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# ANTIOXIDANT CONSTITUENTS FROM THE LEAVES OF *Piper crassipes* Korth ex Miq. GROWING IN MALAYSIA

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**ABSTRACT**. The genus *Piper*, belonging to the Piperaceae family, includes over 2,000 species, many of which are widely recognized for their significant economic and medicinal value. This study aimed to investigate the phytochemicals isolated from the leaf extract of *Piper crassipes.* Soxhlet extraction of the dried powdered leaves was employed to extract the phytochemicals using a polarity gradient of hexane, dichloromethane, and methanol. The phytochemicals were obtained using chromatography techniques and their structures were confirmed by spectroscopic analysis (IR, NMR, and MS) and comparison with the literature. Furthermore, the isolated phytochemicals were evaluated for their DPPH free radical scavenging activity. The isolation successfully led to the identification of ten phytochemicals: asaricin (**1**), chavibetol (**2**), dillapiole (**3**), β-sitosterol (**4**), 5,7-dimethoxyflavone (**5**), 4',5,7-trimethoxyflavone (**6**), N-(3-phenylpropanoyl)pyrrole (**7**), chavicol (**8**), N-isobutyl-(2E,4E,14Z)-eicosatrienamide (**9**), and 4-allyl resorcinol (**10**). Among the isolated phytochemicals, compounds (**8**) and (**10**) showed the highest DPPH radical scavenging activity, with $IC_{50}$ values of 189.3 and 195.8 µmol/L, respectively. This study offers important insights into the antioxidant potential of *P. crassipes* constituents, highlighting further opportunities to explore their nutraceutical and pharmaceutical applications.

**KEY WORDS**: Piperaceae, *Piper crassipes*, Constituent, Phenylpropanoid, Antioxidant

## INTRODUCTION

Foods and medicinal plants are rich sources of natural antioxidants. Numerous biological effects, such as anti-inflammatory, anti-aging, anti-atherosclerosis, and anti-cancer properties, are exhibited by these naturally occurring antioxidants, particularly polyphenols and carotenoids. To investigate prospective sources of antioxidants and encourage their use in functional foods, medications, and food additives, it is essential to extract and evaluate antioxidants from food and medicinal plants efficiently and accurately [1]. The body's natural antioxidant system can scavenge these radicals, maintaining the equilibrium between oxidation and anti-oxidation. Nonetheless, exposure to cigarette smoke, alcohol, radiation, or environmental toxins induces the production of excessive reactive oxygen species (ROS) and reactive nitrogen species (RNS), which disrupt the balance between oxidation and anti-oxidation and result in some chronic and degenerative diseases [2]. Increasing the intake of exogenous antioxidants could ameliorate the

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damage by oxidative stress through inhibiting the initiation or propagation of oxidative chain reaction acting as free radical scavengers, quenchers of singlet oxygen and reducing agents [3]. Butylated hydroxytoluene and butylated hydroxyanisole are synthetic antioxidants that have recently been linked to human health risks. Thus, the search for effective, nontoxic natural compounds with antioxidative activity has been intensified in recent years [4].

Piperaceae is one of the largest basal angiosperm families, widely distributed across both the pantropical and neotropical zones. They are prevalent in tropical Asia, Africa, the Amazon region, and Central America. It is a large family of flowering plants of small trees, shrubs, or herbs. This plant family contains around 4000 species and is classified into five main genera, namely, *Piper*, *Lepianthes*, *Peperomia*, *Trianopiper* and *Macropiper* [5]. The genus Piper has approximately 1500-2000 species of herbs, shrubs, vines, or trees that are extensively spread in Southeast Asia and Oceania, Western and Central Africa, Central and South America. Members of the genus Piper are often used in traditional medicine to treat various illnesses. They are most commonly used to treat chronic bronchitis, asthma, constipation, gonorrhea, paralysis of the tongue, diarrhea, cholera, chronic malaria, viral hepatitis, respiratory infections, stomachache, bronchitis, cough, and tumors [6]. Owing to their valuable pharmacological significance, numerous phytochemical and biological studies on Piper species have been carried out to date, validating their traditional uses from a modern scientific perspective and developing their novel pharmacological activities. The extracts of this genus are rich in phenolics, amides, alkaloids, flavonoids, lignans, neolignans, glycosides, and terpenoids [7-9], all of which possess a wide range of pharmacological activities such antioxidant, antibacterial, antifungal, anti-inflammatory, insecticidal, antiproliferative, cytotoxic, and antiplasmodial properties [10-12].

*Piper crassipes* Korth ex Miq. is locally known as "sireh murai' in Peninsular Malaysia. The species is mainly distributed and native to Southeast Asia, particularly in Singapore, Thailand, Malaysia, India and Myanmar. Generally, *P. crassipes* is similar to *P. ribesioides* but differs by having smaller leaves, rounded floral bracts, and dry fruits which are non-rugulate. It has been used traditionally to treat cough, sinusitis, poor digestion, and throat infection. It also has been used in cooking as herbs and spices [13]. Recently, we have reported the chemical components of the leaf oil of *P. crassipes* [14]. Investigation of the essential oil revealed the presence of 22 components, accounting for 97.8% of the total oil. The analysis revealed the leaf oil consists primarily of phenylpropanoids (78.0%), followed by sesquiterpene hydrocarbons (18.6%) and oxygenated sesquiterpenes (1.2%). The major components of essential oil were chavibetol (59.8%), chavibetol acetate (10.4%), γ-muurolene (5.4%) and germacrene D (4.6%). Furthermore, the leaf oil showed moderate inhibitory effects on acetylcholinesterase and butyrylcholinesterase enzymes with $IC_{50}$ values of 77.2 and 89.2 µg/mL, respectively.

As part of the continuation of our search for bioactive compounds from Piper species, we have investigated the phytochemicals present in the stem and leaves of *P. crassipes.* To our knowledge, no report exists on phytochemical studies concerning the leaves and their antioxidant activity.

## RESULTS AND DISCUSSION

In this study, ten compounds were successfully isolated from the leaves of *P. crassipes*, which were characterized as asaricin (**1**), chavibetol (**2**), dillapiole (**3**), β-sitosterol (**4**), 5,7-dimethoxyflavone (**5**), 4',5,7-trimethoxyflavone (**6**), N-(3-phenylpropanoyl)pyrrole (**7**), chavicol (**8**), N-isobutyl-(2E,4E,14Z)-eicosatrienamide (**9**) and 4-allyl resorcinol (**10**). They were all identified by analyzing their spectroscopic data and comparing them with the reported literature. Their chemical structures are shown in Figure 1.

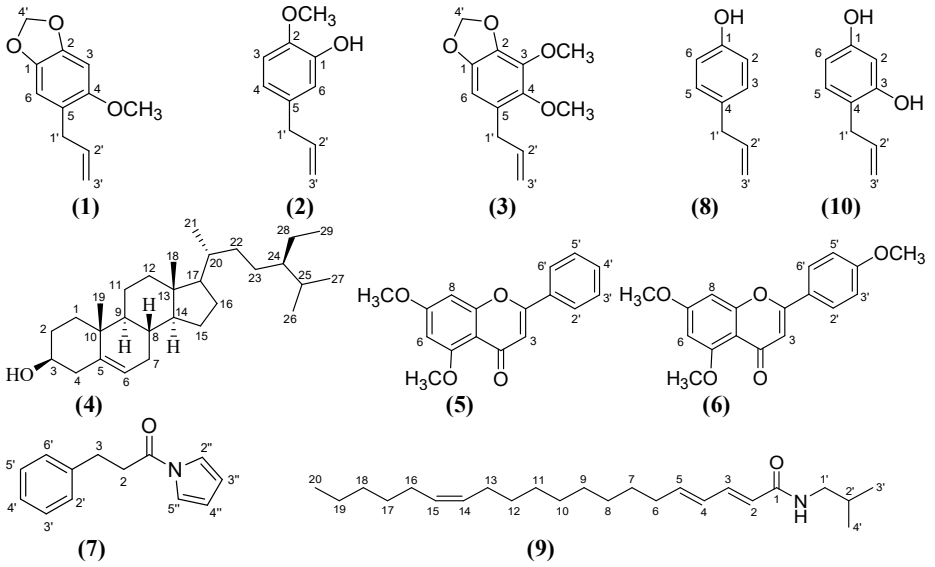

Figure 1. Chemical structures of isolated compounds from *P. crassipes*.

Compound (**1**) was identified as asaricin, it exhibited distinctive signals in its NMR spectrum, confirming its identity. The $^{1}$H NMR spectrum displayed four singlet signals, two of which corresponded to aromatic protons at δ 6.45 (H-3) and 6.57 (H-6). The other two singlets at δ 3.69 (4-$OCH_3$) and 5.81 (H-4') were attributed to methoxy and methylenedioxy protons, respectively. Additionally, a doublet at δ 3.22 (J = 6.6 Hz) and a multiplet at δ 5.86 were associated with two allylic protons, H-1' and H-2', respectively. The allylic protons H-3' exhibited a multiplet signal at δ 4.95. The $^{13}$C NMR spectrum revealed the presence of eleven carbon atoms, including one methoxy carbon at δ 56.6 (4-$OCH_3$), three methine carbons at δ 95.0 (C-3), 109.7 (C-6), 137.2 (C-2'), and three methylene carbons at δ 34.0 (C-1'), 100.9 (C-4'), 115.2 (C-3').

Compound (**2**) was identified as chavibetol. The $^{1}$H NMR spectrum revealed two distinct singlet signals, corresponding to a methoxy group at δ 3.90 (2-$OCH_3$) and a hydroxyl group at δ 5.78 (1-OH). Additionally, doublet signals were observed at δ 3.35 (J = 6.8 Hz), 6.83 (J = 8.1 Hz), and 6.85 (J = 2.3 Hz) attributed to protons at H-1', H-3, and H-6, respectively. Furthermore, the doublet of doublet signal at δ 6.72 (J = 8.3 and 2.3 Hz) was assigned to an aromatic proton at H-4. Furthermore, the multiplet signals at δ 5.12 and 6.00 were identified as H-3' and H-2', respectively. The $^{13}$C NMR spectrum and DEPT spectra revealed the presence of ten carbon atoms, including a methoxy carbon at δ 56.1. Four methine carbons C-3, C-6, C-4, and C-2' resonated at δ 110.8, 115.0, 119.9, and 137.7, respectively. Two methylene carbons were observed at δ 39.8 (C-1') and 115.6 (C-3'), along with three quaternary carbons: C-5 (δ 133.5), C-2 (δ 145.1), and C-1 (δ 145.7).

Compound (**3**) was identified as dillapiole. The $^{1}$H NMR spectrum was closely resembled to the $^{1}$H NMR spectrum of asaricin (**1**), with the addition of a methoxyl group at δ 4.04 (3-$OCH_3$). It was further confirmed by the additional peak at δ 61.3 in the $^{13}$C NMR spectrum, which corresponds to the methoxyl group at C-3.

Compound (**4**) was identified as β-sitosterol (**4**), displaying characteristic signals in its $^{1}$H NMR spectrum, revealing the presence of two singlet signals at δ 0.68 and 1.00, which correspond to two methyl groups at H-18 and H-19, respectively. Additionally, an olefinic proton at H-6 was shown as a doublet signal resonating at δ 5.34 with a coupling constant J = 5.3 Hz. Other doublet

signals resonated at δ 0.80 (J = 6.9 Hz), 0.82 (J = 6.9 Hz), 0.83 (J = 6.9 Hz), and 0.91 (J = 6.5 Hz) were assigned to methyl groups at H-27, H-26, H-29, and H-21, respectively. A multiplet signal at δ 3.52 was assigned to the proton H-3 bearing a hydroxyl group. The $^{13}$C NMR spectrum and DEPT spectra revealed the presence of 29 carbon atoms, comprising of six methyl carbons, nine methine carbons, eleven methylene carbons, and three quaternary carbons.

Compound (**5**) was identified as 5,7-dimethoxyflavone, which exhibited characteristic signals in its $^{1}$H NMR spectrum, confirming the presence of two singlet signals at δ 3.96 and 3.92, attributed to the methoxyl groups located at positions 5-$OCH_3$ and 7-$OCH_3$, respectively. Additionally, a singlet resonance at δ 6.92 was assigned to H-3. Two sets of doublets with J = 2.3 Hz, resonating at δ 6.40 and δ 6.60, correspond to meta-coupled protons H-8 and H-6, respectively. A multiplet signal at δ 7.51, integrating for three protons, was ascribed to the aromatic protons in ring B (H-3', H-4', and H-5'). Furthermore, a doublet of doublets at δ 7.90, with coupling constants J = 7.8 and 1.8 Hz, integrated for two protons from ring B (H-2'/H-6'). The $^{13}$C NMR spectrum displayed signals for seventeen carbons. The DEPT spectra further confirmed these, identifying seven quaternary carbons at δ 109.3 (C-4a), 131.6 (C-1'), 159.9 (C-2), 160.9 (C-7), 160.6 (C-5), 164.1 (C-8a), and 177.6 (C-4), eight methine carbons at δ 92.8 (C-6), 96.2 (C-8), 109.1 (C-3), 125.9 (C-2'/C-6'), 128.9 (C-3'/C-5'), and 131.2 (C-4'), and two methoxyl carbons at δ 55.7 (5-$OCH_3$), and 56.4 (7-$OCH_3$).

Compound (**6**) was identified as 4',5,7-trimethoxyflavone. The $^{1}$H NMR spectrum was closely resembled to the $^{1}$H NMR spectrum of 5,7-dimethoxyflavone (**5**), with an addition of one methoxyl group at δ 3.88 (4'-$OCH_3$). In addition, signals for para-disubstituted moiety were observed as a doublet at δ 7.00 and 7.84 attributed to H-3'/H-5' and H-2'/H-6' of ring B of a flavonoid system. It was further confirmed by the additional peak at δ 56.3 in the $^{13}$C NMR spectrum, which corresponds to the methoxyl group at C-4'.

Compound (**7**) was identified as N-(3-phenylpropanoyl)pyrrole. The $^{1}$H NMR spectrum displayed a multiplet signal at δ 3.13, integrated by four protons, attributed to the methylene protons at H-2 and H-3. A multiplet signal at δ 7.27, integrated for seven protons, was assigned to five aromatic protons (H-2', H-3', H-4', H-5', and H-6') and two pyrrole protons (H-2"/H-5"). Additionally, a triplet signal at δ 6.28 (J = 2.4 Hz) was assigned to the protons H-3"/H-4". The $^{13}$C NMR spectrum revealed the presence of thirteen carbons. Two signals at δ 30.4 and 36.4 corresponded to two methylene carbons at C-2 and C-3, respectively. Additionally, five methines displayed signals at δ 128.7 (C-2', C-6'), 128.4 (C-3', C-5'), and 126.5 (C-4'). A carbonyl carbon (C=O) was observed at δ 169.7, whereas a single quaternary carbon (C-1') was observed at δ 140.2. The pyrrole carbons were represented by the signal at δ 119.0 (C-2" and C-5") and δ 113.2 (C-3" and C-4").

Compound (**8**) was identified as chavicol, displaying characteristic signals in its NMR spectra, confirming the presence of three doublet signals which appeared at δ 3.31 (J = 6.6 Hz), 6.76 (J = 8.6 Hz), and 7.05 (J = 8.6 Hz), assigned to two proton of an allylic skeleton, H-1' and aromatic protons H-3/H-5 and H-2/H-6, respectively. The $^{13}$C NMR and DEPT spectra showed the presence of nine carbons which consist of two quaternary carbons, five methines carbons, and two methylenes carbons. Two quaternary carbons C-4 and C-1 resonated at δ 132.3 and 153.8, respectively. Furthermore, five methines, carbons C-3/C-5, C-2/C-6, and C-2′ resonated at δ 115.2, 129.7, and 137.8, respectively. Meanwhile, two methylenes carbons resonated at δ 39.3 (C-1'), and 115.2 (C-3').

Compound (**9**) was identified as N-isobutyl-(2E,4E,14Z)-eicosatrienamide. The $^{1}$H NMR spectrum revealed the presence of an isobutylamide moiety, evidenced by a doublet signal at δ 0.93 (J = 6.8 Hz) for protons H-3'/H-4', a septet signal at δ 1.81 (J = 6.8 Hz) for proton H-2', and a triplet signal at δ 3.17 (J = 6.4 Hz) for H-1'. A broad singlet at δ 5.47 was determined to be associated with the N-H proton. Furthermore, multiplet signals in the region of δ 1.26–1.33 (integrated for 18H) confirmed the presence of nine methylene groups (C-7 to C-12 and C-17 to C-19). Additional multiplet signals observed at δ 2.03 and 2.16 were assigned to protons H-13/H-

16 and H-6, respectively. Additionally, a doublet signal at δ 5.77 (J = 15.2 Hz) was attributed to proton H-2, while a triplet signal at δ 0.90 (J = 6.8 Hz) was assigned to proton H-20. Two doublets of doublets appeared at δ 7.20 (J = 15.2, 9.6 Hz) and 6.10 (J = 16.4, 9.6 Hz), which corresponded to protons H-3 and H-4. Three doublets of triplets were assigned for protons H-14/H-15 and H-5, appearing at δ 5.35 (J = 9.2, 5.6 Hz) and δ 6.10 (J = 16.4, 6.0 Hz), respectively. The $^{13}$C NMR and DEPT spectra showed the presence of twenty-four carbon atoms, identifying the carbon atoms as one quaternary (C-1), seven methine carbons (C-2', C-2, C-4, C-14, C-15, C-5, and C-3), thirteen methylene carbons (C-19, C-16, C-17, C-13, C-18, C 7-12, C-6, and C-1'), and three methyl carbons (C-20, C-3', and C-4').

Compound (**10**) was identified as 4-allyl resorcinol. The $^{1}$H NMR spectrum was closely resembled to the $^{1}$H NMR spectrum of chavicol (**8**), with an addition of one hydroxyl group at δ 5.29 (3-OH). A doublet signal was observed at δ 3.27 (J = 6.6 Hz) and two multiplet signals which appeared at δ 5.92 and 5.04 were assigned to protons of an allylic skeleton H-1', H-2' and H-3', respectively. A doublet of doublets observed at δ 6.62 (J = 8.0 and 2.1 Hz) was assignable to an aromatic proton of H-6, while the two sets of doublets observed at δ 6.70 (J = 2.1 Hz) and at 6.78 (J = 8.0 Hz) were attributable to aromatic protons, H-2 and H-5, respectively. The $^{13}$C NMR spectrum showed the presence of nine carbons which consist of three quaternary carbons, four methine carbons, and two methylene carbons.

In summary, the chemical constituents of *P. crassipes* from Malaysia were found to be rich in phenylpropanoids. Phenylpropanoids are a diverse group of compounds derived from the carbon skeleton of phenylalanine that are involved in plant defense, structural support, and survival of plants. To the best of our knowledge, all compounds presented have been isolated from *P. crassipes* for the first time. Compounds (**1–10**) have been previously reported as being isolated from several Piper species. Compounds (**1**) and (**7**) have been isolated previously from *P. sarmentosum* [17, 18], compounds (**2**) and (**8**) from *P. betle* [19], and compound (**3**) from *P. hispidum* [20]. Furthermore, compounds (**4**), (**5**), (**6**), and (**10**) were reported previously from *P. caninum* [21] and *P. abbreviatum* [22], while compound (**9**) from *P. stylosum* [23].

2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay is an affordable, quick and easy method for the measurement of antioxidant properties as it uses free radicals to assess the potential of substances. Free radicals are molecules or fragments of independent existence that contain an unpaired electron in the atomic orbital, which are generated by cells during cell-mediated immune and respiration. Radicals are unstable and highly reactive due to the presence of an unpaired electron. Oxygen-containing free radicals, such as hydroxyl, superoxide anion, and hydrogen peroxide, are important in many disease such as cancer, cardiovascular disease, and neurodegenerative disorders. By assessing antioxidant activity, researchers can identify compounds or foods that may help reduce oxidative stress and lower the risk of these diseases [24].

Bioactive compounds, such as phenolic compounds, flavonoids, and amides found within this genus, are powerful antioxidants that complement and enhance the functions of vitamins and enzymes, thereby providing protection against oxidative stress. These compounds may be used in the food industry, mainly in meat products, in which oxidation is one of the main problems related to their deterioration [24]. Based on the relevant potential of plants as sources of antioxidant compounds, in the current study, the isolated compounds from *P. crassipes* were subjected to DPPH radical scavenging activity, and the results are shown in Table 1.

All isolated compounds showed strong antioxidant activity, which gave $IC_{50}$ values in the range of 117.8–215.6 µmol/L. Among them, compounds (**9**) and (**3**) showed the highest antioxidant activity with $IC_{50}$ values of 117.8 and 146.2 µmol/L, respectively. The antioxidant activity of isolated phenylpropanoids (compounds **1**, **2**, **3**, **8**, and **10**) could be due to the presence of phenolic and hydroxyl groups. Previously, a benzene ring where the hydroxyl radical is in the 1,4-orientation allows the oxygen atom to share a positive charge, thereby causing stabilization through delocalization. However, if the hydroxyl radical is in the 1,3-orientation, the oxygen atom is unable to share the charge and this is thought to influence the ability to scavenge the DPPH

radicals. The substitution radical of the 1,2- or 1,4-orientation generally donates an electron to the aromatic ring to activate it, either by the resonance effect or inductive effect. However, a substitution radical in the 1,3-orientation tends to inactivate the ring [25]. In addition, the radical scavenging activity of the isolated phenylpropanoids might also be due to the presence of allylic groups which have the capacity to donate electrons and function as reductones [26].

Table 1. DPPH radical scavenging activity of the selected phytochemicals

| Compounds | $IC_{50}$ (µmol/L) |
|---|---|
| Asaricin (**1**) | 161.8 |
| Chavibetol (**2**) | 215.6 |
| Dillapiole (**3**) | 146.2 |
| 5,7-Dimethoxyflavone (**5**) | 172.9 |
| 4′,5,7-Trimethoxyflavone (**6**) | 157.2 |
| *N*-(3-Phenylpropanoyl)pyrrole (**7**) | 199.2 |
| Chavicol (**8**) | 189.3 |
| *N*-Isobutyl-(2E,4E,14Z)-eicosatrien-amide (**9**) | 117.8 |
| 4-Allyl resorcinol (**10**) | 195.8 |
| Ascorbic acid | 132.9 |

Meanwhile, previous studies in vitro have demonstrated that flavonoids (compounds **5** and **6**) act as hydrogen-donating free radical scavengers, and their function depends on their structural properties, of which there are three key features: 1) the *o*-dihydroxyl (catechol) structure in the B ring, which confers higher stability to radical form; 2) a 2,3-double bond in conjunction with the 4-oxo function in the C ring, which is responsible for electron delocalization; and 3) the 3- and 5-hydroxyl groups responsible for maximal radical scavenging power [27]. Moreover, the activity demonstrated by amides (**7** and **9**) can be attributed to its amino-substituted phenol aromatic amines. Prior investigations have linked amide's antioxidant effects to the inhibition of oxidative damage levels, reducing free radicals and reactive oxygen species. Amide's antioxidative properties are closely associated with its carbon-oxygen five-membered rings and amide structure, inhibiting lipid peroxidation and enhancing glutathione synthesis or transport [28, 29].

## EXPERIMENTAL

### *Plant material*

The leaves of *P. crassipes* were collected from Behrang, Perak (3° 45' N, 101° 34' E) in January 2023. The sample (voucher number - BB-11/23) was identified by Dr. Shamsul Khamis (Universiti Kebangsaan Malaysia) and deposited at UKM Herbarium, UKM.

### *General experimental procedures*

A Soxhlet extraction technique was applied to extract the phytochemicals from the dried leaves of *P. crassipes* using different polarity solvents (n-hexane, dichloromethane (DCM) and methanol). Column chromatography (CC) was performed using Merck silica gel 60 (70-230 mesh) as the stationary phase. Thin layer chromatography (TLC) analysis was performed on Merck precoated silica ($SiO_2$) gel $F_{254}$ plates (0.22 mm thickness) to detect and monitor the presence of compound samples. The spots were visualized under UV light (254 and 365 nm) and sprayed with the reagent vanillin sulfuric acid in MeOH followed by heating. Melting points were measured by comparing them with those reposted in the literature. The $^{1}$H-NMR (500 MHz) and $^{13}$C-NMR (100 MHz) spectra were recorded on a Bruker Avance 500 Spectrometer. Chemical

shifts were reported in ppm and $CDCl_3$ as solvent. The residual solvent was used as an internal standard. The IR spectra were recorded on Perkin Elmer ETR and 1600 spectrophotometer series as KBr discs or thin film of NaCl discs. Mass spectral data were obtained from Orbitrap Exploris 240 Mass Spectrometer.

*Extraction and isolation*

The dried leaves of *P. crassipes* were ground into powder (500 g) and extracted with n-hexane and dichloromethane (DCM) sequentially by Soxhlet extraction. The extract was concentrated using rotary evaporation to afford the crude extracts. The n-hexane extract (PCLH, 20 g) was fractionated by VLC and eluted with n-hexane: DCM: EtOAc to afford 16 major fractions (F1-16). Fractions F2, F4, and F6 were purified by CC and eluted with n-hexane: DCM to afford compound **1** (25 mg), compound **2** (20 mg) and compound **3** (25 mg), respectively. Fractions F8, F13, and F14 were further crystallized to afford compounds **4** (18 mg), **5** (40 mg) and **6** (45 mg), respectively. Purification of the DCM extract (PCLD, 18 g) by CC eluted with n-hexane:EtOAc:MeOH afforded 15 fractions (F1-15). Fraction F9 successfully yielded compound **8** (20 mg), whereas fraction F14 yielded compound **9** (28 mg). Fractions F4 and F11 were further purified by PTLC and successfully afforded compound **7** (8 mg) and compound **10** (8 mg), respectively.

*Asaricin (**1**)*. Yellow solid. $^1$H NMR ($CDCl_3$, 500 MHz): δ 3.22 (2H, d, J = 6.6 Hz, H-1'), 3.69 (3H, s, 4-OCH3), 4.95 (2H, m , H-3'), 5.81 (2H, s, H-4'), 5.86 (1H, m, H-2'), 6.45 (1H, s, H-3), 6.57 (1H, s, H-6); $^{13}$C NMR ($CDCl_3$, 100 MHz): δ 34.0 (C-1'), 56.6 (4-OCH3), 95.0 (C-3), 100.9 (C-4'), 109.7 (C-6), 115.2 (C-3'), 120.83 (C-2), 137.2 (C-2'), 141.0 (C-4), 146.3 (C-1), 152.1 (C-5); EIMS: m/z 192 [$M^+$, $C_{11}H_{12}O_3$] [17].

*Chavibetol (**2**)*. Yellow solid. $^1$H NMR ($CDCl_3$, 500 MHz): δ 3.35 (2H, d, J = 6.8 Hz, H-1'), 3.90 (3H, s, 2-OCH3), 5.12 (2H, m, H-3'), 5.78 (1H, s, 1-OH), 6.00 (1H, m, H-2'), 6.72 (1H, dd, J = 8.3, 2.3 Hz, H-4), 6.83 (1H, d, J = 8.1 Hz, H-3), 6.85 (1H, d, J = 2.3 Hz, H-6); $^{13}$C NMR ($CDCl_3$, 100 MHz): δ 39.8 (C-1′), 56.1 (2-OCH), 110.8 (C-3) , 115.0 (C-6) , 115.6 (C-3′), 119.9 (C-4), 133.5 (C-5), 137.7 (C-2′), 145.1 (C-2), 145.2 (C-1); EIMS: m/z 164 [$M^+$, $C_{10}H_{12}O_2$] [19].

*Dillapiole (**3**)*. Yellow solid. $^1$H NMR ($CDCl_3$, 500 MHz): δ 3.33 (2H, d, J = 6.6 Hz, H-1'), 3.78 (3H, s, 4-$OCH_3$), 4.04 (3H, s, 3-$OCH_3$), 5.07 (2H, m, H-3'), 5.90 (2H, s, H-4'), 5.93 (1H, m, H-2'), 6.37 (1H, s, H-6); $^{13}$C NMR (CDCl3, 100 MHz): δ 33.9 (C-1'), 60.0 (4-OCH3), 61.3 (3-$OCH_3$), 101.1 (C-4'), 102.8 (C-6), 115.5 (C-3'), 126.1 (C-1), 136.0 (C-3), 137.4 (C-4), 137.7 (C-2'), 144.3 (C-5), 144.6 (C-2); EIMS: m/z 223 [$M^+$, $C_{12}H_{14}O_4$] [20].

*β-Sitosterol (**4**)*. White solid. $^1$H NMR ($CDCl_3$, 500 MHz): δ 0.68 (3H, s, H-18), 0.80 (3H, d, J = 6.9 Hz, H-27), 0.82 (3H, d, J = 6.9 Hz, H-26), 0.83 (3H, d, J = 6.9 Hz, H-29), 0.91 (3H, d, J = 6.5 Hz, H-21), 1.00 (3H, s, H-19), 1.02-2.35 (29H, m, overlapping CH/$CH_2$), 3.52 (1H, m, H-3), 5.34 (1H, d, J = 5.3 Hz, H-6); $^{13}$C NMR ($CDCl_3$, 100 MHz): δ 12.0 (C-18/C-29), 18.8 (C-21), 19.0 (C-27), 19.8 (C-19/C-26), 21.1 (C-11), 23.1 (C-28), 24.3 (C-15), 26.2 (C-23), 28.2 (C-16), 29.2 (C-2/C-25), 31.7 (C-7/C-8), 34.0 (C-22), 36.1 (C-20), 36.5 (C-10), 37.3 (C-1), 42.3 (C-4/C-12/C-13), 45.9 (C-24), 50.2 (C-9), 56.8 (C-14/C-17), 71.0 (C-3), 121.7 (C-6), 140.8 (C-5); EIMS: m/z 414 [$M^+$, $C_{29}H_{50}O$] [21].

*5,7 Dimethoxyflavone (**5**)*. White solid. $^1$H NMR ($CDCl_3$, 500 MHz): δ 3.96 (3H, s, 5-$OCH_3$), 3.92 (3H, s, 7-$OCH_3$), 6.40 (1H, d, J = 2.3 Hz, H-8), 6.60 (1H, d, J = 2.3 Hz, H-6), 6.92 (1H, s, H-3), 7.51 (3H, m, H-3'/H-4'/H-5'), 7.90 (2H, m, H-2'/H-6'); $^{13}$C NMR (CDCl3, 100 MHz): δ 56.4 (7-$OCH_3$), 55.7 (5-$OCH_3$), 92.8 (C-6), 96.2 (C-8), 109.1 (C-3), 109.3 (C-4a), 125.9 (C-2'/C-6'),

128.9 (C-3'/C-5'), 131.2 (C-4'), 131.6 (C-1'), 159.9 (C-2), 160.6 (C-5), 160.9 (C-7), 164.1 (C-8a), 177.6 (C-4); EIMS: m/z 282 [$M^+$, $C_{17}H_{14}O_4$] [21].

*4',5,7-Trimethoxyflavone (**6**)*. White solid. $^1$H NMR ($CDCl_3$, 500 MHz): δ 3.95 (3H, s, 5-$OCH_3$), 3.91 (3H, s, 7-$OCH_3$), 3.88 (3H, s, 4'-$OCH_3$), 6.37 (1H, d, J = 2.3 Hz, H-8), 6.57 (1H, d, J = 2.1 Hz, H-6), 6.69 (1H, s, H-3), 7.00 (3H, m, H-3'/H-5'), 7.84 (2H, d, J = 8.9, H-2'/H-6'); $^{13}$C NMR ($CDCl_3$, 100 MHz): δ 56.3 (4'-$OCH_3$), 55.7 (7-$OCH_3$), 55.5 (5-$OCH_3$), 92.3 (C-6), 96.1 (C-8), 107.7 (C-3), 109.2 (C-4a), 114.4 (C-3'/C-5'), 123.9 (C-1'), 127.6 (C-2'/C-6'), 159.8 (C-8a/C-4'), 160.9 (C-5), 162.1 (C-2), 163.9 (C-7), 177.7 (C-4); EIMS: m/z 312 [$M^+$, $C_{18}H_{16}O_5$] [21].

*N-(3-phenylpropanyl)pyrrole (**7**)*. White solid. $^1$H NMR ($CDCl_3$, 500 MHz): δ 3.13 (4H, m, H-2/H-3), 7.27 (7H, m, Ar-H/H-2"/H-5"), 6.28 (2H, t, J = 2.4 Hz, H-3"/H-4"); $^{13}$C NMR ($CDCl_3$, 100 MHz): δ 30.4 (C-2), 36.4 (C-3), 113.2 (C-3"/C-4"), 119.0(C-2"/C-5"), 126.5 (C-4'), 128.4 (C-3'/ C-5'), 128.7 (C-2'/C-6'), 140.2 (C-1'), 169.7 (C-1); EIMS: m/z 199 [$M^+$, $C_{13}H_{13}NO$] [18].

*Chavicol (**8**)*: Yellow liquid. $^1$H NMR ($CDCl_3$, 500 MHz): δ 3.31 (2H, d, J = 6.6 Hz, H-1'), 5.04 (2H, m, H-3'), 5.93 (1H, m, H-2'), 6.76 (2H, d, J = 8.6 Hz, H-3/H-5), 7.05 (2H, d, J = 8.6 Hz, H-2/H-6); $^{13}$C NMR ($CDCl_3$, 100 MHz): δ 39.3 (C-1'), 115.2 (C-3/C-5/C-3'), 129.7 (C-2/C-6), 132.3 (C-4), 137.8 (C-2'), 153.8 (C-1); EIMS: m/z 135 [$M^+H^+$, $C_9H_{10}O$] [19].

N-isobutyl-(2E,4E,14Z)-eicosatrienamide (**9**). Colorless crystalline needles. 1H NMR ($CDCl_3$, 500 MHz): δ 0.90 (3H, t, J = 6.8 Hz, H-20), 0.93 (3H, d, J = 6.8 Hz, H-3'/H-4'), 1.26–1.33 (2H, m, H-7 – H-12, H17–H19), 1.81 (1H, septet, J = 6.8 Hz, H-2'), 2.03 (2H, m, H-16), 2.16 (2H, m, H-6), 3.17 (2H, t, J = 6.4 Hz, H-1'), 5.35 (1H, dt, J = 9.2, 5.6 Hz, H-14/H-15), 5.47 (1H, br. S, N-H), 5.75 (1H, d, J = 15.2 Hz, H-2), 6.10 (1H, dd, J = 16.4, 9.6 Hz, H-4/H-5), 7.20 (1H, dd, J = 15.2, 9.6, Hz, H-3); $^{13}$C NMR ($CDCl_3$, 100 MHz): δ 14.1 (C-20), 20.1 (C-3'/C-4'), 22.6 (C-19), 26.9 (C-16), 26.8-29.3 (C-7–C-12), 28.6 (C-2'), 29.7 (C-17), 31.9 (C-18), 33.0 (C-6), 46.9 (C-1'), 121.7 (C-2), 128.2 (C-40), 129.8 (C-14), 129.9 (C-15), 141.3 (C-5), 143.2 (C-3), 166.3 (C-1); EIMS: m/z 361 [$M^+$, $C_{24}H_{42}NO$] [23].

*4-Allyl resorcinol (**10**)*. Light brown viscous liquid. $^1$H NMR ($CDCl_3$, 500 MHz): δ 3.27 (2H, d, J = 6.6 Hz, H-1'), 5.04 (2H, m, H-3'), 5.29 (1H, s, 1-OH/3-OH), 5.92 (1H, m, H-2'), 6.62 (1H, dd, J = 8.0, 2.1 Hz, H-6), 6.70 (1H, d, J = 2.1 Hz, H-2), 6.78 (1H, d, J = 8.0 Hz, H-5); $^{13}$C NMR ($CDCl_3$, 100 MHz): δ 39.5 (C-1'), 115.4 (C-2), 115.6 (C-3'), 115.7 (C-5), 121.0 (C-6), 133.3 (C-4), 137.6 (C-2'), 141.7 (C-3), 143.5 (C-1); EIMS: m/z 150 [$M^+$, $C_9H_{10}O_2$] [22].

*Antioxidant activity*

The DPPH free radical scavenging assays of phytochemicals were conducted following as a previous method [15-16] with slight modifications. Each sample of stock solution (1.0 mg/L was diluted to a final concentration of 200-25 µmol/L. Then, a total of 3.8 mL of 50 µM DPPH methanolic solution was added to 0.2 mL of each sample solution and allowed to react at room temperature for 30 min. The absorbance of the mixtures was measured at 517 nm. A control was prepared without sample or standard and measured immediately at 0 min. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity, and vice versa. Inhibition of DPPH radical in percent (I%) was calculated as follows:

$$I\% = [A_{blank} - A_{sample} / A_{blank}] \times 100 \quad (1)$$

where $A_{blank}$ is the absorbance value of the control reaction (containing all reagents except the test compound), and $A_{sample}$ is the absorbance value of the test compounds. The sample concentration

that provides 50% inhibition ($IC_{50}$) was calculated by plotting the inhibition percentages against concentration of the sample. All tests were carried out in triplicate and $IC_{50}$ values were reported as means ± SD of triplicates. Ascorbic acid was used as a standard and diluted to the same concentration as the samples.

## CONCLUSION

In the current study, the phytochemical investigation of the leaves of *P. crassipes* yielded five phenylpropanoids, two flavones, two amides, and a terpene. Additionally, the isolation and identification of these phytochemicals may contribute to a more comprehensive understanding of the variety of compounds from this species, which could be utilized as antioxidant agents. Previous studies on the *Piper* genus have shown significant free radical scavenging and antioxidant potential due to the presence of phenylpropanoids. The isolated compounds from these species might have the potential to be applied in pharmaceutical and food industries. This emphasizes the importance of continuing research on these plants, as they may lead to the discovery of new applications for these natural resources, thereby increasing their scientific value.

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