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Aporosa Octandra, A Less Studied Plant Species with Potential Drug Activities-I: Identification of a New Compound from Aqueous Ethanolic Extract of its Stem Bark.

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ABSTRACT*:* This is the first report of occurrence of the compound from Aporosa Octandra. For the first time, the compound 2-Methyl-3-en-butyl-cyclohexyl Phthalate has been isolated and identified from aqueous ethanolic extract of the stem bark of *Aporosa Octandra*. Structure was established by chemical and spectroscopic methods.

KEYWORDS*: Aporosa Octandra*, Euphorbiaceae, aqueous ethanolic extract, 2-Methyl- 3-en-butyl-cyclohexyl Phthalate.

I. INTRODUCTION

Aporosa Octandra locally known as Massania or pata kharalla, kasua is an evergreen tree, 9 to 12 m tall, usually shrubby, young parts rusty-pubescent, leaves sub-leathery; stipules obliquely ovatelanceolate, 4–6 mm; petioles 5–12 mm, with 2 glands at apex. Leaf blade elliptic, narrowly ovate, oblong-elliptic. Male spikes ca. 2.5 cm, the flowers densely clustered; bracts ovate-triangular, ca. 1 mm, pubescent outside; sepals usually 4, oblong-ovate. Stamens 2–4, longer than sepals. Female spikes cylindrical, shorter than the males; sepals 4–6, triangular, margins ciliate; ovary ovoid, densely pubescent, bilocular, with 2 ovules in each locule. Capsules ellipsoidal, 1–1.3 cm, pubescent, 2-seeded; seeds subovate, ca. 9 × 5.5 mm. growing up to 15 metres tall belonging to the family Euphorbiaceae [1].

***Aporosa Octandra*, in Odisha: Massania: bark, leaf and fruits**

The synonyms of this plant are *Aporosa Octandra, Aporosa Dioica, Alnus Dioica Roxb*. Commonly seen in primary forests, grasslands, on sandy, clay or rocky soil, growing up to 1200 m altitude spreading in Uttarakhand, West Bengal, Sikkim, Assam, Meghalaya, Nagaland, Manipur, Mizoram, Tripura, Odisha and Andaman & Nicobar Islands. Pakistan, Nepal, Bhutan, Bangladesh, Myanmar, China, Laos, Cambodia, Vietnam, Thailand, Java, Sumatra, Borneo, Sulawesi and West Malaysia [1], [2] are also the home for the plant.

Scientific literature on the phyto-chemistry and the medicinal values of the plant are scantily available. There are, of course reports on traditional uses in some parts of Asia, including N.E states of India. This plant is used to treat many

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ailments in Mizoram and has been used as a folk medicine for treatment of jaundice, stomach ulcers [3] and colic fever [4]. The plant yields fodder. Timber used for construction of houses. Bark yields a red dye. A decoction of leaves used to dye clothes black in NE. India. The fruits are edible.

However, traditional uses in Asia including North East States of India have been observed in folklores [1]. This plant is used to treat many ailments in Mizoram [5]. The stem bark paste of Aporosa Octandra is applied for curing rheumatism. Stem bark juice is applied on cuts and abscess. Stem bark paste is plastered on the bone fractured area with the help of cloth for one month. This paste was also used for healing of cuts, wounds and itches [6].
The phytochemical analysis [7] showed the presence of tannins, saponins, flavonoids, terpenoids and cardiac glycosides but no phloba- tannins were found. The phenolic content of Aporosa Octandra in both chloroform and ethanol extracts was measured and found to be higher in ethanolic extract than chloroform extract. However, flavonoid content was found to be higher in chloroform extract than ethanolic extract.

In earlier works the DPPH, hydroxyl radical and superoxide anion scavenging properties of chloroform and ethanolic extract of Aporosa Octandra were dose-dependent. Nitric oxide free radical scavenging activity was also found dose-dependent. Therefore, it can be concluded that chloroform and ethanol extract of Aporosa Octandra with potent antioxidant properties may be useful agents in the prevention and treatment of some types of human diseases particularly in cancer.

In this research paper, we report in continuation to our studies on medicinal plants, the isolation and structure elucidation of a new compound(s) from the aqueous ethanolic extract of Aporosa Octandra.

II. EXPERIMENTAL

A. Materials and Methods

A.1 Reagents used

Ethanol, methanol, benzene, petroleum benzene, chloroform, carbon tetrachloride, ethyl acetate and dichloromethane all from CDH, Merck, Ranbaxy were used as such. Thin layer chromatography (TLC) was carried out using silica gel G (Ranbaxy). Chromatographic plates of measured thickness were coated with 0.2 mm silica gel. The plate size was maintained at (20 cm x 5 cm) and (20 cm x 10 cm) for identification and preparative TLC purposes respectively. All chromatograms were developed in a glass chamber (30 x 20 cm2) at room temperature of (23±2) oC and at a relative humidity of approximately 40%.

All the solvents used in this research work viz. ethanol, methanol, benzene, petroleum ether, chloroform, carbon tetrachloride, ethyl acetate and dichloromethane were of highest purity. Thin layer chromatography (TLC) was carried out using silica gel G for identification and preparative TLC purposes.

A.2 Plant Material
Collection
The stem barks of Aporosa Octandra were collected from Gajapati District in Odisha. It is situated within the geographic co-ordinates of 18.45° E- 19.40° W latitude and 85.48° N -84.27° S longitudes with an area of 4325 sq. km and. total population of 575,880 as of 2011. The species was authenticated by Taxonomist in Department of Botany, Khallikote (Autonomous) College, (presently Khallikote University) Berhampur, Odisha and the voucher specimen was deposited in the Herbarium (Ref. No. 3/08).

A.3 Extraction and isolation of compounds

The plant bark of Aporosa Octandra weighing 5.0 Kgs was thoroughly washed with water and shade dried and then ground into powder form. The powdered bark was extracted with ethanol (95%) after keeping tightly for two weeks. It was filtered and then concentrated. The residue was again extracted with hot ethanol using Soxhlet apparatus. It was also filtered and concentrated. The purity of both the extracts was checked by TLC. It was found that both contained two spots at equidistance from the base line. Hence, both the extracts were mixed up and distilled in a hot water bath.

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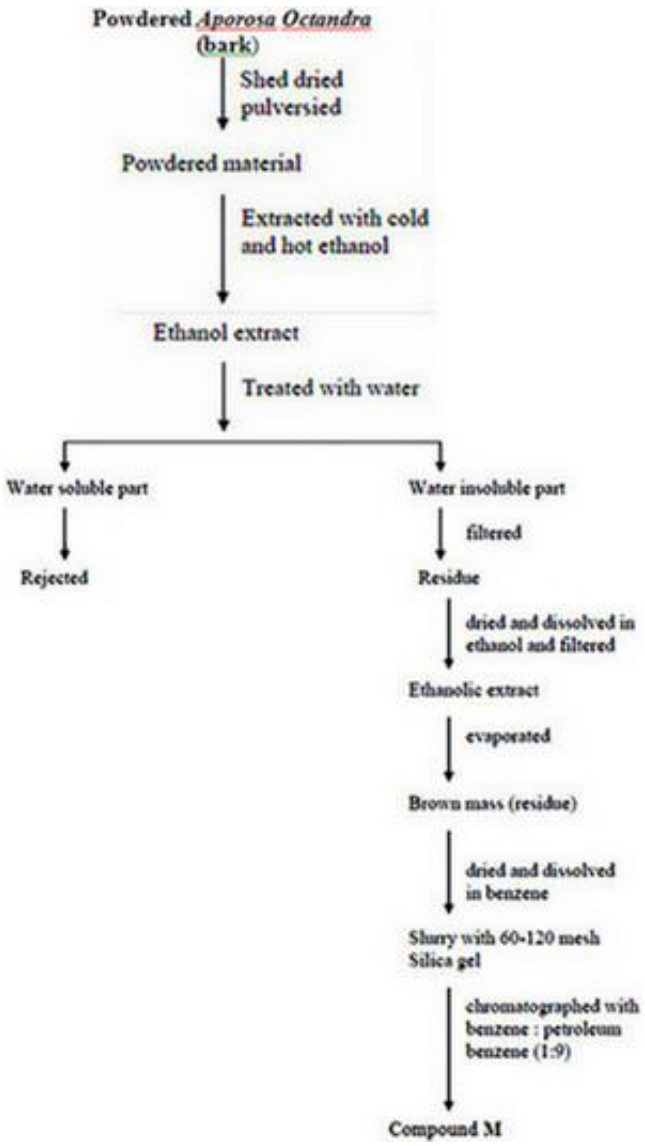
The residue was dark brown in colour. It was then treated with cold water to remove water soluble part which was rejected. The water insoluble part was dried thoroughly, dissolved in ethanol and filtered. The filtrate was treated with dry sodium sulphate to absorb water. After concentration by distillation over hot water bath it was dried in a desiccator over anhydrous calcium chloride. The perfectly dried residue was dissolved in dry benzene and slurry was prepared with 60-120 mesh silica gel.

The separation of the components was done by column chromatography using 60-120 mesh silica gel and benzene: petroleum benzene (1:9) as the eluent. The purity of the fractions was examined by TLC at a regular interval. Eluents were collected in small receivers and the fractions containing same component were mixed together. After removal of the solvent, compound M, a semi

viscous solid with mild odour having low melting point was recovered. The compound was subjected to Mass Spectroscopy and 13C NMR Spectroscopy. The structural elucidation was done by taking mainly the spectral data into consideration.

Above detailed procedure has been schematically presented below (Scheme- 1)



Scheme- 1

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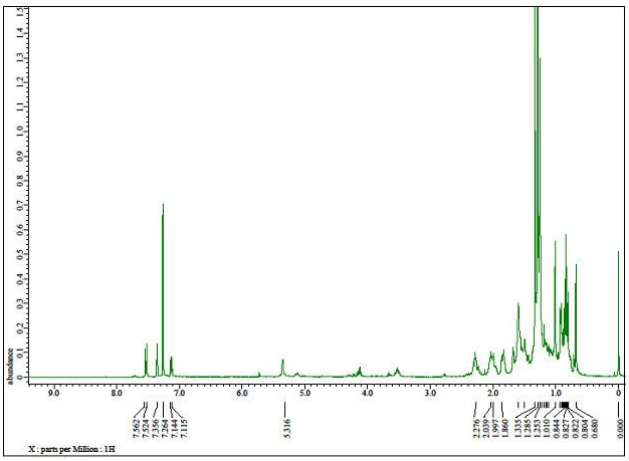
III. RESULTS AND DISCUSSION

A. Spectral Analysis of Compound (M):

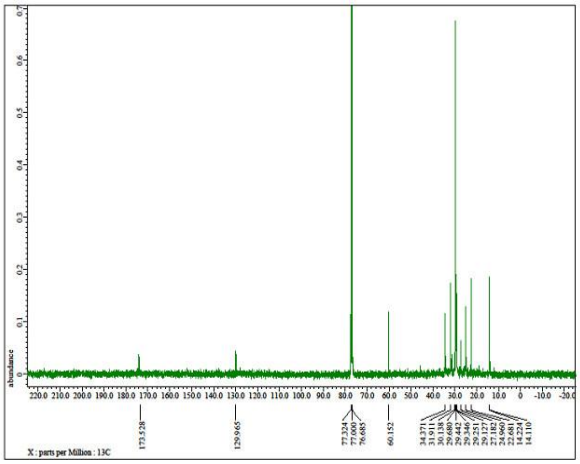
IR(KBr); ύ(cm-1): 2960(C-Hstr), 1740 (>C=O), Cyclic stretch 6 membered: 1715 strong, 1480, 1350, 1250(Aromatic).

1H NMR ($CDCl_3$); ð PPM : 0.866(d, J=7Hz,3H, -CH3), 0.822(d, J=7Hz,3H, -CH_3), 1.616(m, 1H,H12'), 2.000(d, J=10Hz,2H, H4'), 2.112(d, J=10Hz,2H, H3'), 2.254(d, J=10Hz,2H, H5'), 2.271(d, J=9Hz,2H, H6'), 2.290(d, J=9Hz,2H, H2'), 4.101(t, J=7Hz, 1H, H1"), 4.118(d, J=7Hz,2H, H9'), 5.330(d, J=7Hz,1H, H11'), 7.114(t, J=6 Hz,1H, H10'), 7.264(d, 2Hz,1H, H4), 7.524(d, 2Hz,1H, H6). 7.562(d, J=3Hz,1H, H2).
^{13}C NMR ($CDCl_3$); ð PPM : 22.681(C13'), 22.681(C14'), 29.442(C4'), 29.680(C5'), 30.138(C3'), 31.411(C6'), 34.771(C2'), 60.152(C1'), 77.000(C10'), 77.324(C9'), 77.686(C11'), 112.002(C4), 116.125 (C5), 116.136(C3), 129.961(C6), 129.964(C1), 129.965(C2), 175.526(C7'), 175.528(C8').

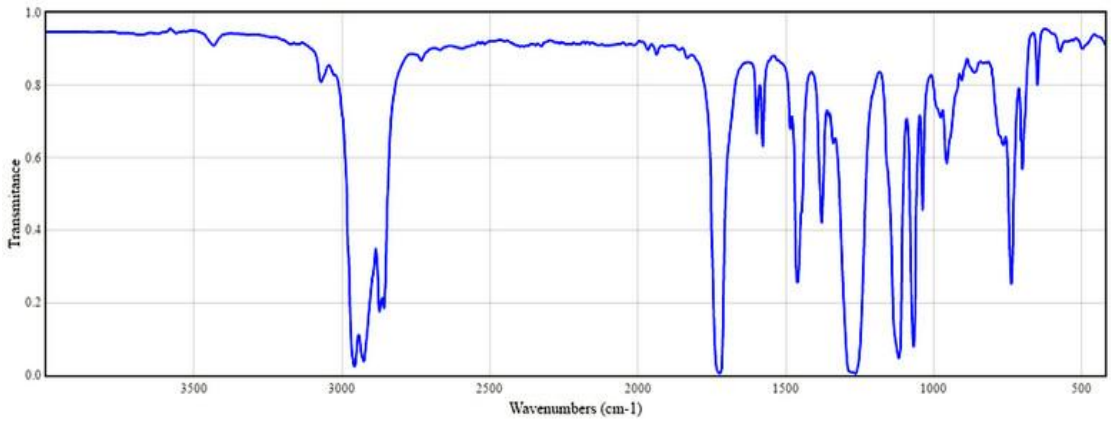
Mass (m/Z): 41, 55, 67, 76, 83, 93, 104, 122, 134, 150(100%), 167,207, 224,257, 314.
Compound M was elucidated to be **$C_{20}H_{26}O_4$**



^{13}C NMR



^{1}H NMR



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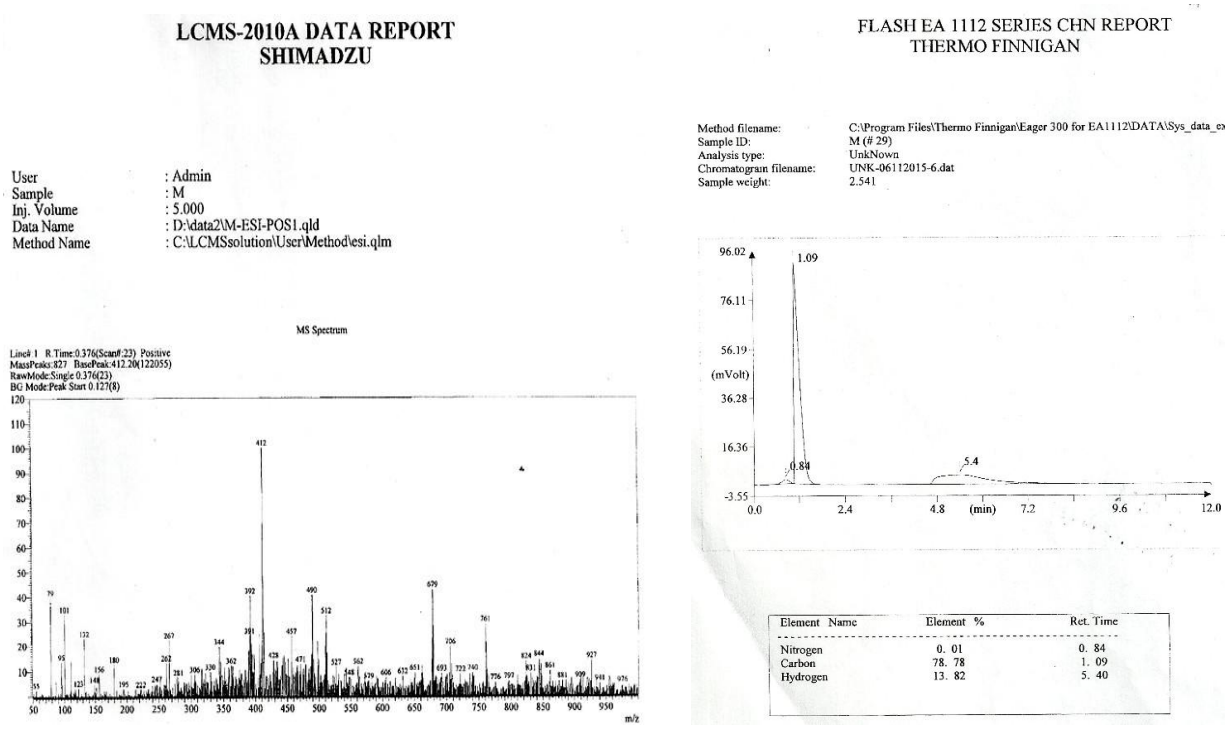
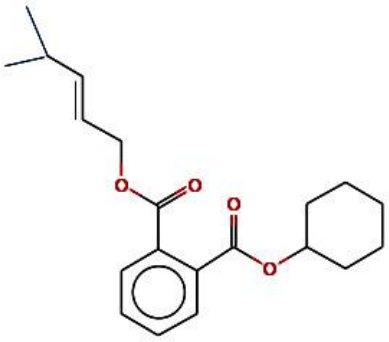


Mass Spectra



2-Methyl-3-en-butyl-cyclohexyl Phthalate: $C_{20}H_{26}O_4$

This is the first report of occurrence of compounds 2-Methyl-3-en-butyl-cyclohexyl Phthalate from Aporosa Octandra, the structures of which has been determined based on the spectral and chemical evidences.

Dr. Duke's Phytochemical and ethnobotanical database shows that earlier works on the Phytochemicals identified by GC-MS from the ethanolic extract of the roots of A. lindleyana [8] reported a phthalate, 1, 2-Benzenedicarboxylic acid, butylcyclohexyl ester ($C_{18}H_{24}O_4$).

IV. CONCLUSION

Our present studies on Aporosa Octandra primarily showed that a very little scientific work has been done and reported on the plant and its medicinal uses, even though it is in use for long by certain isolated tribes of Mizoram, Odisha etc. Further, our work on the bark extract showed the presence of some new chemical substances which need to be explored in addition to the known rich antioxidants, phenolics and flavonoids. A new product could be isolated. The spectral studies proved the isolation of 1,2-benzenedicarboxylic acid, butylcyclohexyl ester.

V. ACKNOWLEDGEMENTS

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