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RESEARCH ARTICLE

ISOLATION AND CHARACTERIZATION OF CONSTITUENTS FROM ETHANOLIC EXTRACT OF *SCINDAPSUS OFFICINALIS* (ROXB.) SCHOTT. FRUITS

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Nature has provided abundant plant wealth for living creatures which possess medicinal virtues. The essential value of a number of plants has long been published and the large numbers of them remain unexplored so far. In the present study, 50% ethanolic extract of fruits of plant *Scindapsus officinalis* (Roxb.) Schott. (Family - Araceae), was purified by column chromatography which yielded three brownish sticky residues *viz.* compound A, B and C. Extracted compounds were further subjected to spectral analysis *i.e.* IR, UV, 1D NMR (^{1}H NMR and ^{13}C NMR) and Mass spectroscopy for structure elucidation and characterization. The findings revealed that the compound A, B and C are piperine, mixture of glycerin and ascorbic acid, and ascorbic acid respectively. Isolation of glycerol and ascorbic acid is a new finding in the present study.

Key words: *Scindapsus officinalis* (Roxb.) Schott, Spectral analysis, Piperine, Glycerine, Ascorbic acid.

INTRODUCTION

Ayurveda stresses on the use of vegetable drugs. Plants are being used as medicine since ancient times (Ajithabai *et al* 2012; Visht and Chaturvedi, 2012). Plants used for traditional medicine, contain a wide range of bioactive constituents that can be used to treat chronic as well as infectious diseases (Duraipandiyan *et al* 2006; Jain *et al* 2011; Dey *et al* 2012; Jain and Argal, 2013; Deb *et al* 2013; Arjariya and Nema, 2014). Extraction and isolation of the bioactive plant constituents has been a challenging job for the researchers. Now a day, the researchers are emphasizing on evaluation and characterization of various plants and plant constituents against a number of diseases based on the traditional claims of the plants given in Ayurveda (Tiwari *et al* 2011). *Scindapsus officinalis* (Roxb.) Schott. (Family - Araceae) is known as Bari-pipli or Gaj-pipli in Hindi and Anaittippilli in Tamil. The plant is a large, stout, epiphytic and perennial climber with adventitious aerial roots growing on trees and rocks (Kirtikar and Basu, 1933; Chatterjee and Pakrashi, 2001). Fruit is very important part of the plant and used in the management of a variety of ailments in both Ayurvedic and Unani system of medicine (**Figure 1**, Nadkarni, 2001; Voigt, 1984).

Fig. 1. Shade dried fruit of *Scindapsus officinalis*

The folklore claims of fruit are diaphoretic, carminative, stimulant, tonic, anthelmintic, aphrodisiac, galactagogue, expectorant, anti-protozoal, appetizer, antidiarrhoeal, anti-diabetic, anticancer, sharpening hearing, cardio tonic and regulating the bowel. It is also used in dysentery, asthma, troubles of the throat, asthma, rheumatism, worm infestations, pharyngopathy, helminthiasis and bronchitis (Kirtikar and Basu, 1999; Nair, 2004; Nadkarni and Nadkarni, 1976). Plant is common in the Midnapore district of West Bengal and cultivated vegetatively for its fruit. A thorough survey of literature reveals that purification of 50% ethanolic extract of fruits of *Scindapsus officinalis* by column chromatography and characterization of purified compounds has not been studied yet. This fact justifies our interest in present study.

MATERIALS AND METHODS

Plant material

The fruits of *Scindapsus Officinalis* (**Figure 1**) were collected from the market [K. Ramaswamy Chetty (KRC), Country Drugs Dealer, Whole sale and retail, Shop No. 117, Rasappa Chetty Street, Park Town, Chennai, India] and authenticated by Dr. P. Jayaraman (Director, Plant Anatomy Research Centre, Chennai). A voucher specimen (No. - PARC/2009/363) has been deposited for further reference.

Extraction procedure

The fruits were shade dried and coarsely powdered. About 300 g powdered drug was extracted successively by cold maceration method with different solvents of increasing polarity *i.e.* hexane, chloroform, ethyl acetate and 50% ethanol. After 72 h of maceration, it was filtered. The marc was dried each time before extraction with next solvent. After complete extraction, the extracts were concentrated by distilling off the solvent and then evaporated to dryness on water bath. Colours of the extracts were observed and percentage yields were calculated on the air-dried basis (Kokate, 1994; Harborne, 1973).

Chromatographic separation

Wet packing was followed for packing of column. The adsorbent was mixed with the mobile phase in a beaker and packed into column. The stationary phase was settled uniform in the column. There was no entrapment of air bubbles and cracking in the column. Fifty percent ethanolic extract was inserted into the column and mixtures of different solvents in a different ratio were used as eluting agent (Harborne, 1973; Houghton and Raman, 1998).

RESULTS AND DISCUSSION

Extraction procedure

The cold maceration process was followed for extraction of fruits by using solvent of low polarity to higher polarity *i.e.* hexane, chloroform, ethyl acetate, 50% ethanol and results are tabulated in **Table 1**. Percentage yield of various extracts was found to be 1.323% (hexane extract), 2.26% (chloroform extract), 0.38% (ethyl acetate extract) and 2.256% (50% ethanolic extract).

Table 1. Percentage yield of successive extracts of *Scindapsus officinalis* fruit

Type of successive extract	Colour	Percentage yield
Hexane extract	Brown	1.323%
Chloroform extract	Brown	2.26%
Ethyl acetate extract	Brown	0.38%
50% Ethanolic extract	Brown	2.256%

Purification and characterization

Twenty two elutes were collected by column chromatographic separation and subjected to Thin Layer Chromatography (TLC). Elute no. 1-11 were colourless and showed no spot. Elute no. 12 - 17 [Ethyl acetate : Ethanol (10:40 up to 00:50)] showed single brownish spot in the solvent system toluene : ethyl acetate : methanol (4:2:2) with Dragendorff's reagent as visualizing agent. They were pooled together, evaporated and the obtained pale brown sticky residue was named as compound A. Elute no. 18-21 [Ethanol : Water (40:10 up to 10:40)] and elute no. 22 [Ethanol : Water (00:50)] showed no spot for TLC. These were mixed on the basis of colour intensity, evaporated and the obtained dark brown and brownish sticky residues were named as compound B and C respectively. The yield was found to be 235 mg (compound A), 24 mg (compound B) and 28 mg (compound C). The compounds were subjected to spectral analysis *viz.* UV, IR, $^{1}H/^{13}C$ NMR and Mass spectroscopy.

Spectral analysis of compound A

The IR spectrum of compound A was obtained using Perkin-Elmer UV-Vis spectrometer Lambda 16 (Germany) which showed different bands at 2962 cm^{-1} (C-H stretching), 1715 cm^{-1} (carbonyl carbon), 1443 cm^{-1} (C-H bending) and 1095, 1022 and 928 cm^{-1} (unsaturation). The UV spectrum exhibited two major absorption peaks between 350-380 nm (Band I) and between 200-220 nm (Band II) showing the presence of hetero atom with double bond (n-π*) and presence of hetero atom with saturation (n-σ*) in the compound respectively. The 1D NMR (^{13}C NMR and ^{1}H NMR) was performed on a Varian INOVA 600 spectrometer and the findings are showed in **Table 2**.

High-resolution ESI mass spectrometry (HR-ESI-MS) was carried out on a JEOL GC mate spectrometer. The electron impact mass spectrum showed the M + 1 peak at *m/z* 274.681 approximately comparable with authentic compound piperine (285.34). The other major fragments were recorded at *m/z* 254.4603, 214.8739, 177.2411, 142.2849 and 89.6805. Rest of the peaks may be due to the impurities (**Figure 2, 3**).

Table 2. NMR data of compound A

S. No.	Signals in ^{13}C NMR	Signals in ^{1}H NMR (δ)	Assignment
1.	166.275	–	Carbonyl carbon
2.	148, 144.7, 143.14, 138, 131, 125, 122, 121, 119	7.4 to 6.5	Unsaturated carbons and aromatics
3.	108, 107, 105, 101.3, 100.688	–	Carbons attached to nitrogen and oxygen atom
4.	43.16, 43.05, 34, 34.01, 26.5, 25.5, 24.1	1.7 to 3.58	Methylene carbons

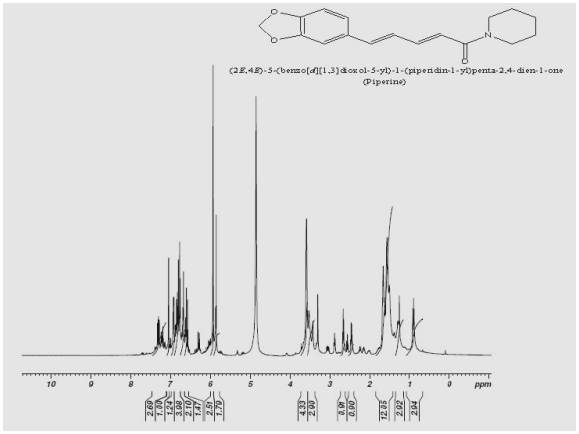

Fig. 2. ^{1}H NMR spectra of compound A

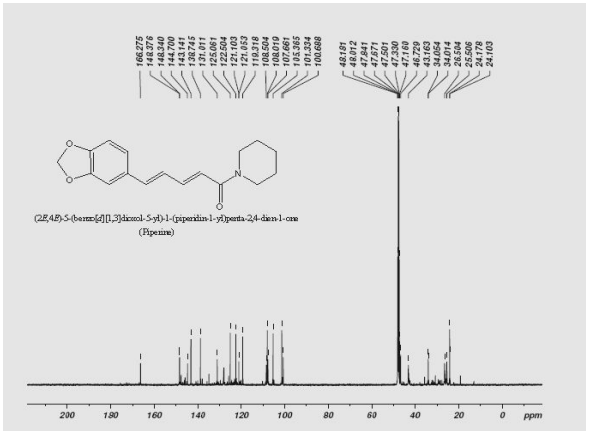

Fig. 3. ^{13}C NMR spectra of compound A

Spectral analysis of compound B

The IR spectrum of compound B showed the bands at 3358 cm^{-1} (-OH group, broad signal), 1663 cm^{-1} (ketone carbonyl group), 1444 cm^{-1} (C-H bending) and 1040 cm^{-1} (unsaturation). The UV spectrum exhibited two major absorption peaks between 260-280 nm (Band I) and between 200-205 nm (Band II) showing the presence of double bond or triple bond (π-π*) and presence of hetero atom with saturation (n-σ*) in the compound respectively. The findings of 1D NMR (^{13}C NMR and ^{1}H NMR) are tabulated in **Table 3**. The electron impact mass spectrum showed the M^{+} (molecular ion) peak nearby at *m/z* 180 and 87.69 approximately comparable with authentic compound ascorbic acid (176.13) and glycerin (92.10). The base peak (100%) was at *m/z* 59.9669. Rest of the peaks may be due to the impurities (**Figure 4, 5**).

Spectral analysis of compound C

The IR spectrum of compound C showed characteristic bands at 3358 cm^{-1} (–OH group, broad band), 1661 cm^{-1} (ketone carbonyl, sharp band), 1419 cm^{-1} (C-H bending) and 1076 cm^{-1} (unsaturation) The UV spectrum exhibited only one absorption peaks between 200-210 nm (Band I) showing the presence of hetero atom with saturation (n-σ*) in the compound. The 1D NMR (^{13}C NMR and ^{1}H NMR) was performed and

findings are tabulated in **Table 4**. High-resolution ESI mass spectrometry (HR-ESI-MS) showed the M^+ (molecular ion) peak at *m/z* 180 comparable with authentic compound ascorbic acid (176.13). The base peak (100%) was at *m/z* 59.9669. The other major fragments were recorded at *m/z* 147.2351. Rest of the peaks were due to the impurities (**Figure 6, 7**).

Table 3. NMR data of compound B

S. No.	Signals in ^{13}C NMR	Signals in ^{1}H NMR (δ)	Assignment
1.	143.927	6.8 to 5.9	Unsaturated carbon
2.	122.98		
3.	61.13 to 72.411	4.9 to 3.2	Carbon with -OH group
4.	47.855, 19.94 to 38.96	2.9 to 1.2	Methylene carbon

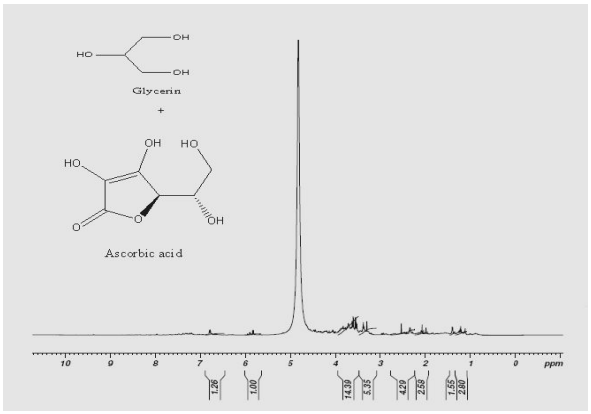

Fig. 4. ^{1}H NMR spectra of compound B

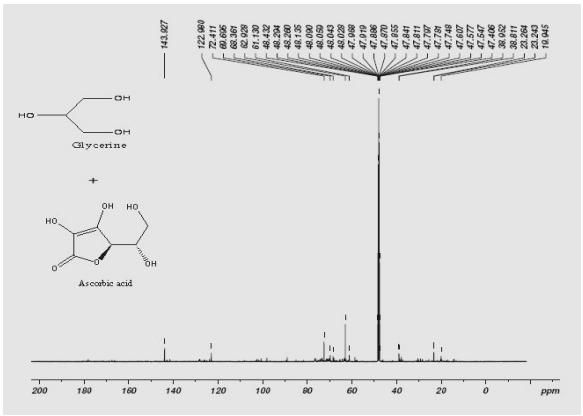

Fig. 5. ^{13}C NMR spectra of compound B

Table 4. NMR data of compound C

S. No.	Signals in ^{13}C NMR	Signals in ^{1}H NMR (δ)	Assignment
1.	143.6 and 123.0	6.8 to 5.9	Unsaturated carbons
2.	69.7 to 62.9	4.9 to 3.2	Carbon with OH group
3.	47.8 to 19.9	2.9 to 1.3	Methylene carbon

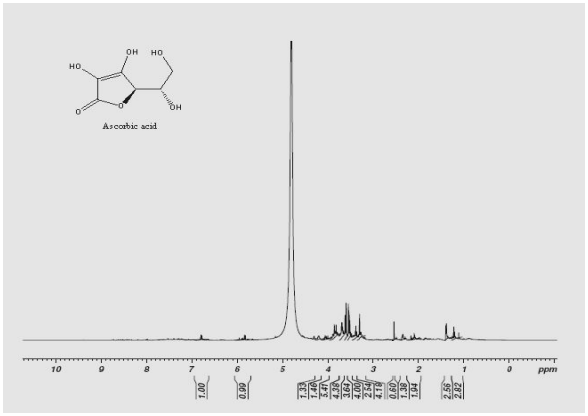

Fig. 6. ^{1}H NMR spectra of compound C

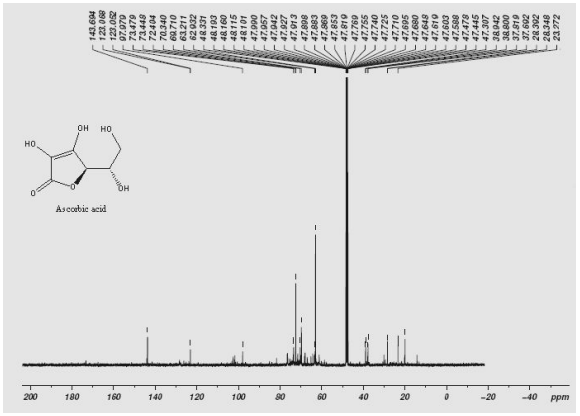

Fig. 7. ^{13}C NMR spectra of compound C

CONCLUSION

Purification of the 50% ethanolic extract by column chromatography resuked in three brownish sticky residues named compound A, B and C which were subjected to spectral analysis *i.e.* IR, UV, 1D NMR (^{1}H-NMR and ^{13}C-NMR) and mass spectroscopy. Based on the systematic spectral study, the purified compounds A, B and C were characterized as piperine, mixture of glycerin and ascorbic acid, and ascorbic acid respectively due to their close resemblances with the standard compounds.

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