Chemical and Biological Studies of Leaf Extract of *Dendrophthoe falcata* Linn.

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(Received: October 10, 2017; Accepted: November 20, 2017; Published (web): December 23, 2017)

ABSTRACT: *Dendrophthoe falcata* (Family Loranthaceae) is used extensively in rural area as a component of ethno-medicine for the treatment of various diseases. In this study, the crude extracts and the fractions obtained from *D. falcata* were investigated for potential chemical constituents and some biological activities. For medicinal properties the antioxidant, brine shrimp lethality and thrombolytic activities have been investigated. The structures of the isolated three compounds were solved by extensive analyses of their high resolution ^{1}HNMR spectroscopic data. They were identified as Lupeol, 3-β-acetoxy-12-ene-11-one and β-sitosterol. For bioactivities, the petroleum ether, dichloromethane, chloroform and aqueous soluble fractions abbreviated as PESF, DCMSF, CSF and AQSF respectively. Brine shrimp lethality bioassay was used to evaluate potential cytotoxic activities, where all fractions showed significant activity with lower LC_{50}. Most significant activity has been observed for methanol extract (LC_{50}= 4.477 μg/ml). AQSF revealed maximum activity in DPPH free radical scavenging assay (IC_{50} = of 43.49 μg/ml). In assay for thrombolytic activity, the methanol extract and its chloroform soluble fraction demonstrated significant efficacy with 32.65% and 32.36% clot lysis, respectively.

Key words: β-amyrin, lupeol, 3 β-acetoxy-12-ene-11-one, β-sitosterol, antioxidant, cytotoxic, thrombolytic.

INTRODUCTION

Plants are considered as the most significant sources to discover and develop drugs which are effective and safer than the other currently available synthetic drugs. Due to the less side effects and better safety margin traditional and folk medicines are gaining popularity over modern therapy. 'Farolla' is Bengali name of the plant which is scientifically known as the *Dendrophthoe falcata* Linn. belongs to the hemiparasitic plant family Loranthaceae. It is used for long time as traditional medicine in India and Bangladesh to treat asthma, wounds, ulcer and pulmonary tuberculosis and it also included in the formulations of aphrodisiac, diuretic, astringent and narcotic traditional medicines. Previous study has shown that *D. falcata* is effective against wound by increasing the tensile strength of the affected area through contraction.[1] Various chemical constituents have been reported from this plant such as β-amyrin acetate, β-sitostirol, stigmasterol, kaempferol, quercetin[2], quercetin–3-*O*-rhamnoside, rutin, myricetin, leucocyanidin and three biologically important cardiac glycosides such as strospeside, odoroside F and neritaloside.[3] Another study reported that leaves extract possesses potent antioxidant activity by inhibiting various oxidizing agents like lipid peroxidation, reduced glutathione, and superoxide dismutase levels.[4] In regular human metabolism different free radicals like hydroxyl, singlet oxygen, hydrogen peroxide, nitric oxide and superoxide radicals, are continuously generated.[5,6] Especially, in living organisms, they play vital roles in cell signaling to inactivate relevant enzymes which consequently damage important cellular components.

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Antioxidants have been proved to resist the destruction of beta-cells[7,8] as well as other cells and inhibit oxidation processes in the human body.[9] Therefore, antioxidants are vital inhibitors of lipid peroxidation and act against oxidative damage or stress of living cells by natural defense mechanism.[10] Another study with ethanolic extract of leaves of this plant has reported significant antioxidant activity.[11] We have tried to explore the antioxidant activity by finding out the specific fraction/compound, which has maximum antioxidant activity. It was our main objective to find out any pharmacologically active compounds and to assess potential biological activities of *D. falcata* leaf extract.

MATERIALS AND METHODS

General experimental procedures. Nuclear magnetic resonance (^{1}H NMR) spectra were recorded on ECA (600 MHz) instrument indeuterated solvent ($CDCl_3$). Preliminary investigations of crude drugs were done by TLC on aluminum plates (20x20 cm) pre-coated with silica gel $60F_{254}$. The chromatogram developed by TLC and PTLC were sprayed with 1% vanillin-sulfuric acid reagent and then heated at 110ºC for 5 minutes. All solvents used were of reagent grade.

Collection of plant. The leaves of *D. falcata* were collected from Sundarban area, Bangladesh in April, 2013 and the plant was verified and identified by an expert taxonomist of Bangladesh National Herbarium.

Extraction and isolation. The leaves were dried under sun light after washing properly with running water to eliminate other particles. Then the dried leaves were ground to a coarse powder by a grinding machine. The powdered plant material (800 gm) was taken in an amber colored bottle and soaked in methanol. The bottle with its contents was sealed and kept for 10 days with intermittent shaking and stirring. After that, the whole mixture was filtered, and the filtrate was concentrated with a rotary evaporator to obtain the crude extract. An aliquant crude extract was then fractionated with column chromatography by using a mixture of petroleum ether and ethyl acetate of increasing polarity. Fractions were screened by TLC and fractions showing similar feature were mixed together. Interesting fractions were further fractionated with gel permeation chromatography by using Sephadex LH-20, preparative TLC (stationary phase – silica gel PF_{254}, mobile Phase: ethyl acetate-Toluene 2:98) of gel permeation chromatographic fractions 97-100 and 134-135 yielded two compound DF-1, DF-5 and DF-6 respectively.

Design of biological investigations. The crude methanol extract was partitioned by modified Kupchan[12] method with petroleum ether, dichloromethane, chloroform and finally water.

Brine shrimp lethality bioassay. Brine shrimp lethality bioassay was used to assess the cytotoxic activity of methanol extract (ME) and its different partitionates i.e. petroleum ether (PESF), dichloromethane (DCMSF), chloroform (CSF) and aqueous soluble (AQSF) fraction according to the procedure of Meyer *et al.*[13] These fractions were separately dissolved in DMSO. The test samples were then applied against *Artemia salina* in a 24 hour assay.

DPPH free radical scavenging activity. A method established by Choi *et al.*[14] was used to determine the DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging activity. For this, 3 ml of methanol solution of DPPH (20 µg/ml) was added into the test tube with 2ml (concentration ranging from 500.0 to 0.977 µg/ml) of plant extract dissolved in methanol and the test tube was incubated for 30 minutes at room temperature for providing possible environment to complete the reaction process and absorbance was measured at 517 nm.

Thrombolytic activity. During assay for thrombolytic activity, dry crude extract (100 mg) along with various partitionates were suspended in distilled water (10 ml) and kept overnight. Then the soluble supernatant was decanted and filtered. As positive control, 100 µl of streptokinase (SK) and as negative non thrombolytic control, 100 µl of distilled water were used.[15] Difference obtained in weight

taken before and after clot lysis was expressed as percentage of clot lysis as shown below:

% of clot lysis = (wt of released clot /clot wt) × 100

RESULTS AND DISCUSSION

Successive chromatographic separation and purification of leaf extract of *D. falcata* yielded several purified compounds from the leaf extract. Among them, the structures of three compounds were solved by extensive analyses of their high resolution ^{1}HNMR spectroscopic data as well as by comparisons with related compounds. They are: lupeol, 3β-acetoxy-12-ene-11-one and β-sitosterol.

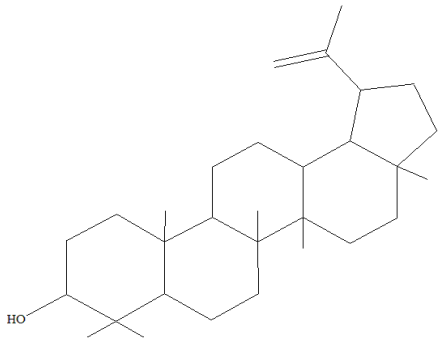
Lupeol (DF-1): White crystals; ^{1}H NMR (600 MHz, $CDCl_3$): δ4.67 and 4.55 (1H, br s each), 1.68 (3H, s, H-30), 0.95 (3H, s, H-27), 1.03 (3H, s, H-26), 0.83 (3H, s, H-25), 0.76 (3H, s, H-24), 0.97 (3H, s, H-23), 0.79 (3H, s, H-28), 2.36 (1H, m, H-19), 3.19 (1H, m, H-3).

3β-acetoxy-olean-12-ene-11one (DF-5): Amorphous solid; ^{1}H NMR (600 MHz, $CDCl_3$): δ5.57 (H-12), 1.33 (methyl), 1.17 (methyl), 1.14 (methyl), 0.86 (4 methyl), 0.83 (methyl), 2.03 (OAc), 4.51 (1H,m, H-3), 2.34 (1H, s, H-9).

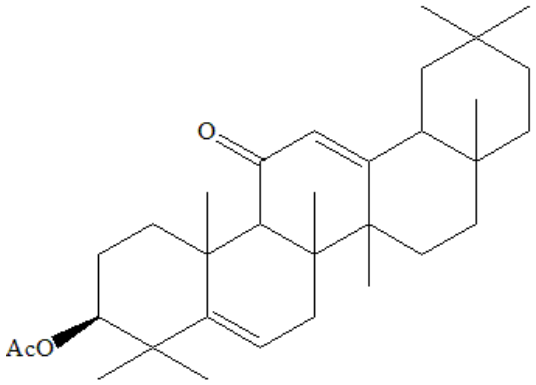
β-sitosterol (DF-6): White crystal; The ^{1}H-NMR spectrum (600 MHz, $CDCl_3$); δ_H 3.50 (1H, m, H-3), δ_H 5.32 (1H, m, H-6), δ_H 0.66 (3H, s, H-13), δ_H 0.99 (3H, s, H-10), δ_H 0.84 (1H, d, H-25) , δ_H 0.82 (1H, d, H-25), δ_H 0.90 (1H, d, H-20), δ_H 0.86 (*J* = 7.5 Hz, t, H-28).

The ^{1}H NMR (600 MHz, $CDCl_3$) spectrum (1H, dd, J-10.8, 5.4 Hz, H-3) of compound DF-1 displayed two olefinic protons at δ4.67 and 4.55 (1H, br. s each), a vinylic methyl at1.68 and six tertiary methyl singlet at 0.95, 1.03, 0.83, 0.76, 0.97 and 0.79. The mulitplet of one proton intensity at δ2.36 was assigned to H-19. Moreover, the spectrum revealed signal at δ3.19 attributable to H-3. On this basis, DF-1 indicates the presence of lupeol.[16]

The ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of compound DF-5 displayed a sharp singlet at δ 5.57 attributable to the olefinic proton (H-12) and eight tertiary methyl groups at δ 1.33, 1.17, 1.14, 0.86 (4 methyl) and 0.83 indicating triterpinoid skeleton. The spectrum further showed an acetyl methyl at δ 2.03 and a multiplet at 4.51 assigned to H-3 bonded to esterified carbon H-9 signal appeared as singlet at δ2.34. Based on the above evidence the structure of this compound was thus established as 3β-acetoxy-olean-12-ene-11one. The ^{1}H NMR data of the compound was not available. However, it was found to be in close agreement with those of 3β-hydroxy-11-oxo-olean-12-enyl palmitate.[17]



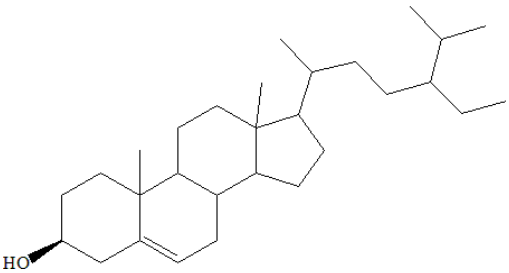
Lupeol



3β-acetoxy-olean-12-ene-11one

The ^{1}H NMR spectrum (600 MHz, $CDCl_3$) of DF-6 revealed a one-proton multiplet at δ 3.50, the position and multiplicity of which was indicative of C-3 protons (H-3) of the steroidal nucleus. The typical signal for H-6 of the steroidal skeleton was evident from a multiplet of one proton intensity at δ 5.32. The ^{1}H-NMR spectrum also displayed signals at δ 0.66 and δ 0.99 (3H each) assignable to two tertiary methyl groups at C-13 and C-10 respectively. The ^{1}H-NMR spectrum showed two doublet centered at δ

0.84 (J = 7.5 Hz) and 0.82 which could be attributed to two methyl groups at C-25. The doublet at δ 0.90 was suggestive of a methyl group at C-20. On the other hand, the triplet at δ 0.86 (J = 7.5 Hz) could be assigned to the primary methyl group attached to C-28. The spectral features are in close agreement to those reported for β-sitosterol Jahan *et al.*[18] Therefore, DF-6 was identified as β-sitosterol.



β-sitosterol

Compounds DF-1, Df-5 and DF-6 were readily identified as lupeol, 3-β-acetoxy-olean-12-ene-11one and β-sitosterol by careful analysis of their high resolution ^{1}H NMR spectral data as well as by comparison with published values.[16-18]

Different partitionates i.e. petroleum ether (PESF), dichloromethane (DCMSF), chloroform (CSF) and aqueous soluble fractions (AQSF) were tested for brine shrimp lethality according to the procedure of Meyer *et al.*[13]. In brine shrimp lethality bioassay, vincristine sulfate (VS) was used as positive control and the LC_{50} was found 0.394 µg/ml for VS. Among the extractives the crude extract demonstrated most significant cytotoxic activity which was evident from the LC_{50} value of 4.477 µg/ml (Table 1).

In table 1, the free radical scavenging capacity of crude extract and various fractions showed significant activity as compared to ASA. The IC_{50} of ASA, ME, PESF, CSF and AQSF were 43.04 ± 1.42 µg/ml, 305.00 ± 4.51 µg/ml, 136.52 ± 3.55 µg/ml, 245.80 ± 3.45 µg/ml, and 43.49 ± 1.12 µg/ml, respectively (Table 1).

In order to get cardioprotective drugs from natural sources, the crude extracts and fractions obtained from *D. falcata* were assessed for thrombolytic activity using 100 µg streptokinase (SK) as positive control (30,000 I.U). In this study, the petroleum soluble fraction (PESF) of *D. falcata* demonstrated highest thrombolytic activity (43.50%) as shown in table 1. However, the other partitionates of methanol extract of *D. falcata* such as dichloromethane (DCMSF), chloroform (CSF) and aqueous (AQSF) soluble fractions showed mild thrombolytic activity.

Table 1. Results of antioxidant, brine shrimp lethality and thrombolytic activities of crude extract and different fractions of *D. falcata*.

Name of fractions	Antioxidant activity (IC_{50} µg/ml)	Brine shrimp lethality bioassay (LC_{50} µg/ml)	Thrombolytic activity (% of clot lysis)
ME	305.00 ± 4.51	4.477 ± 0.83	32.65 ± 3.12
PESF	136.52 ± 3.55	8.041 ± 1.01	43.50 ± 4.02
DCMSF	202.60 ± 2.79	69.098 ± 1.78	17.11 ± 0.89
CSF	245.80 ± 3.45	14.041 ± 1.12	32.36 ± 1.83
AQSF	43.49 ± 1.12	9.281 ± 1.22	11.24 ± 1.16
ASA	43.04 ± 1.42	-	-
VS	-	0.394 ± 0.02	-
SK	-	-	63.11 ± 2.28
Blank	-	-	5.78 ± 0.95

Values are calculated from triplicate experiments and represented as mean ± STD. ME = methanol extract, PESF = petroleum ether soluble fraction, DCMSF= dichloromethane soluble fraction, CSF = chloroform soluble fraction, AQSF= aqueous soluble fraction, ASA= ascorbic acid soluble fraction, VS= vincristine sulphate, SK = streptokinase.

CONCLUSIONS

The methanolic extract of the leaves of *D. falcata* (Family-Loranthaceae) was investigated for isolation of secondary metabolites and determination of bioactivities of this plant. Successive chromatographic separation and purification yielded a total of three compounds. From the biological investigations, it is proved that, the extractives of *D. falcata* showed significant antioxidant, cytotoxic and thrombolytic activities. However, further, studies are required to isolate more bioactive compounds responsible for the activities. The bioactivities shown by the extractives of *D. falcata* rationalize the traditional uses of this plant in various diseases.

Competing interests

The authors declare that they have no competing interests.

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