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Research Article

Metabolite profiling of wild underutilized raspberry (*Rubus pyrifolius*)

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Abstract

Rubus pyrifolius J.E. Smith, one of the *Rubus* species, has been found in Indonesia and collected in the Cibodas Botanical Garden. *Rubus* species are known for their diverse triterpenes, each with unique biological functions. This research aimed to analyse the metabolite profiles of *R. pyrifolius* using n-hexane and methanol solvents. GC-MS analysis was used to identify volatile and semi-volatile compounds in the *R. pyrifolius* extracts, with compound identification based on NIST 17 through GC/MS mass spectra analysis. The results showed that the total number of identified phytochemical compounds in methanol and n-hexane extracts of *R. pyrifolius* were 115 and 174, respectively. Methanol extracts from *R. pyrifolius* flower, young leaf, mature leaf, and young stem exhibited 46, 20, 15, and 34 compounds, respectively. In contrast, n-hexane extracts from *R. pyrifolius* flower, young leaf, mature leaf, and young stem contained 35, 47, 29, and 63 compounds, respectively. These compounds were classified into seven phytochemical groups: alkanes, alkenes, cyclic ethers, diterpenes, fatty acids, triterpenes, and vitamin E. Furthermore, only carboxylic esters, ergosterols, esters, fatty alcohols, and phenols were found in n-hexane extracts. The methanol extract showed seven significant phytochemical groups, including linolenic acid, phthalate esters, phytol, phytosterol, sterol lipids, terpenoids, and triterpenoids. *R. pyrifolius* possesses a variety of bioactive phytochemical profiles that are relevant in the field of phytopharmaceuticals. Nevertheless, further research is essential to determine their biological activity.

Keywords: fatty acids; GC-MS; metabolite profiling; *Rubus pyrifolius*; terpenoids

Introduction

Plants are still an important component of traditional health treatments in developing countries, although many ethnobotanical reports have not been supplemented with proper research. For example, although it is a critical starting point for planning a reasonable use of medicinal plants (Rahman *et al*., 2019; Heinrich, 2008), more than a complete identification of secondary metabolites is frequently inadequate. At the same time, precise profiling of the many herbal medications obtained from a single plant species is important for standardization, preventing adulteration, and evaluating prospective applications of less value or less accessible plant material (Smillie and Khan, 2009). Rubus is a genus in the Rosaceae family, which is a subfamily of the Rosoideae. *Rubus*, one of the biggest plant genera with over 740 species, is native to six continents and

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adaptable in many habitats (Alice and Campbell, 1999; Hummer, 2010). Rubus has been classified into 12 subgenera (Focke, 1910). Additionally, 46 species of Rubus have been reported in the Malesia Region, with 25 species spread throughout Indonesia (Kalkman, 1993). This genus contains sugars, vitamins, secondary metabolites (such as triterpenoids, flavonoids, and polyphenols), and numerous compounds with anti-aging characteristics, including antioxidant, anti-elastase, anti-tyrosinase, anti-collagenase, anti-UV, anti-inflammatory, and wound healing properties (Bravo *et al*., 2016; Zeng *et al*., 2018).

Rubus species have been recognized for their extensive variety of triterpenes, each with distinct biological functions (Desmiyati *et al*., 2021; Mei *et al*., 2016; Ono *et al*., 2016; Yu *et al*., 2019). Certain Rubus species exhibit antioxidant, anti-bacterial, anti-elastase, anti-collagenase, and anti-thrombotic properties, particularly effective against cancer and inflammatory diseases. Moreover, they are rich in polyphenols and flavonoids. Petreanu *et al*. (2015) identified compounds like 5,7-dihydroxy-6,8,4'-trimethoxyflavonol, 5-hydroxy-3,6,7,8,4'-pentamethoxyflavone, and tormentic acid in the leaves of *Rubus rosifolius*, all of which hindered ovarian cancer cell growth. Similarly, Kanegusuku *et al*. (2007) isolated 28-methoxy-tormentic acid from *R. rosifolius* herb hexane extract, demonstrating its analgesic properties. Traditional folk remedies involve infusing raspberry plant leaves, specifically *Rubus idaeus*, with boiling water. These leaves can be used topically as antibiotics and anti-inflammatories. Raspberries might also offer benefits in early-stage type II diabetes and hypertension (Cheplick *et al*., 2007). Raspberry extract contains specific polyphenols such as anthocyanins, ellagitannins, and ellagic acid, or combinations with other compounds like ascorbic acid and carotenoids for synergistic effects, can effectively inhibit the growth of cancer cells in vitro. Raspberry extract has shown anti-proliferative properties, effectively suppressing the development of human tumor cells in the colon, prostate, breast, and mouth (Wedge *et al*., 2001; Cerda *et al*., 2005; Seeram *et al*., 2006; Ross *et al*., 2007; McDougall *et al*., 2008; Bowen-Forbes *et al*., 2010). The main species of European blackberry plant, *R. fruticosus* leaves, have been applied as an astringent, antidiarrheic, and hypoglycemic medication, as well as an anti-inflammatory therapy for the oral cavity and throat mucous membranes (Rocabado *et al*., 2007). The traditional therapeutic usage of Rubus in Southeast Asia is quite similar to that of *Rubus* in Europe. The astringent and tonic characteristics of the leaves and roots are used to treat diarrhea. In Indonesia, the roots are chewed with additional components to treat digestive ailments. Fresh leaves eaten with toasted coconut are supposed to treat thrush. Combined with betel nut (*Areca catechu*), they are considered antitussive and a treatment to prevent miscarriage (PROSEA).

Rubus species are distinguished by their ability to synthesize and accumulate ellagitannins, while other physiologically active compounds such as flavonoids, anthocyanins, and triterpenoids have been found in recent years. More than 100 species of the genus Rubus have been chemically studied to obtain and determine the active components of the plants (Bhuyan and Dutta, 2021). Because increasing research shows that specific chemical components of Rubus species might inhibit tumor formation, finding such components may be a useful, non-invasive technique for reducing cancer rate and severity. Analysing the chemical composition of different components of medicinal plants, which underlies their various pharmacological effects, holds significant importance in medicinal chemistry. Through this method, we can systematically assess and confirm the traditional applications, pharmacological effects, and therapeutic possibilities associated with these plant constituents. *R. pyrifolius,* one of the Rubus species, has been found in Indonesia and collected in the Cibodas Botanical Garden (Surya *et al*., 2018). However, no prior phytochemical on the non-polar and polar constituents of *R. pyrifolius* has been documented. As a result, examining the chemical components is important for verifying the plant's use as a traditional phytomedicine. This study aimed to identify the metabolite profiles of *R. pyrifolius* in n-hexane and methanol extract to see the variations in bioactive compounds in flowers, leaves, and stems.

Materials and Methods

Plant materials

Rubus pyrifolius (young leaves, mature leaves, young stem, and flower) were collected from Cibodas Botanical Garden (West Java, Indonesia) with collection number IVB. 37a. Plant was identified by Muhammad Imam Surya and refer to Kalkman (1993) (Figure 1).

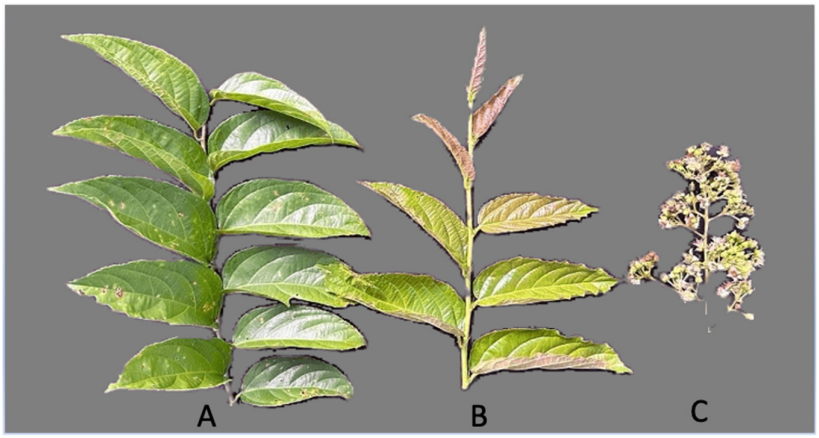

Figure 1. *Rubus pyrifolius* (A) mature leaves (B) young leaves (C) flowers

Preparation of plant extracts

Each part of *Rubus pyrifolius* (young leaves, mature leaves, young stem, and flower) was cleaned and dried in an oven at 40 °C. The dried leaves were chopped into small pieces and then smoothed into powder using an electric blender. Each of 5 grams of *R. pyrifolius* powder was macerated using 100 mL of methanol and n-hexane, then incubated and shaken at 150 rpm for 24 h. After that, the extract was filtered using Whatman No. 1 filter paper. The filtrate was placed in a porcelain cup and concentrated by air drying until the weight of the extract was constant. The crude extract was obtained and stored at 4 °C for further use.

GC/MS analysis

GC/MS analysis was performed to identify some of the potent volatile and semi-volatile compounds in *R. pyrifolius* extract. The Retention time was used to differentiate between different chemicals. The 25 mg of concentrated n-hexane, methanol extracts were redissolved in the respective solvents, vortexed properly and filtered through 0.22 mm syringe filter. One microlitre aliquot of the sample solution was injected into the GC/MS system for the requisite analysis. The bioactive compounds of methanol and n-hexane plant crude extracts were identified by GC/MS Column type 19091S-433: 93.92873 DB-5MS UI 5% phenyl methyl silox, dimension: 30 m x 250 µm x 0.25 µm. Initial temperature: 150 °C; hold time: 2 min. Post run, 300 °C with retention time (Rt) total for 30 min.

Identification of bioactive compounds

The identification of compounds was based on NIST 17 (MassHunter\Library\NIST17.L) by analysing the mass spectra of the detected compounds through GC/MS. Finally, the compound name and molecular formula of the individual compounds were determined further using NIST WebBook and PubChem. The biological activity was determined by comparing with Dr. Duke's Phytochemical and Ethnobotanical database (Duke's, 2013) and literature reviews.

Results

The GC/MS chromatogram showed the relative amounts of different compounds as a function of retention time. The heights of the peaks represent how much of each component is present in *Rubus pyrifolius* extract. The mass spectrometer examines these compounds at various elution times to determine their characteristics and structures. Larger compounds break into smaller ones, creating peaks at various mass-to-charge (M/Z) ratios. These unique mass spectra act like a fingerprint for each compound, and reference data libraries can aid in their identification (Szarka *et al*., 2008). The GC/MS chromatogram of *R. pyrifolius* flower methanol extract showed various peaks resulting from 46 chemical compounds, as shown in Tabel 1 and Figure 2. The most abundant compound was stigmasta-3,5-diene (21.78%), followed by gamma-sitosterol (12.63%), gamma-sitostenone (8.60%), supraene (5.48%), hexanedioic acid, dioctyl ester (1.83%), and heptadecane (1.86%). Meanwhile, n-hexane extracts of the *R. pyrifolius* flowers produced 35 compounds, and the major chemical compound was di-isononyl phthlate (6.01%), followed by heneicosane (5.64%), tetracosane (4.19%), 1,2-benzenedicarboxylic acid, dinonyl ester (2.58), hexanedioic acid, and bis(2-methylpropyl) ester (2.20%).

Table 1. Identified compounds from GC/MS analysis for *R. pyrifolius* flower (similarity 80-100)

Compounds	Nature compound	Molecular formula	Flower methanol extract		Flower hexane extract	
			RT	Area %	RT	Area %
2,4-Di-tert-butylphenol	Phenol	$C_{14}H_{22}O$	-	-	14.548	0.21
Hexanedioic acid, bis(2-methylpropyl) ester	Fatty acid	$C_{14}H_{26}O_4$	-	-	16.602	2.20
3-Eicosene, (E)-	Alkene	$C_{20}H_{40}$	-	-	19.841	0.39
5-Eicosene, (E)-	Alkene	$C_{20}H_{40}$	-	-	21.694	0.39
Diisooctyl adipate	Carboxylic ester	$C_{22}H_{42}O_4$	-	-	23.382	5.14
1-Hexacosene	Alkene	$C_{26}H_{52}$	-	-	24.996	0.40
Di-isononyl phthlate	Carboxylic ester	$C_{26}H_{42}O_4$	-	-	26.798	6.01
1,2-Benzenedicarboxylic acid, dinonyl ester	Fatty acid	$C_{26}H_{42}O_4$	-	-	26.899	2.58
Heneicosane	Alkane	$C_{21}H_{44}$	-	-	27.214	5.64
Tetracosane	Alkane	$C_{24}H_{50}$	-	-	28.827	4.19
Nonacos-1-ene	Alkene	$C_{29}H_{58}$	-	-	28.903	0.19
Cyclononasiloxane, octadecamethyl-	Terpenoid	$C_{18}H_{54}O_9Si$	17.509	0.15		
Hexadecanoic acid, methyl ester	Fatty acid	$C_{17}H_{34}O_2$	19.173	1.95		
9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)-	Linolenic acid	$C_{19}H_{32}O_2$	20.862	3.14	-	-
Methyl stearate	Fatty acid	$C_{19}H_{38}O_2$	21.089	0.94	-	-
.γ.-Sitosterol	Phytosterol	$C_{29}H_{50}O$	21.794	12.63	-	-
Stigmasta-3,5-diene	Triterpene	$C_{29}H_{48}$	22.173	21.78	-	-
Heptadecane	Alkane	$C_{17}H_{36}$	22.614	2.63	-	-
Eicosane	Alkane	$C_{20}H_{42}$	23.370	1.83	27.945	0.51
Hexanedioic acid, dioctyl ester	Fatty acid	$C_{22}H_{42}O_4$	23.370	1.83	-	-
Tetracosamethyl-cyclododecasiloxan	Alkane	$C_{24}H_{72}O_{12}Si_{12}$	24.882	1.26	-	-
.γ.-Sitostenone	Phytosterol	$C_{29}H_{48}O$	26.281	8.60	-	-
Supraene	Triterpenoid	$C_{30}H_{50}$	26.596	5.48	-	-
1-Octadecene	Alkene	$C_{18}H_{36}$	27.189	1.54		
dl-.α.-Tocopherol	Vitamin E	$C_{29}H_{50}O_2$	29.180	0.91	-	-
Neophytadiene	Diterpen	$C_{20}H_{38}$	29.331	1.97		

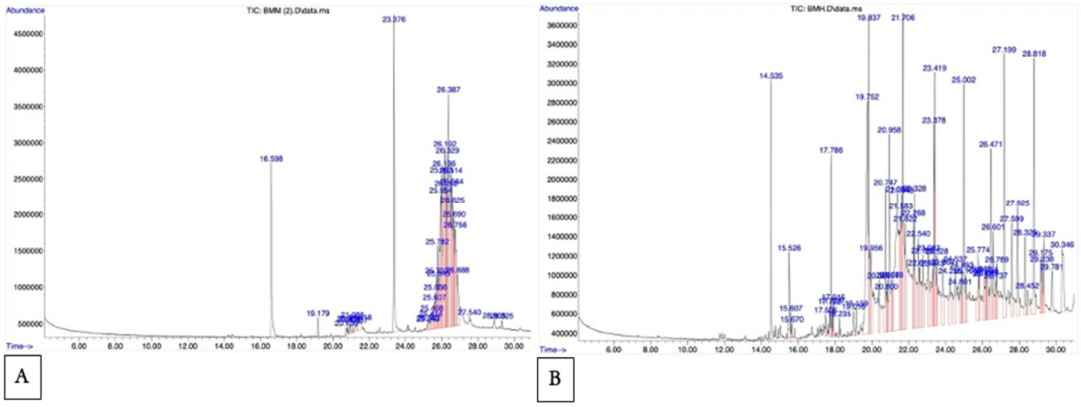

Figure 2: GC/MS chromatogram of organic compounds from *R. pyrifolius* (A) flower methanol extract, (B) flower n-hexane extract

The GC/MS analysis of the methanolic extract of *R. pyrifolius* young leaves and mature leaves led to identifying 20 and 15 chemical compounds, respectively (Table 2, Table 3 and Figure 3). The most abundant compound in young leaves was 3,7,11,15-tetramethyl-2-hexadecen-1-ol (14.47%), followed by hexadecanoic acid, methyl ester (10.83%), neophytadiene (9.57%), hexanedioic acid, dioctyl ester (6.11%), 9,12,15-octadecatrienoic acid, methyl ester (Z, Z, Z) (5.94%), and methyl stearate (4.15%). A significant compound in mature leaves was neophytadiene (22.51%), followed by stigmast-4-en-3-one (11.98%), squalene (7.94%), and vitamin E (3.40%). Furthermore, the GC/MS analysis of the n-hexane extract of young leaves and mature leaves produced 47 and 29 chemical compounds, respectively. The most abundant compound in young leaves was eicosane (15.12%), followed by octadecane (8.65%), 3-eicosene, (E)- (4.14%), 1-tetracosene (3.51%), cyclooctacosane (3.25%), and 1-hexacosene (2.83%). Meanwhile, the most abundant compound in mature leaves was 1-docosene (15.04%), followed by hentriacontane (14.10%), tetracosane (8.11%), squalene (4.63%), and 2,4-di-tert-butylphenol (4.43%).

Table 2. Identified compounds from GC/MS analysis for *R. pyrifolius* young leaves (similarity 80-100)

Compounds	Nature compound	Molecular formula	Young leaves methanol extract		Young leaves hexane extract	
			RT	Area %	RT	Area %
2,4-Di-tert-butylphenol	Phenol	$C_{14}H_{22}O$	-	-	14.548	2.45
1-Octadecene	Alkene	$C_{18}H_{36}$	-	-	17.799	1.08
3-Eicosene, (E)-	Alkene	$C_{20}H_{40}$	-	-	19.841	4.14
Hexadecanoic acid, propyl ester	Fatty acid	$C_{19}H_{38}O_2$	-	-	20.748	2.59
Nonacos-1-ene	Alkene	$C_{29}H_{58}$	-	-	21.694	3.95
n-Propyl 9,12,15-octadecatrienoate	Fatty acid	$C_{21}H_{36}O_2$	-	-	22.324	1.29
Octadecanoic acid, propyl ester	Fatty acid	$C_{21}H_{42}O2$	-	-	22.538	0.94
1-Tetracosene	Alkene	$C_{24}H_{48}$	-	-	23.408	3.51
Cyclotetracosane	Alkane	$C_{24}H_{48}$	-	-	24.996	3.50
1-Heptadecene	Fatty alcohols	$C_{17}H_{34}$	-	-	25.777	1.12
Squalene	Triterpene	$C_{30}H_{50}$	-	-	26.596	1.44
1,19-Eicosadiene	Alkene	$C_{20}H_{38}$	-	-	26.785	2.19
Octadecane	Alkane	$C_{18}H_{38}$	-	-	27.201	8.65
Eicosane	Alkane	$C_{20}H_{42}$	-	-	28.814	15.12
dl-.α.-Tocopherol	Vitamin E	$C_{29}H_{50}O_2$	-	-	29.167	1.17
1-Hexacosene	Alkene	$C_{26}H_{52}$	-	-	29.772	1.39
Neophytadiene	Diterpen	$C_{20}H_{38}$	18.253	9.57	-	-
Hexadecanoic acid, methyl ester	Fatty acid	$C_{17}H_{34}O_2$	19.186	10.83	-	-

9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	Fatty acid	$C_{19}H_{32}O_2$	20.862	5.94	-	-
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Phytol	$C_{20}H_{40}O$	20.950	14.47; 14.31	-	-
Methyl stearate	Fatty acid	$C_{19}H_{38}O_2$	21.089	4.15	-	-
Hexanedioic acid, dioctyl ester	Fatty acid	$C_{22}H_{42}O_4$	23.370	6.11	-	-

Table 3. Identified major compounds from GC/MS analysis for *R. pyrifolius* mature leaves (similarity 80-100)

Compounds	Nature compound	Molecular formula	Mature leaves methanol extract		Mature leaves hexane extract	
			RT	Area %	RT	Area %
1-Docosene	Alkene	$C_{22}H_{44}$	-	-	21.693	15.04
1-Hexacosene	Alkene	$C_{26}H_{52}$	-	-	24.995	1.37
1-Octadecene	Alkene	$C_{18}H_{36}$	-	-	29.785	1.21
1,19-Eicosadiene	Alkene	$C_{20}H_{38}$	-	-	28.323	2.68
2,4-Di-tert-butylphenol	Phenol	$C_{14}H_{22}O$	-	-	14.548	4.43
3-Eicosene, (E)-	Alkene	$C_{20}H_{40}$	-	-	19.841	1.45
3-Methylene-7,11-dimethyl-1-dodecene	Alkene	$C_{15}H_{28}$	-	-	27.630	3.38
9-Octadecene, (E)-	Alkene	$C_{18}H_{36}$	-	-	17.799	0.48
Cyclotetracosane	Alkane	$C_{24}H_{48}$	-	-	23.407	1.26
dl-.α.-Tocopherol	Vitamin E	$C_{29}H_{50}O_2$	-	-	29.180	2.33
Hentriacontane	Alkane	$C_{31}H_{64}$	-	-	28.827	14.10
Nonacos-1-ene	Alkene	$C_{29}H_{58}$	-	-	26.470	2.29
Pentadecafluorooctanoic acid, octa decyl ester	Fatty acid	$C_{26}H_{37}F_{15}O_2$	-	-	27.919	3.41
Tetracosane	Alkane	$C_{24}H_{50}$	-	-	27.188	8.11
Neophytadiene	Diterpen	$C_{20}H_{38}$	18.266;	22.51	18.240	0.28
Stigmast-4-en-3-one	Sterol lipids	$C_{29}H_{48}O$	26.256	11.98	-	-
Squalene	Diterpene	$C_{30}H_{50}$	26.609	7.94	26.596	4.63
Vitamin E	Vitamin E	$C_{29}H_{50}O_2$	29.167	3.40	-	-

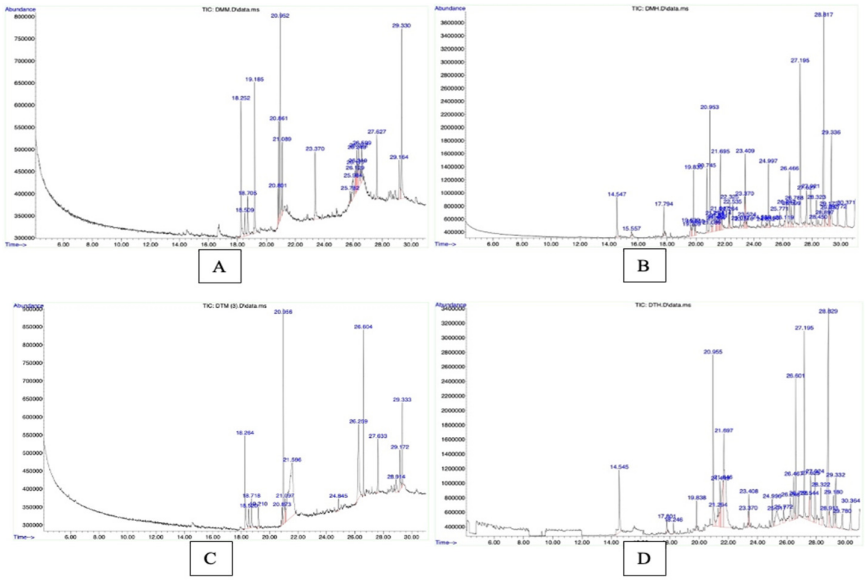

Figure 3. GC/MS chromatogram of organic compounds from *Rubus pyrifolius* (A) young leaves methanol extract, (B) young leaves n-hexane extract, (C) mature leaves methanol extract, (D) mature leaves n-hexane extract

The GC/MS analysis of methanol and n-hexane young stem extracts revealed 34 and 63 compounds, respectively (shown in Table 4 and Figure 4), the majority compounds of methanol young stem extract which were hexanedioic acid, bis(2-ethylhexyl) ester (7.29%), di-isononyl phthlate (7.66%), hexanedioic acid, bis(2-methylpropyl)ester(5.85%), and the majority chemical compounds detected in n-hexane young stem extracts were 2-methyl-Z,Z-3,13-octadecadienol (8.56%), n-hexadecanoic acid (6.54%), 5-eicosene, (E)- (4.61%), eicosane (4.34%), 3-eicosene, (E)-(4.31%). Hexadecanoic acid, methyl ester and 1-octadecene were found in methanol and n-hexane young stem extracts.

Table 4. Identified major compounds from GC-/MS analysis for *R. pyrifolius* young stem (similarity 80-100)

Compounds	Nature compound	Molecular formula	Young stem methanol extract		Young stem hexane extract	
			RT	Area %	RT	Area %
2,4-Di-tert-butylphenol	Phenol	$C_{14}H_{22}O$	-	-	14.535	2.68
2-Tetradecene, (E)-	Alkene	C14H28	-	-	15.531	0.93
3-Eicosene, (E)-	Alkene	$C_{20}H_{40}$	-	-	19.841	4.3
Hexadecanoic acid, propyl ester	Fatty acid	$C_{19}H_{38}O_2$	-	-	20.748	1.89
n-Hexadecanoic acid	Fatty acid	$C_{16}H_{32}O_2$	-	-	20.862	6.54
10,13-Octadecadienoic acid, methyl ester	Fatty acid	$C_{19}H_{34}O_2$	-	-	20.799	0.39
2-Methyl-Z, Z-3,13-octadecadienol	Fatty alcohol	$C_{19}H_{36}O$	-	-	21.391	8.56
5-Eicosene, (E)-	Alkene	$C_{20}H_{40}$	-	-	21.706	4.61
trans, trans-9,12-Octadecadienoic acid, propyl ester	Fatty acid	$C_{21}H_{38}O_2$	-	-	22.273	1.72
n-Propyl 9,12,15-octadecatrienoate	Fatty acid	$C_{21}H_{36}O_2$	-	-	22.324	2.15
Octadecanoic acid, propyl ester	Fatty acid	$C_{21}H_{42}O_2$	-	-	22.538	1.41
1-Tetracosene	Alkene	$C_{24}H_{48}$	-	-	23.307	0.94
1-Hexacosene	Alkene	$C_{26}H_5$	-	-	24.806	0.72
Nonadecyl pentafluoropropionate	Esters	$C_{22}H_{39}F_5O_2$	-	-	24.995	3.25
Octadecane	Alkane	$C_{18}H_{38}$	-	-	25.777	1.29
Tetracosamethyl-cyclododecasiloxan	Alkane	$C_{24}H_{72}O_{12}Si_{12}$	-	-	25.840	0.64
Squalene	Triterpene	$C_{30}H_{50}$	-	-	26.596	1.13
Oxirane, hexadecyl-	Cyclic ether	$C_{18}H_{36}O$	-	-	26.785	0.64
Tetracosane	Alkane	$C_{24}H_{50}$	-	-	27.201	3.42
δ-Tocopherol	Vitamin E	$C_{27}H_{46}O_2$	-	-	27.604	1.49
Octacosyl trifluoroacetate	Esters	$C_{30}H_{57}F_3O_2$	-	-	27.919	1.62
1,19-Eicosadiene	Fatty acids	$C_{20}H_{38}$	-	-	28.323	1.04
γ-Tocopherol	Vitamin E	$C_{28}H_{48}O_2$	-	-	28.449	0.54
Eicosane	Alkane	$C_{20}H_{42}$	-	-	28.814	4.34
Vitamin E	Vitamin E	$C_{29}H_{50}O_2$	-	-	29.180	0.68
Ergost-5-en-3-ol, (3.β.)-	Ergosterols	$C_{28}H_{48}O$	-	-	30.352	1.91
Hexanedioic acid, bis(2-methylpropyl) ester	Fatty acid	$C_{14}H_{26}O_4$	16.602	5.85	-	-
Hexadecanoic acid, methyl ester	Fatty acid	$C_{17}H_{34}O_2$	19.173	0.39	19.160	0.33
Methyl stearate	Fatty acid	$C_{19}H_{38}O_2$	21.089	0.28	-	-
Hexanedioic acid, bis(2-ethylhexyl) ester	Fatty acid	$C_{22}H_{42}O_4$	23.383	7.29	-	-
Di-isononyl phthlate	Phthalate esters	$C_{26}H_{42}O_4$	26.130	7.66	-	-
1-Octadecene	Alkene	$C_{18}H_{36}$	27.541	0.14	17.787	1.53

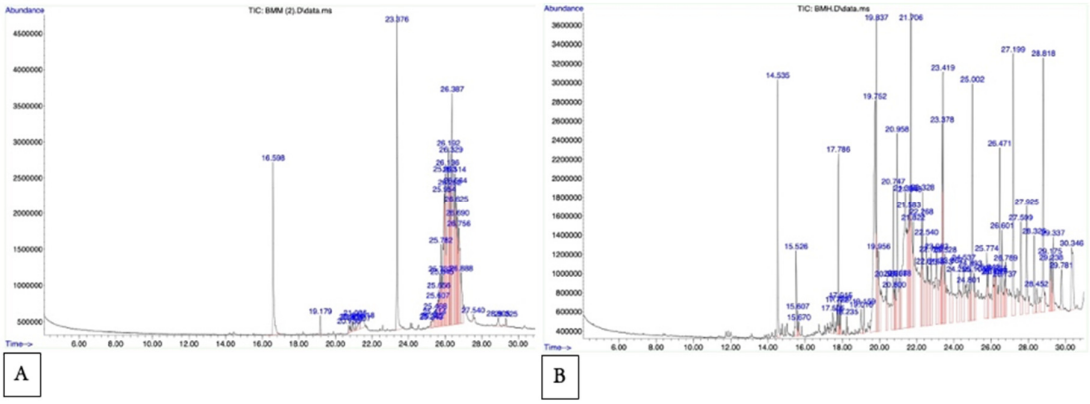

Figure 4. GC/MS chromatogram of organic compounds from *R. pyrifolius* (A) young stem leaves methanol extract, (B) young stem n-hexane extract

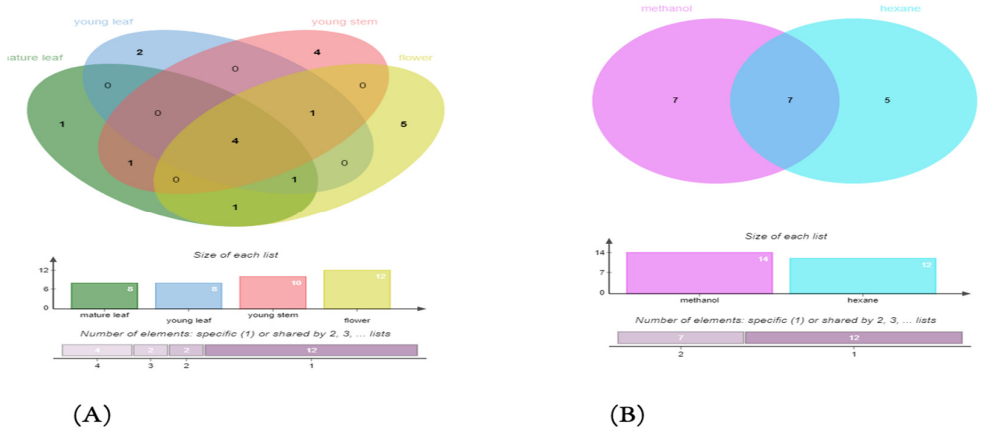

Figure 5. Venn diagram showing (A) the number of common natural metabolite compounds in all four *R. pyrifolius* organs and (B) phytochemical groups detected specifically in methanol and n-hexane solvents

A Venn diagram analysis revealed different phytochemical groups in each organ of *R. pyrifolius* (shown in Figure 5A). Four common phytochemical groups (alkene, fatty acid, phenol, and vitamin E) were identified in flowers, young leaves, mature leaves, and young stems. Additionally, five phytochemical groups were exclusively found in flowers (carboxylic esters, linolenic acids, phytosterols, terpenoids, and triterpenoids); two phytochemical groups were detected to young leaves (fatty alcohols and phytols); one phytochemical group was solely present in mature leaves (sterol lipids); and four phytochemical groups were detected in young stem (ergosterols, esters, fatty alcohols, and phthalate esters).

The n-hexane and methanol extracts contained seven common phytochemical groups: alkanes, alkenes, cyclic ethers, diterpenes, fatty acids, triterpenes, and vitamin E. Additionally, five significant phytochemical groups were exclusively detected in n-hexane extracts, i.e., carboxylic esters, ergosterols, esters, fatty alcohols, and phenols. Conversely, within methanol extracts, seven major phytochemical groups were identified—namely, linolenic acid, phthalate esters, phytol, phytosterol, sterol lipids, terpenoids, and triterpenoids (in Figure 5.B).

Many of the identified compounds possess valuable biological activities. Terpenoids exhibit antibacterial, antifungal, anti-inflammatory, and antioxidant effects, while hydrocarbons (alkanes and alkenes) have demonstrated anti-inflammatory, anti-cancer, and anti-diabetic properties. Steroids show prophylactic, antioxidant, antibacterial, anti-diarrheal, anti-inflammatory, and anti-hyperglycemic activities. Fatty acids and

derivatives are recognized for their antioxidant, antimicrobial, nematocidal, anticarcinogenic, and antihypertensive properties (as shown in Table 6).

Table 6. Biological activity of identified compounds from GC/MS analysis of methanol and hexane extracts of *R. pyrifolius)*

Compounds	Total content of compounds (%)				Biological activity/ Function (references)
	Flower	Young leaves	Mature leaves	Young stem	
δ-Tocopherol				1.49	Antioxidant, antitumor, hypocholesterolemic (Duke, 2013)
γ-Sitostenone	8.60				Glucose uptake and promote insulin, activates proteins in the insulin signal transduction pathway (Kumar *et al*., 2021)
γ- Sitosterol	12.63				Prophylactic activity, antioxidant, antibacterial, anti-diarrhoeal, anti-inflammatory, anti-hyperglycemic activity (Mathi et al., 2015; Senthil *et al*., 2016; Mohammed *et al*., 2016; Sahi 2016)
γ-Tocopherol				0.54	Antineoplastic (Cooney *et al*., 1993, anticarcinogenesis (Takahashi *et al*., 2009), anti-inflammatory (Reiter *et al*., 2007, antioxidant (Brigelius-Flohe *et al*., 2021)
1-Docosene			15.04		Antibacterial properties (Sahar and Aida 2018)
1,19-Eicosadiene		2.19	2.68	1.04	Anti-inflammatory, anti-cancer, and anti-diabetic properties.
2-Methyl-Z, Z-3,13-octadecadienol				8.56	Anti-protozoal, anti-cancer activity (Carballeira *et al*., 2012); Wilson *et al*., 2014)
2,4-Di-tert-butylphenol	0.21	2.45	4.43	2.68	Antioxidant, anti-inflammatory (Choi *et al*., 2013; Nair *et al*., 2018), insecticidal and nematicidal (Chen *et al*., 2015), antibacterial (Aissaoui *et al*., 2019), antiviral (Leila *et al*., 2019), and antifungal (Sang and Kim 2012)
3-Eicosene, (E)-	0.39	4.14	1.45	4.31	Antibacterial activity (Lulamba *et al*., 2021)
3,7,11,15-Tetramethyl-2-hexadecen-1-ol		14.47; 14.31			Anti-insecticidal, anti-inflammatory, anti-tuberculosis, antibacterial, and antioxidant properties (Rao *et al*., 1998, Cooney *et al*., 1993)
5-Eicosene, (E)-	0.39	0.83		1.72	Antibacterial activity (Lulamba *et al*., 2021)
9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)-	3.14	5.94			Antimicrobial, antioxidant and anticancer activities (Wei *et al*., 2011)
dl-alpha-Tocopherol	0.91	1.17	2.33		Antitumor properties (Birringer *et al*., 2010; Schubert *et al*., 2018)
Eicosane	1.83	15.12		4.34	Antibacterial (Boussaada *et al*., 2008), antifungal, antitumor, larvicidal, antimicrobial cytotoxic (Sunita *et al*., 2017)
Heneicosane	5.64				Antimicrobial activity (Vanitha *et al*., 2020)
Hentriacontane			14.10		Anti-tumor (Takahashi *et al*.,1995), anti-inflammatory (Das and Himaja 2014), larvicidal effects (Sowmiya *et al*., 2017), antioxidant (Kim *et al*., 2011)
Hexadecanoic acid, methyl ester	1.95	10.83		0.33	Antimicrobial (Shaaban *et al*., 2021)
Methyl stearate	0.94	4.15		0.28	Antimicrobial activity (Suresh *et al*., 2014; Pinto *et al*., 2017)

n-Hexadecanoic acid				6.54	Antibacterial, anti-inflammatory (Sharma and Goel 2018; Zayed *et al*., 2019; Alghamdi and Ababutain 2019), antioxidant, antiandrogenic, pesticidal, nematocidal (Kim and Choi 2019), hypocholesterolemic, anti-cancer (Balamurugan *et al*., 2011; Balamurugan *et al*., 2012)
Neophytadiene	1.97	9.57	7.83		Analgesic, anti- inflammatory, antimicrobial, and antioxidant effect (Shajib *et al*., 2015; Fatima *et al*., 2017; Swamy *et al*., 2017)
Octadecane		8.65		1.29	Antimicrobial, antioxidant, anti-cancer, hypoglycaemic activities (Prajna *et al*., 2016; Sunita *et al*., 2017), anti-inflammatory and antiallergic properties (Zhang *et al*., 2019), α-glucosidase inhibitors (Rosa *et al.,* 2023).
Octadecanoic acid, propyl ester		0.94		1.41	Anti-inflammatory, hypocholesterolemic and antiarthritic (Rani *et al*., 2009; Uma *et al*., 2009; Ponnamma and Manjunath 2012)
Squalene		1.44	4.63	1.13	Hormone precursors in animals and plants, chemopreventive agent, antibacterial, antioxidant, antitumor (Kim and Karadeniz 2012), anti-cancer (Lozano-Grande et al., 2018), immunostimulant, lipoxygenase-inhibitor (Swamy *et al*., 2017; Sharma and Goel, 2018; Bose *et al*., 2018, Zayed *et al*., 2019)
Stigmast-4-en-3-one			11.98		Antimicrobial, hypoglycaemic activity, antiprostatic (Udobre *et al*., 2015)
Stigmasta-3,5-diene	21.78				Anti-inflammatory, anti-tumor, anti-oxidative, and anti-microbial activities (Khan *et al*., 2016)
Supraene	5.48				Anticancer, antioxidant, detoxicant bioactivities, anticholesterolemia antiatherosclerotic (Feng *et al*., 2018)
Tetracosane	4.19		8.11	3.42	Antioxidant (Paudel *et al*., 2019), cytotoxicity against HT-29 colon cancer cells (Uddin *et al*., 2012).
Vitamin E			3.40	2.15	Anti-inflammatory and antioxidant functions (Li *et al*., 1998)

Discussion

GC/MS analysis of the plant extract

This study represents the first work effort to explore the metabolites profiling of *R. pyrifolius* through GC/MS analysis. The chemical composition of the extract was studied using GC/MS as a first step towards determining the nature of bioactive components and identifying the major components in *R. pyrifolius*. The findings reveal that the diversity of metabolites in this plant differs depending on the organ examined. The discovered metabolites are classified as alkanes, alkenes, cyclic ethers, diterpenes, triterpenes, fatty acids, carboxylic esters, ergosterols, fatty alcohols, phenols, and vitamin E. Consequently, these metabolites have the potential to fulfil various essential roles. Extraction of plants was carried out using both polar (methanol) and non-polar (n-hexane) solvents to obtain compounds based on their polarity. The non-polar n-hexane solvent was used to extract lipophilic components, primarily fatty acid methyl esters (FAME), with traces of hydrocarbons and terpenes. A polar solvent was used to extract a wide range of bioactive compounds, including phenolic compounds, flavonoids, alkaloids, and organic acids. This approach is particularly effective for extracting compounds with polar or ionic functional groups, as they tend to dissolve well in polar solvents.

In this study, the major compounds discovered in the n-hexane extracts of *R. pyrifolius* (flowers, young leaves, mature leaves, and young stem) are di-isononyl phthalate, heneicosane, diisooctyl adipate, tetracosane, octadecane, eicosane, cyclotetracosane, 3-eicosene, (E)-, 1-docosene, hentriacontane, and 2-methyl-z, z-3,13-octadecadienol, followed by minor compound such as hexanedioic acid, bis(2-methylpropyl) ester, 1,2-benzenedicarboxylic acid, dinonyl ester, 2,4-di-tert-butylphenol, dl-.α.-tocopherol, and squalene. This compound belongs to the class carboxylic esters and hydrocarbon (alkanes and alkenes), fatty acid, terpenoid, and steroid derivatives. These phytochemical compounds were dominating in hexanes extract. It is obvious that the most prevalent chemical components in non-polar solvent extracts are lipophilic. Earlier studies investigating lipophilic extracts from Pinus bark using GC and GC/MS techniques discovered four major groups of components: fatty acids and alcohols, monoterpenes and sesquiterpenes, resin acids, as well as steroids and triterpenoids (Masendra *et al*., 2017). Our results confirm the findings of Hana *et al*. (2020) that the hydrocarbon compounds found in *Chrysanthemum* flowers include hexadecene, tetratetracontane, heneicosane, tetratriacontane, octacosane, and eicosane. Additionally, young stem hexane extracts of *R. pyrifolius* contain n-hexadecanoic acid compounds in small amounts of 6.54%. This result contrasts with Adu *et al*. (2022), who reported that n-hexadecanoic acid (11.77%) was found in the hexane leaf extract and stem extract of *Diospyros villosa* in high proportions.

The methanolic extract of *R. pyrifolius* (flower, young leaves, mature leaves, and young stem) produced more phytochemical group compounds than n-hexane extract, which might be attributed to solvent polarity. The main bioactive compounds were stigmasta-3,5-diene, squalene, γ.-sitosterol, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, neophytadiene, stigmast-4-en-3-one, and hexanedioic acid, bis(2-ethylhexyl) ester, respectively. Stigmasta-3,5-diene, a naturally occurring triterpenoid found in various plant sources, comprised the highest area in the methanol flower extract at 21.78%. It is a major component in many plant oils and is utilized in the production of medicines, perfumes, cosmetics, and more. According to Khan *et al*. (2016), stigmasta-3,5-diene exhibits multiple biological properties, including anti-inflammatory, antioxidant, and antimicrobial effects. Furthermore, our results showed that the main bioactive compound belongs to the class triterpene, phytosterol, phytol, diterpene, sterol lipids, and fatty acid. This result was supported by Narender *et al*. (2012) and Alara *et al*. (2021), who reported that methanol was the best solvent for extracting a wide range of plant components and that the polarity of the solvent used for extraction may explain the difference in compound content, because the increased solubility of compounds in polar solvents allows for a higher yield in extracts derived from them. Additionally, factors such as climate, soil conditions, harvest season, storage conditions, environmental factors (temperature, pH), and the extraction method can also contribute to variations in compound composition.

Triterpenoids are the most common terpenoids found in Rubus species. Our results showed that *R. pyrifolius* contains five phytochemical groups that were exclusively found in flowers, including carboxylic esters, linolenic acids, phytosterols, terpenoids, and triterpenoids. *R. pyrifolius* flower have abundant terpenoid compounds, such as stigmasta-3,5-diene, neophytadiene, squalene, and gamma-sitosterol. These compounds belong to the terpenoid classes of triterpenes and diterpenes, as well as phytosterols. Triterpenoids constitute a broad and diversified class of active natural compounds derived from squalene. Similarly, Desmiaty *et al*. (2021) reported that the active components in *Rubus fraxinifolius* leaves are triterpenoids, while Ruan et al. (2001) reported that the chemical components of *Rubus ursinus* include stigmasta-5,22-dien-3-ol, β-sitosterol, and β-sitosterol-3-D-glucose. Additionally, Guo and Yang (2005) conducted a study on the chemical components of *Rubus chingii* fruit, revealing the presence of isolated compounds such as ursolic acid, 2α-hydroxyursolic acid, and β-sitosterol. Moreover, two phytochemical groups were detected to young leaves, such as fatty alcohols and phytols. According to van den Brink and Wanders (2006), in nature, phytol is the aliphatic chain of chlorophyll molecules, which represents around one-third of the mass of both chlorophylls a and b, or about 0.2% of the wet weight of green plants. Phytol may also be considered a novel class of pharmaceuticals for the treatment of

chronic inflammatory diseases. Phytol, an acyclic diterpene alcohol, could potentially be considered a precursor for the commercial production of vitamin E (Ogunlesi *et al*., 2009).

In addition, our results revealed the presence of four phytochemical groups in young stems (ergosterols, esters, fatty alcohols, and phthalate esters), while one specific phytochemical group, sterol lipids (specifically stigmast-4-en-3-one), was exclusively found in the mature leaves of *R. pyrifolius*. Our result aligns with the findings of Pham *et al*. (2021), who reported the detection of stigmast-4-en-3-one in both leaves and stems of *Dysoxylum tpongense*. Additionally, Marín *et al*. (2020) reported the presence of sterol (stigmasterol) in *Clusia minor* leaf extracts. Notably, common plant sterols, such as sitosterol and stigmasterol, which share a similar structure, have been identified as significant sterol components. β-sitosterol, in particular, has been associated with cardiovascular protection, primarily by enhancing the antioxidant defense system and effectively reducing blood cholesterol levels in individuals (Loizou *et al*., 2010).

The chemical compounds 1-octadecene, 2,4-di-tert-butylphenol, and 3-eicosene were found in all extracts of *R. pyrifolius* at various retention times. Neophytadiene can be found in all extracts of the rubus plant except in young stems, whereas stigmasta-3,5-diene is exclusively found in flower extract. The bioactive chemical profile of all eight extracts was found to be quite distinct. Those discovered to be similar have a wide range of existences.

Conclusions

The current study has successfully identified 115 and 174 phytochemical compounds in the flower, young leaf, mature leaf, and young stem extracts of *Rubus* pyrifolius using GC/MS analysis with methanol and n-hexane solvents. Methanol extracts from *R. pyrifolius* flower, young leaf, mature leaf, and young stem exhibited 46, 20, 15, and 34 compounds, respectively. In contrast, n-hexane extracts from *R. pyrifolius* flower, young leaf, mature leaf, and young stem contained 35, 47, 29, and 63 compounds, respectively. These compounds were classified into seven phytochemical groups: alkanes, alkenes, cyclic ethers, diterpenes, fatty acids, triterpenes, and vitamin E. Furthermore, only carboxylic esters, ergosterols, esters, fatty alcohols, and phenols were found in n-hexane extracts. On the other hand, the methanol extract showed seven significant phytochemical groups, including linolenic acid, phthalate esters, phytol, phytosterol, sterol lipids, terpenoids, and triterpenoids. In order to explore this potential further, it is recommended that individual phytochemical profiles be isolated and subjected to biological activity, which could yield promising results in the development of innovative pharmaceuticals.

Authors' Contributions

Conceptualization (LI and MIS), writing—original draft preparation (LI 0p-); Writing - review and editing curation (LI and MIS); Formal analysis, Investigation, Methodology (LI and MIS). All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Adu OT, Naidoo Y, Lin J, Adu TS, Sivaram V, Dewir YH, El-Banna AN (2022). Phytochemical screening and biological activities of *Diospyros villosa* (L.) De Winter leaf and stem-bark extracts. Horticulturae 8:945. *https://doi.org/10.3390%2Fplants12040769*

Aissaoui N, Mahjoubi M, Nas F, Mghirbi O, Arab M, Souissi Y, Hoceini A, Masmoudi AS., Mosbah A, Cherif A (2019). Antibacterial potential of 2, 4-di-tert-butylphenol and calixarene-based prodrugs from thermophilic *Bacillus licheniformis* isolated in Algerian hot spring. Geomicrobiology Journal 36:53-62. *https://doi.org/10.1080/01490451.2018.1503377*

Alara OR, Abdurahman NH, Ukaegbu CI (2021). Extraction of phenolic compounds: A review. Current Research in Food Science 4:200-214. *https://doi.org/10.1016%2Fj.crfs.2021.03.011*

Alghamdi AI, Ababutain IM (2019). Phytochemical screening and antibacterial activity of *Eucalyptus camaldulensis*'s leaves and bark extracts. Asian Journal Scientific Research 12(2):202-210. *http://dx.doi.org/10.3923/ajsr.2019.202.210*

Alice LA, Campbell CS (1999). Phylogeny of Rubus (rosaceae) based on nuclear ribosomal DNA internal transcribed spacer region sequences. American Journal of Botany 1:81-97.

Balamurugan R, Duraipandiyan V, Ignacimuthu S (2011). Anti-diabetic activity of γ-sitosterol isolated from *Lippia nodiflora* L. in streptozotocin induced diabetic rats. European Journal of Pharmacology 667:410-418. *https://doi.org/10.1016/j.ejphar.2011.05.025*

Balamurugan R, Stalin A, Ignacimuthu S (2012), Molecular docking of γ-sitosterol with some targets related to diabetes. European Journal of Medicinal Chemistry 47(1):38-43. *https://doi.org/10.1016/j.ejmech.2011.10.007*

Bhuyan B, Dutta A (2021). A Review on the phytochemical, pharmacological and traditional profile on the Rubus genus in North Eastern and Western Parts of India. Current Trends in Pharmaceutical Research 8(1).

Birringer, M, Lington D, Vertuani S, Manfredini S, Scharlau D, Glei M, Ristow M (2010). Proapoptotic effects of long-chain vitamin E metabolites in HepG2 cells are mediated by oxidative stress. Free Radical Biology & Medicine 49:1315-1322. *https://doi.org/10.1016/j.freeradbiomed.2010.07.024*

Boussaada O, Ammar S, Saidana D, Chriaa J, Chraif I, Daami M, Helal AN, Mighri Z (2008). Chemical composition and antimicrobial activity of volatile components from capitula and aerial parts of *Rhaponticum acaule* DC growing wild in Tunisia. Microbiological Research 163:87-95. *https://doi.org/10.1016/j.micres.2007.02.010*

Bowen-Forbes CS, Zhang Y, Nair MG (2010). Anthocyanin content, antioxidant, anti-inflammatory and anticancer properties of blackberry and raspberry fruits. Journal of Food Composition and Analysis 23(6):554-560. *https://doi.org/10.1016/j.jfca.2009.08.012*

Bravo K, Alzate F, Osorio E (2016). Fruits of selected wild and cultivated Andean plants as sources of potential compounds with antioxidant and anti-aging activity. Industrial Crops and Products 85:341-352. *http://dx.doi.org/10.1016/j.indcrop.2015.12.074*

Brigelius-Flohé R (2021). Vitamin E research: Past, now and future. Free Radical Biology and Medicine 177:381-390. *https://doi.org/10.1016/j.freeradbiomed.2021.10.029*

Carballeira NM, Cartagena M, Sanabria D, Tasdemir D, Prada CF, Reguera RM, Balaña-Fouce R (2012). 2-Alkynoic fatty acids inhibit topoisomerase IB from *Leishmania donovani*. Bioorganic & Medicinal Chemistry Letter 22(19):6185-9. *https://doi.org/10.1016/j.bmcl.2012.08.019*

Cerda B, Tomas-Barberan F.A, Espin JC (2005). Metabolism of antioxidant and chemopreventive ellagitannins from strawberries, raspberries, walnuts, and oak-aged wine in humans: Identification of biomarkers and individual variability. Journal Agricultural and Food Chemistry 53:227-235. *https://doi.org/10.1021/jf049144d*

Chen Y, Dai G (2015). Acaricidal, repellent, and oviposition-deterrent activities of 2,4-ditert-butylphenol and ethyl oleate against the carmine spider mite *Tetranychus cinnabarinus*. Journal of Pest Science 88:645-655. *https://doi.org/10.1007/s10340-015-0646-2*

Cheplick S, Kwon Y, Bhowmik P, Shetty K (2007). Clonal variation in raspberry fruit phenolics and relevance for diabetes and hypertension management. Journal of Food Biochemistry 31:656-679. *https://doi.org/10.1111/j.1745-4514.2007.00136.x*

Choi SJ, Kim JK, Kim HK, Harris K, Kim CJ, Park GG, Park CS, Shin DH (2013). 2,4-Di-tert-butylphenol from sweet potato protects against oxidative stress in PC12 cells and in mice. Journal of Medicinal Food 16:977-983. *https://doi.org/10.1089/jmf.2012.2739*

Cooney RV, Franke AA, Harwood PJ, Hatch-Pigott V, Custer LJ, Mordan LJ (1993). Gamma-tocopherol detoxification of nitrogen dioxide: superiority to alpha-tocopherol. Proceedings of the National Academy of Sciences of the United States of America 90(5):1771-5. *https://doi.org/10.1073%2Fpnas.90.5.1771*

Das M, Himaja M (2014). Phytochemical screening, GC-MS analysis and biological activities of *Ipomoea eriocarpa* leaf extracts. International Journal of Pharmacy and Pharmaceutical Sciences 6(4):592-594.

Desmiaty Y, Hanafi M, Saputri FC, Elya B, Rifai EA, Syahdi RR (2012). Two triterpenoids from *Rubus fraxinifolius* leaves and their tyrosinase and elastase inhibitory activities. Science Report 11:20452. *https://doi.org/10.1038/s41598-021-99970-x*

Duke's (2013). Phytochemical and ethnobotanical databases. Retrieved 2023 Agust 25 from: *https://phytochem.nal.usda.gov*

Feng S, Gan L, Yang CS, Liu AB, Lu W, Shao P, Dai Z, Sun P, Luo Z (2018). Effects of stigmasterol and β-sitosterol on nonalcoholic fatty liver disease in a mouse model: a lipidomic analysis. Journal of Agricultural and Food Chemistry 66(13):3417-3425. *https://doi.org/10.1021/acs.jafc.7b06146*

Focke, WO (1910). Species Ruborum. Bibliotheca botanica E. Schweizerbartsche Verlagsbuchhandlung, Stuttgart.

Hana, PN, Nurchayati Y, Budihastuti R (2020). Efek naungan dan umur tanaman terhadap pertumbuhan dan profil metabolit bunga krisan (*Chrysanthemum* sp.) [Effect of shade and age of plants on growth and profile of *Chrysanthemum* metabolites. Buletin Anatomi dan Fisiologi 5(1):8-17.

Heinrich M (2008). Ethnopharmacy and natural product research—Multidisciplinary opportunities for research in the metabolomic age. Phytochemistry Letters 1:1-5. *https://doi.org/10.1016/j.phytol.2007.11.003*

Hummer KE (2010). Rubus pharmacology: Antiquity to the present. HortScience 45(11):1587-1591. *https://doi.org/10.21273/HORTSCI.45.11.1587*

Joshi N, Sah GC, Mishra D (2013). GC-MS analysis and antimicrobial activity of essential oil of *Senecio pedunculatus*. IOSR Journal of Applied Chemistry 6:49-51.

Kalkman C (1993). Rosaceae. Flora Malesiana-Series 1 Spermatophyta 11(2):227-351. National Herbarium of the Netherlands.

Kanegusuku M, Sbors D, Bastos ES, de Souza MM, Cechinel-Filho V, Yunes RA, Delle Monache F, Niero R (2007). Phytochemical and analgesic activity of extract, fractions and a 19-hydroxyursane-type triterpenoid obtained from *Rubus rosaefolius* (Rosaceae). Biological & Pharmaceutical Bulletin 30(5):999-1002. *https://doi.org/10.1248/bpb.30.999*

Khan K, Firdous S, Ahmad A, Fayyaz N, Nadir M, Rasheed M, Faizi S (2016). GC-MS profile of antimicrobial and antioxidant fractions from *Cordia rothii* roots, Pharmaceutical Biology 54(11):2597-2605. *https://doi.org/10.3109/13880209.2016.1172320*

Kim DY, Choi BY (2019). Costunolide—A bioactive sesquiterpene lactone with diverse therapeutic potential. International Journal of Molecular Sciences 20(12):2926. *https://doi.org/10.3390/ijms20122926*

Kim SJ, Chung WS, Kim SS, Ko SG, Um JY (2011). Anti- inflammatory effect of *Oldenlandia diffusa* and its constituent, hentriacontane, through suppression of caspase-1 activation in mouse peritoneal macrophages. Phytotherapy Research 25(10):1537-1546. *https://doi.org/10.1002/ptr.3443*

Kim SK, Karadeniz F (2012). Biological importance and applications of squalene and squalane. Advance Food and Nutrition Research 65:223-233. *https://doi.org/10.1016/b978-0-12-416003-3.00014-7*

Kumar KJS, Lin C, Tseng YH, Wang SY (2021). Fruits of *Rosa laevigata* and its bio-active principal sitostenone facilitate glucose uptake and insulin sensitivity in hepatic cells via AMPK/ PPAR-γ activation. Phytomedicine Plus 1:100109. *https://doi.org/10.1016/j.phyplu.2021.100109*

Leila A, Lamjed B, Roudaina B, Najla T, Taamalli A, Jellouli S, Mokhtar Z (2019). Isolation of an antiviral compound from Tunisian olive twig cultivars. Microbial Pathogenesis. 128:245-249. *https://doi.org/10.1016/j.micpath.2019.01.012*

Li BZ, Wang BG, Jia ZJ (1998). Pentacyclic triterpenoids from *Rubus xanthocarpus*. Phytochemistry 49(8):2477-2481. *https://doi.org/10.1016/S0031-9422(98)00161-7*

Loizou S, Lekakis I, Chrousos GP, Moutsatsou P (2010). Beta-sitosterol exhibits anti-inflammatory activity in human aortic endothelial cells. Molecular Nutrition& Food Research 54:551-558. *https://doi.org/10.1002/mnfr.200900012*

Lozano-Grande MA, Gorinstein S, Espitia-Rangel E, Dávila-Ortiz G, Martínez-Ayala AL (2018), Plant sources, extraction methods, and uses of squalene. International Journal of Agronomy 2018:13. *https://doi.org/10.1155/2018/1829160*

Lulamba TE, Green E, Serepa-Dlamini MH (2021). *Photorhabdus* sp. ETL antimicrobial properties and characterization of its secondary metabolites by Gas Chromatography–Mass Spectrometry. Life 11:787. *https://doi.org/10.3390/life11080787*

Manilal A, Sujith S, Sabarathnam B, Kiran GS, Selvin J, Shakir C, et al (2011). Biological activity of the red alga *Laurencia brandenii.* Acta Botanica Croatica: 70:81-90. *https://doi.org/10.2478/v10184-010-0001-x*

Marín RM, de Oca Porto RM, Paredes MEH, Alarcón AB, Balmaseda IH, del Valle RM, Paz Lopes MT, Guerra IR (2018). GC/MS Analysis and bioactive properties of extracts obtained from *Clusia minor* L. Leaves. Journal of Mexican Chemical Society 62(4). *https://doi.org/10.29356/jmcs.v62i4.544*

Mathi P, Das S, Nikhil K, Roy P, Yerra S, Ravada SR, Bokka VR, Botlagunta M (2015). Isolation and characterization of the anticancer compound piceatannol from *Sophora Interrupta* Bedd. International Journal of Preventive Medicine 6:101. *https://doi.org/10.4103%2F2008-7802.167181*

McDougall GJ, Ross HA, Ikeji M, Stewart D (2008). Berry extracts exert different antiproliferative effects against cervical and colon cancer cells grown in vitro. Journal of Agricultural and Food Chemistry 56(9):3016-3023. *https://doi.org/10.1021/jf073469*n

Mei QX, Chen XL, Xia X, Fang ZJ, Zhou HB,Gao, YQ, Dai WB, Jiang RW (2016). Isolation and chemotaxonomic significance of chemical constituents from *Rubus parvifolius*. Chinese Herbal Medicine 8(1):75-79. *https://doi.org/10.1016/S1674-6384(16)60011-4*

Mohammed GJ, Al-Jassani MJ, Hameed IH (2016). Anti-bacterial, antifungal activity and chemical analysis of *Punica grantanum* (pomegranate peel) using GC-MS and FTIR spectroscopy. International Journal of Pharmacognosy and Phytochemical Research 8(3):480-494.

Narender PD, Rao BG, Rao ES, Rao TM, Praneeth VS (2012). Quantification of phytochemical constituents and *in vitro* antioxidant activity of *Mesua Ferea* leaves. Asian Pacific Journal of Tropical Biomedicine 2:539-542. *http://dx.doi.org/10.1016/S2221-1691(12)60269-X*

Ogunlesi M, Okiei W, Ofor E, Osibote AE (2009). Analysis of the essential oil from the dried leaves of *Euphorbia hirta* Linn (Euphorbiaceae), a potential medication for asthma. African Journal Biotechnology 8:7042-7050. *http://www.academicjournals.org/AJB*

Ono M, Yasuda S, Nishi K, Yamamoto K, Fuchizaki S, Higuchi S, Komatsu H, Okawa M, Kinjo J, Yoshimitsu H, Nohara T (2016). Two new triterpenoids from the seeds of blackberry (*Rubus fructicosus*). Natural Product. Research 30(8):904-911. *https://doi.org/10.1080/14786419.2015.1076820*

Paudel MR, Chand MB, Pant B, Pant B (2019). Assessment of antioxidant and cytotoxic activities of extracts of *Dendrobium crepidatum*. Biomolecules 9(9):478. *http://dx.doi.org/10.3390/biom9090478*

Petreanu M, Ferreira EK, Sagaz AP, Vendramini-Costa DB, Ruiz AL, De Carvalho JE, Campos A, Cechinel Filho V, Delle Monache F, Niero R (2015). Uncommon trimethoxylated flavonol obtained from *Rubus rosaefolius* leaves and its antiproliferative activity. Evidence-Based Complementary Alternative Medicine 2015:341216. *https://doi.org/10.1155/2015/341216*

Pinto MEA, Araújo SG, Morais MI, Sá NP, Lima CM, Rosa CA, Siqueira EP, Johann S, Lima LARS (2017). Antifungal and antioxidant activity of fatty acid methyl esters from vegetable oils. Anais da Academia Brasileira de Ciências 89:1671-1681. *https://doi.org/10.1590/0001-3765201720160908*

Plant Resources of South-East Asia No 2: Edible fruits and nuts. *Rubus* L. PROSEA Foundation, Bogor, Indonesia. *https://prosea.prota4u.org/view.aspx?id=8*

Ponnamma and Manjunath 2012 Ponnamma SU, Manjunath K (2012). GC-MS analysis of phytocomponents in the methanolic extract of *Justicia wynaadensis* (Nees) *T. anders*. International Journal of Pharma and Bio Sciences 3(3):570-576.

Rahman S, Ul Haq F, Ali A, Khan MN, Shah SMZ, Adhikhari A, El-Seedi HR, Musharraf SG (2019). Combining untargeted and targeted metabolomics approaches for the standardization of polyherbal formulations through UPLC-MS/MS Combining untargeted and targeted metabolomics approaches for the standardization of polyherbal formulations through UPLC–MS/MS. Metabolomics 15:116. *https://doi.org/10.1007/s11306-019-1582-6*

Reiter E, Jiang Q, Christen S. Anti-inflammatory properties of alpha- and gamma-tocopherol. Molecular Aspects of Medicine 28(5-6):668-691. *https://doi.org/10.1016%2Fj.mam.2007.01.003*

Rocabado GO, Bedoya LM, Abad MJ, Bermejo P (2008). Rubus - A Review of its phytochemical and pharmacological Profile. Natural Product Communications 3(3):423-436. *https://doi.org/10.1177/1934578X0800300319*

Rosa D, Elya B, Hanafi M, Khatib A, Surya MI (2023). In vitro and in silico screening analysis of *Artobotrys sumatranus* leaf and twig extracts for α-glucosidase inhibition activity and its relationship with antioxidant activity. Scientia Pharmaceutica 91(2):1-19. *https://doi.org/10.3390/scipharm91010002*

Ross HA, McDougall GJ, Stewart D (2007). Antiproliferative activity is predominantly associated with ellagitannins in raspberry extracts. Phytochemistry 68(2):218-228. *https://doi.org/10.1016/j.phytochem.2006.10.014*

Sahar WMH, Aida HS (2018). GC/MS identification and applications of bioactive seaweed extracts from Mediterranean coast of Egypt. The Egyptian Journal of Aquatic Biology and Fisheries 22(5):1-21 *http://dx.doi.org/10.21608/ejabf.2018.17952*

Sahi NM (2016). Evaluation of insecticidal activity of bioactive compounds from Eucalyptus citriodora against *Tribolium castaneum*. International Journal of Pharmacognosy and Phytochemical Research 8(8):1256-1270.

Sang MK, Kim KD (2012). The volatile-producing *Flavobacterium johnsoniae* strain GSE09 shows biocontrol activity against *Phytophthora capsici* in pepper. Journal of Applied Microbiology 113:383-398. *https://doi.org/10.1111/j.1365-2672.2012.05330.x*

Schubert M, Kluge S, Schmölz L, Wallert M, Galli F, Birringer M, Lorkowski S (2018). Long-chain metabolites of vitamin E: Metabolic activation as a general concept for lipid-soluble vitamins? Antioxidants 7:10. *http://dx.doi.org/10.3390/antiox7010010*

Seeram NP, Lee R, Scheuller HS, Heber D (2006). Identification of phenolic compounds in strawberries by liquid chromatography electrospray ionization mass spectroscopy. Food Chemistry 97:1-11. *https://doi.org/10.1016/j.foodchem.2005.02.047*

Senthil J, Rameashkannan MV, Mani P, Jayaseelan T, Dinesh KG (2016). Antidiabetic activities of *Ipomoea sepiaria* (Koenig Ex. Roxb) ethanolic leaves extracts in streptozotocin induced diabetic rats. International Journal of Chemistry and Pharmaceutical Science 4(5):247-250.

Shaaban MT, Ghaly MF, Fahmi SM (2021). Antibacterial activities of hexadecanoic acid methyl ester and green-synthesized silver nanoparticles against multidrug-resistant bacteria. Journal of Basic Microbiology 61(6):557-568. *https://doi.org/10.1002/jobm.202100061*

Sharma RK, Goel A (2018). Identification of phytoconstituents in *Lawsonia inermis* Linn. leaves extract by GC-MS and their antibacterial potential. Pharmacognasy Journal 10(6):1101-1108. *https://doi.org/10.5530/pj.2018.6.187*

Smillie TJ, Khan IA (2010). Comprehensive approach to identifying and authenticating botanical products. Clinical Pharmacology and Therapeutics 87:175-186. *https://doi.org/10.1038/clpt.2009.287*

Sowmiya R, Balasubramani G, Deepak P, Aiswarya D, Ravikumar S, Prasannakumar S, Perumal P (2017). Characterization and screening of in vitro antimalarial and larvicidal activities of selected seaweeds from southeast coast of India against *Plasmodium falciparum* and *Anopheles stephensi*. Journal of Coastal Life Medicine 5(6):242-248. *https://doi.org/10.12980/jclm.5.2017J7-35*

Sunita A, Manju S (2017). Phytochemical examination and GC-MS analysis of methanol and ethyl acetate extract of root and stem of *Gisekia phamaceoides* Linn. (Molluginaceae) from Thar desert, Rajasthan, India. Research Journal of Pharmaceutical, Biological and Chemical Sciences 8(4):168-174.

Suresh A, Praveenkumar R, Thangaraj R, Oscar FL, Baldev E, Dhanasekaran D, Thajuddin N (2014). Microalgal fatty acid methyl ester a new source of bioactive compounds with antimicrobial activity. Asian Pacific Journal of Tropical Disease 4(S2):S979-S984. *https://doi.org/10.1016/S2222-1808(14)60769-6*

Surya MI, Suhartati S, Ismaini L, Lusini D, Anggraeni S, Normasiwi N, Asni and Sidiq MAB (2018). Fruits nutrients content of five species of wild raspberries (*Rubus* spp.) from Indonesian Mountain's Forest. Journal Tropical of Life Sciences 8(1):75-80. *https://doi.org/10.11594/jtls.08.01.13*

Swamy MK, Arumugam G, Kaur R, Ghasemzadeh A, Yusoff MM, Sinniah UR. (2017). GC-MS based metabolite profiling, antioxidant and antimicrobial properties of different solvent extracts of Malaysian *Plectranthus amboinicus* leaves. Evidence-Based Complementary Alternative Medicine 1517683. *https://doi.org/10.1155/2017/1517683*

Takahashi C, Kikuchi N, Katou N, Miki T, Yanagida F, Umeda M (1995). Possible anti-tumor-promoting activity of components in Japanese soybean fermented food, natto: Effect on gap junctional intercellular communication. Carcinogenesis 16(3):471-476. *https://doi.org/10.1093/carcin/16.3.471*

Takahashi S, Takeshita K, Seeni A, Sugiura S, Tang M, Sato SY, Kuriyama H, Nakadate M, Abe K, Maeno Y, Nagao M, Shirai T (2009). Suppression of prostate cancer in a transgenic rat model via gamma-tocopherol activation of caspase signaling. Prostate 69(6):644-651. *https://doi.org/10.1002/pros.20915*

Tayade AB, Dhar P, Kumar J, Sharma M, Chauhan RS, Chaurasia OP, Srivastava RB (2013). Chemometric profile of root extracts of *Rhodiola imbricata* Edgew. with hyphenated gas chromatography mass spectrometric technique. PLoS One 8(1):e52797. *https://doi.org/10.1371/journal.pone.0052797*

Uddin SJ, Grice D, Tiralongo E (2012). Evaluationof cytotoxic activity of patriscabratine, tetracosane and various flavonoids isolated from the Bangladeshi medicinal plant *Acrostichum aureum*. Pharmaceutical Biology 50:1276-1280. *https://doi.org/10.3109/13880209.2012.673628*

Udobre AS, Etim EI, Udobang JA, Udoh (2015). Antimicrobial activity of stigmast-4-en-3-one and 2,4-Dimethylhexane isolated from *Nauclea latifolia*. International Journal of Phytopharmacy Research 6(2):65-68.

Uma B, Prabhakar K, Rajendran S (2009). Invitro antimicrobial activity and phytochemical analysis of *Ficus religiosa* L. and *Ficus bengalensis* L. against diarrhoeal enterotoxigenic *E. coli*. Ethnobotanical Leaflets 2009:4. *https://opensiuc.lib.siu.edu/ebl/vol2009/iss4/7*

Vanitha V, Vijayakumar S, Nilavukkarasi M, Punitha VN, Vidhya E, Praseetha PK (2020). Heneicosane—A novel microbicidal bioactive alkane identified from *Plumbago zeylanica* L. Industrial Crops and Products 154:112748. *https://doi.org/10.1016/j.indcrop.2020.112748*

Wedge DE, Meepagala KM, Magee JB, Smith SH, Huang G, Larcom LL (2001). Anticarcinogenic activity of strawberry, blueberry, and raspberry extracts to breast and cervical cancer cells. Journal of Medicinal Food 4(1):49-51. *https://doi.org/10.1089/10966200152053703*

Wei LS, Wee W, Siong JYF, Syamsumir DF (2011). Characterization of antimicrobial, antioxidant, anticancer properties and chemical composition of Malaysian *Andrographis paniculata* leaf extract. Pharmacology Online 2:996-1002.

Wilson MK, Baguley BC, Wall C, Jameson MB, Findlay MP (2014). Review of high-dose intravenous vitamin C as an anticancer agent. Asia Pacific Journal of Clinical Oncology 10(1):22-37. *https://doi.org/10.1111/ajco.12173*

Yoon MA, Jeong TS, Park DS, Xu MZ, Oh HW, Song KB, Lee WS, Park HY (2006). Antioxidant effects of quinoline alkaloids and 2,4-di-tert-butylphenol isolated from *Scolopendra subspinipes*. Biological Pharmaceutical Bulletin 29:735-739. *https://doi.org/10.1248/bpb.29.735*

Yu G, Luo Z, Wang W, Li Y, Zhou Y, Shi Y. (2019). *Rubus chingii* Hu: A Review of the phytochemistry and pharmacology. Frontiers in Pharmacology 16(10):799. *https://doi.org/10.3389%2Ffphar.2019.00799*

Zayed MZ, Wu A, Sallam SM (2019). Comparative phytochemical constituents of *Leucaena leucocephala* (Lam.) leaves, fruits, stem barks, and wood branches grown in Egypt using GC-MS method coupled with multivari- ate statistical approaches. BioResource 14(1):996-1013.

Zeng HJ, Liu Z, Wang YP, Yang D, Yang R, Qu LB (2018). Studies on the anti-aging activity of a glycoprotein isolated from Fupenzi (*Rubus chingii* Hu.) and its regulation on klotho gene expression in mice kidney. International Journal of Biological Macromolecules 119:470-476. *https://doi.org/10.1016/j.ijbiomac.2018.07.157*

Zhang H, Zhang L, Peng LJ, Dong XW, Wu D, Wu VC, Feng FQ (2012). Quantitative structure-activity relationships of antimicrobial fatty acids and derivatives against *Staphylococcus aureus*. Journal of Zhejiang University Science B13(2):83-93. *https://doi.org/10.1631%2Fjzus.B1100049*