Constituents of the leaves extracts of *Crassocephalum cepridioides* (Benth.) Moore

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The leaves of *Crassocephalum cepridioides* (Benth.) Moore has been investigated for its chemical constituents. Germacrene D, caryophyllene oxide and linolenic acid ethyl ester have been identified using GC-MS from petroleum ether extract. The chloroform extract has been subjected to open column chromatography to get a new compound, a polymer of pent-3-one, along with pentacontane, β-sitosterol and β-sitosterol-α-D-glucoside.

**Keywords:** *Crassocephalum cepridioides*, GC-MS, flavour constituents

*Crassocephalum cepridioides* (Benth.) (Asteraceae.) is used traditionally in Manipur as cure for many ailments. The leaves are used for treatment of ulcer, controlling hypertension and enhancing the immune system. The leaves are taken in raw form with meals. The research done on the *C. cepridioides* species of Indonesia reported Na, K, Mg, Ca, Fe, Zn. Phytochemical screening of *C. cepridioides* has been done on the species from South Africa and antioxidant, anti-inflammatory, cytotoxicity of the crude extracts have been performed and the ethanol extract showed maximum activity. Other *Crassocephalum* species were also studied. Essential oil obtained from leaves of *C. mannii* was analyzed by GC and GC/MS. The aqueous extract and the alkaloid fraction of *C. bauchiense* produced a significant antinociceptive effects in the acetic acid, formalin, glutamate, capsaicin and hot plate tests. In continuation to our studies on medicinal plants of Manipur the present work has been taken up. Herein is reported chemical constituents of *C. cepridioides* identified by GC-MS and spectroscopic data of isolated compounds separated by open column chromatography.

**Results and Discussion**

**Identification of compounds from the leaves of *C. cepridioides* by GC-MS**

The leaves of *C. cepridioides* were extracted with petroleum ether by Soxhlet extraction to yield yellowish transparent oil with high fragrance. The GC-MS of the petroleum ether extract showed the presence of several compounds of which six compounds were identified by matching their mass spectra with the spectra of reference compounds in NIST library spectral data. The GC-MS chromatogram is shown in Figure 1.

The peak with highest intensity (retention time 16.32 min) was found to be unknown as it did not show any matching compound (Figure 1). The sweet fragrant petroleum ether extract was due to the major components, 1–6.

A) Compound 1 (Figure 2) at retention time 11.09 min. Formula C_{12}H_{24}, Molecular weight 204. Mass spectral data 105(100), 119(76), 91(70), 161(47), 162(7), 204(6), 205(1).

C) Compound 2 (Figure 3) at retention time 14.09 min. Common name—Caryophyllene oxide. Formula C_{15}H_{24}O, Molecular weight 220. Mass spectral data 67(100), 91(86), 96(72), 105(63), 109(40), 123(25), 131(14), 145(7), 205(5), 220(3).

D) Compound 3 (Figure 4) at retention time 13.17 min. Formula C_{11}H_{16}O_{2}, Molecular weight 602. Mass spectral data 67(100), 111(90), 152(15), 180(12).

E) Compound 4 (Figure 5) at retention time 17.85 min. Formula C_{29}H_{46}O_{2}, Molecular weight 426. Mass spectral data 55(100), 129(52), 157(66), 185(46), 213(48), 299(12), 400(6).

F) Compound 5 (Figure 6) at retention time 20.34 min. Formula C_{20}H_{34}O_{2}, Molecular weight 306. Mass spectral data 79(100), 91(68), 93(39), 121(36), 135(30), 149(30), 173(14), 261(6).

G) Compound 6 (Figure 7) at retention time 19.05 min. Formula C_{27}H_{46}O_{4}, Molecular weight 428. Mass spectral data 81(100), 67(82), 95(74), 123(36), 149(30), 149(12), 429(2).

Similar studies have reported aromatic compounds analysed by GC-MS. Eighty-eight components were investigated using GC–MS from *Cistus albidus* (L). Malic acid, β-caryophyllene (spicy, woody), cembrene (woody) and caryophyllene oxide (spicy)
were investigated using GC/MS in Sumach (*Rhus coriaria* L.)\(^\text{10}\). Seventy-five compounds were identified from *Nepet-acilicica boiss*. Ex Benth. by GC–MS\(^\text{11}\). GC–MS study of extract of *Cyrtocarpa procera* Kunth led to the identification of 1,3-propyl-dipentadecanoate, 3-hydroxy-propyl-9-octadecenoate, pentadecylbenzene, eicosyl-benzene, docosane, heptacosane, dotriacontane and 2,6,10-trimethyl-tetradecane\(^\text{12}\). The volatile organic compounds from *Artemisia fragrans* Willd. were investigated using headspace solid-phase micro-extraction (HS-SPME) coupled to gas chromatography\(^\text{13}\). A GC/MS technique was used to obtain the primary metabolite profiling of *Scutellaria baicalensis*\(^\text{14}\). Monoterpenic-rich oil dominated by (Z)-β-ocimene and acyclic unsaturated ketones from *Tagetes bipinnata* was analyzed by GC/MS\(^\text{15}\). The GC-Ms analysis of petroleum ether extract of seeds of *Piper nigrum* Linn. resulted in the identification of fourteen compounds\(^\text{16}\).
Identification of compounds from the leaves of *C. cepridioides* by open column chromatography

Open column chromatography of the chloroform extract of the leaves after the oils were extracted with petroleum ether yielded compounds 7-10.

Compound 7 (pentacontane, Figure 8), white solid, m.p. 58-60°C, was isolated from the chloroform extract by elution with 2% EtOAc in petroleum ether and was found to be a hydrocarbon, C_{50}H_{102}. IR spectrum of 8 showed characteristic absorption bands at 2916, 2848, 1462, 723 cm\(^{-1}\). The \(^1\)H NMR (400 MHz, CDCl\(_3\)) showed the presence of a quartet at \(\delta_H 1.6\) (CH\(_2\)) and methyl protons as triplet at \(\delta_H 0.89\).

Compound 8 (Figure 9), white solid, m.p. 78-82°C, was isolated from the chloroform extract by elution with 2% EtOAc in petroleum ether and was found to be a saturated ketone, C_{40}H_{62}O_{10}. IR spectrum of 9 showed characteristic absorption bands at 2917, 2896, 1708, 1462, 1298, 937, 729 cm\(^{-1}\). The \(^1\)H NMR (400 MHz, CDCl\(_3\)) showed the presence of a triplet at \(\delta_H 2.35\) (CH\(_2\)), and a multiplet at \(\delta_H 1.63\) (CH\(_2\)) and methyl protons as triplet at \(\delta_H 0.88\). The \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) showed peaks at \(\delta_C 14.12, 22.70, 24.70, 31.94\) and 33.87.

Compound 9 (Figure 10), m.p. 128-132°C, colourless solid showing steroidal nature was indicated by Libermann-Burchard (LB) test. The IR spectrum of...
showed characteristic absorption bands at 3424 cm$^{-1}$ and 1659 cm$^{-1}$ for hydroxyl and olefinic groups as β-sitosterol. On the basis of physical constant, IR, $^1$H NMR, $^{13}$C NMR with DEPT spectral data; and by comparing with the authentic sample, 9 was established as β-sitosterol$^{17}$. Compounds 10 (Figure 11), white solid, was isolated from the chloroform extract by elution with 7% EtOAc in petroleum ether and was found to be a steroid indicated by Libermann-Burchard (LB) test, C$_{40}$H$_{62}$O$_{10}$. IR spectrum of 10 showed characteristic absorption bands at 3381, 2940, 1463, 1368, 1074, 1027 cm$^{-1}$. On the basis of physical constant, IR, $^1$H NMR, $^{13}$C NMR with DEPT spectral data; and by comparing with the authentic sample, 10 was established as β-sitosterol-α-D-glucoside$^{18}$.

**Experimental Section**

**Apparatus**

The analyte from petroleum ether extract was monitored using a Thermo Fisher GC-MS system with FID as detector and nitrogen as carrier gas. The column used was TG-5MS with stationary phase, 5% methyl polysiloxane from Thermo Scientific, 30m × 0.25 mm × 0.25 μm (Non polar column). Oven temperature was increased from 70°C to 270°C with flow of carrier gas at 1.0 mL/min. The peaks obtained were compared with the NIST-MS-2005 library spectral data with software WIN AMT. Melting points were recorded by capillary tube method and are uncorrected. The IR spectra were recorded on a Shimadzu FT-IR spectrophotometer. The mass spectra were recorded in MALDI-TOF Mass Spectrometer. $^1$H, $^{13}$C and 2D NMR spectra were recorded on a FT-NMR Bruker Avance (500 MHz for $^1$H NMR and 125 MHz for $^{13}$C NMR) in CDCl$_3$, at ambient temperature with TMS as internal standard. Silica gel (60-120 mesh) was used for open column chromatography and silica gel–G for thin layer chromatography.

**Chemicals**

Nitrogen 99.998%, was from Premier Cyrogenics Ltd Assam. All other chemicals, sulphuric acid, acetic anhydride, iodine, silica gel (60-120 mesh), silica gel-G, petroleum ether, chloroform, methanol and ethyl acetate were of analytical grade and were purchased from Merck.

**Preparation of plant materials**

Leaves and young buds of *Crassocephalum cepridioides* (Benth.) Moore were collected from North Eastern part of Manipur. A specimen (000207), collected and identified by Prof. P. Kumar Singh, Department of Life Sciences, Manipur University was deposited in Manipur University Museum of Plants, Manipur, India.

The dried leaves of *C. cepridioides* were ground to powder form. The extraction of 1.4 kg of ground leaves was carried out with Soxhlet condensation apparatus with light petroleum ether, chloroform, methanol, n-butanol and distilled water successively. After filtration, the different extraction solvents – petroleum ether, chloroform and methanol were evaporated to dryness under reduced pressure to leave a pasty mass. The petroleum ether extract was injected in a Thermo Fisher GC-MS system with FID as detector and nitrogen as carrier gas. The column used was
TG-5MS with stationary phase, 5% methyl polysiloxane from Thermo Scientific. Oven temperature was increased from 70°C to 270°C with flow of carrier gas at 1.0 mL/min. The peaks obtained were compared with the NIST-MS-2005 library spectral data with software WIN AMT. and compounds 1-5 were obtained as major components along with an unknown compound19.

GC and GC/MS operation conditions
The highest peak capacity was achieved when the column temperature was programmed at 10°C per min to 70°C for 2 min and then from 70°C to 270°C, which was maintained for 5 min in a split-less mode. The carrier gas velocity was 1 mL/min. Injector temperature was optimised at 280°C. Injection volume was 1 µL.

Conclusions
Our results have indicated that the fragrant smell of the yellowish transparent oil have been from the compounds 1-6 isolated with GC-MS, out of which the main component was not identified. This work opens perspective for further isolation of the compound with retention time 16.32 min, with open column. The traditional medicinal use was manifested from the bioactive sitostiol and its glucoside isolated from the plant.

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