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Phytochemical Investigation of the Rhizomes of *Sansevieria Roxburghiana*

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ABSTRACT

The present study reports the phytochemical investigation of the rhizomes of *Sansevieria roxburghiana* belonging to the family Dracanaceae. Nine phytoconstituents have been reported namely Palmitic acid, 6,4-dihydroxy-3-propen chalcones, 4-propenoxy-7-hydroxyanthocyanidines, Caftaric acid, Isorhamnetin-3-O-β-D-glucopyranoside, Di-(2-ethylhexyl) phthalate, Buphanidrine, Gallic acid and Di-isobutyl phthalate. These isolated compounds are reported for the first time from the rhizomes of *Sansevieria roxburghiana.*

Keywords: *Sansevieria roxburghiana,* Buphanidrine, Dracanaceae

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INTRODUCTION

Sansevieria roxburghiana is a herbaceous perennial perennial plant with short fleshy stem and stout root stock, occurring in eastern coastal region of India belonging to the family Dracanaceae. It is commonly referred to as bowstring hemp, piles root and Manjinanaru in Kannada (Vernacular)[1]. *Sansevierians* are popular garden or indoor plants with long rhizomes and fibrous roots having ability to flourish under low light conditions and requiring mineral attention[2]. A number of spices such as *Sansevieria cylindrical, Sansevieria ehrenbergii, Sansevieria guineensis, Sansevieria longiflora, Sansevieria roxburghiana, Sansevieria trifasciata and Sansevieria zeylanica* are grown as ornamental plants[3]. Extensive literature survey revealed that many phytoconstituents like Alkaloids, Triterpenoids, Steroids, Phthalates, Flavonoids, saponins and mucilage from the whole plant, leaves and other species of *Sansevieria*.[4-9] It was also learnt that no substantial work on rhizomes of *S. roxburghiana* was carried out. Hence an effect was made to investigate the phytoconstituents from the ethanolic extract of the rhizomes of *S. roxburghiana*.

MATERIALS AND METHODS

All melting points were recorded using Biotechnics India apparatus (Model No: BTI-38) and were uncorrected. IR spectra of the compounds were recorded using KBr pellet method on, Bruker α- T apparatus at SET'S College of Pharmacy, Darwad. ^{1}HNMR (200MHz) spectra of the compounds were carried out on Amx-200 liquid state PMR spectroscopy using $CDCl_3$ as a solvent at AstraZeneca Pharma India Ltd, Bangalore. Mass spectra were recorded on ECMS 2010 PLUSH 200 MHz Shimadzu at RL FINE CHEM UNIT-II, Bangalore. TLC was carried out using Aluchrosep Silica Gel 60/UV254 from S.D. Fine Chemicals Private Limited, Mumbai. Column Chromatography was carried out using Glass Column with Glass stopcock, 30 x 600 mm from Merck Private Limited, Mumbai. All the chemicals and reagents used were obtained from either S.D. Fine Chemicals Private Limited, Bombay, India or Molychem, Mumbai.

Collection and Authentification:

The rhizomes of *S. roxburghiana* were collected from Gomantak Ayurveda Mahavidyalaya and Research Centre, Shiroda, Goa during November 2011. The plant sample was authenticated by Prof. G. I. Hukkeri, Associate Professor in Botany, Dhempe College of Arts and Science, Miramar, Panaji, Goa.[10]

Preparation of ethanolic extract:

The rhizomes of *S. roxburghiana* were collected, washed and dried in shade. The dried rhizomes

were powdered (400 gm) and exhaustively extracted by maceration with ethanol (95%) for three days. After three days, ethanol layer was decanted off. The process was repeated for three times. The solvent from the total extract was distilled off and the concentrate was evaporated to a syrupy consistency using rotary vacuum evaporator (25 rpm; 60°C) and then evaporated to dryness (22.45g).[11]

Preliminary phytochemical screening

Preliminary Phytochemical studies were performed for testing the different phytoconstituents present in the ethanolic extracts. The tests were performed & results are tabulated in table 1.[12,13]

Table 1: Result of qualitative tests for phytoconstituents

Sr.No	Tests	Inference
1	Alkaloids	+ve
2	Glycosides	+ve
3	Carbohydrates	-ve
4	Flavonoids	+ve
5	Proteins	+ve
6	Tannins	+ve
7	Resins	-ve
8	Saponins	+ve
9	Triterpenoids	+ve
10	Steroids	+ve

Isolation of phytoconstituents

The ethanolic extract (12g) was dissolved in chloroform (10ml) and adsorbed onto silica (15g) after evaporation of the solvent it was loaded onto a silica gel column (200-400 mesh, 170g). The column was first eluted with 100% petroleum ether (60-80°C), followed by petroleum ether (60-80°C): chloroform graded mixtures (95:5, 90:10, 80:20, 70:30, 60:40 and 50:50), then with 100% chloroform followed by graded mixtures of chloroform: ethyl acetate (95:5, 90:10, 80:20, 70:30, 60:40 and 50:50), 100% ethyl acetate and finally by graded mixtures of ethyl acetate: methanol(99:1, 98:2, 97:3, 96:4, 95:5 and 90:10).

The elutions were monitored by TLC (Silica gel-G; visualization by vanillin-sulphuric acid reagent heated at 110°C). Each time 10ml were collected and identical elutes (TLC monitored) were combined and concentrated to 5ml and kept aside.

Elution carried out with chloroform (100%) resulted a single spot on TLC ($CHCl_3$, 100%). After removing the solvent a white oily liquid resulted, which was designated as compound 1 (50mg).

Elution carried out with chloroform: ethyl acetate (95:5) resulted a single spot on TLC (chloroform: ethyl acetate, 95:5). After removing the solvent a yellow orange powder resulted, the product was designated as compound 2(190mg).

Elution carried out with chloroform: ethyl acetate (70:30) resulted a single spot on TLC (chloroform: ethyl acetate, 70:30). After removing the solvent a yellow powder resulted, the product was designated compound 3 (200 mg).

Elution carried out with chloroform: ethyl acetate (60:40) resulted a single spot on TLC (chloroform: ethyl acetate, 60:40). After removing the solvent a yellowish white liquid resulted, the product was designated as compound 4 (200 mg).

Elution carried out with chloroform: ethyl acetate (50:50) resulted a single spot on TLC (chloroform: ethyl acetate, 50:50). After removing the solvent a yellow amorphous powder resulted, the product was designated as compound 5 (180 mg).

Elution carried out with ethyl acetate: methanol (99:1) resulted a single spot on TLC (ethyl acetate: methanol, 99:1). After removing the solvent a yellow viscous liquid resulted, the product was designated as compound 6 (50 mg).

Elution carried out with ethyl acetate: methanol (96:4) resulted a single spot on TLC (ethyl acetate: methanol, 96:4). After removing the solvent brown flakes resulted, the product was designated as compound 7 (500mg).

Elution carried out with ethyl acetate: methanol (95:5) resulted a single spot on TLC (ethyl acetate: methanol, 95:5). After removing the solvent a yellowish white solid resulted, the product was designated as compound 8 (155 mg).

Elution carried out with ethyl acetate: methanol (90:10) resulted a single spot on TLC (ethyl acetate: methanol, 90:10). After removing the solvent a yellowish viscous liquid resulted, the product was designated as compound 9 (145 mg).

The concentration of other elutes gave only brown resinous masses, which were not processed further.[10]

RESULTS AND DISCUSSIONS

Phytochemical screening of the ethanolic extract of *S. roxburghiana* led to the presence of phytoconstituents like Alkaloids, Glycosides, Flavonoids, Proteins, Tannins, Saponins, Triterpenoids and Steroids.

The chemical investigation led to the isolation of nine compounds from the ethanolic extract of rhizomes of *S. roxburghiana.* The isolated components are Palmitic acid, 6,4-dihydroxy-3-propen chalcones, 4-propenoxy-7-hydroxyanthocyanidines, Caftaric acid, Isorhamnetin-3-O-β-D-glucopyranoside, Bis-(2-ethylhexyl) phthalate, Buphanidrine, Gallic acid and Di-isobutyl phthalate.

Compound 1(Palmitic acid): m.p.: 62°C**;** IR (KBr): 3410.38 cm^{-1}(br, OH), 2924.52 cm^{-1} (C-H str. of CH_3), 2856.69 cm^{-1} (C-H str. of CH_2), 1721.26 cm^{-1} (C=O str.), 1456.13 cm^{-1} (C-H deformation in CH_3), 1029.85 cm^{-1}(C-H deformation in CH_2), ^{1}HNMR ($CDCl_3$): δ 0.85 to δ 0.97 (m,3H, terminal methyl group), δ1.25 to δ1.56 (m, 26H, 13XCH_2), δ2.03 (s, 2H, 1xCH_2).The GC-MS spectra showed molecular ion peak at m/z 256 (M^+) which was consistent with molecular formula $C_{16}H_{32}O_2$.

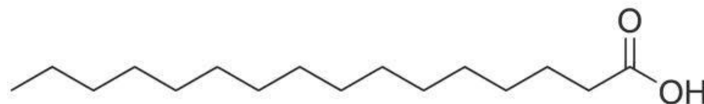

Figure 1: Palmitic acid

Compound 2 (6,4-dihydroxy-3-propen chalcones): IR (KBr):3361.90 cm^{-1}(br, OH), 2939.70 cm^{-1} (C-H str. of CH_3), 2835.31 cm^{-1} (C-H str. of CH_2), 1722.18 cm^{-1} (C=O str.), 1656.36 cm^{-1} (C=C str.); ^{1}HNMR ($CDCl_3$): δ7.713 (S, 1H, H-α) , δ7.696 (d,1H, H-β), δ 7.687 (s, 5H, H-2, H-2', H-6'), δ7.532 (d, 1H, H-4), δ7.548 (m, 1H, H-5), δ5.344 (s, 1H, OH), δ7.504 (m, 2H, H-3', H-5'), δ5.367 (s, 1H, OH), δ4.340 (d, 1H, H-7'), δ4.308 (d, 1H, H-8'), δ1.26 (s, 1H, H-9')**.** The GC-MS spectra showed molecular ion peak at m/z 280 (M^+) which was consistent with molecular formula $C_{18}H_{16}O_3$. In the mass spectra, a loss of a proton from the parent ion produces the peak at m/z 279 for $C_{18}H_{15}O_3$ due to proton migration. Further the compound undergoes α – cleavage from the carbonyl to produce the base peak at m/z 149($C_9H_9O_2$).[14]

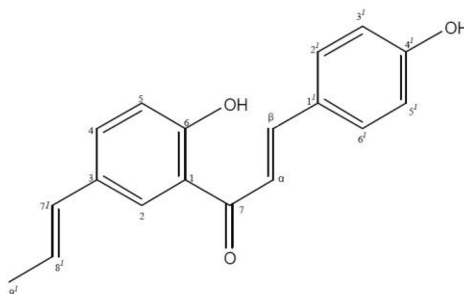

Figure 2: 6,4-dihydroxy-3-propen chalcones

Compound 3(4' propenoxy 7-hydroxy anthocyanidines): IR (KBr): 3349.57 cm^{-1}(br, OH), 2941.32 cm^{-1} (C-H str. of CH_3), 2832.64 cm^{-1} (C-H str. of CH_2), 1654.08 cm^{-1} (C=C str.), 1028.70 cm^{-1} (C-O str.); ^{1}HNMR ($CDCl_3$): δ 7.522 (m, 2H, H-3, H-8) ; δ7.532 (m,2H, H-5, H-6); δ 7.547 (m, 2H, H-6', H-2'); δ7.698 (m, 6H, H-3', H-5'); δ4.274 (s, 1H,H-8'); δ4.206 (s, 1H,H-7'); δ1.257 (d, 2H, H-4); δ1.328 (d, 3H,H-9'). The GC-MS spectra showed molecular ion peak at m/z 280 (M^+) which was consistent with molecular formula $C_{18}H_{16}O_3$. In the mass spectra, a loss of a proton from the parent ion produces the peak at m/z 279 for $C_{18}H_{15}O_3$, while detachment of

the propenoxy group gives the peak at m/z 56 for C_3H_5O. Fragmentation of the flavonoid heterocyclic nucleus with the hydroxyl group produces the base peak at m/z 149 for $C_9H_9O_2$.[14]

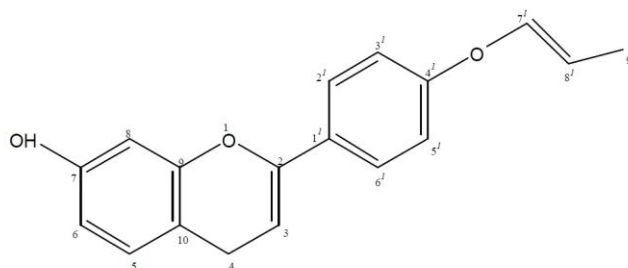

Figure 3: 4' propenoxy 7-hydroxy anthocyanidines

Compound 4(Caftaric acid): b.p.:615°C; IR (KBr): 3387.33 cm^{-1}(br, OH); 2923.91 cm^{-1} (C-H str.of CH_3); 2858.84 cm^{-1} (C-H str.of CH_2); 1723.83 cm^{-1} (C=O str.); 1456.70 cm^{-1} (C=C str.); 1076.80 cm^{-1}(C-O str.); ^{1}HNMR ($CDCl_3$): δ 7.69 (s, 1H,H-7'); δ 7.19(s,1H, H-5'); δ 7.15(s, 1H, H-6'); δ 7.14(s, 1H, H-2'); δ 7.11(s, 1H, H-8'); δ 4.21 to δ 4.31(m,2H H-2,H-3); δ 4.07 to δ 4.10(d, 5H, OH). The GC-MS spectra showed molecular ion peak at 311 $(M-H)^-$ which was consistent with molecular formula $C_{13}H_{12}O_9$. In mass spectra the fragment of caffeic acid gave peak at m/z 177 ($C_9H_8O_4$) and tartaric acid gave fragment ion peak at m/z 149 ($C_4H_6O_6$) which was also the characteristics base peak for caftaric acid. In addition, there was a low signal produced by the decarboxylation of caffeic acid at m/z 135. [15,16]

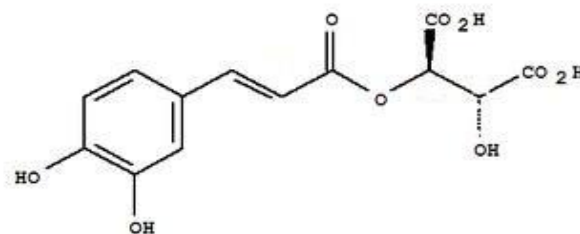

Figure 4: Caftaric acid

Compound 5 (Isorhamnetin-3-o-β-d-glucopyranoside): b.p.: 268°C; IR (KBr): b.p.; 3387.33 cm^{-1}(br, OH); 2923.91 cm^{-1} (C-H str.of CH_3); 2858.84 cm^{-1} (C-H str.of CH_2); 1723.83 cm^{-1} (C=O str.); 1456.70 cm^{-1} (C=C str.); 1076.80 cm^{-1}(C-O str.);^{1}HNMR ($CDCl_3$): δ 7.11 to δ7.70 (m, 4H,H-8,H-6, H-2', H-6'); δ 4.07 to δ 4.34 (m, 7H,OH); δ 3.94 (s, 3H, $-OCH_3$); δ 2.02 to δ 2.38 (s, 2H, H-2, H-3); δ 3.70 to δ 3.88 (m, 5H, H-2'',H-3'', H-4'', H-5'', H-6''); δ 3.67 (s,2H, H-7). The GC-MS spectra showed molecular ion peak at m/z 479 (M^+) which was consistent with molecular formula $C_{22}H_{23}O_{12}$. In the mass spectra loss of a glucose molecule from the parent ion produces the peak at m/z 316 corresponding to isorhamnetin, while fragment ion peaks at m/z 151 and m/z 107 were due to ring a and a*of isorhamnetin.[17]

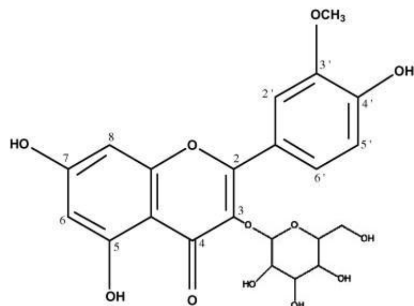

Figure 5: Isorhamnetin-3-o-β-d-glucopyranoside

Compound 6 (Bis(2-ethylhexyl)phthalate): b.p.: 320°C; IR (KBr): 2923.80 cm^{-1} (C-H str.of CH_3); 2857.80 cm^{-1} (C-H str.of CH_2); 1724.80 cm^{-1} (C=O str.); 1409.28 cm^{-1} (methylene C-H bending); 1126.84 cm^{-1} (C-O str.); ^{1}HNMR ($CDCl_3$): δ 7.739 (m, 2H,H-1,H-4); δ 7.712 (m, 2H,H-6,H-5); δ 2.347 (t,4H, H-1', H-1''); δ 2.311 (t, 2H, H-2', H-2''); δ 4.230 (d, 4H, H-7', H-7''); δ 1.257 to δ 1.455 (m, 12H, H-3', H-4', H-5', H-3'', H-4'', H-5''); δ 0.896 (m, 12H, H-6', H-8', H-6'', H-8''). The GC-MS spectra showed molecular ion peak at 391 $(M+H)^+$ which was consistent with molecular formula $C_{24}H_{38}O_4$. The mass spectra showed the fragment ion peaks at m/z 149 and 167 characteristic of alkyl phthalates and fragment ions at m/z 279 and 261 characteristic of mono-(2-ethylhexyl)phthalate and phthalic acid respectively suggested that the alkyl phthalate is bis-(2-ethylhexyl) phthalate. The base peak is the protonated phthalic anhydride ($C_8H_5O_3$) m/z 149.[18,19]

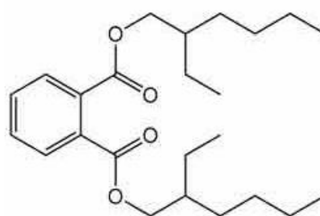

Figure 6: Bis(2-ethylhexyl)phthalate

Compound 7(Buphanidrine): IR (KBr): 3446.64 cm^{-1} (N-H str); 1716.63 cm^{-1} (C=O str); 1585.73 cm^{-1} (C=C str); 1451.25 cm^{-1} (C=C str); 1294.67 cm^{-1} (C-O str); 1037.81 cm^{-1} (C-N Vibration); ^{1}HNMR ($CDCl_3$): δ 6.569 (s, 1H, H-10); δ 6.614 to δ 6.798 (m, 2H, H-1, H-2); δ 4.074 to δ 4.163 (m, 6H, H-6α, H-6β, H-4 α, H-4β, H-3, H-4a); δ 3.964 (d, 6H, -OCH3); δ 4.184 (s, 2H, epoxy); δ 2.779 (s,1H, H-12 endo); δ 2.634 (s, 1H, H-12 exo); δ 2.247 (s, 1H, H-11 exo); δ 1.993 (s, 1H, H-11 endo). The GC-MS spectra showed molecular ion peak at 316 $(M+H)^+$ which was consistent with molecular formula of $C_{18}H_{21}NO_4$. The fragment due to the loss of methoxy group from the parent molecule is of Buphanisine m/z 285 is indicative and considered characteristic of Buphanidrine.[20]

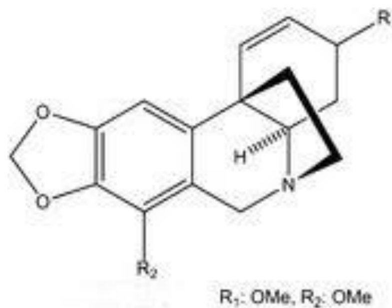

Figure 7: Buphanidrine

Compound 8 (Gallic acid): m.p. : 248°C; IR (KBr): 3402.71 cm^{-1} (br, OH); 1724.80 cm^{-1} (C=O str); 1600.88 cm^{-1} (C=C str); 1456.23 cm^{-1} (C=C str); 1280.46 cm^{-1} (C-O str); ^{1}HNMR ($CDCl_3$): δ 7.68 to δ 7.73 (m, 3H, H-1, H-2, H-6); δ4.23 TO δ 4.20 (t, 3H, OH). The GC-MS spectra showed molecular ion peak at m/z 169 $(M-H)^-$ which was consistent with molecular formula $C_7H_6O_5$. The mass spectra of gallic acid produces fragment ion peak at m/z 125 due to the loss of CO_2 from the parent ion. This pattern of fragmentation was characteristic feature of hydroxybenzoic acid derivatives like other phenolic acids.[21]

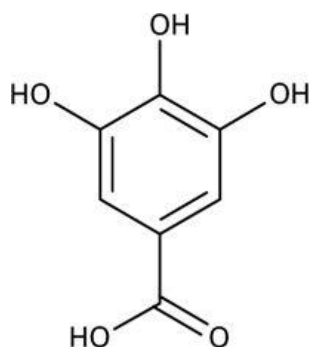

Figure 8: Gallic acid

Compound 9 (Di isobutylphthalate): b.p.:320°C; IR (KBr): 2923.75 cm^{-1} (C-H str. of CH_3); 2857.62 cm^{-1} (C-H str. of CH_2); 1724.80 cm^{-1} (C=O str.); 1600.88 cm^{-1} (C=C str); 1456.23 cm^{-1} (C-H deformation in CH3); 1280.46 cm^{-1} (C-O str); 1075.70 cm^{-1} (C-H deformation in CH2); 856.69 cm^{-1} (C-H Bending of Ar.); ^{1}HNMR ($CDCl_3$): δ 7.71 (m, 2H,H-1,H-6); δ 7.52 (m, 2H,H-4,H-5); δ 4.07 (t,4H, H-1, H-1'); δ 2.31 (t, 2H, H-2, H-2''); δ 0.97 to 1.64 (m, 12H, H-3', H-4', H-3'', H-4''). The GC-MS spectra showed molecular ion peak at 279$(M+H)^+$ which was consistent with molecular formula $C_{16}H_{22}O_4$.The mass spectra showed fragment ion peaks indicated at m/z 149 and 167, are considered characteristic of alkyl phthalates and used in their characterization. The ions at m/z 223 suggested that the alkyl phthalate is Di isobutyl phthalate. The ion peak at 149 is due to the protonated phthalic anhydride ($C_8H_5O_3$).[22]

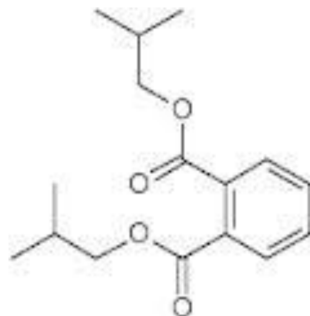

Figure 9: Di isobutylphthalate

CONCLUSION

The chemical investigation led to the isolation of nine compounds from the ethanolic extract of rhizomes of *S. roxburghiana*. The isolated components are Palmitic acid, 6,4-dihydroxy-3-propen chalcones, 4-propenoxy-7-hydroxyanthocyanidines, Caftaric acid, Isorhamnetin-3-O-β-D-glucopyranoside, Di-(2-ethylhexyl) phthalate, Buphanidrine, Gallic acid and Di-isobutyl phthalate. The constituents isolated and characterized from the rhizomes of *S. roxburghiana* can be categorized under Fatty acid, Tannins, Flavonoids (Flavonol Glycosides, chalcones and Anthocyanidines), Phenolic compounds, Phthalates and an Alkaloids. The above compounds were isolated for the first time from the rhizome S. *roxburghiana,* however only phthalates were previously reported from the leaves of *S. roxburghiana.*[5]

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