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Isolation and Characterization of Potential Cytotoxic Leads from *Ambrosia maritima* L. (Asteraceae)

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Abstract
Medicinal plants have long been recognized as an indispensable source for new leads. In this study, *Ambrosia maritima* L. (Asteraceae) was subjected to chromatographic (TLC & PTLC) and spectroscopic analysis (UV, IR, GC-MS & NMR) to isolate and elucidate its active constituents. Two promising antitumor leads namely; di(2-ethylhexyl) phthalate and 13-hexyloxacyclotridec-10-en-2-one, had been, unprecedentedly, isolated and characterized from the Brine shrimp Lethality Assay (BSLA) active dichloromethane extract.

Keywords: *Ambrosia maritima*, spectroscopic analysis, antitumor leads, di (2-ethylhexyl) phthalate, 13-hexyloxacyclotridec-10-en-2-one.

1. Introduction
Natural products continue today in the never-ending quest to provide new lead compounds [1]. Hereby, much interest has been directed towards medicinal plants, as a renewable source of compounds, in order to fulfill human health necessities by making the disease curable [2]. Cancer is one of the most common diseases that threaten peoples' health. Contrary to conventional anticancer therapy, treatment strategy had been oriented towards traditional medicine with a hope to develop effective and safe drugs [3].
Ambrosia maritima L., belongs to family Asteraceae, is a widely distributed plant in African countries and is locally known as Damsissa [4, 5]. Traditionally, it is used to cure gastrointestinal disturbance, abdominal pain, kidney inflammation and renal colic. In addition, its therapeutic properties extend to include antimolluscicidal, antimalarial and antitumor activities [6, 7]. Being of diverse curative properties, this plant species has captured the attention of researchers to explore its ability in providing new molecules. As a part of our on-going drug discovery of the biologically assessed plant species, we now report the isolation and identification of potential cytotoxic leads from the BSLA active *A. maritima* dichloromethane extract.

2. Materials and Methods
2.1. Plant material
Ambrosia maritima's whole plant was collected from Khartoum, Sudan during March 2012. The plants species were taxonomically authenticated by Dr. Haidar Abdalgadir and their voucher specimens had been deposited at the Herbarium of Medicinal and Aromatic Plants Research Institute (MAPRI), Sudan.

2.2. Equipments
Chromatographic analysis was carried out using silica gel GF_{245}, S.D. fine chemical limited; Bombay, India. Ultraviolet (UV) and Infra-red (IR) spectra were recorded on Shimadzu spectrometers. Nuclear Magnetic Resonance (NMR) spectra were measured on Bruker AMX 500 spectrometer (500MHz) in $CDCl_3$ with tetramethylsilane (TMS) as internal standard. Gas chromatography-mass spectrometry (GC-MS) was determined on Shimadzu QP 2010 spectrophotometer (EI detector).

2.3. Preparation of plant extract
The shade dried, powdered material (100 g) was soaked in dichloromethane (DCM) and then in methanol (80%). Maceration process was repeated, several times, for each solvent to

complete the extraction process. The obtained extracts were filtered and air-dried at room temperature. The Brine Shrimp Lethality Assay (BSLA) [8] was adopted to assess the cytotoxicity for both extracts.

2.4. Isolation and structural elucidation of compounds

The potent cytotoxic extract, *A. maritima* DCM, was purified by preparative thin-layer chromatography (PTLC). The elution was commenced with toluene: ethyl acetate (93:7) on silica gel GF_{254} (20×20 cm, 0.05 cm thickness). The obtained sub-fractions were analyzed by Thin layer chromatography (TLC) on silica gel GF_{254} (0.25 mm thickness) using toluene: ethyl acetate (93:7) and vanillin-sulphuric acid as visualization reagent followed by heating at 110 °C. The obtained isolates were identified and characterized by various chromatographic and spectroscopic techniques.

3. Results and Discussion

3.1. Structure Elucidation of SF1

SF1 (14 mg) was obtained as transparent liquid. The UV spectrum showed a band, characteristic for conjugated benzene moiety, at λ_{max} = 275 nm. The IR spectrum showed bands at 2957 and 2853 cm^{-1} which were assigned to methylene and methyl stretching, respectively. On the other hand, the methylene C–H bending appeared at 1445 cm^{-1}. The bands at 1730 and 1122 cm^{-1} were attributed to carbonyl of conjugated ester and C–O stretching, respectively. The gas chromatogram of SF1 (Fig.1.a) indicates a major peak at retention time of 47.9 minutes. The mass spectrum of SF1 (Fig. 1.b) indicates the molecular ion peak $[M+H]^+$ ion at *m/z* 279 and the base peak at *m/z* 149 characteristic for mono(2-ethylhexyl) phthalate (MEHP).

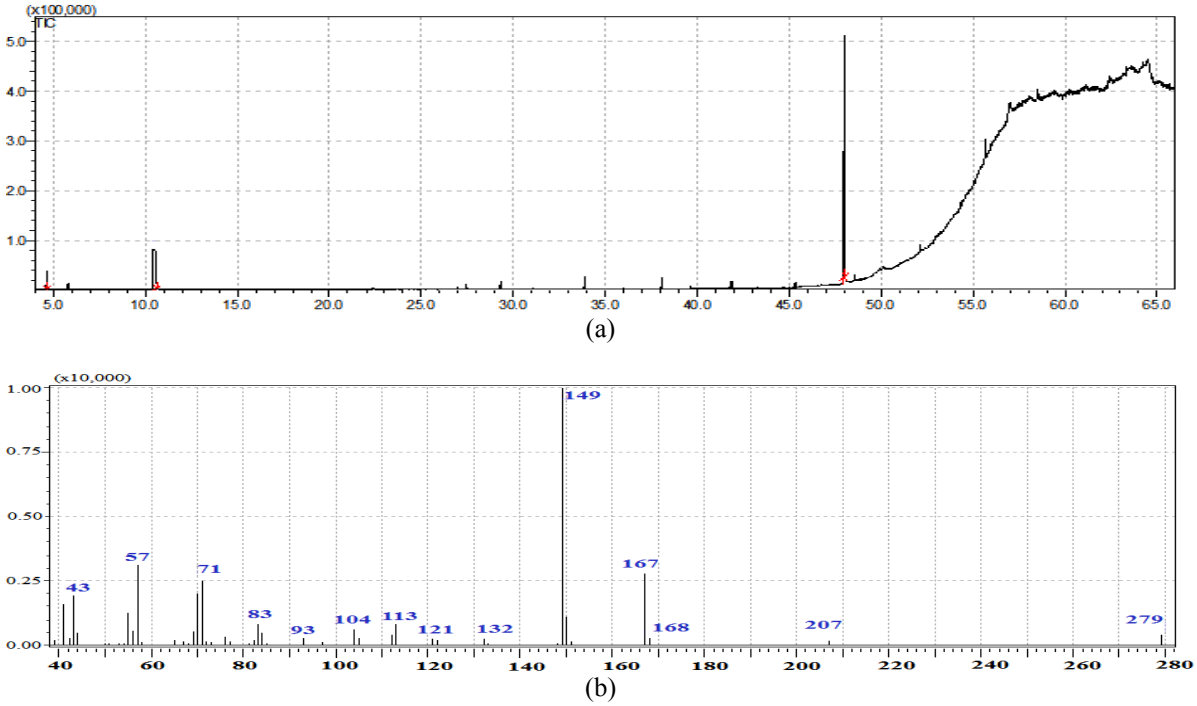

Fig 1: (a) Gas chromatogram and (b): Mass spectrum of SF1

The ^{1}H NMR (Fig. 2) showed aryl protons at δ 7.55 and 7.73 ppm. The existence of two peaks integrated for four protons indicate chemical equivalency, which was apparent only in di(2-ethylhexyl) phthalate (DEHP). 2D proton-carbon HMQC analysis showed that these aryl protons were correlated to respective carbon signals at δ 131.2 (C-a/ C-a') and 129.1 (C-b/C-b'), respectively. The carbon oxygen-related methylene protons signals were found further upfield in the ^{1}H-NMR spectrum (δ 4.25, 2H), correspondent with a carbon resonance at δ 68.5 (C-e/ C-e').The methine protons were resonant at δ 1.65 (2H), correspondent with a carbon resonance at δ 38.8(C-f/ C-f'). Both the methylene (H-e) and methine (H-f) protons were seen to share pronounced COSY contours with each other, in accordance with their vicinal relationship, confirming the branched nature of the alkyl chain of the ester as opposed to the straight chain present in the isomeric dioctyl phthalate (DOP) [9]. Furthermore, three-bond HMBC correlation clearly linked the methylene proton (H-e, e') to the ester carbonyl (δ 167.8, CO), thus precluding the possibility of the 1-methylheptyl ester isomer [9]. The other multiplet at 0.95–1.44 ppm accounted for the remaining methyl and methylene group protons of DEHP.

The ^{13}C-NMR spectrum (Fig. 3) also indicated the presence of aryl ester carbonyl groups (δ 167.8, CO) and the C-c/C-c' quaternary carbons (δ 132.5), thus accounting for the 24-carbon skeleton of DEHP (Fig. 4).

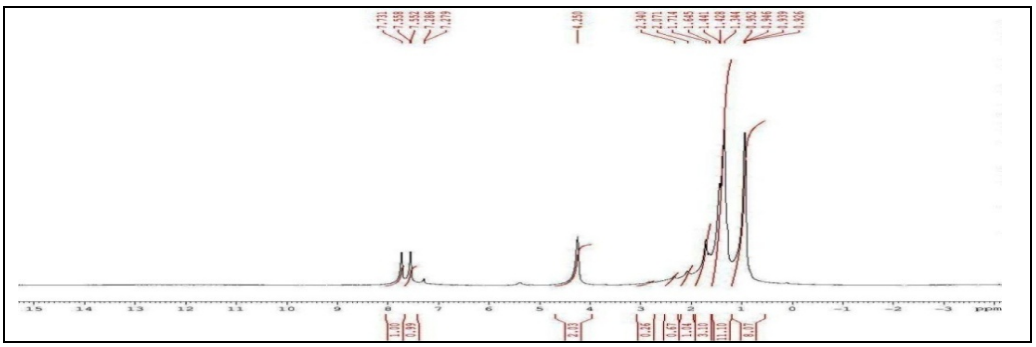

Fig 2: ^{1}H NMR spectrum

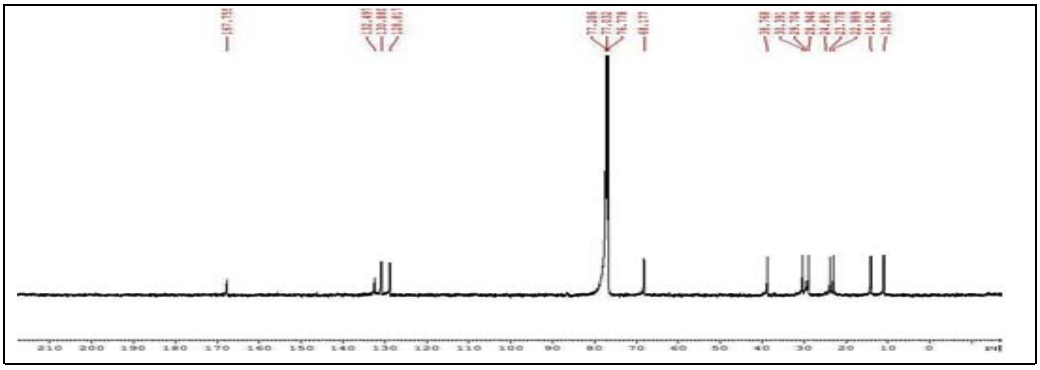

Fig 3: ^{13}C NMR spectrum

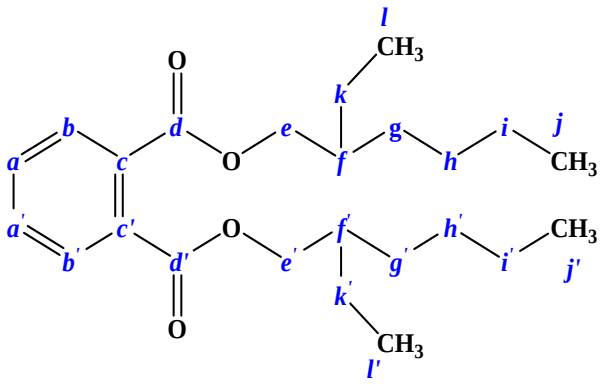

Fig 4: di(2-ethylhexyl)phthalate

3.2. Characterization of SF2

Compound SF2 (31 mg) was obtained as yellow crystals. The GC chromatogram (Fig. 5) revealed the presence of different compounds. Nevertheless, the MS spectrum (Fig. 6.a) of peak 4, at retention time 39.6 minutes, was characterized. Gratifyingly, both NIST and WILEY libraries proposed only 13-hexyloxacyclotridec-10-en-2-one (Fig. 6.b), whose fragmentation pattern was in a full agreement with that of the proposed compound.

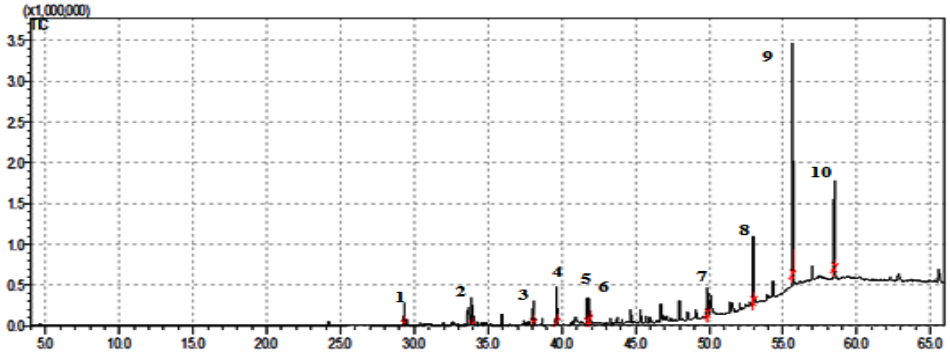

Fig 5: Gas chromatogram of SF2

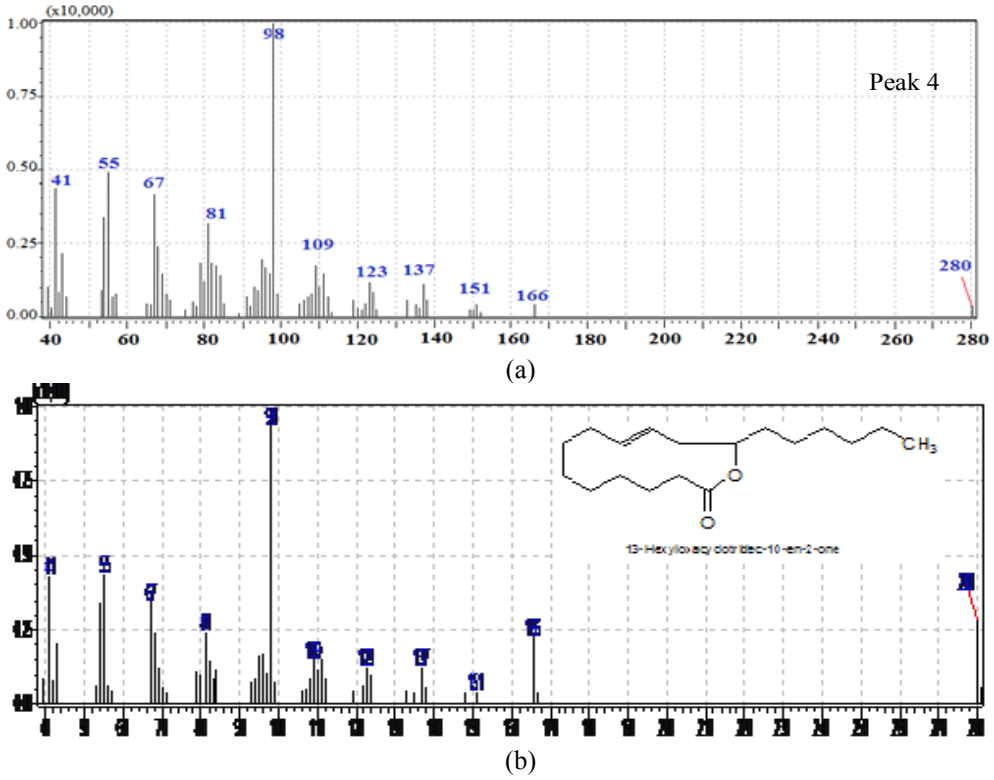

Fig 6: Fragmentation pattern of peak 4 (R_t=39.6 min) with standard hits libraries. (b): Mass spectra of Peak 4, (c): Standard hits from NIST & WILEY libraries.

It is worth noting that NIST and WILEY libraries could not match the fragmentation patterns of the other peaks with any of the known compound in the database. Thus, a part from DEHP and 13-hexyloxacyclotridec-10-en-2-one some novel antitumor agents are possibly present.

4. Conclusion

Two promising antitumor leads, di (2-ethylhexyl) phthalate and 13-hexyloxacyclotridec-10-en-2-one, had been isolated and characterized, from *Ambrosia maritima* L., for the first time. Needless to say, further study is important to unravel their cytotoxic activity.

5. Acknowledgment

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