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Chemical and biological studies of *Kalanchoe pinnata* (Lam.) growing in Bangladesh

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ABSTRACT

Objective: To isolate compounds from *K. pinnata* and elucidate their structures and to explore preliminary antioxidant, antimicrobial, cytotoxic and thombolytic activities of extractives of the plant. **Methods:** The methanol extract of whole plant of *K. pinnata* has been subjected to different chromatographic separation and purification processes to isolate the secondary metabolites. The structures of the isolated compounds have been elucidated by extensive NMR studies. The free radical scavenging activity of the crude extract and its different Kupchan fractions were determined on stable radical DPPH. *In vitro* antimicrobial activity was determined by the disk diffusion method. Cytotoxicity screening has been performed against *Artemia salina*. Total phenolics content, membrane stabilizing activity and thombolytic activities were assessed by following established protocol. **Results:** The isolated compounds were identified as glut-5(6)-en-3-one, taraxerone, 3β-friedelanol, β-amyrin-3-acetate, 3,5,7,3',5'-pentahydroxyflavone and β-sitosterol. The chloroform soluble fraction showed potent antioxidant activity of (IC_{50}= 80.0 μg/mL) and significant cytotoxicity, while the crude extract demonstrated noticeable total polyphenol content (149.24 mg of GAE/gm of extractive), moderate membrane stabilizing activity and inhibition of clot lysis of blood. **Conclusions:** The obtained results rationalize the folkloric use of the plant and can be further investigated to isolate the active compounds responsible for the biological activities.

1. Introduction

Kalanchoe pinnata (Lam., syn. *Bryophyllum pinnatum*, *B. calycinum*; Local name: Pathorkuchi, Coughpatha; English name: Air plant; Family: Crassulaceae) is an herb found ubiquitously in Bangladesh. It has tall hollow stems, fleshy dark green leaves that are distinctly scalloped and trimmed in red, and bell-like pendulous flowers[1]. *Kalanchoe pinnata (K. pinnata)* has become naturalized in temperate regions of Asia, Australia, New Zealand, West Indies, Macaronesia, Mascarenes, Galapagos, Melanesia, Polynesia, and Hawaii. It is also widely distributed in the Philippines, where it is known as *katakataka* or *kataka-taka* which means astonishing or remarkable[1,2]. The leaves of *K. pinnata* have a variety of uses in the traditional system of medicine in Bangladesh. They are eaten for diabetes, diuresis, dissolving kinney stones, respiratory tract infections, as well as applied to wounds, boils, and insect bites[1]. It is useful for preventing alcoholic, viral and toxic liver damages. The aqueous extract of this plant have shown anti-inflammatory, anti-diabetic, anti-tumor and cutaneous leishmanicidal activities[3-6].

Previous phytochemical investigations of *K. pinnata* led to the isolation of bryophillin A that showed strong anti-tumor activity, and bersaldegenin-3-acetate and bryophillin C which exhibited insecticidal properties[4]. Besides, 1-octen-3-O-α-L-arabinopyranosyl-(1-6)-β-glucopyranoside[7], 24-alkyl-Δ-25-sterol[8], quercetin-3-O-α

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-L-arabinopyranosyl-(1-2)- α -L-rhamnopyranoside[9] have also been reported from this plant.

As a part of our systematic studies on medicinal plants of Bangladesh[10,11], we studied *K. pinnata* and we, herein, report isolation of compounds for the first time from this plant. The preliminary antioxidant, antimicrobial, cytotoxic activities are also reported here.

2. Materials and methods

2.1. General experimental procedure

NMR spectra were recorded using a Bruker AMX-400 (400 MHz for 1H and 100 MHz for ^{13}C) instrument in deuterated chloroform and the δ values for ^{1}H and ^{13}C spectra were referenced relative to the residual non-deuterated solvent signals.

2.2. Plant material

The whole plants of *K. pinnata* were collected from Narsingdi in November 2009 and was identified by the experts of Bangladesh National Herbarium where a voucher specimen (DACB Accession number-35468), representing this collection has been deposited.

2.3. Chemicals

1,1-Diphenyl-2-picryl-hydrazyl (DPPH) and ascorbic acid were purchased from Sigma Chemical Co. Ltd, (St. Louis, MO, USA). Lyophilized streptokinase vials (1 500 000 I.U.) were obtained from Beacon Pharmaceutical Ltd. Bangladesh. All other chemicals and reagents were of analytical grade.

2.4. Extraction and isolation

The air dried and powdered material (450 g) was extracted with 2 L of methanol in a large flask at room temperature for 15 days with occasional shaking and stirring. The whole mixture was then filtered off through a filter paper and the filtrate thus obtained was concentrated at 40 ℃ with a rotary evaporator. A portion (5.0 g) of the concentrated methanol extract was fractionated by the modified Kupchan partitioning protocol[12] to afford petroleum ether (700 mg), carbon tetrachloride (400 mg), chloroform (900 mg) and aqueous (2.8 g) soluble materials.

A portion of the crude methanolic extract (10.0 g) was subjected to vacuum liquid chromatography (VLC) over silica gel 60H (100-200 mesh). The column was eluted with petroleum ether, followed by mixtures of petroleum ether and ethyl acetate, then with ethyl acetate and finally with ethyl acetate and methanol mixtures of increasing polarities. Depending on the TLC behaviors, fractions 1-4A and 13A-16 eluted with 3%-15% ethyl acetate in petroleum ether and 5%-30% methanol in ethyl acetate, respectively were selected for further investigation.

Fractions 1-4A were mixed together and further subjected to column chromatography over silica gel (Kieselgel, mesh 70-230) using petroleum ether and ethyl acetate mixtures of increasing polarities. A total of 50 fractions were collected. Depending on TLC behaviors, fractions 12, 17, 18, 20, 35 were selected for further purification. Evaporation of solvents from each of these provided white crystalline mass. Repeated washings with ethyl acetate allowed to remove the colored impurities and provided Glut-5(6)-en-3-one (compound 1, 5.6 mg), Taraxerone (compound 2, 6.1 mg), 3 β -Friedelanol (compound 3, 4.5 mg), β -Amyrin-3-acetate (compound 4, 6.5 mg) and 3,5,7,3 ' ,5 ' -Pentahydroxyflavonoe (compound 5, 4.3 mg). VLC fractions 13A-16 were bulked and subjected to gel permeation chromatography over Sephadex (LH-20) using *n*-hexane-dichloromethane-methanol (2:5:1) as the mobile phase. A total of 40 fractions were collected. Preparative thin layer chromatography of column fraction 25, over silica gel using 10% ethyl acetate in petroleum ether yielded β -Sitosterol (compound 6, 6.5 mg).

2.4.1. Glut-5(6)-en-3-one

White crystals, m.p. 208-210 ℃[13-14]; 1H NMR (400 MHz, $CDCl_3$): δ 5.69 (1H, m, H-6), 0.82 (3H, s), 0.96 (3H, s), 1.00 (3H, s), 1.03 (3H, s), 1.07 (3H, s), 1.17 (3H, s), 1.23 (3H, s), 1.24 (3H, s); 13C NMR (100 MHz, $CDCl_3$): see Table 1.

Table 1.

^{13}C NMR (100 MHz) spectral data of compounds 1, 2, 4 and 6 in $CDCl_3$.

Position	1	2	4	6
1	18.2	38.7	39.0	37.3
2	27.8	34.5	27.9	31.6
3	215.5	217.7	79.9	72.4
4	40.8	47.9	39.1	42.2
5	141.6	56.1	55.2	140.8
6	122.0	20.3	18.5	122.6
7	23.6	35.5	33.2	31.5
8	47.4	39.2	40.0	31.5
9	34.8	49.1	47.0	50.2
10	49.6	36.1	37.0	36.4
11	34.6	17.8	17.8	21.5
12	30.3	38.1	124.0	40.0
13	39.3	38.1	139.0	42.4
14	37.8	158.0	42.8	56.8
15	32.1	117.5	28.1	24.2
16	36.0	37.0	26.0	28.3
17	30.1	37.9	34.0	56.3
18	43.0	49.2	59.8	12.0
19	34.8	41.0	39.2	19.4
20	20.2	29.1	39.7	36.4
21	33.1	33.9	31.6	18.9
22	38.9	33.4	41.6	33.8
23	28.9	26.5	28.6	25.8
24	25.5	21.7	15.9	48.3
25	16.2	15.1	15.0	26.1
26	19.6	30.2	16.7	18.7
27	18.4	25.9	23.4	18.8
28	32.1	30.3	28.5	22.8
29	34.5	33.7	23.5	12.2
30	32.1	21.8	21.5	-

2.4.2. Taraxerone

White crystals; m.p. 242-245 ℃[15]; 1H NMR (400 MHz, CDCl3): δ 5.58 (1H, dd, *J*=8.0, 3.2 Hz, H-15), 2.57 (1H, ddd, *J*=16.0, 7.6, 3.2 Hz, Ha-2), 2.33 (1H, ddd, *J*=16.0, 6.4, 3.6 Hz, Hb-2), 0.83 (3H, s), 0.91(3H, s), 0.92 (3H, s), 0.96 (3H, s), 1.07 (3H, s), 1.08 (3H, s), 1.09 (3H, s), 1.14 (3H, s); 13C NMR (100 MHz, $CDCl_3$): see Table 1.

2.4.3. 3β-Friedelanol

White amorphous powder; m.p. 242-244 ℃[16]; 1H NMR (400 MHz, CDCl3): δ 1.90 (1H, dt, *J*=10.4, 2.4 Hz, Ha-2), 1.74 (1H, dt, *J*=12.8, 3.2 Hz, Ha-6), 3.74 (1H, m, H-3), 0.97 (s, H_3-24), 0.80 (s, H_3-25), 0.99 (s, H_3-26), 1.01 (s, H_3-27), 1.17 (s, H_3-28), 0.95 (s, H_3-29), 1.00 (s, H_3-30).

2.4.4. β-Amyrin-3-acetate

White crystalline mass; m.p. 241-243 ℃[17]; 1H NMR (400 MHz, $CDCl_3$): δ 5.18 (1H, t, *J*=3.2, Hz, H-12), 4.50 (1H, dd, *J*=8.0, 3.0 Hz, H-3), 2.04 (3H, s, CH_3-COO), 0.83 (3H, s), 0.87 (2×CH_3), 0.88 (2×CH_3), 0.96 (3H, s), 0.97 (3H, s) and 1.13 (3H, s). The ^{13}C NMR (100 MHz, $CDCl_3$): see Table 1.

2.4.5. 3,5,7,3',5'-Pentahydroxyflavonoe

Colorless gum; 1H NMR (400 MHz, CD3OD): δ 6.21 (1H, d, *J*=2.0 Hz, H-6), 6.38 (1H, d, *J*=2.0 Hz, H-8), 7.34 (1H, d, *J*=2.4 Hz, H-2'), 6.91(1H, d, *J*=8.4 Hz, H-5'), 7.76 (1H, d, *J*=2.8 Hz, 6').

2.4.6. β-Sitosterol

Colorless mass; 1H NMR (400 MHz, $CDCl_3$): d 3.52 (1H, m, H-3), 5.35 (1H, d, J=6 Hz, H-6), 0.68 (3H, s, H3-18), 1.01 (3H, s, H3-19), 0.93 (3H, d, J=6.4 Hz, H_3-21), 0.83 (3H, d, J=7.2 Hz, H3-26), 0.82 (3H, d, J=7.2 Hz, H_3-27), 0.85 (3H, t, J=8 Hz, H_3-29); ^{13}C NMR (100 MHz, $CDCl_3$): see Table 1.

2.5. Free radical scavenging activity

The free radical scavenging activities (antioxidant capacity) of the plant extracts on the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) were determined by the method of Brand-Williams[18]. The antioxidant potential was assayed from the bleaching of purple colored methanol solution of DPPH radical by the plant extract as compared to that of BHT by a UV-vis spectrophotometer at 517nm. Inhibition free radical DPPH in percent (I%) was calculated as follows:

$$(I\%)=(1-A\text{sample}/A\text{blank})\times 100$$

where Ablank is the absorbance of the control reaction (containing all reagents except the test material) and Asample is the absorbance of the sample. Extract IC_{50} was calculated from the graph plotted by inhibition percentage against extract concentration.

2.6. Antimicrobial activity

The *in vitro* antimicrobial activity of the extractives was determined by the standardized disk diffusion method, the details of the procedure could be found elsewhere[19-20]. The bacterial (*Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Sarcina lutea, Staphylococcus aureus, Escherichia coli, Salmonella typhi, Salmonella Paratyphi, Shigella boydii, Shigella dysenteriae, Pseudomonas aeruginosa, Vibrio mimicus, Vibrio parahaemolyticus*) and fungal (*Aspergillus niger, Candida albicans, Sacharomyces cerevacae*) strains used in this experiment were collected as pure cultures from the Institute of Nutrition and Food Science, University of Dhaka. The activity of the test agent was determined by measuring the diameter of zone of inhibition expressed in millimeter.

2.7. Cytotoxicity screening

DMSO solutions of all the extractives were applied against *Artemia salina* in a one-day *in vitro* assay[21,22]. For the experiment, 4 mg of each of the Kupchan fractions was dissolved in DMSO and solutions of 400, 200, 100, 50, 25, 12.50, 6.25, 3.13, 1.56, 0.78 μg/mL were prepared by serial dilution. Vincristine sulphate and DMSO were used as the positive and negative control, respectively.

2.8. Total phenolics content (TPC)

TPC of methanolic extract of *K. pinnata* was determined by employing established protocol described by Skerget et al.[23] involving Folin-Ciocalteu reagent as oxidizing agent and gallic acid as standard[24]. In the alkaline condition phenol ionizes completely. When Folin-Ciocalteu reagent is used in this ionized phenolic solution the reagent will readily oxidize the phenols. Usual color of Folin-Ciocalteu reagent is yellow and after the oxidation process the solution becomes blue. The intensity of the color change is measured with a spectrophotometer at 760 nm. The absorbance value will reflect the total phenolic content of the compound[25]. The TPC was expressed as mg of GAE (gallic acid equivalent)/100 g of the dried extract.

2.9. Membrane stabilizing activity

The membrane stabilizing activity was assessed by using hypotonic solution and heat induced hemolysis of erythrocyte, by following established protocol[26,27].

2.10. Thrombolytic activity

The thombolytic activity of extractives was evaluated by the method of Prasad and collaborators[28,29] by using streptokinase as standard.

3. Results

The whole plant of *K. pinnata* provided a total of six compounds (1-6). The structures of these compounds were determined as glut-5(6)-en-3-one, taraxerone, 3β-friedelanol, β-amyrin-3-acetate, 3,5,7,3',5'-pentahydroxyflavonoe and β-sitosterol by extensive analysis of spectral and physical data (Table 1) as well as by comparison with those of structurally related compounds.

The methanol extract of *K. pinnata* and its petroleum ether, carbon tetrachloride, chloroform and aqueous soluble fractions showed significant antioxidant and cytotoxic activities (Table 2), but weak inhibition of antimicrobial growth. The crude methanolic extract of *K. pinnata* also demonstrated the presence of high phenolic contents but it showed moderate membrane stabilizing and thrombolytic activities (Table 2). It is clearly evident that *K. pinnata* exhibited some bioactivities which are in accordance with the folk uses of the plant in various diseases.

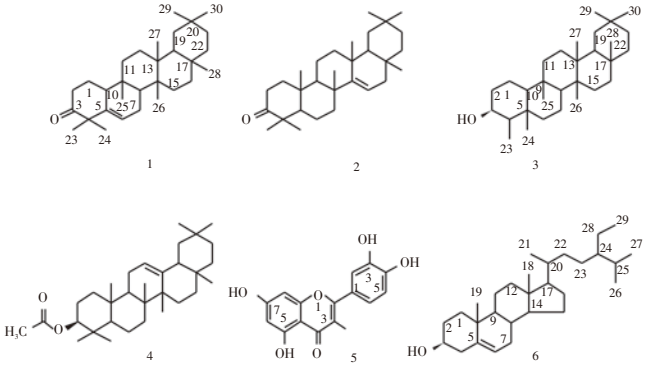

Figure 1.

The methanolic crude extract of *K. pinnata* and its Kupchen fructions were subjected to screening for free radical scavenging (DPPH) activity using BHT as reference standard. In this investigation, the chloroform soluble partitionate of *K. pinnata* showed the highest free radical scavenging activity with IC_{50} value 80.00 μg/mL. The crude methanol extract of *K. pinnata* also revealed moderate free radical scavenging activity (IC_{50}=130.0 μg/mL) (Table 2).

As a part of discovery of cardio protective drugs from natural sources the methanolic extracts of *K. pinnata* was assessed for thrombolytic activity. Addition of 100 μL SK, a positive control (30 000 I.U.), to the clots and subsequent incubation for 90 minat 37 ℃, showed 66.77% lysis of clot. Here, distilled water was treated as negative control which exhibited negligible percentages of lysis of clot (3.80%) whereas the methanolic extract of *K. pinnata* demonstrated thrombolytic activity by 16.41%. The mean difference in the percentage of clot lysis the between positive and negative controls was found to be statistically significant.

In the brine shrimp lethality bioassay, the LC_{50} values of crude methanol extract and its petroleum ether, chloroform, carbon tetrachloride and aqueous soluble fractions were found to be 8.32, 6.31, 1.32, 1.26 and 1.12, respectively. However, varying degree of lethality to *Artemia salina* was observed with exposure to different dose levels of the test samples.

Table 2.

Free radical scanvenging, cytotoxic, thrombolytic activities and total phenolic content of *K. pinnata*

Sample	IC_{50} (μg/mL)	Cytotoxic activity LC_{50} (μg/mL)	Total phenolic content (g of GAE/100 g of dried extract)	% Clot lysis
BHT	24.0	–	–	–
VS	–	0.44	–	–
SK	–	–	–	66.77±0.66
ME	130.0	8.32	149.24	16.42±1.20
PESF	350.0	6.31	–	–
CTSF	180.0	1.32	–	–
CFSF	80.0	1.26	–	–
AQSF	170.0	1.12		

BHT=tert-butyl-1-hydroxytoluene; VS=Vincristine sulphate; SK=Streptokinase; ME=Methanolic extract of *K. pinnata*; PESF= Petroleum ether soluble fractions; CTSF= Carbon tetrachloride soluble fraction; CFSF=Chloroform soluble fraction; AQSF=Aqueous soluble fractions of crude methanolic extract.

4. Discussion

Compound 1 was obtained as white crystals, which melted at 208-210 ℃. This was identical to that reported for glut-5(6)-en-3-one[13-14]. The 1H NMR spectrum of 1 displayed an olefinic proton signal at δ 5.69 (1H, m) which could be assigned to H-6 of a pentacyclic triterpenoid-type carbon skeleton. It also showed eight three proton singlets at δ 0.82, 0.96, 1.00, 1.03, 1.07, 1.17, 1.23 and 1.24, which could be attributed to eight methyl groups present in the molecule. The ^{13}C NMR spectrum of this revealed the presence of 30 carbon resonances. This further confirmed the presence of a triterpene skeleton. The DEPT-135 experiment was very informative, which exhibited signals for 8 methyl, 10 methylenes and 4 methine carbons. Thus, it had 8 quartnary carbons. The ^{13}C signal at δ 215.5 could be ascribed to the ketonic (>C=O) functionality at C-3. On the basis of the above spectral data, compound 1 was characterized as glut-5(6)-en-3-one (1). The identity of this compound as glut-5(6)-en-3-one (1) was further confirmed by comparison of its spectral data with previously reported values[13,14] as well as by co-TLC with authentic sample.

Compound 2 was obtained as white crystals. This melted at 242-245 ℃, which was identical to that published for taraxerone[15]. The 1H NMR spectrum of 2 showed a typical signal of one proton intensity at δ 5.61 (dd, *J*=8.0, 3.2 Hz), the chemical shift and splitting pattern of which was characteristic for an olefinic proton in a taraxerol-type triterpenoid carbon skeleton. The pair of double doublets centered at δ 2.57 (1H, ddd, *J*=16.0, 7.6, 3.2 Hz) and 2.33 (1H,

ddd, *J*=16.0, 6.4, 3.6 Hz) could be attributed to Ha-2 and Hb-2, respectively. The 1H NMR spectrum also showed eight methyl singlets at δ 0.83, 0.91, 0.92, 0.96, 1.07, 1.08, 1.09 and 1.14. The absence of an oxymethine proton in the 1H NMR spectrum and the presence of a carbon signal at 217.6 ppm in the 13C NMR spectrum confirmed the presence of a ketonic group in this molecule. The ^{13}C NMR spectrum of compound 2 displayed the presence of 30 carbon resonances. This furthers substantiated the presence of a triterpene skeleton. The DEPT-135 experiment differentiated these carbon signals into 8 methyl, 10 methylenes, 4 methine carbons. Thus, compound 2 had 8 quartnary carbons. Careful interpretation of the 1H and ^{13}C NMR data allowed to characterize compound 2 as taraxerone (taraxa-14-en-3-one), the identity of which was further substantiated by comparison with published values[15].

Compound 3 was obtained as white amorphous powder. This powder decomposed at 242-244 ℃, which was in close agreement to that of 3β-friedelanol[16]. The 1H NMR spectrum of 3 appeared a pair of doublet of triplets centered at δ 1.90 (1H, dt, *J*=10.4, 2.4 Hz) and 1.74 (1H, dt, *J*=12.8, 3.2 Hz) which could be assigned to Ha-2 and Ha-6, respectively. The presence of a multiplet δ 3.74 indicated an oxymethine proton at H-3 position. The chemical shift and splitting of this proton signal was typical for 3β-friedelanol type triterpenoid skeleton. The 1H NMR spectrum also showed one three proton doublets at δ 0.95 (H_3-23) and seven three proton singlets at δ 0.97 (H_3-24), 0.80 (H_3-25), 0.99 (H_3-26), 1.01 (H_3-27), 1.17 (H_3-28), 0.95 (H_3-29) and 1.00 (H_3-30). On this basis, compound 3 was characterized as 3β-friedelanol (3). The identity of 3 as 3β-friedelanol was further confirmed by comparison of its spectral data with literature values[16].

Compound 4 was obtained as white crystalline mass, with m.p. of 241-243 ℃. This was identical to that published for β-amyrine-3-acetate[17]. The 1H NMR spectrum of 4 reveled a triplet (*J*=3.2 Hz) of one proton intensity at δ 5.18 characteristic for H-12 and a downfield oxymethine proton at δ 4.50 (1H, dd, *J*=8.0, 3.0 Hz). The downfield resonance of this signal demonstrated that C-3 was esterified. This was supported by a methyl group singlet at δ 2.04 (CH_3CO). The 1H NMR spectrum also showed eight methyl signals at δ 0.83 (3H, 1×CH_3), 0.87 (6H, 2×CH_3), 0.88 (6H, 2×CH_3), 0.96 (3H, s), 0.97 (3H, s) and 1.13 (3H, s). The ^{13}C NMR spectrum of compound 4 was very informative, which revealed the presence of 32 carbon atoms including signals for a carbonyl and acetyl methyl group. Thus, compound 4 was characterized as β-amyrin-3-acetate. Its identity was confirmed by comparison of its spectral data with reported values[17], as well as co-TLC with authentic sample.

The 1H NMR spectrum of compound 5 exhibited a pair of doublets (*J*=2.0 Hz) at δ 6.38 (1H) and 6.21 (1H) characteristic for H-8 and H-6, respectively, in a flavonoid type compound. The spectrum also demonstrated two doublets centered at δ 6.91 (1H, *J*=8.4 Hz), and δ 7.34 (1H, *J*=2.4 Hz) and a double doublet (*J*= 8.4, 2.4 Hz) at δ 7.76. These resonances suggested the presence of a 1,3,5-trisubstituted aromatic ring. On the basis of the above spectral data, compound 5 was characterized as 3,5,7,3',5'-pentahydroxyflavone, the identity of which was further supported by comparison of its spectral data with reported values[30].

Compound 6 was obtained as colorless gum. The 1H NMR spectrum of 6 allowed a one proton multiplet at d 3.52, the position and multiplicity of which was indicative of H-3 of the steroid nucleus. The typical H-6 of the steroidal skeleton was evident as a doublet (*J*=6.0 Hz) at d 5.35 that integrated for one proton. The spectrum further revealed two singlets at d 0.68 and 1.01 (3H each) assignable to two tertiary methyl groups at C-13 and C-10, respectively. Two doublets centered at d 0.82 (J=7.2 Hz) and 0.83 (J=7.2 Hz) could be attributed to two methyl groups at C-25. The doublet at d 0.93 (J=6.4 Hz) was demonstrative of a methyl group at C-20. On the other hand, the triplets (J=8 Hz) of three-proton intensity at d 0.85 could be assigned to the primary methyl group attached to C-28. The ^{13}C NMR spectrum of compound 6 was very informative, which revealed the presence of 29 carbon atoms. The DEPT-135 experiment showed signals for 6 methyl, 11 methylenes and 9 methine carbons. Thus, it had 3 quaternary carbons. The above spectral features are in close agreement to those observed for β-sitosterol[31]. Co-TLC with an authentic sample further confirmed its identity as β-sitosterol.

The antimicrobial activity of extracts from *K. pinnata* was examined in the present study. The zone of inhibition produced by the crude methanol extract and its petroleum ether, carbon tetrachloride, chloroform, and aqueous soluble partitionates was determined at a concentration of 400 μg/disc. The chloroform soluble partitionate only showed very weak activity against the test organisms having the zone of inhibition of 7 mm (data not shown).

The methanol extract of *K. pinnata* was tested for total phenolic content by using Folin-Ciocalteu reagent. Based on the absorbance values of the various extract solutions by colorimetric analysis, the total phenolics of different extracts were determined and compared with the standard solutions of gallic acid.

The extractives of *K. pinnata*, at concentration 1.0 mg/mL, significantly protected the lysis of human erythrocyte membrane induced by hypotonic solution and heat, as compared to the standard acetyl salicylic acid (0.10 mg/mL). In hypotonic solution-induced haemolysis, the crude methanolic extract inhibited 68.34% haemolysis of RBCs as compared to 71.9% produced by acetyl salicylic acid. On the other hand, in heat induced haemolysis, this extract inhibited 52% haemolysis of RBCs as compared to 42.20% demonstrated by acetyl salicylic acid (at 0.10 mg/mL).

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