phenazine, 2-methoxy-	Unknown
4.	Acetamide, n-(3,3- diphenylpropyl) –	Unknown
5.	Cyclododecanone, 2-methylene-	Unknown
6.	Tridecanoic acid, 12-oxo-	Unknown
7.	(5-Nitrohex-1-enyl) benzene	Unknown
8.	9-Methyltricyclo [4.2.1.1 (2,5) ] deca-3,7-diene-9, 10-diol	Unknown
9.	Cyclopropane carboxamide, 2- cyclopropyl-2-methyl-N-(1-cyclopropyethyl) –	Unknown
10.	Trispiro [ (3,4-diazatetracyclo [5.5.1.0 (2,6). 0 (8,12) ] trideca-3,10-diene)-5,9,13-tricyclopropane	Unknown
11.	7-Ergosterol	Unknown
12.	2,3-bis (tetradecanoyloxy) propyl myristate	Unknown
13.	Pyridazin-3 (2H)-one, 4,5-dihydro-6-(4-fluorophenyl) –	Unknown

Stigmasterol (C<sub>29</sub>H<sub>48</sub>O) is a phytosterol or plant lipid that has a chemical structure similar to cholesterol (49) and is most commonly found in food (50). To be precise, stigmasterol belongs to the subclass of stigmastanes. Stigmasterol helps prevent anti-inflammatory (42,43), anti-tumor (45), anti-cancer, & anti-allergic (51), anti-diabetic (44) by lowering cholesterol in the blood (52). Even in animal studies, stigmasterol can

produce ovalbumin by reducing oxidative stress and can inhibit the expression of vascular cell adhesion molecules (53). Stigmasterol has also been found in the leaves of *R. Tuberosa* L., which shows benefits (1).

Stigmast-5-en-3-ol and 7-ergosterol are phytosterol group compounds found in the leaves of *R. Tuberosa* L. Stigmast-5-en-3-ol belongs to stigmasterol derivatives which are also in the

stigmastanes subclass. In contrast to 7-ergosterol, which is precisely derived from the subclass ergosterol. Although the leaves of *R. Tuberosa* L. contain only a tiny amount of phytosterols, the presence of these compounds is very beneficial for humans.

Compounds as most compounds (15.41%) include unsaturated triterpenoid compounds (54) found in plant oils that are often used for the manufacture of medicines (22,55,56), dietary supplements (57), industrial cosmetics (54,58). Squalene can protect the skin from UV rays, increase skin flexibility, moisturize human skin, and increase the absorption of other substances in the human body (59,60). Squalene also has pharmacological properties, such as preventing cholesterol and cardiovascular diseases, and has anti-oxidant, anti-tumor, and anti-cancer effects, such as ovaries, breasts, and lungs (58). Several studies have proven that Squalene contains hypolipidemic action in the blood and liver in spinach plants (61,62).

Vitamin E (C<sub>29</sub>H<sub>50</sub>O<sub>2</sub>) is tocopherol, a lipid product of plant and animal origin, and is specifically included in lipophilic anti-oxidants used in food packaging, cosmetics, and the biomedical field (63). Vitamin E is useful for preventing anti-oxidants (39), anti-inflammatory (40), and antibacterial (41). Vitamin E in animals such as fish serves to protect biological membranes and lipoproteins against oxidation (64), such as studies in *O. niloticus* (65), *Micropterus salmoides* (66), *Salmo caspius* (67). Through this study, many compounds still have not been reported for biological activity. These compounds should be further researched so that people from various parts of the world know the essential benefits of each compound and can maximize the utilization of *R. Tuberosa* L. leaves in life.

### CONCLUSION

GC-MS analysis carried out using ethanol extract found 18 compounds in the leaves of *R. Tuberosa* L. from various classes of compounds. The dominant compound is Squalene which is very beneficial as an anti-oxidant. Phytosterols are also found in the leaves of *R. Tuberosa* L. but in small amounts. Only five compounds whose

biological activity has been reported. This suggests that the leaves of *R. Tuberosa* L. still require further research regarding the biological activity of each compound contained.

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### Competing Interests

The authors declare that they have no competing interests.

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