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Research Article

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Chemistry and bioactivity of compounds of genus *Schumacheria* and its close chemotaxonomic relationship to the genus *Dillenia*

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ABSTRACT

The extracts of different plant parts of Schumacheria were subjected to several chromatographic fractionations. These extracts yielded fifteen known compounds whose structures revealed that all three species contained taraxerol, betulinaldehyde, betulinic acid, β-sitosterol, 3-O-α-L-arabinosyloleanolic acid and β-sitosterol-3-O-β-D-glucopyranoside; the extracts of S. angustifolia and S. alnifolia gave betulin; betulonic acid, (6β)-6-hydroxy-3-oxolup-20(29)-en-28-oic acid, sorbifolin and epicatechin were only found in the extracts of S. castaneifolia. Kaempferol, 7-O-methylkaempferol, catechin and gallocatechin were isolated from the extracts of S. angustifolia. Bioactivity determination of these compounds revealed that (6β)-6-hydroxy-3-oxolup-20(29)-en-28-oic acid exhibited antibacterial activity against both S. aureus and E. coli; 3-O-α-L-arabinosyloleanolic acid showed antibacterial activity and toxicity to brine shrimps. The genus Dillenia was found to be closely related to the genus Schumacheria because of the presence of oleanene-type triterpenoids.

Keywords: *Schumacheria*, Antioxidant, Antibacterial, Antifungal, Cytotoxic, Oleanene-type triterpenoids.

INTRODUCTION

Sri Lanka is a plant biodiversity hot spot with 25 % of its flowering plants being endemic. The independent diversity of Sri Lankan flora in comparison to peninsular India has led to speculation that during the continental drift, Sri Lanka may have experienced a higher degree of impoverishment, which would have contributed to the facilitation of speciation of new taxa on the other hand. Among the lower plants such as lichens the recent reports of new species are being discovered frequently, indicating that their diversity may be as high as the higher plants [1, 2]. Sri Lankan plants have been tested for biological activity with promising results [3, 4]. In addition, the structural diversity among Sri Lankan higher and lower plants are typified by the discovery of naphthaquinones [5], butanolides [6], alkaloids, [7, 8], quinonemethide triterpenoids [9], compounds with iron chelating function [10, 11, 12, 13, 14], and phenolic acids and ketones [15, 16] possessing a variety of bioactivities.

Schumacheria is a Sri Lankan endemic plant genus belonging to the family Dilleniaceae and consist of three species, *S. castaneifolia* Vahl., *S. angustifolia* Hook.f. & Thomson and *S. alnifolia* Hook.f. & Thomson [17]. They are morphologically distinct and are distributed over the western slope of the southern montane rainforest [18]. They have evolved about 100 to 120 million years ago in Gondwanaland and are considered as relic plants [19].

The present study was carried out to investigate the chemistry and the bioactivity of the compounds isolated from the genus *Schumacheria* and also to determine the chemotaxonomic relationships of its three species with the other genera of the family Dilleniaceae.

EXPERIMENTAL SECTION

General conditions

Melting points of the isolated compounds were determined using a Stuart Scientific electrothermal melting point apparatus. UV spectral data was obtained using a UV-160, SHIMADZU UV-Visible spectrophotometer. FT-IR spectral data was obtained by an IR-Prestige-21(200VCE), SHIMADZU FT-IR Spectrophotometer on KBr pellets. The ^{1}H-NMR, ^{13}C-NMR, ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC, and ^{1}H-^{1}H t-ROECY spectral data were obtained using a VARIAN 600 MHz. Mass spectral data was obtained with electrospray ionization (ESI) method using a Escquire-LC_00085. Analytical thin layer chromatography (TLC) using silica gel 60 F_{254} MERCK on aluminium sheets and the preparative thin layer chromatography using silica gel 60 PF_{254} MERCK on glass plates were carried out. Medium pressure liquid chromatography, flash chromatography and gravity column were carried out using silica gel – 60 (0.040 – 0.063 mm) (230 – 400 mesh ASTM) MERCK.

As visualizing spray reagents for TLC, anisaldehyde reagent [glacial acetic acid (120 ml), H_2O (250 ml), anisaldehyde (6 ml) and conc. H_2SO_4 (9 ml)] was sprayed and heated. Antioxidant compounds of MPLC fractions were visualized on TLC using DPPH (1,1-diphenyl-2-picrylhydrazyl) 4.0×10^{-3} mol dm^{-3} in methanol.

Plant collection

S. castaneifolia were collected Illukkovita (Southern Province), *S. alnifolia* from Maskeliya (Central Province) and *S. angustifolia* from Hiniduma (Southern Province). The specimens have been deposited and each specimen was compared and confirmed the identification with the available specimens at the National Herbarium; Royal Botanical gardens; Peradeniya.

Extraction and fractionation

Leaves, stem-bark, root-bark and flowers were separately collected cleaned, air dried and ground. Then, each of them was separately and sequentially extracted into hexane, CH_2Cl_2 and methanol at 27 ºC. Each extract was evaporated at 30 ºC in vacuum to obtain the solid extracts. Each CH_2Cl_2 extract (10.0 g) was subjected to MPLC fractionation using a gradient solvent system starting from hexane and increasing the polarity gradually by mixing CH_2Cl_2 and methanol, until the polarity reached the CH_2Cl_2: methanol (9:1), to give several fractions. The methanol extracts (10 g) of *Schumacheria* were separately subjected to solvent partition with ethyl acetate: 10 % aqueous methanol and ethyl acetate portions (1.0 g) were subjected to size exclusion chromatography using Sephadex LH 20 and eluted with isocratic solvent system (ethyl acetate: methanol; 1:1) to isolate compounds.

Isolation of compounds

Isolation of taraxerol (1)

The 3rd combined fraction (53.0 mg), obtained from the MPLC of the CH_2Cl_2 extract of *S. castaneifolia* stem-bark was subjected to flash chromatography on silica-gel (60.0 g) using a gradient elution method starting with hexane to CH_2Cl_2 to give taraxerol (10 mg; 0.1 %). Similarly, taraxerol (7 mg; 0.06 %) was also isolated from the 1st combined MPLC fraction (20 mg) of the CH_2Cl_2 extract of stem-bark of *S. alnifolia* using identical chromatographic conditions. The obtained spectral data (^{1}H-NMR) were identical to those reported for taraxerol [20].

Isolation of betulinaldehyde (2) and β-sitosterol (3)

The 6th and 4th combined MPLC fraction obtained from the leaf (745.2 mg) and stem-bark (35.0 mg) CH_2Cl_2 extract of *S. castaneifolia* were subjected to flash chromatography separately on silica-gel (60.0 g) using a gradient elution method starting with hexane to CH_2Cl_2 to give betulinaldehyde (**2**) (70 mg; 0.7 %), and β-sitosterol (**3**) (78.2 mg; 0.8 %). Similarly, betulinaldehyde and β-sitosterol were isolated from the initial combined MPLC fractions of *S. angustifolia* and *S. alnifolia* leaf and stem-bark CH_2Cl_2 extracts using identical chromatographic techniques (Table 1). The obtained spectral data (^{1}H-NMR) were identical to those reported for betulinaldehyde [21]. Compound **3** exhibited identical R_f to an authentic sample of β-sitosterol upon Co-TLC. The obtained spectral data (^{1}H-NMR) were identical to those reported for β-sitosterol [22].

Isolation of betulonic acid (4)

The 5th combined fraction (105.0 mg) obtained from MPLC of the CH_2Cl_2 extract of stem-bark of *S. castaneifolia* was subjected to flash chromatography on silica-gel (60.0 g) using a gradient elution method starting with hexane to CH_2Cl_2 to give betulonic acid (**4**) (10 mg; 0.1 %). The obtained spectral data (^{1}H-NMR) were identical to those reported for betulonic acid (**4**) [22].

Isolation of betulin (5) and betulinic acid (6)

The 4th (1.5 g) and 7th (756 mg) combined fractions obtained from MPLC of the CH_2Cl_2 extract of leaf and stem-bark of *S. angustifolia* and 7th (3.2 g), 8th (1.2 g) and 4th (327 mg), 5th (1.5 g) fractions obtained from MPLC of the

CH_2Cl_2 extracts of leaf and stem-bark of *S. alnifolia* were separately subjected to flash chromatography on silica-gel (60.0 g) using a gradient elution method starting with hexane: dichloromethane (2:8) and the polarity was gradually increased up to methanol: dichloromethane (5:95) to give betulin (**5**) and betulinic acid (**6**) (Table 1).

Similarly, betulinic acid (**5**) was isolated from the 8th (2.3 g) and 6th (3.2 g) combined fraction obtained from MPLC of the CH_2Cl_2 extract of leaf and stem-bark of *S. castaneifolia* using identical chromatographic techniques (Table 1). The obtained spectral data (^{1}H-NMR) were identical to those reported for betulin (**5**) [23] and betulinic acid (**6**) [24].

Isolation of (6β)-6-hydroxy-3-oxolup-20(29)-en-28-oic acid (7)

The 9th combined fraction (317.6 mg) of leaves and 7th combined fraction (0.253 mg) of stem-bark obtained after the MPLC fractionation of CH_2Cl_2 extract of *S. castaneifolia* were subjected to flash chromatography separately on silica-gel (60.0 g) using a gradient elution method starting with dichloromethane to methanol:dichloromethane (5:95) to give (6β)-6-Hydroxy-3-oxolup-20(29)-en-28-oic acid (**7**) (Table 1). The obtained spectral data (^{13}C-NMR) were identical to those reported for (6β)-6-hydroxy-3-oxolup-20(29)-en-28-oic acid (**7**) [25].

Isolation of 3-O-α-L-arabinosyloleanolic acid (8)

The 10th combined fraction (519.0 mg) of leaves, 8th combined fraction (701.0 mg) of the stem-bark of *S. castaneifolia*, 6th combined fraction (112.0 mg) of leaves, 9th combined fraction (291.0 mg) of stem-bark of *S. angustifolia* and 9th combined fraction (600.0 mg) of leaves of *S. alnifolia* obtained after the MPLC fractionation of each CH_2Cl_2 extracts, were separately subjected to flash chromatography on silica-gel (60.0 g) using a gradient elution method starting with ethyl acetate to ethyl acetate:methanol (5:96) to give 3-O-α-L-arabinosyloleanolic acid (**8**) [26].

Isolation of β-sitosterol-3-O-β-D-glucopyranoside (9)

The 10th combined fraction (519.0 mg) of leaves, 7th combined fraction (74.0 mg) of leaves of *S. castaneifolia*, 10th combined fraction (56.0 mg) of stem-bark of *S. angustifolia* and 9th combined fraction (600.0 mg) of leaves of *S. alnifolia* obtained after the MPLC fractionation of each CH_2Cl_2 extracts, were separately subjected to flash chromatography on silica-gel (60.0 g) using a gradient elution method starting with ethyl acetate to ethyl acetate: methanol (5:96) to give β-sitosterol-3-O-β-D-glucopyranoside (**9**) (Table 1). The obtained spectral data (^{1}H-NMR) were identical to those reported for β-sitosterol-3-O-β-D-glucopyranoside (**9**) [27].

Isolation of sorbifolin (10)

The ethyl acetate portion (1.5 g) of leaf methanol extract (10.0 g) of *S. castaneifolia* after a solvent partition with 10 % aqueous methanol and ethyl acetate mixture (250.0 ml) was subjected to flash chromatography on silica-gel (60.0 g) using a isocratic solvent system of ethyl acetate: methanol (99:1) to give sorbifolin (**10**) (Table 1). The obtained spectral data (^{1}H-NMR) were identical to those reported sorbifolin (**10**) [28].

Isolation of catechin (11), epicatechin (12), gallocatechin (13), 7-O-methylkaempferol (14) and kaempferol (15)

The ethyl acetate portion (1.0 g) of flower methanol extract (10.0 g) of *S. castaneifolia* was dissolved in minimum amount of ethyl acetate: methanol (1:1) and fractionated using an isocratic solvent system (ethyl acetate: methanol; 1:1) in a Sephadex LH20 column (height = 30.0 cm, diameter = 2.5 cm). Obtained fractions were combined according to the TLC patterns after visualizing with anisaldehyde. The second combined fraction was subjected to further fractionations using the same column and solvent system to give catechin (**11**) and epicatechin (**12**) mixture (1:4) (The ^{1}H-NMR analysis confirmed the catechin and epicatechin ratio in the mixture by giving 1:4 on proton signals). Similarly, catechin (**11**), gallocatechin (**13**), 7-O-methylkaempferol (**14**) and kaempferol (**15**) were also isolated from the ethyl acetate portion (0.9 g) of flower methanol extract (10.0 g) of *S. angustifolia*, using identical chromatographic techniques (Table 1). The obtained spectral data (^{1}H-NMR) were identical to those reported catechin (**11**), epicatechin (**12**) [29], gallocatechin (**13**) [30], 7-O-methylkaempferol (**14**) [31] and kaempferol (**15**) [32].

Bioassay on compounds

Isolated compounds of the genus *Schumacheria* were separately subjected to bioassays. The antioxidant activity was determined using DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging method and the antioxidant activity of the compounds were expressed as the IC_{50} values as described by Budzianowski *et al* (2006) [33].

Cytotoxic activity of each compound was determined using the brine shrimp assay as mentioned in Rahman *et al*. (2005) [34]. The LC_{50} value of each compound was determined with 95 % confidence intervals using the software Minitab®16. As positive control (4*S*)-4-methyl-2-(11-dodecynyl)-2-butenolide [35] isolated from the genus *Hortonia* was carried out and the negative control was carried out with DMSO (1 % v/v) in seawater.

Compounds isolated from genus *Schumacheria* was screened against *Staphylococcus aureus*, (NCTC 8532) and *Escherichia coli* (NCTC 10418) and also against the fungal strain, *Aspergillus niger* (wild type) to determine the antimicrobial activity. The determination of antifungal susceptibility was carried out using agar well diffusion method [36], and the antibacterial activity was determined using the pour plate method [37] with a concentration series range from 100, 75, 50, 25 and 10 ppm by dissolving the compounds in dimethylformamide (DMF) (1 mg/ml). Data were expressed as minimum inhibitory concentration (MIC) in ppm.

RESULTS AND DISCUSSION

Compounds isolated from the different plant parts of *Schumacheria* were evaluated for cytotoxic, antioxidant, antibacterial and antifungal activities. Compounds (**2**), (**4**), (**5**), (**6**) and (**7**) showed only moderate activity in cytotoxicity and the antioxidant assays and in both assays. Betulinaldehyde (**2**) and betulonic acid (**4**) exhibited antibacterial activity with a MIC of 100 ppm against *S. aureus* but did not show any activity against *E. coli* and the *A. niger*. Betulin (**5**) exhibited antibacterial activity against *S. aureus* (MIC 75 ppm); it also exhibited activity against *E. coli* (MIC 100 ppm). Betulinic acid (**6**), however, did not show any antibacterial activity. (6β)-6-hydroxy-3-oxolup-20(29)-en-28-oic acid (**7**) exhibited the highest antibacterial activity against *S. aureus* and *E. coli* with a MIC at 50 ppm.

The highest cytotoxic activity was exhibited by 3-O-α-L-arabinosyloleanolic acid (**8**) giving a LC_{50} value of 7.6 ± 0.6 ppm in the brine shrimp assay; it also exhibited antibacterial activity (MIC 75 ppm) against *S. aureus* and *E coli* (MIC 100 ppm) with no considerable activities in the antioxidant assay and antifungal assays. β-Sitosterol-3-O-β-D-glucopyranoside (**9**) only exhibited antibacterial activity against *S. aureus* with a MIC value of 100 ppm and the sorbifolin (**10**), on the other hand, exhibited antioxidant activity giving IC_{50} value at 187.2 ± 75.9 ppm. The mixture of catechin: epicatechin (**11** and **12**) (1:4) exhibited the highest antioxidant activity (IC_{50} 3.7 ± 0.1 ppm).

In Dilleniaceae, the genus *Dillenia* has been reported to be genetically closest to *Schumacheria* [37]. Chemotaxonomically the most important finding is the presence of the oleanene-type triterpenoid 3-O-α-L-arabinosyloleanolic in the genus *Schumacheria*. In Dilleniaceae, oleanene-type triterpenoids and *seco*-A-ring triterpenoids with oleanene based structures have been reported only in genus *Dillenia: Dillenia papuana* Martelli [39], *Dillenia serrata* Thunb. [40], *Dilenia philippinensis* Rolfe [41, 42].

CONCLUSION

Phytochemically, *S. castaneifolia* deviated from *S. alnifolia* and the *S. angustifolia*. Although taraxerol (**1**), betulinaldehyde (**2**), β-sitosterol (**3**), betulinic acid (**5**), 3-O-α-L-arabinosyloleanolic acid (**8**) and β-sitosterol-3-O-β-D-glucopyranoside (**9**) were found in all three species of *Schumacheria*, betulonic acid (**4**), (6β)-6-hydroxy-3-oxolup-20(29)-en-28-oic acid (**7**) was only found in *S. castaneifolia*. On the other hand, betulin (**5**) was found only in *S. angustifolia* and *S. alnifolia.* Sorbifolin (**10**), catechin (**11**) and epicatechin (**12**) were found in the methanol extract of *S. castaneifolia* flowers and catechin (**11**), gallocatechin (**13**) and 7-O-methylkaempferol (**14**) were found in the methanol extract of *S. angustifolia* flowers. The highest antibacterial compound (6β)-6-hydroxy-3-oxolup-20(29)-en-28-oic acid (**7**) was isolated from the extracts of *S. castaneifolia* which gave a MIC of 50 ppm against both *S. aureus* and *E. coli*. The highest cytotoxic activity was exhibited by the 3-O-α-L-arabinosyloleanolic acid (**8**) (LC_{50} 7.6 ± 0.6 ppm); it also exhibited moderate antibacterial activity (MIC 75 ppm) against *S. aureus* and *E. coli* (MIC 100 ppm) with no considerable activities in the antioxidant assay and antifungal assays. The presence of betulinic acid and derivatives are common to the family Dilleniaceae and they are also found in all the three species of *Schumacheria*. The isolation of taraxerol, (6β)-6-hydroxy-3-oxolup-20(29)-en-28-oic acid (**7**), 3-O-α-L-arabinosyloleanolic acid (**8**) and β-sitosterol-3-O-β-D-glucopyranoside (**9**) constitute the first report of these compounds in the family Dilleniaceae. The oleanilic acid derivatives found in the family Dilleniaceae are restricted to the genus *Dillenia.* The isolation of oleanene type triterpenoid 3-O-α-L-arabinosyloleanolic acid (**8**) in all three species of *Schumacheria* showed close relationship with the genus *Dillenia.* Interestingly, the potent bioactivity of the *S. castaneifolia* flower extracts corroborates with the ethnopharmacological claims that the use of its flower extracts can cure oral aphthous; in addition, leaves of *S. castaneifolia* is used as a treatment for snake bites in ethnomedicine [43].

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Table 1. The isolated compounds, amounts and their (w/w) percentages respect to the dry weight of each plant parts of *Schumacheria*.

Plant species	*S. castaneifolia*				*S. angustifolia*			*S. alnifolia*	
Plant part	Flowers	Leaf		Stem-bark	Flowers	Leaf	Stem-bark	Leaf	Stem-bark
Extract	MeOH	MeOH	CH_2Cl_2	CH_2Cl_2	MeOH	CH_2Cl_2	CH_2Cl_2	CH_2Cl_2	CH_2Cl_2
Compound	Isolated amounts (mg) (w/w %, respect to the weight of the dry plan parts)								
Taraxerol (**1**)	-	-	-	10(0.002)	-	-	+	-	7(0.001)
Betulinaldehyde (**2**)	-	-	70(0.01)	12(0.002)	-	13(0.009)	7(0.003)	79(0.01)	70(0.01)
β-Sitosterol (**3**)	-	-	78(0.01)	+	-	20(0.01)	17(0.006)	276(0.05)	70.4(0.01)
Betulonic acid (**4**)	-	-	+	10(0.002)	-	-	-	-	-
Betulin (**5**)	-	-	-	-	-	12(0.008)	47(0.02)	51(0.009)	79(0.01)
Betulinic acid (**6**)	-	-	1724(0.29)	950(0.16)	-	175(0.11)	126(0.05)	539(0.10)	1269(0.23)
(6β)-6-Hydroxy-3-oxolup-20(29)-en-28-oic acid (**7**)	-	-	56(0.01)	47(0.008)	-	-	-	-	-
3-O-α-L-Arabinosyloleanolic acid (**8**)	-	-	233(0.04)	227(0.04)	-	38(0.02)	40(0.01)	150(0.03)	+
β-Sitosterol-3-O-β-D-glucopyranoside (**9**)	-	-	102(0.02)	+	-	18(0.01)	17(0.006)	92(0.02)	+
Sorbifolin (**10**)	+	10(0.01)	-	-	-	-	-	-	-
Catechin (**11**)	300(0.40) Mixture	+	-	-	160(0.30)	-	-	-	-
Epicatechin (**12**)		+	-	-	-	-	-	-	-
Gallocatechin (**13**)	-	-	-	-	136(0.25)	-	-	-	-
7-O-Methylkaempferol (**14**)	-	-	-	-	69(0.13)	-	-	-	-
Kaempferol (**15**)	-	-	-	-	78(0.14)	-	-	-	-

"-" Not isolated and absence on TLC; "+" Presence on TLC and not isolated