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Short Communication

Isolation and characterization of two prenylated flavonoids from the stem bark of *Artocarpus hirsutus* Lam.

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The genus *Artocarpus* (Moraceae) is a rich source of prenylated flavonoids with diverse pharmacological properties. Among its members, *Artocarpus hirsutus*—endemic to the Western Ghats of India—is traditionally used in folk medicine but remains underexplored phytochemically. This study reports the isolation and structural elucidation of two prenylated flavonoids from the acetone extract of the stem bark of *A. hirsutus*. The compounds, designated AHSB (1) and AHSB (2), were isolated by silica gel column chromatography and characterized using UV, IR, mass spectrometry, and advanced NMR techniques, including 1D and 2D experiments. Compound AHSB (1) was identified as Artonin J, and compound AHSB (2) was characterized as Artonin E. Both compounds possess flavonoid skeletons substituted with prenyl groups, and their structural features were confirmed through HMBC correlations. These findings expand the phytochemical profile of *A. hirsutus* and highlight its potential as a source of bioactive flavonoids for pharmacological applications.

Keywords: *Artocarpus hirsutus*, Artonin E, Artonin J, Moraceae, Prenylated flavonoid

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Introduction

The genus *Artocarpus* belongs to the family *Moraceae*, which represents large evergreen trees and involves the life and traditions of people of various cultures of Asia. The parts of various species, such as fruits, roots, and leaves, have been used as traditional medicines to treat various diseases. The species under this genus is noted as an abundant source of isoprenylated flavonoids, a class of compounds whose chemistry and biological properties have attracted much attention[1,2]. In the Western Ghats of India, three species of *Artocarpus*, namely *Artocarpus gomezianus, Artocarpus heterophyllus*, and *Artocarpus hirsutus*, have been identified. *Artocarpus heterophyllus,* commonly called the jackfruit tree or pala in Tamil, is native to tropical Asia and grows in the Western Ghats in India, while *Artocarpus hirsutus,* commonly called the wild jack, is endemic to the Western Ghats. *Artocarpus* gomezianus is also native to South Asia. The fruit and seeds of these plants have culinary use, while the medicinal potential is high due to a large number of bioactive molecules. The timber of all three species is found to be used in making furniture.

Prenylated flavonoids and related compounds were identified from thirteen species of Indonesian *Artocarpus*[3]. A review on the Phytochemistry, pharmacology, and traditional uses of the *Artocarpus* genus[4,5], and an updated review on the constituents and pharmacology of the *Artocarpus* genus provide details of reports on the *Artocarpus* genus[6]. The structure-activity relationship, biosynthesis, and metabolism of prenylated flavonoids in *Moraceae* plants have been reviewed to explore the untapped potential of these constituents and their utilisation for promoting human health[7]. Studies on the structure-activity relationships of several flavonoids and stilbenes of *A. incisus* suggested that compounds having the 4-substituted resorcinol skeleton have potent tyrosinase inhibitory ability. The extract of *Artocarpus incisus* showed the strongest tyrosinase inhibitory activity, which was equivalent to kojic acid[8].

Artocarpus hirsutus, also known as wild jack, is mainly distributed in the South Western Ghats of Peninsular India and is endemic to the Western Ghats. Various parts of this tree are well documented in the third volume of Hortus Malabaricus, the oldest comprehensive printed book on the natural plant wealth of Asia. *A. hirsutus* has so far not been reported for any medicinal preparation in Ayurveda. However, it has been involved in the folk medicinal practices of Kerala from time immemorial, as evidenced by Rheede's Hortus Malabaricus[9]. The decoction of roots and bark cures diarrhoea. Folkloric reports highlight the use of this tree's dried-up and warmed leaves to ease pain and rigidity of the knees when placed over it. The powdered leaves mixed with white camphor (*Cinnamomum camphor*) and made into a paste are said to cure venereal buboes, and dried leaves with the root of

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Curcuma (*Curcuma longa*), made into a paste, continuously applied to the affected part, completely stops chronic haemorrhage.

The stem wood extract yielded major flavonoids cudraflavone A, cycloartocarpin, and artocarpin, which were tested for anti-acne activity[10,11]. Cudraflavone C[12], and the prenylflavone artocarpin[13] from *Artocarpus hirsutus* are reported as promising inhibitors of pathogenic, multidrug-resistant *S. aureus*, including persisters and biofilms. The leaves are used traditionally in treating buboes and hydrocele, whereas the leaves, fruits, and barks help to cure diarrhoea, skin disease, and hemorrhage. The paste of bark ash in coconut oil finds use in the cure of *Tinea cruris* (dhobi's itch). Topical application of stem bark infusion is found to be effective in healing sores, cracks, and pimples, whereas the fruit juice of *A. hirsutus* induces appetite and relieves the pains of hemorrhage. Studies on pylorus-ligated rats demonstrate that *A. hirsutus* stem bark extract reduces gastric secretory volume, acidity, and ulceration. The leaf extract has analgesic and anti-inflammatory potential[14]. The micromorphological parameters, HPLC, and HPTLC fingerprints of leaf and stem wood extracts of *A. hirsutus* have been reported[15], and the findings of the study may augment standardization data on the plant.

Although there are many reports on the phytochemicals and biological studies of the *Artocarpus* species[16], reports on the constituents of *Artocarpus hirsutus* are few. This paper reports the isolation and structural elucidation of two prenylated flavonoids from *A. hirsutus.*

Materials and Methods

The stem bark of *A. hirsutus* was collected from the natural reserve forest of Siruvani, Coimbatore district, Tamil Nadu in April 2008. The dried plant material (2 kg) was defatted using petroleum ether and then extracted with acetone. Column chromatography of the acetone extract of *A. hirsutus* stem bark was carried out to isolate the compounds. The residue from the acetone extract of the stem bark of *A. hirsutus* (56 g) was dissolved in a minimum quantity of methanol and made into a slurry with minimum amount of silica gel. The slurry was subjected to chromatographic separation over a column of silica gel (400 g) built in petroleum ether. The column was eluted with i) petroleum ether, ii) petroleum ether and ethyl acetate mixtures with increasing amounts of ethyl acetate.

Melting points were measured on an electrical melting point apparatus (Joshiba) and were uncorrected. Ultraviolet (UV) spectra were recorded on a Systronics 2202 UV Doublebeam spectrophotometer. Infrared (IR) spectra were recorded on a Shimadzu FT-IR (4000-400) spectrophotometer. The ^{1}H NMR, ^{13}C NMR, DEPT 90, DEPT 135, ^{1}H-^{1}H COSY, HSQC, and HMBC spectra were recorded on a Bruker Avance III 500MHz spectrometer. Deuterated chloroform ($CDCl_3$), methanol (CD_3OD), dimethyl sulphoxide (DMSO-d_6), and acetone (CD_3COCD_3) were used as solvents for recording NMR spectra. Mass spectra were recorded in a JEOL GCMATE II GC-MS instrument. AR grade solid sodium acetate and aluminium chloride were used as shift reagents. Shift reagents were added to the methanolic solution of the compounds to study the shift in λ_{max} in the UV spectrum and correlate the position of the hydroxyl groups of flavone moieties. All the chemicals and reagents used were of AR grade. All the solvents used for chromatography were purified by standard procedures.

Results and Discussion

Two compounds designated as AHSB (1) and AHSB (2) were obtained by column chromatography of the acetone extract of the stem bark of *A. hirsutus*. AHSB (1) is *A. hirsutus* stem bark compound (1), and AHSB (2) is *A. hirsutus* stem bark compound (2). AHSB (1) was obtained from the column chromatographic analysis of the acetone extract of *A. hirsutus* bark on elution with petroleum ether: ethyl acetate (90:10) as a brown solid. The brown solid was again subjected to re-column chromatography over silica gel and on elution with a petroleum ether: ethyl acetate (92: 8) mixture, a yellow powdery solid was obtained (55 mg). The compound gave a dark grey colour when reacted with an ethanolic ferric chloride solution, indicating the presence of polyhydroxy groups. The TLC of AHSB (1) showed an R_f of 0.84 in petroleum ether: ethyl acetate (2:3).

In the characterization of flavonoidal compounds, it is usual to adopt the use of shift reagents[17] while recording the UV spectrum of the flavonoidal compounds. The use of UV shift reagents are a non-destructive and informative method for determining the hydroxylation pattern in flavonoids. It is especially useful in natural product chemistry and phytochemical analysis, where flavonoid standards may not always be available.

A shift in the UV spectral absorption bands of flavonoidal compounds on the addition of specific reagents called shift reagents provides useful information on the position and nature of hydroxyl groups in the flavonoid class of compounds. The common shift reagents used are sodium methoxide (NaOMe). Sodium acetate (NaOAc), aluminium chloride, and boric acid. The UV spectrum of compound (1) (Fig. 1) showed peaks at 253 nm (band II) and 356 nm (band I), indicative of a flavonoid moiety. There was a new band formation at 330 nm on the addition of sodium acetate, showing the presence of a 7-hydroxyl substituent in the flavonoid structure. A shift of band II by 40 nm was also observed with the addition of aluminium chloride ($AlCl_3$), revealing the presence of a chelated 5-hydroxy substituent in the compound. The IR spectrum of the compound showed characteristic absorption for the hydroxyl group at 3429 and 1023 cm^{-1}. The characteristic absorption for the presence of the carbonyl group was also seen at 1649 cm-1 (Fig. 2).

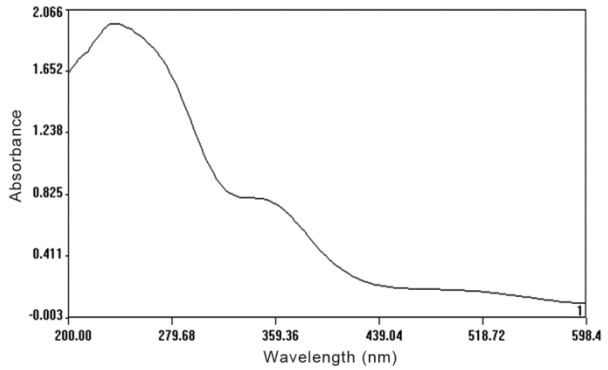

Fig. 1 — UV Spectrum of AHSB (1).

The ^{1}H NMR spectrum showed two sharp singlets at δ 6.68(1H) and δ 6.26(1H), indicating the two isolated protons at C-6 and C-8 of a flavone skeleton. A triplet at δ 5.28 (1H, J=7 Hz) and a broad doublet at δ 3.48 (d, 2H, J=7 Hz) characteristic of a prenyl group were observed. Two broad multiplets were seen at δ 3.20 and δ 2.35 and are due to the H-11 protons. Three sharp singlets characteristic of the methyl protons at H-20, H-19, and H-14, H-15 were seen at δ 1.78 (3H), 1.65 (6H), and 1.32 (3H), respectively (Fig. 3). The expanded scale ^{1}H NMR spectra are represented by Figs. 3a and b.

The ^{13}C NMR spectrum showed 25 carbon signals (Table 1) corresponding to four methyls, two methylene, four methine, and fifteen quaternary carbons (Fig. 4 and 4a). The ^{1}H-^{1}H COSY spectrum showed the correlation between the triplet at δ 5.28 (1H, J=7Hz) and the doublet at δ 3.48 (2H). This coupling is characteristic of a prenyl group. The other significant couplings seen are between the signals at δ 3.4 (1H) and at δ 3.20 and δ 2.3, thus revealing the ABM system of signals at H-11 and H-12, respectively (Fig. 5).

The HMBC spectrum of **(1)** (Fig. 6) showed the relevant long-range correlations. The protons of the methylene at δ 3.40 and δ 5.28 (C-11) showed cross peaks with δ 111.9 (C-3), δ180.3 (C-4), and δ 160.5 (C-2), indicating the linkage of the methylene group at C-3. This methylene proton also showed cross peaks with δ 46.2 (C-12), δ 128.2 (C-6'), and δ 92.4 (C-13). Analysis of the HMBC spectrum revealed that the methine at δ 46.2 is adjacent to the quaternary carbon at δ 128.2 (C-6') and δ 92.4 (C-13), indicating that the C-12 carbon is attached to C-6'. The methine

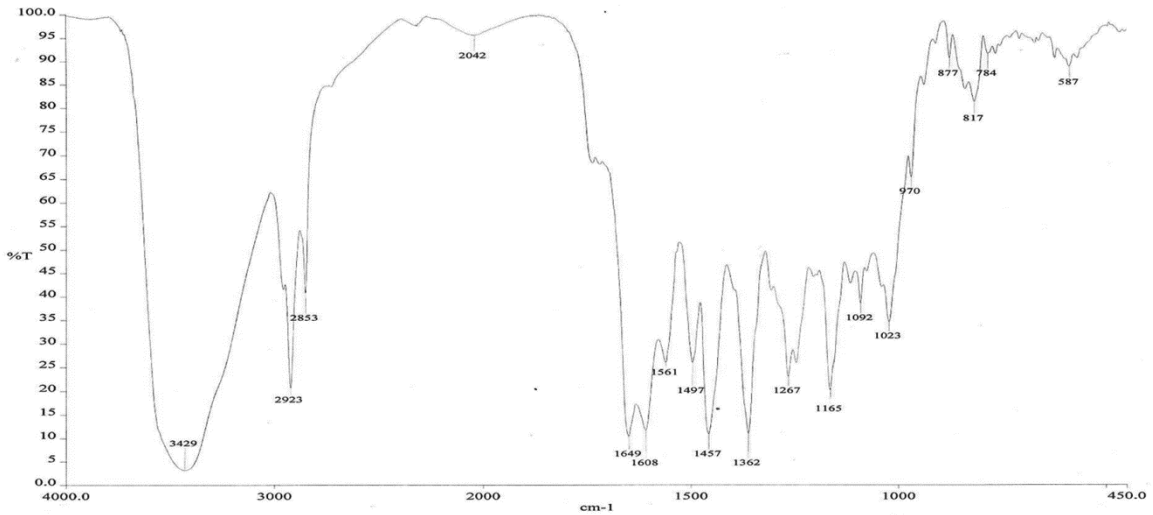

Fig. 2 — IR Spectrum of AHSB (1).

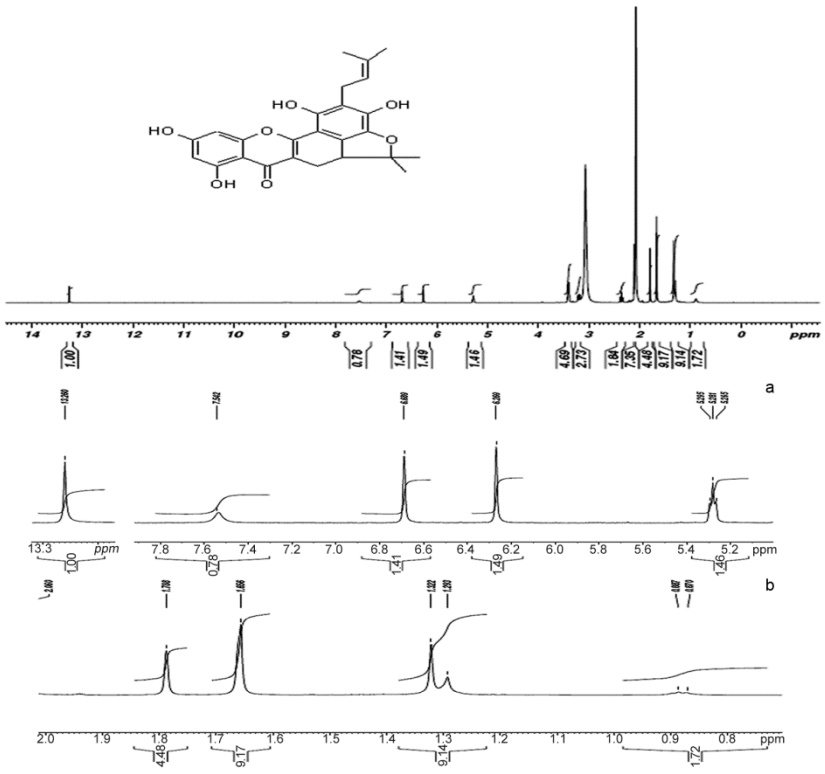

Fig. 3 — ^{1}H NMR Spectrum of AHSB (1), a) Expanded Scale (δ 5.2-7.8) ^{1}H NMR Spectrum of AHSB (1), and b) Expanded Scale (δ 0.6-1.9) ^{1}H NMR Spectrum of AHSB (1).

Table 1 — ^{13}C NMR Chemical Shifts of Isolated Compounds AHSB (1) and AHSB (2)

Carbon Position	δ (ppm) AHSB (1)	AHSB (2)
2	160.5	161.3
3	111.9	120.6
4	180.3	182.4
5	163.7	161.8
6	98.4	98.7
7	162.3	159.0
8	94.3	100.8
9	156.5	152.3
10	103.5	104.5
11	19.5	23.5
12	46.2	121.2
13	92.4	131.5
14	27.4	24.4
15	21.9	16.2
16	22.3	114.3
17	122.9	126.7
18	130.4	77.73
19	17.1	27
20	25.1	27
1'	103.7	110.3
2'	147.3	148.7
3'	117.6	103.8
4'	143.9	148.5
5'	137.2	138.0
6'	128.2	115.8

at δ 92.4 (C-13) was seen correlating to methyl groups at δ 21.9 (C-15) and δ 21.4 (C-16), methylene at δ 19.5 (C-11), methine at δ 46.2 (C-12) and quaternary carbon at δ 128.2 (C-6') confirming the position of this methine (δ 92.4) at C-13. The prenyl group was seen correlating with the C-2' (δ 147.3), C-4' (δ 143.9), and C-3' (δ 117.6) of the B ring. The signals at δ 147.3 and δ 143.9 indicated hydroxyl-attached carbons. Hence, the prenyl group is attached at the C-3' position in the B ring.

The mass spectrum indicated a molecular ion peak at 436 corresponding to the molecular formula $C_{25}H_{24}O_7$, which confirms the proposed structure of compound (1) (Fig. 7).

The spectroscopic data obtained for the compound AHSB (1) were found to be identical to those reported for Artonin J (Fig. 8) isolated from the root bark of *Artocarpus heterophyllus* of Moraceae[18].

Artonin J is a prenylated xanthone of the flavonoid family, earlier reported from *Artocarpus integer*, *Artocarpus teysmannii*, and *Artocarpus heterophyllus*, and is considered to be a flavonoid lipid molecule with promising anti-inflammatory, antioxidant, and potential anticancer properties. Research indicates it may also inhibit cathepsin K, which is potentially

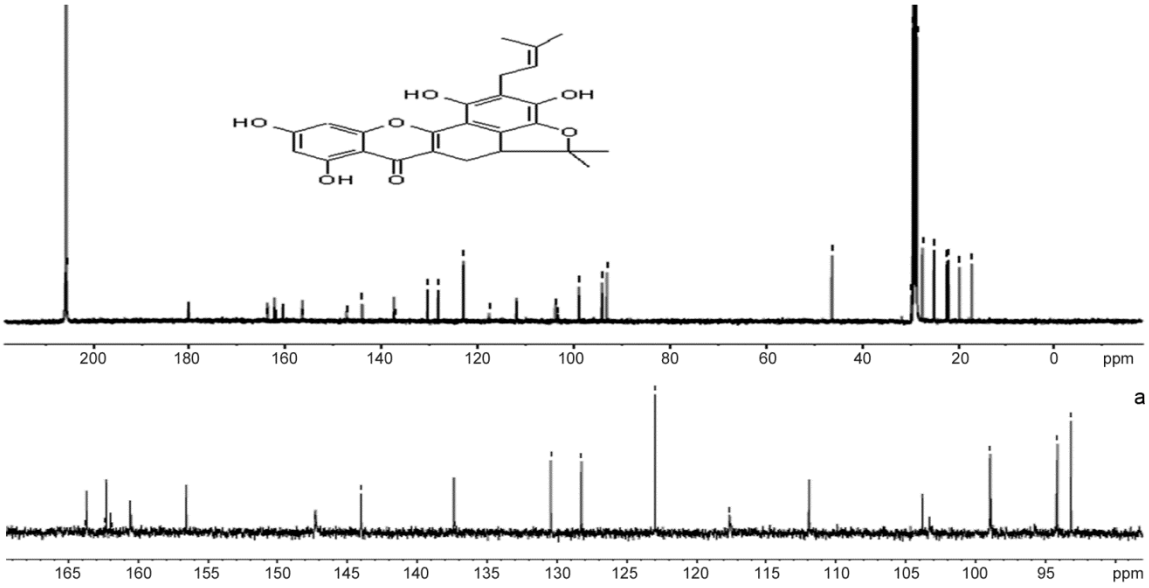

Fig. 4 — ^{13}C NMR Spectrum of AHSB (1), a) Expanded Scale ^{13}C NMR Spectrum of AHSB (1).

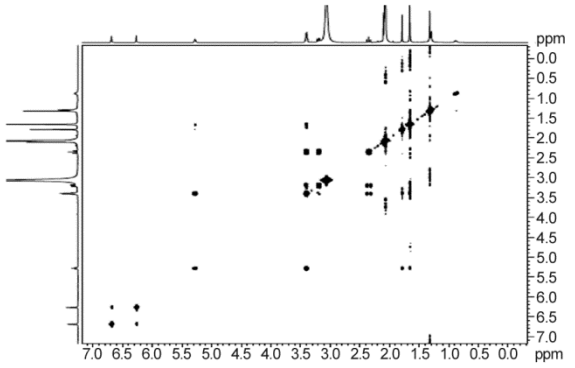

Fig. 5 — ^{1}H-^{1}H COSY Spectrum of AHSB (1).

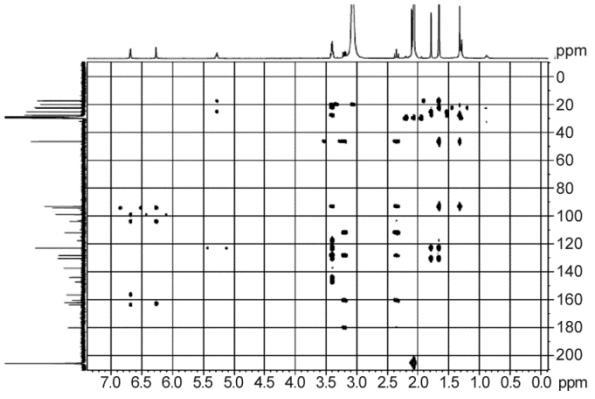

Fig. 6 — HMBC Spectrum of AHSB (1).

useful in preventing bone resorption. It finds mention in the Human Metabolome Database; however, there are no systematic scientific reports.

Compound (2), designated as AHSB (2), was obtained as a yellow powder on elution from the column using petroleum ether and ethyl acetate (4:1). The UV spectrum (Fig. 9) exhibited a bathochromic shift of 10nm with $AlCl_3$, indicating the presence of a chelated carbonyl group and 5-hydroxyl substituent. A bathochromic shift of 12 nm was observed with boric acid, indicating an ortho dihydroxy system in the B ring of the flavonoidal structure. No shift in the UV band was observed with sodium acetate, indicating that the 7-hydroxyl group of the flavonoid moiety is not free. The IR spectrum of compound AHSB (2) showed a peak at 3382.9 cm^{-1}, which is characteristic of a hydroxyl group. A strong peak at 1652 cm^{-1} was seen, indicating the presence of a chelated carbonyl group (Fig. 10).

The ^{1}H NMR spectrum of compound AHSB (2) showed two sharp singlets at δ 1.45(9H) and δ 1.61 (3H), revealing the presence of 4 methyl groups in the compound (Fig. 11 and 11a).

The ^{1}H NMR of AHSB (2) revealed the presence of a prenyl group by a broad doublet at δ 3.14 (d, 2H, J = 7.0 Hz), which was attributable to the methylene protons at C-9. The presence of these methylene protons was supported by the DEPT-135 spectrum. The methine of the prenyl group was seen as a triplet at δ 5.12 (t, 1H, J = 7.0 Hz) and is attributed to the vinyl proton at C-10. The methyl signals of the prenyl group at H-12 and H-13 appeared as two singlets at δ 1.45 and δ 1.57, respectively. The two protons in the 2, 2-dimethyl chromene ring were seen as two doublets at δ 5.62 (1H, J=10Hz) and δ 6.64 (1H, J=10Hz), representing the H-17 and H-16,

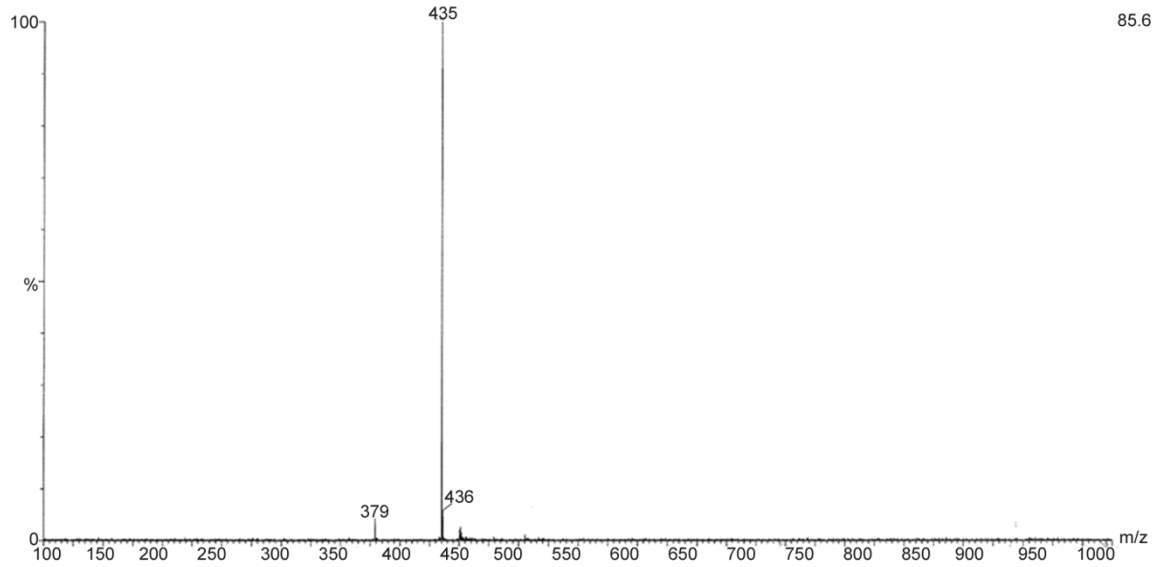

Fig. 7 — Mass Spectrum of AHSB (1).

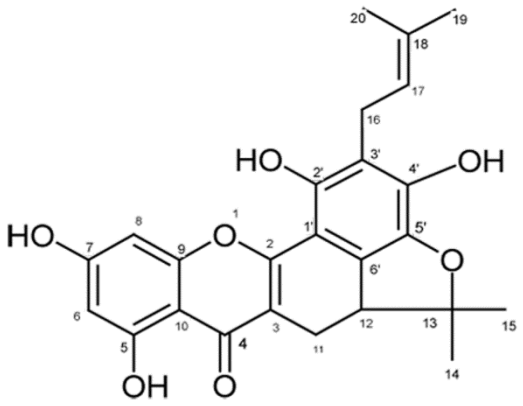

Fig. 8 — Chemical Structure of AHSB (1)

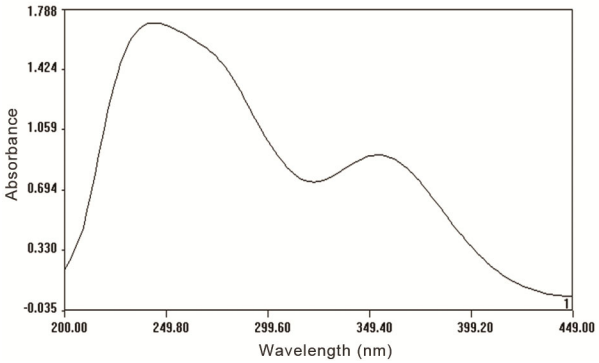

Fig. 9 — UV Spectrum of AHSB (2)

respectively. Isolated aromatic protons in the structure appeared as three singlets at δ 6.14, δ 6.58, and δ 6.87 due to H-6, H-3', and H-6', respectively.

The ^{13}C NMR spectrum AHSB (2) (Fig. 12) showed characteristic signals indicative of a flavonoid moiety. The signal at δ 160.57 was indicative of C-2 of the flavonoid. Other characteristic signals indicative of C-9 (δ 156.79) and C-3 (δ 180.31) were seen. From ^{13}C NMR spectral data (Table 1) and DEPT spectrum, it was seen that compound AHSB (2) has 25 carbon atoms with six methine, one methylene, four methyl, and fourteen quaternary carbons.

The HMBC (Fig. 13) measurements of compound (2) showed a correlation between the signal for H-11 at δ 3.14 (d, 2H, J = 7.0 Hz) with the signals at δ 182.49 (C-4), 161.30 (C-2), 120.62 (C-3), 121.31 (C-12) and 131.54 (C-13) indicating that the prenyl group is attached to the C-3 of the flavone skeleton. The mass spectrum (Fig. 14) showed the molecular ion peak at m/z 436, thus confirming the molecular mass of the compound.

Compound (2) was identified as Artonin E (Fig. 15) based on its spectral data and comparison of its physical properties and spectroscopic data with the compound isolated from *Artocarpus altilis*[19], and *Artocarpus communis*[20].

Artonin E[21] isolated from *Artocarpus rigida* Blume expresses antimicrobial activity comparable with the antibiotic kanamycin sulfate at the same concentration[22]. Artonin E and related prenylated flavonoids from the medicinal plant *Artocarpus elasticus* demonstrate the ability to trigger apoptosis of different types of cancer cells[23]. Artonin E, isolated from the root bark of *Artocarpus Rigida*, was evaluated for anticancer activity[24].

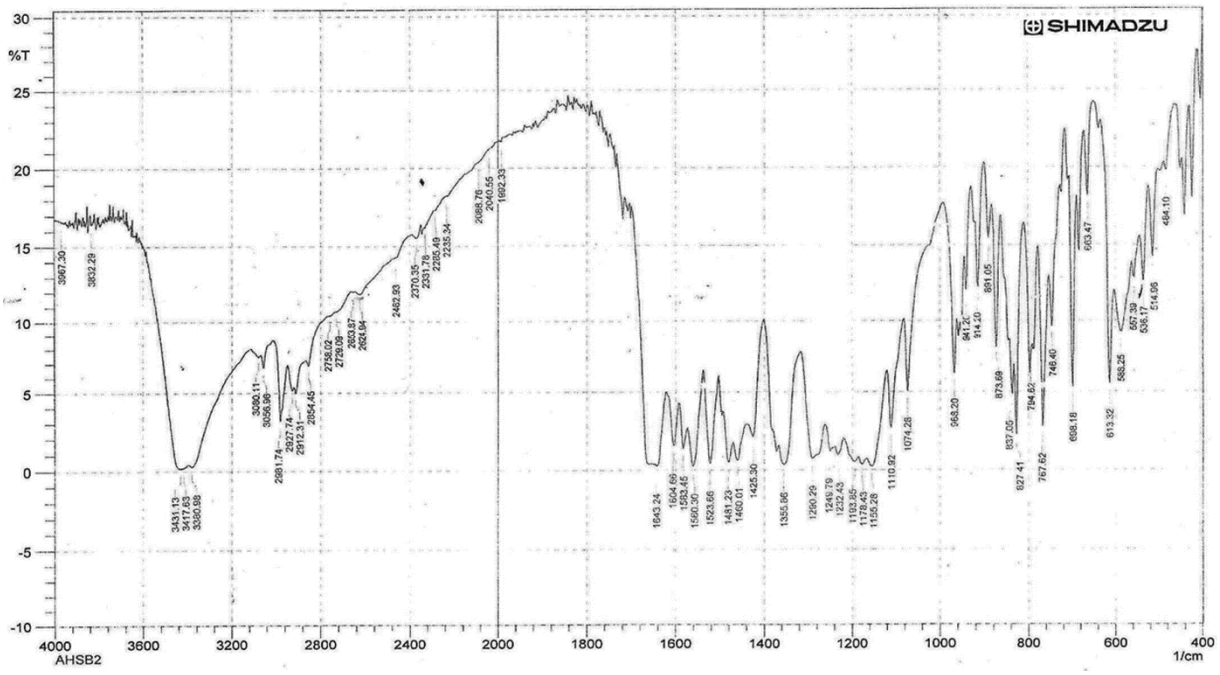

Fig. 10 — IR Spectrum of AHSB (2)

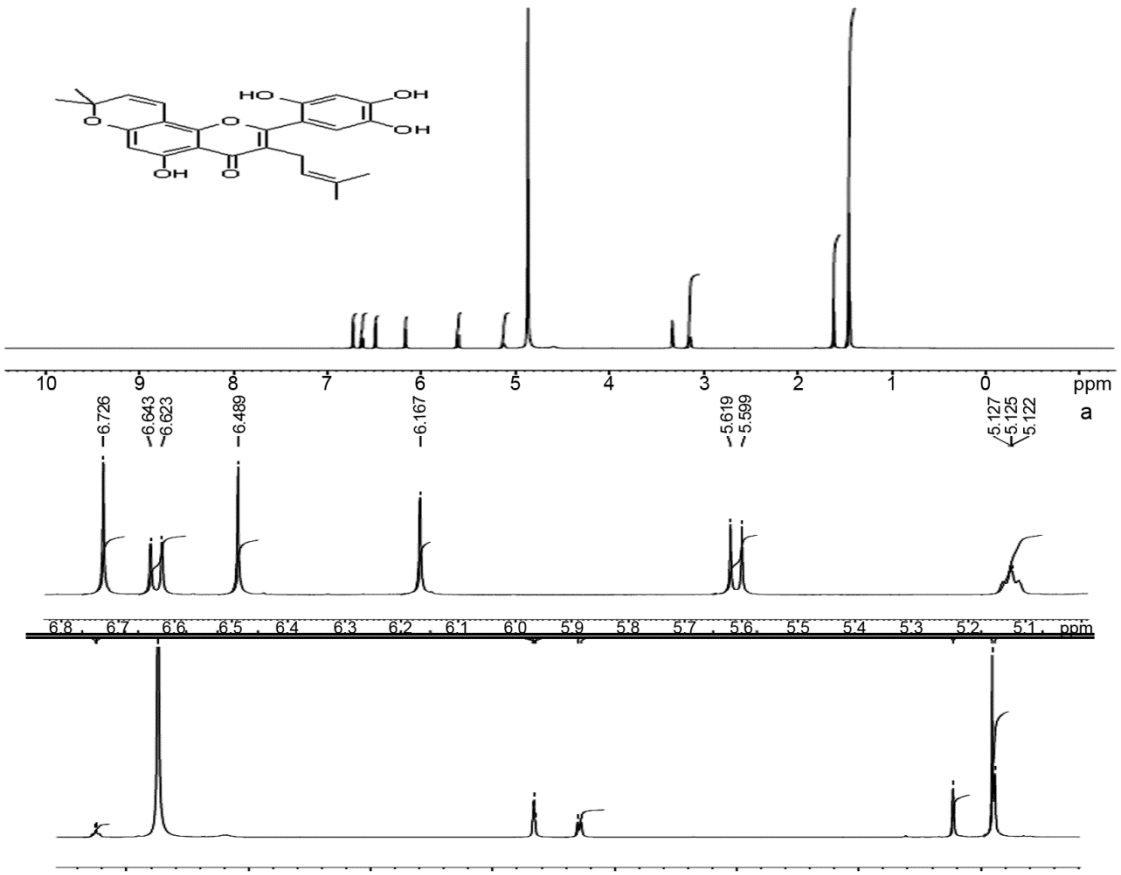

Fig. 11 — ^{1}H NMR Spectrum of AHSB (2), a) Expanded Scale ^{1}H NMR Spectrum of AHSB (2)

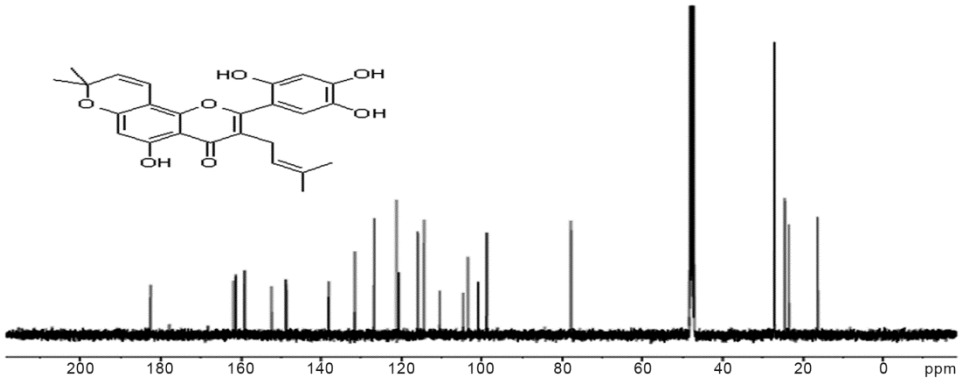

Fig. 12 — ^{13}C NMR Spectrum of AHSB (2)

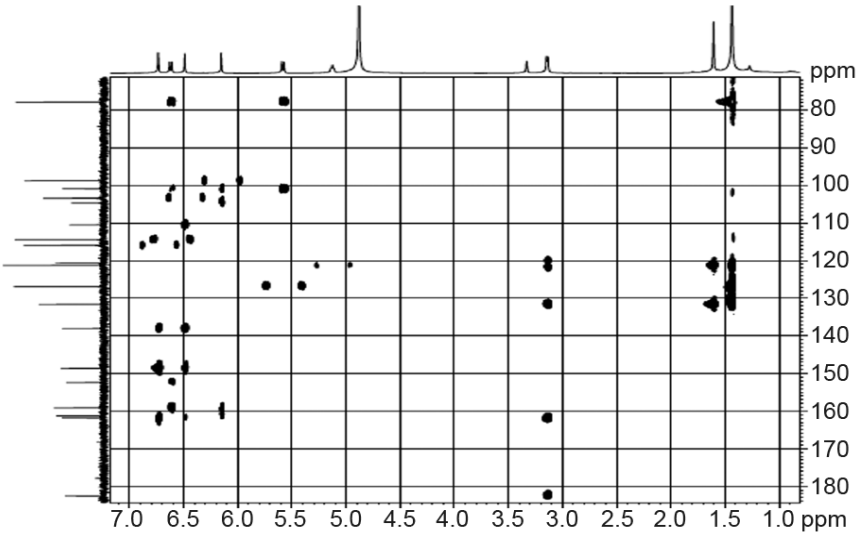

Fig. 13 — HMBC Spectrum of AHSB (2)

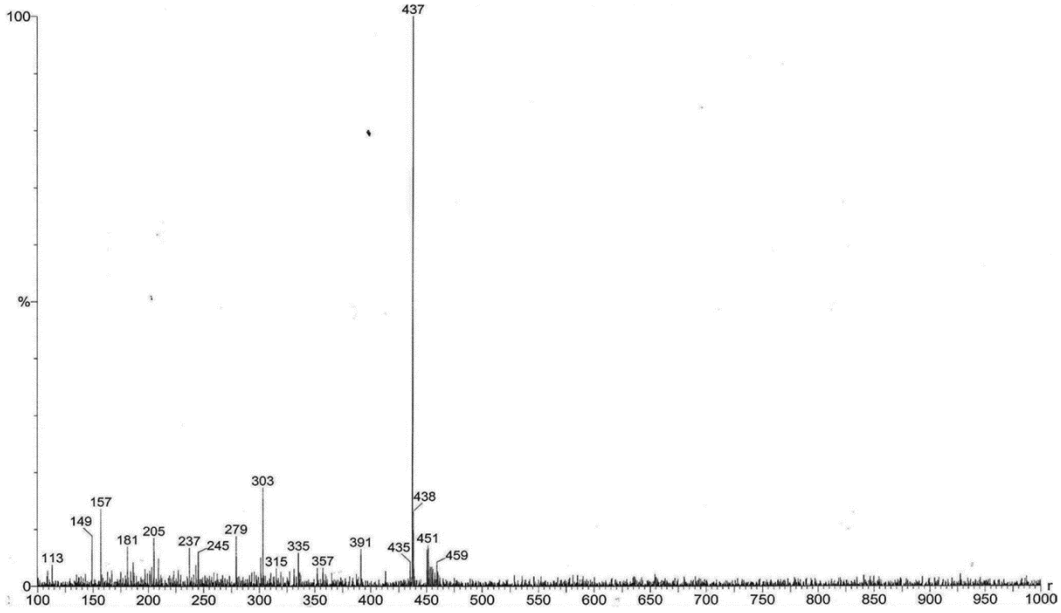

Fig. 14 — Mass Spectrum of AHSB (2)

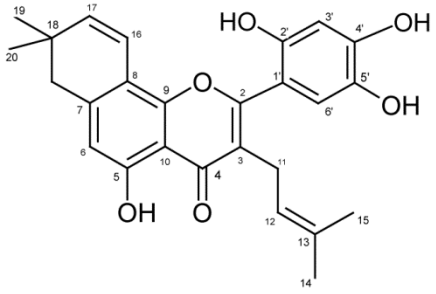

Fig. 15 — Chemical Structure of AHSB (2)

Analytical data

Compound AHSB (1): Artonin J was obtained as yellow powder petroleum ether: ethyl acetate 90:10; Yield: 55 mg; IR (KBr) 3429, 2923, 1649, 1608, 1497, 1457, 1362, 1267, 1165, 1023 cm^{-1}; EI-MS *m/z*: 436 [M^+, $C_{25}H_{24}O_7$]; ^1H NMR (500 MHz, CD_3COCD_3, δ): 6.68(s,1H, H-8), 6.26 (s, 1H, H-6), 5.28 (t, 1H, J=7Hz, H-16), 3.40 (m, 3H, H-16, H-12), 3.20(m, 1H, J=10Hz, H-11a), 2.35(m, 1H, J=10Hz, H-11b), 1.78(m, 3H, H-20), 1.65(s, 6H, H-19, H-14), 1.32(s, 3H, H-15); ^{13}C NMR (125 MHz, CD_3COCD_3, δ): Table 1.

Compound AHSB (2): Artonin E was obtained as yellow powder petroleum ether: ethyl acetate 4:1; Yield: 950 mg; IR (KBr) 3431, 1643, 1604, 1560, 1523, 1481, 1355, 1290, 1155, 968, 827, 767 cm^{-1}; EI-MS *m/z*: 437 [M+1 peak] calculated $C_{25}H_{24}O_7$; ^1H NMR (500 MHz, CD_3OD, δ): 6.72(s, 1H, 6.72), 6.64(d,1H, J=10Hz, H-16), 6.48(s, 1H, H-3'), 6.16 (s, 1H, H-6), 5.61 (d, 1H, J=10Hz, H-17), 5.12 (t, 1H, H-12), 3.15 (d, 2H, H-11), 1.61 (s, 3H, H-14), 1.45 (s,9H, H-15, H-19, H-20); ^{13}C NMR (125 MHz, CD_3OD, δ): Table 1

Conclusion

The present phytochemical investigation on the acetone extract of the stem bark of *A. hirsutus* (family Moraceae) has resulted in the successful isolation of two prenylated flavonoids. Comprehensive spectroscopic analyses, including IR, mass spectrometry, and detailed 1D and 2D NMR studies, enabled the structural elucidation of these compounds as Artonin J and Artonin E. Notably, Artonin E was isolated in appreciable yield from the acetone extract of the stem bark, highlighting *A. hirsutus* as a promising and underexplored natural source of bioactive prenylated flavonoids. The presence of Artonin E, known for its diverse biological activities, including anticancer and antimicrobial properties, underscores the therapeutic potential of this endemic species. This finding adds to the limited phytochemical data available for *A. hirsutus* and opens new avenues for targeted pharmacological investigations. Future studies should focus on evaluating the bioactivity profiles of the isolated compounds, particularly in the context of their anticancer, antimicrobial, and anti-inflammatory effects.

Conflict of interest

The authors declare no conflict of interest.

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