Two new stereoisomeric antioxidant triterpenes from *Potentilla fulgens*

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**A R T I C L E   I N F O**

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**A B S T R A C T**

The roots of *Potentilla fulgens* have been used for a long time as a folk remedy for many ailments without having information on its pharmacological action. Of the various extracts prepared by partitioning of the methanol extract, the ethyl acetate fraction was found to possess better antioxidant and cytotoxic activities. The degree of reduction in cloning efficiencies of MCF-7 cell lines was more with ethyl acetate than with hexane fraction of the root-extract. Hence, this fraction was further purified and nine compounds, including two new ursane type triterpenoids Fulgic acid A and Fulgic acid B, were identified and characterized. Other compounds were identified as ursolic acid, euscaphic acid, corosolic acid, epicatechin, catechin, p-hydroxybenzaldehyde and gallic acid. Chemical structures were elucidated by spectroscopic methods, especially ESIHRMS and 2D NMR techniques. The new compounds showed good antioxidant activity and therefore this plant can be a source of natural antioxidants.

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**1. Introduction**

The genus *Potentilla* includes about 500 species, herbs or small shrubs with rhizomes and belongs to family Rosaceae. The genus evolves from the Latin term *potens* meaning “powerful” which is in reference to the curative properties of some species. The plants find their natural abode in temperate, arctic and alpine zones of the Northern hemisphere while a few species have been found in high mountains of the tropics and in South America [1]. Various species of genus *Potentilla* are used in Ayurveda, Unani, Siddha, Chinese and Tibetan systems of medicine [2]. Several reports in the literature have indicated the presence of polyphenolic compounds in the plants of this genus suggesting that these plants can be explored for their potential antioxidant properties. Polyphenols isolated from these plants are of immense importance both in medicine and nutrition for their potent antioxidant capacity and protective effects on human health and diseases including neoplastic diseases [3].

*Potentilla fulgens* (Vajradanti) is a therapeutically and commercially important member of this genus. The plant is generally distributed at higher altitudes in western Himalayan region, Khasi hills and in eastern Himalaya in Meghalaya, India [4]. Traditionally, the root stock is edible and is effective against high blood pressure. In several areas of North-East India, the root juice is taken as a remedy for stomach ailments, peptic ulcers, cough and cold and other respiratory problems. Root powder is consumed to strengthen the teeth and as a treatment to gingivitis and pyorrhea. Owing to its importance in managing various tooth ailments, the species is commercially utilized by the Vicco Laboratories, India for manufacturing Vicco Vajradanti toothpaste and powder. The pieces of roots are also chewed along with betel, composed of raw areca nut, locally called Kawai, and betel leaf [5].
2. Materials and methods

2.1. General

HPLC analysis was carried out on Phenomenex C_{18} column (250 × 4.6 mm) connected to a Shimadzu (USA manufacturing Inc.) HPLC system consisting of a model LC-10AT VP fitted with SIL-20AC autosampler and SPD-M10A VP photodiode array detector. Princeton SPHER-C_{18} column (250 × 10 mm) was used for isolation of compounds. The 1H and 13C NMR spectra were recorded on Ultrashield 400 NMR spectrometer (Bruker, Germany). The optical rotations were measured on Autopol IV polarimeter (Rudolph, USA). Mass spectra were recorded on a LCQ (Thermo-Quest, USA). The IR spectra were performed on a FTIR system ( Nicolet, USA). Absorbances were recorded on Multiskan (Thermo scientific) 96 well plate reader. The extracts/compounds were dried on rotary vacuum evaporator (Buchi, Switzerland).

2.2. Chemicals and reagents

All chemicals and solvents used for extraction and purification were of laboratory reagent grade. All chromatographic purifications were performed with silica gel #60–120 and silica gel G whereas all TLC analysis was performed on silica gel coated (Merck Kieselgel 60 F254, 0.2 mm thickness) plates. HPLC grade acetonitrile (JT Baker) and ultra pure water (Elga®) were used for sample preparation and in HPLC mobile phases. For antioxidant assays, 1,1-diphenyl-2-picrylhydrazyl (DPPH\(^{\cdot}\)). Trolox, 2,2-azinobis-(3-ethylbenz thiazoline-6-sulfonic acid) (ABTS\(^{\cdot}\)), potassium persulfate, trichloroacetic acid, potassium ferricyanide, and ferrous chloride were purchased from Loba Chemie, Mumbai, India, ferric chloride from Spectorchem, Mumbai, India, 3-(4,5-dimethyl thiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and quercetin dihydrate were purchased from Sigma Aldrich and l-ascorbic acid from HiMedia.

2.3. Cell lines and culture medium

MCF-7 (human breast cancer) cell line was purchased from the National Center for Cell Science (Pune, India). Cells were grown in Dulbecco’s MEM (DMEM, Invitrogen–GIBCO), supplemented with 10% fetal calf serum (Invitrogen–GIBCO), 100 U/ml penicillin and 100 mg/ml streptomycin (Invitrogen–GIBCO) and 2 mM l-glutamine (Invitrogen–GIBCO).

2.4. Plant material

P. fulgens roots were collected from Shillong peak forest area of Meghalaya state in India. A voucher specimen was deposited in the herbarium of the Department of Botany, North-Eastern Hill University, Shillong (accession number 11906). The roots were air-dried in the shade and crushed to a coarse powder.

2.5. Extraction and isolation

The plant material (1 kg root material) was dried and ground to yield a coarse powder. The root powder was macerated with methanol at 35–37 °C for 72 h to yield 400 g methanol extract. The concentrated extract was then suspended in water–methanol mixture (80:20) and partitioned with hexane, chloroform, ethyl acetate and butanol, in the same order. Solvent–solvent partitioning yielded 8 g hexane extract, traces of chloroform extract, 293 g ethyl acetate and 30 g of butanol extract. Ethyl acetate extract (40 g) was chromatographed on silica gel (silica gel #100–200, Column H: 30 cm, i.d.: 7 cm; Eluent: hexane–ethyl acetate (0% to 90% EtOAc) then chloroform–methanol gradients (1% to 50% MeOH)) to yield five major fractions. Fraction 1 (2.28 g) was column chromatographed using hexane-ethyl acetate to give compound 1 (30 mg), compound 2 (8 mg) and compound 3 (6 mg). Fraction 2 (2.6 g) which was eluted with 5% EtOAc in hexane was a mixture of two compounds, which were resolved on HPLC using Phenomenex C_{18} (250 × 4.6 mm, 5 μm) column and isocratic ACN/H_{2}O, 75:25 mobile phase at a flow rate of 1 mL/min. It furnished two compounds, compound 4 and compound 5. Fraction 3 (15 g) on TLC analyses showed presence of compound 6 and compound 7 which were identified as Epicatechin and catechin by co-TLC with authentic sample available in laboratory. Fraction 4 (5 g) yielded compound 8 and compound 9 after chromatography on silica gel.

2.6. Antioxidant assays

2.6.1. DPPH radical scavenging assay

Free radical scavenging activity of the test compounds/extracts was determined by the DPPH assay [13]. Briefly, 200 μL reaction mixture contained methanolic solution of DPPH (190 μL) and 10 μL of different concentrations of test
compounds/extracts in methanol. The reaction mixture was incubated for 1 h at 27 °C and the absorbance was measured at 517 nm. The change in absorbance with respect to the control (containing DPPH only without sample, expressed as 100% free radicals) was calculated as percentage scavenging and the IC50 value was determined. Ascorbic acid and trolox were used as positive controls. The ability to scavenge DPPH radicals was calculated as follows:

\[
\% \text{ Inhibition} = \left( \frac{OD_{\text{blank}} - OD_{\text{test}}}{OD_{\text{blank}}} \right) \times 100
\]

ODblank is the absorbance of blank; ODsample is the absorbance of test (extract/compound).

The IC50 value was obtained by plotting the DPPH scavenging percentage of each sample against the sample concentration. The experiment was performed in triplicate for each concentration of the individual sample.

2.6.2. ABTS** radical scavenging assay

ABTS** radical scavenging activity of the test compounds was determined using an ABTS** decolorization assay [14]. To the ABTS** liquid substrate system 2.45 mM potassium persulfate was added in a stoichiometric ratio of 1:0.5 (v/v). The mixture was allowed to stand in the dark at 27 °C for 8 h. In the ABTS** solution (190 μL) different concentrations of test compounds/extracts (10 μL) were added. The reaction mixture was incubated for 6 min at 27 °C and the absorbance was measured at 734 nm. The change in absorbance with respect to the control (containing ABTS** solution only without sample, expressed as 100% free radicals) was calculated as percentage scavenging and the IC50 value was calculated. The known antioxidants ascorbic acid and trolox were used as positive controls.

2.6.3. Reducing power assay

The reducing power assay was done by reported protocol [15,16]. Briefly each extract/compound (0.125–1 mg) was dissolved in 1.0 mL of DMSO. To this extract solution, phosphate buffer (0.2 M, 2.5 mL, pH 6.6) was added followed by addition of a solution of potassium ferricyanide (1%, 2.5 mL). The reaction mixture was incubated in water bath at 50 °C for 20 min. After incubation trichloroacetic acid (10% w/v, 2.5 mL) was added to the reaction mixture and was centrifuged at 7500 rpm (5471 g) for 10 min. Distilled water (2.5 mL) and a solution of ferric chloride (0.1%, 0.5 mL) were added to an aliquot of the upper layer (2.5 mL). Finally the absorbance of the reaction mixture was measured at 700 nm. Increased absorbance of the reaction mixture indicates greater reducing power. The experiment was performed in triplicate. Quercetin dihydrate was used as a positive standard.

2.6.4. MTT antioxidant assay

MTT antioxidant assay was performed as per the reported procedure [17]. Briefly a stock solution of test extracts/compounds (1 mg/mL) in DMSO and an aqueous solution of MTT (1 mg/mL) were prepared. In a capped glass vial (5 mL) mixture of aqueous solution of MTT (190 μL) and different concentrations of test compound/extract (10 μL) was vortexed. To it methanol (200 μL) was added and was vortexed again. The reaction mixture was incubated at 37 °C for 6 h. After the incubation, 200 μL of the reaction mixture was taken in 96-well cell culture plate, and the absorbance was read at 570 nm. Each sample was assayed in triplicate and the average absorbance was noted. In control the test solution was replaced with 10 μL of methanol. Quercetin dihydrate was used as a positive standard.

2.7. Clonogenic cell survival assay

The cell survivality was evaluated using a clonogenic assay in MCF-7 cancer cell lines. Cells plated at two cell densities (2000 and 3000 cells) onto three 25 cm² flasks each and for untreated controls, four flasks were plated at one cell density (1000 cells). Five hours after plating, the cells were exposed for 24 h with 15 to 120 μg/ml of the test samples. Cells were washed twice with the medium and finally flasks were incubated in CO2-incubator with fresh DMEM with 10% fetal calf serum for 8 to 10 days. The colonies were fixed and stained with 0.2% crystal violet in 70% ethanol. Colonies with a minimum of 50 cells were counted as the progeny of a viable cell.

2.8. Statistics

The data obtained from clonogenic survival assay are represented as a sigmoidal fit curve with a linear X scale, the function used was Boltzmann. The results are expressed as mean plus or minus standard deviation (SD).

3. Results and discussion

Of the various extracts prepared by partitioning of the methanol extract viz. hexane, ethyl acetate, butanol and aqueous extract, the ethyl acetate fraction was found to possess both the antioxidant and cytotoxic activities. Hence, this fraction was selected for further purification of pure compounds. Nine compounds (Fig. 1), including two new compounds, were identified and characterized from the ethyl acetate extract of the roots of P. fulgens.

3.1. Structure elucidation of compounds 4 and 5

Compound 4: 9 mg (0.0009% yield) was obtained as a white powder with [α]D50 −89.9 (c 0.34, CHCl3). IR spectrum revealed the presence of carboxyl group (1692 cm⁻¹) and hydroxyl group (3422 cm⁻¹). The molecular formula was established as C30H48O5 on the basis of ESIHRMS data at m/z: 511.3393[M + Na]⁺ (calcd for m/z 511.3399 [M + Na]⁺) and at 493.3293 [M + Na-H2O]⁺. Combined analysis of the 1H, 13C and DEPT-135 NMR spectroscopic data revealed the presence of seven CH3 (δ 10.4, 16.5, 17.5, 17.7, 20.4, 21.6, 22.9 and 28.5); nine CH2 (δ 17.9, 21.5, 25.4, 27.4, 27.9, 32.8, 33.2, 34.8 and 42.3); five CH (δ 40.4, 48.1, 50.7, 66.6 and 78.9), six sp³ quaternary carbons (δ 38.3, 38.8, 41.7, 42.5, 43.4 and 83.1), two sp² quaternary carbons (δ 131.36 and 140.1) and one carboxyl group (δ 176.5). Absence of an olefinic proton signal at δ 5.5–5.2 ppm in 1H NMR and the corresponding carbon signal at δ 128–126 ppm was indicative of the absence of a trisubstituted double bond at the usual position of ursane type triterpenes. Furthermore, 13C and DEPT-135 suggested a double bond between two quaternary carbons. Presence of deshielded carbon signals at δ 66.5, 78.8 and 83.1 ppm was indicative of the attachment of a hydroxyl group at these positions. Exact position of the double bond
and hydroxyl groups was elucidated using detailed analyses of the correlations observed in the $^1$H–$^1$H COSY, HSQC, HMBC and NOESY spectrum. $^1$H–$^1$H COSY spectrum presented correlation between the proton signals resonating at $\delta$ 4.02 and 3.43, indicating their adjacent location (Fig. 2). Appearance of one doublet signal at $\delta$ 1.08 for methyl group in $^1$H NMR was supported by the presence of its COSY correlation with proton at $\delta$ 2.56 (H-19). The HMBC spectrum depicted the correlations for seven methyl groups. Methyl signal at $\delta$ 1.01 (H-23) was correlated with tertiary carbons at $\delta$ 78.8 (C-3) and 48.2 (C-5), quaternary carbon at $\delta$ 38.8 (C-4) and a primary carbon at $\delta$ 21.7 (C-24). Similar correlations were observed for the methyl signal at $\delta$ 0.84 (H-24). It showed correlation with C-3, C-4, C-5 and C-23. These correlations confirmed the position of two germinal methyl groups C-23 and C-24 at C-4 of skeleton. The methyl signal at $\delta$ 0.91 (3H, H-25) presented correlations with secondary carbon at $\delta$ 42.3 (C-1), a quaternary carbon at $\delta$ 38.8 (C-10) and a tertiary carbon at $\delta$ 50.7 (C-9). The common correlation by protons at $\delta$ 0.91 (H-25) and 0.81 (H-26) with C-9 confirmed the position of two methyl groups, C-25 and C-26 at the positions C-10 and C-8 of the ursane nucleus. Other correlations for H-26 with quaternary carbon at $\delta$ 41.7 (C-8) and quaternary carbon at $\delta$ 42.5 (C-14) also supported its location at C-8. For methyl signal at $\delta$ 1.13 (H-27) correlations were observed with quaternary carbon at $\delta$ 41.7 (C-8), 42.5 (C-14) and 140.1 (C-13) and also with the secondary carbon at $\delta$ 27.4 (C-15). All these correlations...
were indicative of the methyl group (C-27) at C-14. Both methyl signals at δ 1.08 (H-29) and 1.38 (H-30) showed correlations with carbon signals at δ 83.1 (C-20) and 40.3 (C-19). In addition, correlations were observed between the H-29 and carbon signal at δ 131.4 (C-18) and between H-30 and carbon signal at δ 32.8 (C-21). These two correlations were helpful in assignments of the C-29 and C-30 positions for methyl groups on the nucleus. All these correlations confirmed the presence of seven methyl groups at C-23, C-24, C-25, C-26, C-27, C-29 and C-30 along with double bond between C-13 and C-18. Chemical shift values are presented in Table 1. A search on SciFinder database showed only one ursane compound with C-20 hydroxy group [18] and one with C(13,18) double bond. However, no compound possessing both of these features has been retrieved on the SciFinder.

Compound 5: 10 mg (0.0010% yield) was obtained as a white powder with [α]$_D^{20}$ = -91.1 (c 0.34, CHCl$_3$). The molecular formula was established as C$_{30}$H$_{48}$O$_5$ on the basis of ESIHRMS data at m/z: 511.3396 [M + Na]$^+$ (calcd for m/z 511.3399 [M + Na]$^+$) and at 493.3293 [M + Na-H$_2$O]$^+$. Comprehensive analysis of the $^1$H, $^{13}$C and DEPT-135 NMR spectroscopic data revealed that there was a close agreement between the chemical shifts of both the compounds except in the values of ring A. The differences were observed for H-1, H-2 and H-3 and the corresponding carbon signals. This suggested a similar planar structure of both the compounds. Evaluation of the 2D spectra was helpful in assessment of the difference between the two molecules. A similar pattern was observed for the COSY and HMBC correlations as were found in compound 4. A difference was seen between coupling constants in $^1$HNMR and the crosspeaks in NOESY spectra of the two which confirmed the relative stereochemistry of compounds 4 and 5. In compound 4, a dddd due to protons resonating at δ 4.02 (H-2) displayed coupling constant values of 11.8 Hz (H-1/β-H-2), 4.3 Hz (H-1/α-H-2) and 3.2 Hz (H-2/H-3) which indicated trans, cis and cis coupling of the protons respectively. The coupling constant values for ddd at δ 3.71 (H-2) were 11.9 Hz (H-1/β-H-2), 9.08 Hz (H-1/α-H-2), 9.08 Hz (H-2/H-3) and 4.4 Hz (H-1/β-H-2) suggesting the trans, trans and cis arrangements of these protons in compound 5. Moreover the NOESY spectrum of compound 4 presented the cross-peaks between H-2 and H-3 while the same were absent in case of compound 5. This leads to the assignment of cis configuration of the two protons, H-2 and H-3 in compound 4 and trans arrangement in compound 5. Furthermore, triterpenoid literature suggests that the chemical shifts associated with the carbons C-2/C-3 are around 66/79 for α/α placement of two hydroxyl groups at these positions, around 71/78 for β/β and around 68/83 for α/β arrangements [19–21]. The aforementioned

### Table 1

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<td>δ$_H$ ppm</td>
<td>δ$_C$ ppm</td>
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<td>1.22 (overlapped with H-5, 22, 15)</td>
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<td>1.32 (m)</td>
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A Choudhary et al. / Fitoterapia 91 (2013) 290–297
NOESY interpretations along with the coupling constant values and literature comparison suggested that the compound 4 has α/α hydroxyl groups at C-2/C-3 while the other stereoisomer 5 has α/β arrangement for them. Other key NOESY correlations were observed between H-19/H-30 suggesting α positioning of the C-29 methyl group and β placement of C-30 methyl group. Key NOESY correlations are presented in Fig. 2.

Compounds 4 and 5 possess a rearranged ursane type carbon skeleton in which the double bond existing between C-12/C-13 has shifted to C-13/C-18 positions.

A plausible biogenetic pathway is shown in Fig. 3. It suggests that these compounds might be derived from germanicenyl carbocation by a sequence of hydride shifts to introduce double bond between C-13 and C-18. Once the nucleus is formed, the Cyt P450 monoxygenases may act on the molecule to introduce hydroxyl and carboxyl functionalities [22,23].

Compounds 1, 2, 3, 6, 7, 8, and 9 were characterized as ursolic acid [24], euscaphic acid [25], corosolic acid [26], epicatechin, catechin, p-hydroxy benzaldehyde and gallic acid by analysis of spectral data and comparison with literature.

3.2. Antioxidant activity

Antioxidants exert their action by different mechanisms including, suppression of formation of reactive oxygen species, scavenging the free radicals, chelation of metal ion responsible for free radical generation and protecting the antioxidant enzymes in the body. The classical antioxidant action is generally based upon hydrogen atom transfer and single electron transfer by an antioxidant molecule. Secondly, the structural features like solubility, partition coefficient, bond dissociation energy, and ionization potential are a set of parameters that determine the overall efficacy of an antioxidant. Because of the multiple reaction mechanisms and different structural characteristics of the test compounds, no single assay can be used to reflect the antioxidant potential of a molecule [27]. Various extracts prepared by partitioning from the methanol extract of P. fulgens i.e. hexane extract (HE), ethyl acetate extract (EAE), butanol extract (BE), aqueous extract (AE) and the triterpene acids were evaluated in various antioxidant assays, DPPH and ABTS assay (hydrogen atom transfer based), reducing power assay (single electron transfer based) and MTT assay. Antioxidant potential of triterpene acids has been reported in the literature. Ursolic acid has been reported to provide significant protection against UV-induced lipid peroxidation, oxidative stress and DNA damage by preventing the oxidation of guanine bases and enhancing the levels of antioxidant enzymes [28]. Corosolic acid on the other hand is known to reduce oxidative stress by reducing the levels of thio- barbituric acid-reactive substances and 8-hydroxydeoxyguanosine (oxidative stress bio-markers) [29]. Euscaphic acid is a potential anti-inflammatory molecule that inhibits lipopolysaccharide induced inflammatory responses by reducing production of nitric oxide, prostaglandin E2, Cyclooxygenase-2, TNF-α and IL-1β. Inhibition of these mediators is an important mechanism for avoiding the generation of reactive oxygen species [30]. Owing to the involvement of different mechanisms in the antioxidant activity of triterpene acids, the two new compounds were evaluated for their antioxidant activity. The antioxidant activity of other phenolic compounds isolated in this study is well documented in literature [31–33].

3.2.1. Radical scavenging activity

Lipid peroxidation involves free radical chain reaction generating peroxide radicals. The radical scavengers react with and quench peroxide radicals to terminate the chain reactions. DPPH and ABTS radicals scavenging assays are among the most common spectrophotometric methods used to determine antioxidant capacity. Antioxidants reduce the oxidative damage to the cellular components caused by reactive oxygen species and hence preventing chronic diseases. The ethyl acetate fraction obtained from the methanol extract was shown to exhibit significant inhibition against free radicals. Comparatively less activity was observed in butanol fraction while hexane and aqueous extracts were found to have minimal activity. Thus, the ethyl acetate fraction was selected for isolation of molecules that could account for its antioxidant activity.

Out of the five isolated triterpene acids, 1, 4 and 5 showed better inhibitory activity than 2 and 3 against free radicals in DPPH and ABTS assay. The results are depicted in Table 2. Compound 1 was the most potent with IC_{50} of 5.3 ± 0.2 μM and 7.8 ± 0.4 μM in DPPH and ABTS assay respectively. Compound 4 showed comparatively better antioxidant activity than 5 (Fig. 4). The IC_{50} for compound 4 was found to be 9.8 ± 0.5 μM and 19.9 ± 0.3 μM in DPPH assay and ABTS assay while for compound 5, IC_{50} values were 11.6 ± 0.7 μM and 23.3 ± 0.3 μM respectively. The free radical scavenging activity of these compounds could be attributed to the presence of free hydroxyl and the carboxyl groups in these compounds. Compounds 4 and 5 demonstrated comparatively better activity than 2. This difference can be due to variability in the position of hydroxyl group which is
present at C-20 in 4 and 5 and at C-19 in compound 2. The free hydroxyl groups are capable of transferring hydrogen atom to scavenge free radicals.

3.2.2. Reducing power assay

The reducing power of the compounds is due to their single electron donation capacity which in turn is based on deprotonation and ionization potential of the reactive functional groups. In the reducing power assay, there is reduction of the Fe³⁺/ferricyanide complex to its ferrous form in the presence of antioxidants. Tested compounds did not show activity at micromolar concentration.

3.2.3. MTT antioxidant assay

The MTT assay is a colorimetric assay for measuring the activity of the mitochondrial enzymes present in healthy cells. The assay involves monitoring the absorbance of purple formazan formed as the enzymatic reduction product of MTT. The MTT antioxidant assay utilizes the redox reaction between MTT and natural antioxidants to assess their antioxidant potential. A dose response curve was plotted and is presented in Fig. 4A. Quercetin dihydrate was used as a positive control. Ethyl acetate extract has showed comparable activity as compared to the positive standard while the two compounds 4 and 5 showed good antioxidant activity than compounds 2 and 3.

3.3. Clonogenic cell survival assay

A dose-dependent reduction in the cloning efficiency was observed in ethyl acetate and hexane treated MCF-7 cell lines (Fig. 4B). However, the degree of reduction in cloning efficiencies was more with ethyl acetate than with hexane fraction of the root-extract. The inhibition concentration (IC₅₀) value deduced from the Sigmoidal fit graph and it was 48.38 and 73.65 μg/ml for ethyl acetate and hexane fractions, respectively. Such reduction in cloning efficiencies was not seen with compounds 4 and 5.

4. Conclusion

In present study, the ethyl acetate soluble fraction from the roots of P. fulgens showed good inhibitory activity against the free radicals. Furthermore, a dose-dependent reduction in the cloning efficiency was also observed in ethyl acetate and hexane treated MCF-7 cell lines. However, the degree of reduction in cloning efficiencies was more with ethyl acetate than with hexane fraction of the root-extract. Thus, the ethyl acetate fraction showing good results in both the assays was selected for phytochemical investigations. From the ethyl acetate extract nine compounds, including two new compounds were isolated. The two new compounds exhibited good antioxidant activity but no reduction in the cloning efficiency was observed in MCF-7 cell lines on treatment with these pure isolates. The plausible biogenetic pathway for the two new compounds has been proposed. The antioxidant activity of the plant can attributed to the presence of these compounds along with other phenolics and hence the plant can be considered as a source of natural antioxidants for food and nutraceutical products.

Conflict of Interest

None.

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Appendix A. Supplementary data

The 1H-, 13C-, 2D-NMR and ESIHRMS data are available as supplementary information. Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.fitote.2013.09.008.

References