Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(4): 1744-1747
Received: 10-05-2019
Accepted: 12-06-2019

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Phytochemical studies and fatty acid analysis of the stems of *Polyalthia suberosa* (Roxb.)

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Abstract
In the present study, stigmasterol was isolated from the petroleum ether extract of the stems of *Polyalthia suberosa* Roxb. which had further been characterized by spectroscopic analysis. The fatty acid composition was also analyzed afterward using Gas Liquid Chromatography and the fractions of free and bound fatty acids were isolated. The amount of bound fatty acids was found to be higher than that of free fatty acids.

Keywords: *Polyalthia suberosa*, stigmasterol, fatty acids, composition, bound and free fatty acids

Introduction
Plants, an inseparable part of human life, have long been existing since the early geological past on this earth. From the early stages of human civilization, plants, specially medicinal plants have been playing a pioneering role for the welfare of human being. Bangladesh has a very rich biodiversity. It contains more than 500 medicinal plants having various therapeutic uses [1]. *Polyalthia suberosa* Roxb. (locally known as Boro Challi), a member of Annonaceae family, is a widely distributed plant in Bangladesh. The genus *Polyalthia* includes about 120 species occurring mainly in Africa, South and South-Eastern Asia, Australia, and New Zealand [2]. The plant is well known for its traditional uses as a bitter tonic, abortifacient, febrifuge, and cure of sorption stings all over the world. It usually appears as a shrub or small tree growing up to a height of 2 to 4 meters. Leaves are oblong to narrowly oblong-obovate, 5 to 11 centimeters long; flowers are solitary, pale-yellow, about 1 centimeter long or less and fruits are ovoid or globose, 4 to 5 millimeters long, purple, fleshy and edible [3]. Along with Bangladesh the plant widely occurs in India, China, Malaysia, Myanmar, Sri Lanka, Thailand, and Vietnam.

A number of medicinal uses have been reported about this plant which includes adaptogenic, anti-HIV replication and antibacterial activities. The bark has been considered febrifuge, astringent, analgesic and laxative, and seeds as a diuretic, soporific and sedative. Plant mucilage has also been reported to have diverse applications as thickening, binding, disintegrating, suspending, emulsifying and gelling agents [4].

The literature review has already revealed the presence of medicinally active compounds as alkaloids, flavonoids, phenols, sterols, triterpenes and also micronutrients from different parts of *Polyalthia suberosa* [5,6]. The presence of carbohydrate, sugar, protein, ascorbic acid and moisture has also been reported [6].

The furans, 1-(2-furyl) pentacosa 16, 18-diyne and 23-(2-furyl) tricosa-5,7-diynoic acid along with the alkaloids kalasinamide, N-*trans*-feruloyltyramine and N-*trans*-coumaroyltyramine isolated from the stems of *Polyalthia suberosa* have been found to show antiviral activity [7, 8]. Leaves have been found to contain α- and β-amyrin, lupeol, β-sitosterol, stigmasterol and campesterol, stem bark to yield alkaloids, oxostephanine and lanuginosine [9].

Triterpene suberosol isolated from *Polyalthia suberosa* leaves and stems was found to show anti-HIV activity [10]. Different extracts of the leaves of *Polyalthia suberosa* have also shown significant amounts of antibacterial, antifungal, analgesic, antidiarrhoeal, cytotoxic and antioxidant activities [11, 12].

Therefore, based on the information obtained from sufficient literature survey an attempt has been undertaken to study the phytochemical constituents and fatty acid composition of the stems of *Polyalthia suberosa*.

Hence, the present study deals with the isolation and characterization of phytochemicals and analysis of fatty acids from the pet-ether extract of the plant *Polyalthia suberosa*.

Materials & Methods

Sample collection

The stems of the plant *Polyalthia suberosa* were collected from Savar, Dhaka. A voucher specimen of this was deposited in the Bangladesh National Herbarium (BNH) having Accession No. DACB 38380. After collection, the plant stems were cleaned, dried at room temperature and then in an oven at below 45 °C and finally ground to powder using a cyclotec grinding machine (200 meshes). The powders were stored in airtight bottles and used throughout the investigation.

Extraction

The stem powder (~480 g) of *Polyalthia suberosa* was extracted with petroleum ether (b.p. 60-80 °C) in refluxing apparatus. The extract was filtered and evaporated to dryness separately using a rotary evaporator (Stuart, UK) under reduced pressure. The amount of petroleum ether (b.p 60-80 °C) extract was found to be 0.85 g per 100 g of dry powder.

Isolation and characterization of compounds from petroleum ether

The crude petroleum ether (b.p 60-80 °C) extract was subjected to TLC screening to find out the type of compounds present in the extract. TLC analysis of the petroleum ether extract showed several spots in iodine chamber and vanillin-sulfuric acid spray on TLC plate. The dry mass of petroleum ether extract (4.1 gm) was subjected to column chromatography over column grade silica gel (Kiesel gel 60G). The column was first eluted with 100% petroleum ether (b.p 40-60 °C) followed by mixtures of petroleum ether with an increasing amount of dichloromethane, then eluted with a mixture of ethyl acetate and finally with methanol when thirty eight fractions were obtained. Each of the fractions was monitored by TLC and the fractions of similar behaviors were combined together and marked as P-1 to P-8. From TLC analysis the fraction P-4 was found to be a single compound. The fraction (P-4) was concentrated and allowed to stand for several days till the fraction yielded a white solid compound and it was then marked as A (23 mg).

Analysis of fatty acids

Both free fatty acids (FFA) and bound fatty acids (BFA) were identified and isolated from petroleum ether (b.p 40-60 °C) extracts of the powdered stems of *Polyalthia suberosa* Roxb. The amounts of FFA and BFA were found to be 13 mg and 106 mg, respectively per 100 g of dry powder. A portion (5 mg) of both the bound and free fatty acids were converted into their methyl esters and further analyzed by GLC (Shimadzu 9A, Column BP-50, detector-FID, at 170° to 270 °C, rising temperature 4 °C/min for 30 minutes) [13, 14]. The results are given in Table 1.

Results and Discussion

Characterization of compound A

Compound A (~23.0 mg) was a colorless crystalline solid having R_f value: 0.55 (in 100% DCM) and its melting point was found to be 128-130ºC. It was soluble in dichloromethane.

FT-IR (400-4000 cm^{-1}) Data

ν (cm^{-1}): 3437 (O-H stretching); 2935 and 2859 (sp^3 C-H stretching); 1645 (>C=C< stretching); 1462 (-CH_2 bending); 1376 (-CH_3 bending); 1059 and 1243 (C-O stretching); 802 and 964 (sp^2 C-H out-of-plane bending).

^{1}H-NMR (400 MHz, in $CDCl_3$) Data

δ (ppm): 0.686 and 0.996 (s, methyl protons H-18 and H-19 at C-13 and C-10; 3.514 (m, >CH- proton at C-3); 5.342 (br, s, olefinic proton at C-6); 5.05 and 5.151 (m, olefinic protons at C-22 and C-23); 0.833 and 0.793 (d, >CH- protons at C-20 and C-25); 1.242 (br, s, methyl protons at C-29); 1.985 (br, s, -OH proton at C-3); 1.017- 2.323 (different -CH_2- and >CH- protons).

^{13}C-NMR (100 MHz, in $CDCl_3$) Data

δ (ppm): 140.76, 121.74, 138.32 and 129.31 (olefinic carbons at C-5, C-6, C-22 and C-23); 42.29 and 36.53 (quarternary carbons at C-13 and C-10); 21.23, 21.10, 19.0, 19.41, 12.06 and 12.0 (methyl carbons at C-27, C-21, C-26, C-19, C-18 and C-29); 71.85 (>CH-OH carbon at C-5); 42.34, 39.71, 37.28, 31.93, 31.65, 28.92, 25.42, 23.10 and 21.10 (methylene carbons at C-4, C-12, C-1, C-7, C-2, C-16, C-28, C-15 and C-11); 56.89, 56.09, 51.26, 50.16, 40.49, 31.93, 31.65 (methine carbons at C-14, C-17, C-24, C-9, C-20, C-25 and C-8).

From the physical characteristics and spectral data FT-IR, ^{1}H-NMR and ^{13}C-NMR of the compound A and comparing with the published data of ^{1}H-NMR and ^{13}C-NMR of stigmasterol, the structure of the compound A was established as stigmasterol [15]. According to the literature review, though phytosterols have previously been isolated from other parts of *Polyalthia suberosa* but the isolation of stigmasterol as a medicinally active natural compound from the plant stems has not been reported earlier.

Stigmasterol has long been being investigated due to its pharmacological prospects such as cytotoxicity, antitumor, hypoglycemic, antimutagenic, antioxidant, anti-inflammatory and CNS effects [16, 17].

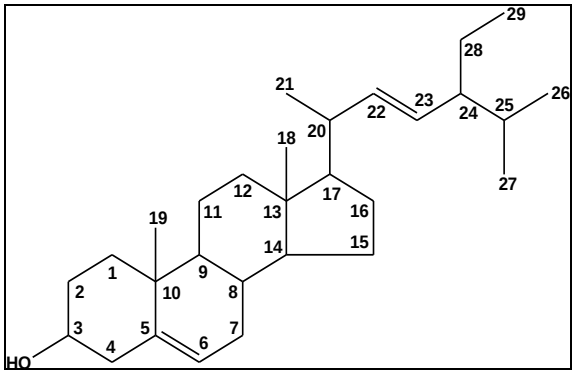

Fig 1: Structure of compound A as stigmasterol

Fatty acid analysis

The fatty acids of the plant were converted into their methyl esters and analyzed by GLC. The fatty acids present in the plant were identified and their relative percentages were determined (Table 1) by comparison with the retention time of the standard samples. The proportion of BFA (106 mg/100 g of dry plant powder) was found to be higher than the FFA (13 mg/100 g of dry plant powder) which indicated that only a small percentage of fatty acids existed as free state and the remaining fatty acids were associated with lipids or esterified with other organic compounds. The analysis of bound fatty acids showed that *Polyalthia suberosa* contains the highest proportion of oleic acid (53.24%), and the lowest proportion of arachidic acid (5.28%). Others as palmitic, stearic and behenic acids are present with an intermediate percentage of 24.70, 9.82 and 6.96%. Palmitic acid is a major saturated acid present in leaf lipids and also occurs in some seeds' oils like

palm oil [19]. Oleic acid is common in soybean, palm, corn, linseed, coconut, and cottonseed oil [19]. Stearic acid is found in animal fats [18].

The analysis of free fatty acids showed that arachidic acid is the most abundant (39.81%) fatty acid present in free form in the stems of *Polyalthia suberosa.* Palmitic, oleic and stearic acids are the other fatty acids present in free form with an intermediate percentage of 31.26, 12.87 and 16.06%. Except for oleic acid, all other fatty acids were found to be saturated.

Table 1: Amount and relative percentages of BFA and FFA isolated from *Polyalthia suberosa* stems

Amount of Petroleum ether extract*	Amount of Bound fatty acids (BFA)*	Amount of Free fatty acids (FFA)*	Name of fatty acids	Bound fatty acids (Relative percentages)	Free fatty acids (Relative percentages)
0.6	0.1061	0.013	Palmitic acid	24.70	31.26
			Oleic acid	53.24	12.87
			Stearic acid	9.82	16.06
			Arachidic acid	5.28	39.81
			Behenic acid	6.96	-

*Value expressed in g/100 g of dry powder

The fatty acid analysis of the plant stems of *Polyalthia suberosa* indicated that the percentage of saturated fatty acids was much higher compared to that of unsaturated one which resembles many edible oils [20]. Fatty acids have been found to be useful to lessen effects of allergies and autoimmune conditions, inflammation of arthritis, preventing atrophy, yeast infections, metastasis of certain cancers, heart problems and development of the retina and visual cortex [19]. From all of these studies, it can be concluded that this plant is beneficial to health both as diet and having bioactive properties. So further investigation on this plant may contribute more to the field of medicine.

Conclusion

Based on the results of present study and literature review, it can be concluded that the stems of *Polyalthia suberosa* Roxb. possess a remarkable amount of medicinally active compounds which are responsible for high bioactivity that can serve as a potential source of the drug. Hence, further phytochemical and pharmacological studies on this plant are necessary to be undertaken to understand the nature and activity of more drug active compounds in a much better way.

Acknowledgement

Authors are thankful to University Grants Commission of Bangladesh for necessary financial support for this work.

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