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Full Length Research Paper

Screening of antioxidant activity, total phenolics and gas chromatography-mass spectrophotometer (GC-MS) study of ethanolic extract of *Aporosa lindleyana* Baill

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The present study was carried out for the identification of phytochemicals present in the roots of *Aporosa lindleyana* and also to evaluate the total phenol, total flavonoids and antioxidant activity. Total phenol was carried out by Folin Ciocalteu method and the phenolic content was 31.20 mg/100 g of gallic acid equivalent (GE) and the flavonoid content was 203.10±0.9. Antioxidant activity was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) method and the roots of *A. lindleyana* showed 19.91 mg/100 g of ascorbic acid equivalent antioxidant capacity (AEAC). The gas chromatography-mass spectrophotometer (GC-MS) study was also carried out and it showed the presence of phytochemicals like 1,2-benzenedicarboxylic acid, diphenyl ester (RT: 11.97), pthalic acid, bis (7 methylocytyl) ester (RT: 20.86) and squalene (RT: 24.70).

Key words: *Aporosa lindleyana*, antioxidant, flavonoids, gas chromatography-mass spectrophotometer (GC-MS), 2,2-diphenyl-1-picrylhydrazyl (DPPH).

INTRODUCTION

Aporosa lindleyana belongs to the family of Euphorbiaceae, is a much branched, evergreen, glabrous tree, grown in India and Sri Lanka. It possesses antioxidant activity (Badami et al., 2005) and hepatoprotective effect (Ramakrishnan et al., 2010) and also having antihyperglycemic effect (Jayakar and Suresh, 2003). So far, very few research works were carried out in this plant. *A. lindleyana* Baill have much medicinal properties such as diuretic, antiviral and a good analgesic. Its leaves are used to treat diabetics. Its bark and roots were used to treat headache, fever and jaundice. Decoctions of roots are used to treat insanity, seminal loss and excessive thirst.

The preliminary phytochemical studies reveal the presence of phytosterol, alkaloids and flavonoids. Polyphenolic compounds have high antioxidant potential, the antioxidant activity of *A. lindleyana* was investigated by employing various *in vitro* tests. The present study was carried out to investigate the antioxidant activity, flavonoids and total phenolic content of *A. lindleyana*. In addition, chemical constituents of *A. lindleyana* was analyzed by gas chromatography-mass spectrophotometer (GC-MS).

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MATERIALS AND METHODS

Collection and processing of plant material

The roots of *A. lindleyana* was collected from Keeriparai, Kanyakumari District, Tamilnadu during the month of January 2009. The specimen was identified by Dr. V. Chelladurai, Taxonomist, Department of Ayurvedic Sciences, Tirunelveli District. The roots were cleansed and shade dried for a weak and grounded into uniform powder. 1 g of plant material was added to 20 ml of aqueous ethanol (20% v/v) for 18 h at room temperature. The

Table 1. Total phenols, flavonoids and antioxidant activity in the roots of *A. lindleyana.*

S/N	Parameter analysed	Value obtained
1	Total Phenols (mg/100 g) GE*	31.20±0.2
2	Total flavonoids (mg/100 g) GE*	203.10±0.9
3	Antioxidant activity ((mg/100 g) AEAC**	19.91±0.5

The values are mean value of three replicates, *Gallic acid equivalent, **Ascorbic acid equivalent antioxidant capacity.

extracts was filtered and used for the estimation of total phenols and antioxidant activity.

Total phenols

0.5 ml of freshly prepared sample was taken and diluted with 8 ml of distilled water. 0.5 ml of Folin Ciocalteu reagent (1 N) was added and kept at 40°C for 10 min. 1.0 ml of sodium carbonate (20%) was added and kept in dark for one hour. The color was read at 650 nm using shimadzu UV-1650 spectrophotometer (Malick et al., 1980). The same procedure was repeated for all standard gallic acid solution and standard curve obtained. The sample concentration was calculated as gallic acid equivalent (GE).

Total flavonoids

0.5 ml of ethanolic extract of sample is diluted with 3.5 ml of distilled water at zero time and 0.3 ml of 5% sodium nitrate was added to the tubes. After 5 min, 0.3 ml of aluminium chloride (10%) was added to all the tubes. At the 6^{th} min, 2 ml of sodium hydroxide (1 M) was added to the mixture. Immediately, the contents of the reaction mixture were diluted with 2.4 ml of distilled water and mixed thoroughly, after which absorbance of the mixture was then determined at 510 nm versus a prepared blank. Gallic acid was used as the standard compound for quantification of total flavonoids as mg/100 g (Zhisen et al., 1999).

Antioxidant activity

0.1 ml of the freshly prepared sample was taken in the test tubes. 6.0 ml of 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution (0.1 mM) was added and the tubes kept in dark for one hour. The color was read at 517 nm. The difference in the optical density (O.D) of DPPH solution and DPPH solution + sample was calculated. The decrease in O.D with sample addition is used for the calculation of the antioxidant activity. Ascorbic acid standards were prepared in different concentration and antioxidant activity was determined as ascorbic acid equivalent antioxidant capacity (AEAC) mg/100 g of sample (Koleva et al., 2002).

GC-MS analysis

Preparation of extract

The ratio of *A. lindleyana* was shade dried and 20 g of the powdered roots was soaked in 95% ethanol for 12 h. The extract was filtered through Whatmann filter paper No. 41 along with 2 g sodium sulfate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with sodium sulphate was wetted with 95% ethanol. The filtrate was then concentrated by bubbling nitrogen gas into the solution. The extract contained both polar and nonpolar phytocomponents of the plant material used. 2 µl of this solution was employed for GC-MS analysis (Merlin et al., 2009).

GC analysis

GC-MS analysis was carried out on a GC clarus 500 perkin Elmer system comprising a AOC-20i autosampler and gas chromatography interfaced to a mass spectrophotometer (GC-MS) instrument employing the following condition. Column Elite – 1 fused silica capillary column (30 × 0.25 mm ID × IEM df, composed of 100% trimethyl poly siloxane) operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1 ml/min and an injection volume of 0.5 EI was employed (split ratio of 1:1) injector temperature (280°C). The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10 C/min to 200°C, then 5 C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectrum was taken at 70 eV; a scan interval of 0.5 s fragments from 40 to 550 Da.

Identification of components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

RESULTS AND DISCUSSION

Total phenol and flavonoid content

Plant polyphenols, a diverse group of phenolic compounds (flavonols, anthocyanins, phenolic acids, etc.) possess an ideal structural chemistry for free radical scavenging activity. Antioxidative properties of polyphenols arise from their high reactivity as hydrogen or electron donors from the ability of the polyphenol derived radical to stabilize and delocalize the unpaired electron (chain-breaking function) and from their potential to chelate metal ions (termination of the Fenton reaction) (Rice-Evans et al., 1996).

The flavonoid contents of the extracts were expressed in terms of gallic acid equivalent (Table 1). Total phenolic content of the ethanolic extract of *A. lindleyana* root was

Table 2. Phytochemicals and their activity identified in the ethanolic extract of the roots of *A. lindleyana* by GC-MS.

S/N	RT	Name of the compound	Molecular formula	MW	Peak area (%)	Nature of compound	**Activity
1	9.00	1,3-Dioxolane-2-heptanenitrile, à-methyl-ë-oxo-2-phenyl-	$C_{17}H_{21}NO_3$	287	1.16	Aromatic nitrile compound	Antimicrobial
2	11.97	1,2-Benzenedicarboxylic acid, diheptyl ester	$C_{22}H_{34}O_4$	362	3.10	Plasticizer compound	Antimicrobial, antifouling
3	13.08	1,2-Benzenedicarboxylic acid, butyl cyclohexyl ester	$C_{18}H_{24}O_4$	304	1.15	Plasticizer compound	Antimicrobial, antifouling
4	20.86	Phthalic acid, bis(7-methyloctyl) ester	$C_{26}H_{42}O_4$	418	10.46	Plasticizer compound	Antimicrobial, antifouling
5	21.38	4-Methoxymethoxy-hex-1-ene	$C_8H_{16}O_2$	144	4.79	Alkene compound	No activity reported
6	24.70	Squalene	$C_{30}H_{50}$	410	32.34	Triterpene	Anticancer, antimicrobial, antioxidant, chemo preventive pesticide, anti- tumor sunscreen
7	28.77	Silane, 1,4-phenylenebis[trimethyl-	$C_{12}H_{22}Si_2$	222	16.25	Aromatic silica compound	No activity reported
8	32.40	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-	$C_{13}H_{22}OSi_2$	250	30.76	Ketone compound	No activity reported

**Source: Dr. Duke's: Phytochemical and ethnobotanical databases.

31.20 mg/100 g of GE. The highest value of phenolic content indicates that the plant has high antioxidant activity.

The antioxidant properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper, and inhibition of enzymes responsible for free radical generation (Benavente-Garcia, 1997). Depending on their structure, flavonoids are able to scavenge practically all known reactive oxygen species (ROS). Total flavonoid content of ethanolic extract of *A. lindleyana* roots was 203.10 mg/100 g of GE.

GC-MS study

The GC-MS study of roots of *A. lindleyana* has shown many phytochemicals which contributes to the medicinal activity of the plant (Table 2 and Figure 1). The major component present in the roots of *A. lindleyana* was 1,3 –Dioxolane – 2 – hepanenitrile a'-methyl-e-oxo-2-phenyl (RT:9.00), 1,2-Benzene dicarboxylic acid, diphenyl ester (RT: 11.97), 1,2 – Benzene dicarboxylic acid butyl cyclohexyl ester (RT:13.08)- (Figure 2) and phthalic acid (RT:20:86) , 4- Methoxymethoxy hex-1-ene, Squalene (RT: 24.70) - (Figure 3), Silane 1,4, phenylene bis(trimethyl), 2,4,6 cycloheptatrien -1-ene-3,5 bis-trimethyl silyl found in the roots of *A. lindleyana*.

Squalene plays a major role in the protection and enhancement of human skin. Like glutathione (GSH), Squalene is one of the few antioxidants manufactured within the body, both of which detoxify, help balance our protective metabolisms and protect us from other threats. Squalene also has anticancer and blood cholesterol lowering effects (Nakagawa et al., 1985).

In our study, Squalene, an antioxidant found in the ethanolic extract, may give antioxidant properties of the *A. lindleyana*.

Conclusion

Our present study showed that the root extract was rich in antioxidants, phenolics and flavonoids. The GC-MS study also proved many phytochemicals such as Squalene, Pthalic acid and 1,2 benzene dicarboxylic acid, butyl cyclohexyl ester etc., which contributes to the activities like antioxidant, antimicrobial and antifouling activity.

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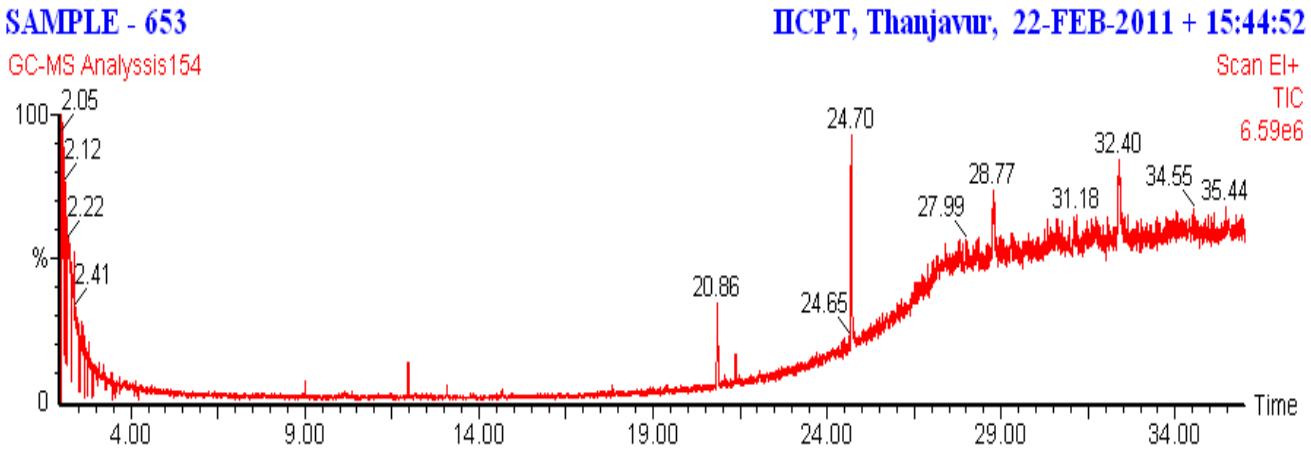
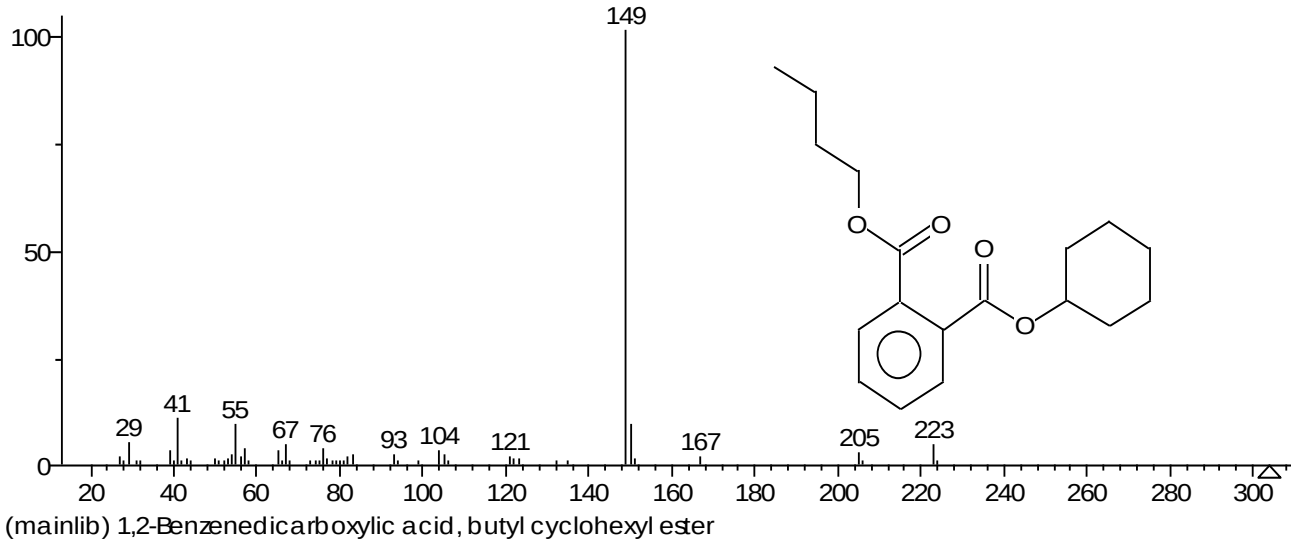
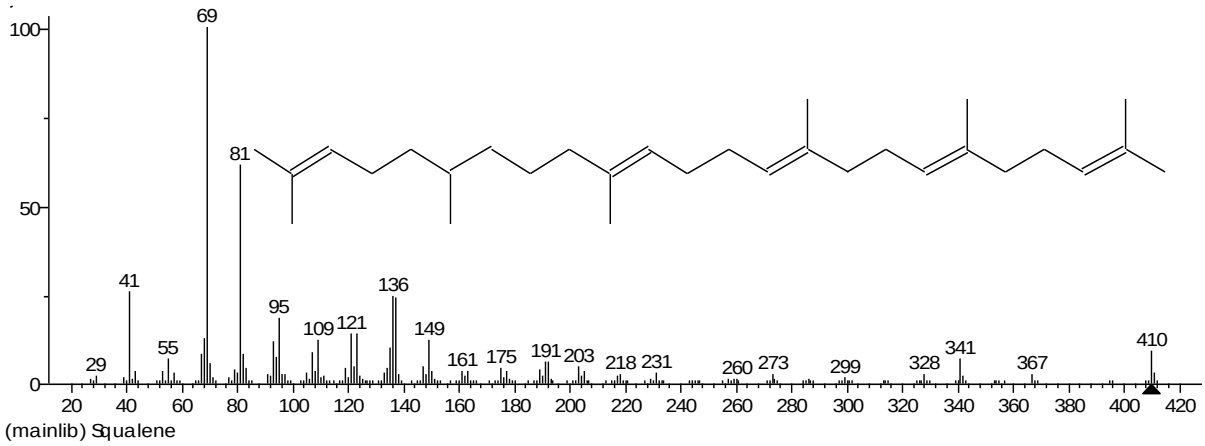


Figure 1. Chromatogram of *A. lindleyana* roots by GC-MS.



Name: 1,2-Benzenedicarboxylic acid, butyl cyclohexyl ester
Formula: $C_{18}H_{24}O_4$
MW: 304 CAS#: 84-64-0 NIST#: 75987 ID#: 87207 DB: mainlib
Other DBs: None
Contributor: RADIAN CORP
10 largest peaks:
149 999 | 41 104 | 150 92 | 55 89 | 29 50 |
223 44 | 67 44 | 57 37 | 76 37 | 65 30 |
Synonyms:
1.Phthalic acid, butyl cyclohexyl ester
2.Butyl cyclohexyl phthalate
3.Cyclohexyl butyl phthalate
4.Elastex 50B
5.1-Butyl 2-cyclohexyl phthalate #

Figure 2. Mass spectrum of 1,2-Benzenedicarboxylic acid, butyl cyclohexyl ester.



Name: Squalene
Formula: $C_{30}H_{50}$
MW: 410 CAS#: 7683-64-9 NIST#: 227620 ID#: 27655 DB: mainlib
Other DBs: None
Contributor: Japan AIST/NIMC Database- Spectrum MS-NW-8230
10 largest peaks:
69 999 | 81 612 | 41 257 | 136 243 | 137 240 |
95 184 | 121 139 | 123 137 | 68 124 | 149 119 |
Synonyms:
1.2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-
2.Skvalen
3.Spinacene
4.Supraene
5.(6E,10E,14E,18E)-2,6,10,15,19,23-Hexamethyl-2,6,10,14,18,22-tetracosahexaene

Figure 3. Mass spectrum of squalene.

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