Alpha Tocopherol - A New Report from *Memecylon* Species

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Bharathi, *et al.*: α-Tocopherol from *Memecylon* Species

The genus *Memecylon* L. (Melastomataceae), an important source of traditional medicine is mainly distributed in the Western Ghats region of Karnataka. In Indian ethnomedical practices, this plant is reported to be effective for skin and viral diseases including herpes and chickenpox. The objective of the present study was to screen *Memecylon* species namely *Memecylon umbellatum*, *M. edule*, *M. talbotianum*, *M. malabaricum* and *M. wightii* for the bioactive compounds with special reference to skin diseases. Leaf samples were dried and extracted using methanol. Thin-layer chromatography analysis of methanol extracts of five *Memecylon* species showed single band with RF value 0.61 detected at 260 nm. This band was eluted and subjected to high performance liquid chromatography. Profiles of *Memecylon* species confirmed the presence of α-tocopherol on comparison with the standard. Among five *Memecylon* species, *M. malabaricum* (0.724%) and *M. talbotianum* (0.362%) showed highest α-tocopherol concentration. For the first time α-tocopherol was reported from *Memecylon* species. Further, the structure of α-tocopherol was confirmed by liquid chromatography-mass spectrometry and nuclear magnetic resonance spectroscopy analysis. Presence of α-tocopherol (vitamin E) in *Memecylon* species validates the traditional use of *Memecylon* species in the treatment of skin diseases.

Key words: *Memecylon* species, HPLC, α-tocopherol

Medicinal plants play a major role in the discovery of new therapeutic agents for drug development. India is a rich source of medicinal plants and market value of all therapeutically important plant species is increasing day by day[1]. Traditionally, natural products have been a source of lead molecules in drug discovery. However, natural products have been de-emphasized as high throughput screening resources in the present, because of difficulty in obtaining high quality natural products. New technologies such as mass spectrometry, nuclear magnetic resonance spectroscopy (NMR) and other spectroscopic techniques can greatly facilitate structure elucidation process for creation of high quality natural product libraries[2].

The genus *Memecylon* (Melastomataceae), is used for medicinal purposes in Asia-Pacific regions. It consists of more than 300 species. In India, the genus is represented by about 40 species out of which 21 are endemics. The Western Ghats is the major centre of *Memecylon* diversity. In Ayurveda and Siddha, several *Memecylon* species are reported to be used by tribals in the treatment of skin disorders, herpes, chickenpox, stomach disorders, leucorrhoea, polyuria, menorrhagia, dysentery and also in the treatment of bacterial infections and inflammation[3]. Several pharmacological activities such as, antimicrobial, antiinflammatory, antidiabetic properties including some of the phytoconstituents such as, apigenin, isorhamnetin-3-O-glycoside-7-O-glycoside etc. are reported from *Memecylon* species[4-6].

In the current study, methanol extracts of *Memecylon* species were selected as bioactive fractions. Thin layer chromatography (TLC) screening and high performance liquid chromatography (HPLC) analysis showed the active compound is highly polar, based on mobile phase in both types of chromatography in five species of *Memecylon*. Alpha-tocopherol was detected in aerial parts of five *Memecylon* species from the TLC and HPLC analysis with reference standard and relative concentration of α-tocopherol was calculated in five *Memecylon* species. Further the compound

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identification was confirmed by liquid chromatography-mass spectrometry (LC-MS) and NMR analysis.

Five *Memecylon* species namely, *M. umbellatum* Burm, *M. edule* Roxb, *M. talbotianum* Brandis, *M. malabaricum* Clarke and *M. wightii* Thwaites were collected from different parts of Karnataka. Shade dried leaves were powdered in an electric blender and extracted with methanol using Soxhlet apparatus. Extracts of the species were collected and tested for different bioactive potentials namely, antioxidant, antiinflammatory, and antimicrobial potentials. Only methanol extracts showed significant activity. Therefore, methanol extracts of *Memecylon* species were selected for further studies.

TLC of methanol extracts of *Memecylon* species was carried out on silica gel (TLC silica gel 60 mesh, 20×20, 0.5 mm, Merck and Co, Inc) with methanol:acetone (8.7:1.3) solvent system. The methanol extract was spotted and the solvent front was allowed to run for approximately 16 cm. The running lane was then dried thoroughly; elution of compound was detected at 260 nm. The bands corresponding to active compound by initial screening were scraped out and the compounds were washed out of silica gel with methanol. The obtained methanol solutions were filtered and subjected to HPLC analysis for further purification. A TLC bioactive fraction of *Memecylon* species extract was subjected to HPLC. Analytical HPLC (Waters, 10 ATVP) was performed with a reversed phase C18 column using LC-10-ATVP double unit pumps. The analytical chromatography was carried out under isocratic conditions with mobile phase of methanol and water (99:1) using a flow rate 1.0 ml/min and chromatogram recorded at wavelength of 292 nm using a SPD-10AVP multi-wavelength detector. Sample were dried and dissolved in methanol.

Concentration of α-tocopherol in different *Memecylon* species was calculated relative to the standard based on the comparison of peak area in HPLC profiles of samples and standard vitamin E. Bioactive HPLC fraction of *M. talbotianum* was injected to LC-MS (Waters Acquity, Milford, MA) Series UPLC/SYNAPT G_2 HDMS with electrospray ionization) connected with a waters C18 column (particle diameter 5 µm, 150×4.6 mm i.d.). Mobile phase: acetonitrile:water (75:25) containing 0.1% formic acid with a flow rate of 1.1 ml/min.

The NMR spectrum of the purified compound of *M. talbotianum* was recorded on a Bruker Avance-III 400 Hz spectrometer equipped with BBFO probe and connected with VT Unit (temp. range from −80°+130°). ^{1}H NMR was recorded in deuterated dimethyl sulfoxide (DMSO) and 2D data were acquired in CD3 OD at a temperature of 300 K.

The TLC analysis of methanol extracts of five *Memecylon* species showed single band with RF value 0.61 detected at 260 nm (fig. 1). This band was filtered and subjected to HPLC analysis for further purification.

HPLC profiles of α-tocopherol (standard) and α-tocopherol of *Memecylon* species were given in fig. 2. Standard α-tocopherol showed the retention time (R_t) at 16.668 min. R_t of α-tocopherol in *M. umbellatum*, *M. edule*, *M. talbotianum*, *M. malabaricum* and *M. wightii*, were 16.719, 16.162, 16.317, 17.006, and 16.8 min, respectively. *M. malabaricum* and *M. talbotianum* showed highest α-tocopherol concentration (0.724 and 0.362%) compared to other *Memecylon* species. In *M. umbellatum*, *M. edule* and *M. wightii* α-tocopherol was present in minute concentrations of 0.181, 0.125 and 0.146% (Table 1).

Among five *Memecylon* species, *M. malabaricum* and *M. talbotianum* showed highest concentration of α-tocopherol. Hence the bioactive fractions of these two plant species were purified using preparative HPLC and then subjected to LC-MS for the detection and confirmation of bioactive molecule. Among these two fractions, *M. talbotianum* fraction showed a single major peak in LC-MS with a molecular mass of 429.391 (ion positive mode, fig. 3). Hence this fraction was subjected to NMR spectra analysis for confirming the structure of the molecule.

The NMR spectral analysis of LC-MS fraction of mass 429.391 from *M. talbotianum* confirms that the compound is α-tocopherol. This is the first report

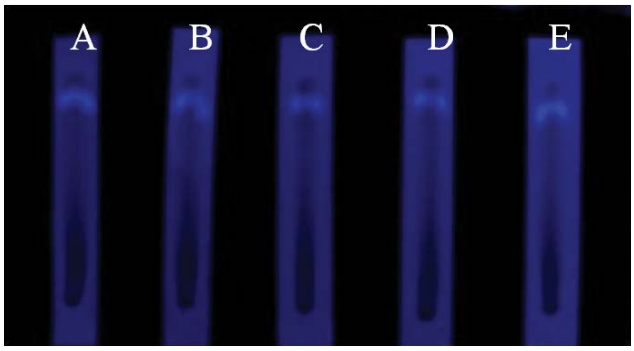

Fig. 1: TLC banding pattern of methanol extracts of *Memecylon* species
A: *M. umbellatum*, B: *M. edule*, C: *M. talbotianum*, D: *M. malabaricum* and E: *M. wightii*

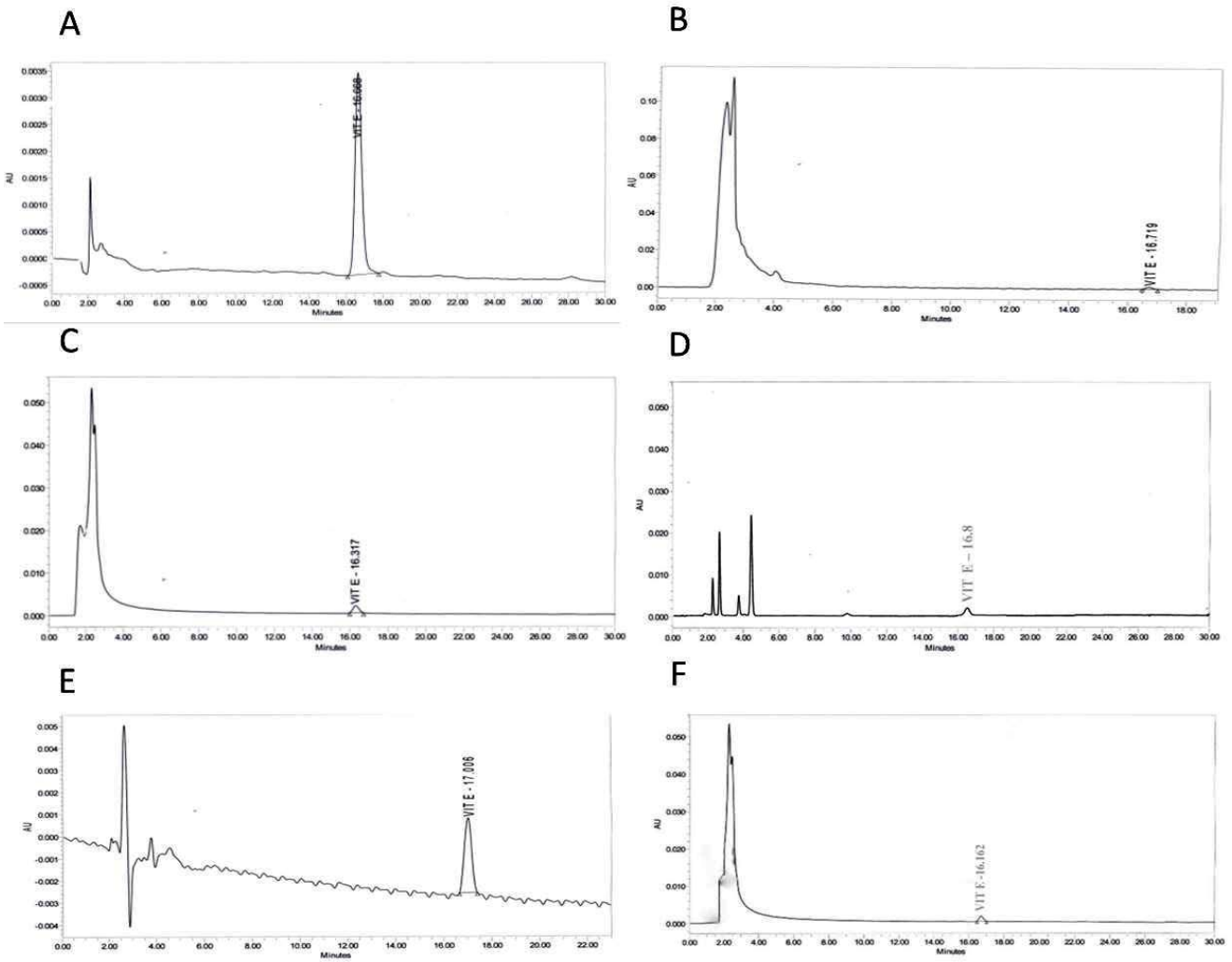

Fig. 2: HPLC profiles of five *Memecylon* species
A: HPLC profile of standard α-tocopherol; B, C, D, E and F are the HPLC profile of *M. umbellatum* with relative concentration (0.181), *M. edule* (0.724), *M. talbotianum* (0.362), *M. malabaricum* (0.146) and *M. wightii* (0.125), respectively

TABLE 1: RELATIVE CONCENTRATION OF α-TOCOPHEROL IN *MEMECYLON* SPECIES

Memecylon species	Relative concentration of α-tocopherol (%)
M. umbellatum	0.181
M. malabaricum	0.724
M. talbotianum	0.362
M. edule	0.146
M. wightii	0.125

In *M. umbellatum*, *M. edule* and *M. wightii*, α-tocopherol was present in minute concentrations

for α-tocopherol in *M. talbotianum*. The ^{1}H-NMR chromatogram of *M. talbotianum* was reported in fig. 4. Further, to confirm the structural arrangements of the *M. talbotianum*, ^{1}H-NMR and 13C-NMR data revealed 50 protons and 29 carbons as confirmed by deuterium exchange (fig. 4A and 4B). The ^{1}H-NMR and 13C-NMR data are expressed as follows.

^{1}H-NMR (400MHz, DMSO-d^6): δ 7.18 (s, 1H, H-26), 2.38 (t, 2H, H-3), 1.97(s, 3H, H-28), 1.93 (s, 3H, H-25), 1.90 (s, 3H, H-27), 1.57 (q, 2H, H-2), 1.4 (m, 2H, H-11) 1.29-1.37 (m, 4H, H-12, 14, 18), 1.17 (m, 8H, H-13, 15, 16, 20), 1.103 (s, 3H, H-12), 0.97 (s, 7H, H-17, 19, 21, 22), 0.73-0.76 (t, 12H, H-29, 30, 31, 23) (fig. 4A).

13C-NMR (100MHz, DMSO-d^6): δ 145.59 (C-8), 144.45 (C-5), 122.75 (C-4), 121.73 (C-10), 120.30 (C-9), 116.67 (C-7), 73.8 (C-1), 37.1-37.5 (C-11, 13, 15, 17, 19, 21), 32.5 (c-14), 31.7 (C-18), 27.8 (C-2), 24.6 (C-22), 24.2 (C-20), 22.7 (C-22,23), 22.6 (C-29, 30), 20.8 (C-3), 19.65 (C-25, 27, 28) (fig. 4B).

Traditional knowledge about the therapeutic potential of plants is necessary to isolate and identify biologically active products from plants[7]. Therefore, isolation and identification of bioactive compounds present in a crude

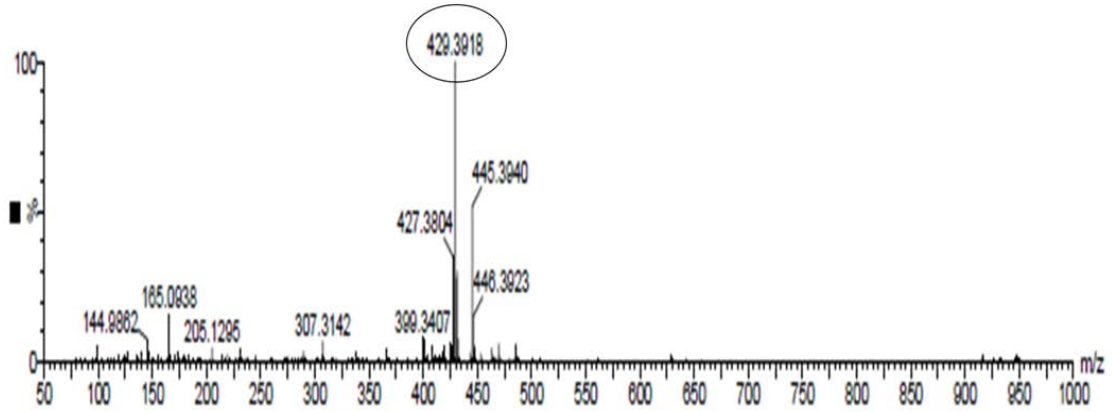

Fig. 3: LC-MS analysis of bioactive HPLC fraction of *M. talbotianum*
***M. talbotianum* fraction showed a single major peak in LC-MS with a molecular mass of 429.391 (ion positive mode)**

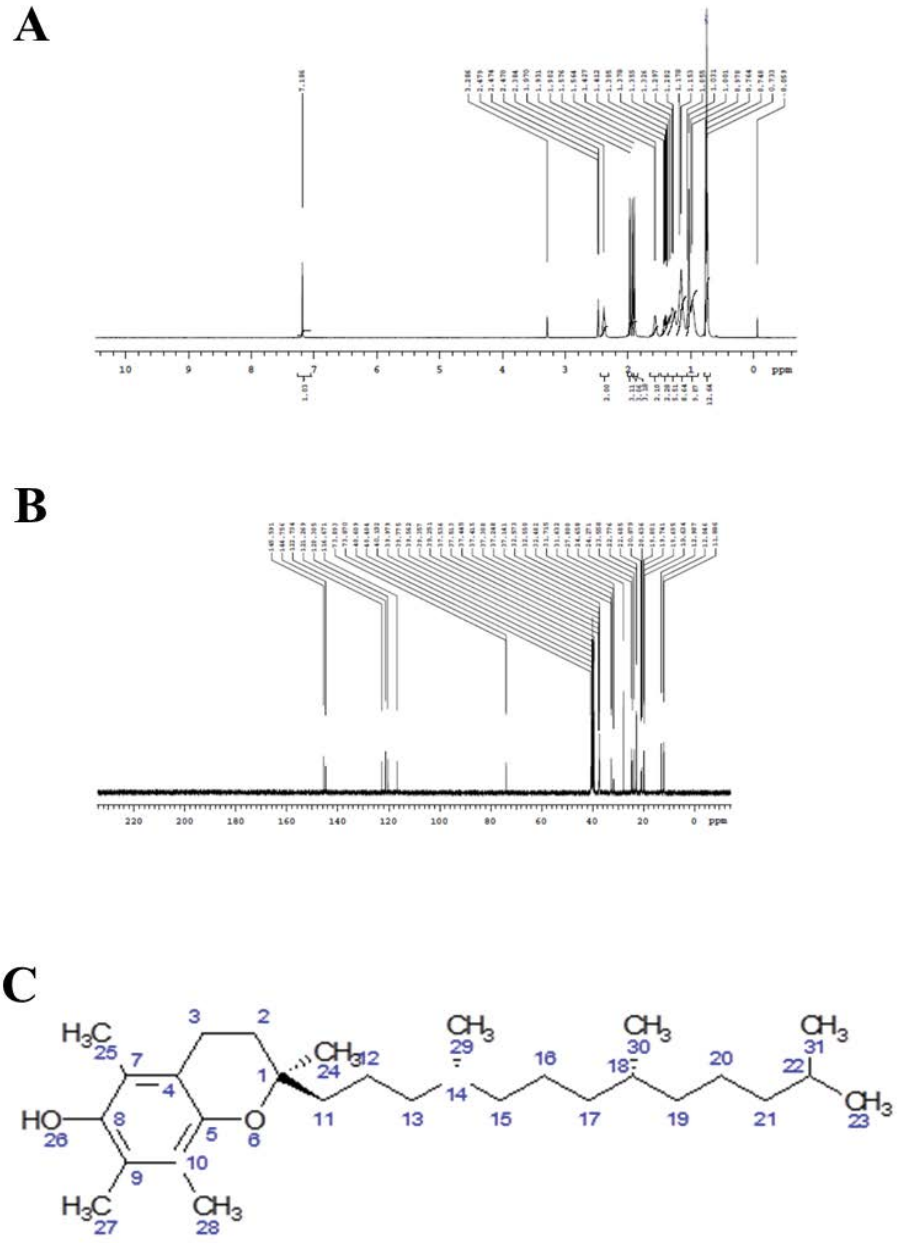

Fig. 4: 1H NMR, 13C NMR spectra of bioactive fraction and the structure of α-tocopherol isolated from the *M. talbotianum*
(A) ^{1}H NMR, (B) 13C NMR spectra, (C) chemical structure of α-tocopherol

extract serve as the building block for the development of new type of therapeutics with new mechanisms of action[8]. Traditionally, natural products have been a source of lead molecules in drug discovery. However, natural products have been de-emphasized as high throughput screening resources in the present, because of difficulty in obtaining high quality natural products. New technologies such as mass spectrometry, NMR and other spectroscopic technique can greatly facilitate structure elucidation process for creation of high quality natural product libraries. In the current study, methanol extracts of *Memecylon* species were selected as bioactive fractions. TLC and HPLC analysis showed the active compound is α-tocopherol. α-tocopherol is also identified in several other plants such as *Cyamopsis tetragonoloba*, *Moringa oleifera*, *Stevia rebaudiana*, *Millingtonia hortensis* and *Jasminum sambac* through GC-MS analysis[9-13].

For the first time α-tocopherol was reported in five *Memecylon* species in the present study. Higher concentration of α-tocopherol was detected in *M. malabaricum*, *M. talbotianum*. The higher α-tocopherol concentration might be one of the reasons for the superior bioactive property of *M. malabaricum* and *M. talbotianum* than other *Memecylon* species. HPLC method is easy, precise and showed better resolution of α-tocopherol in different *Memecylon* species. Further the compound identification was confirmed by LC-MS and NMR analysis. Since α-tocopherol is preferentially absorbed and accumulated in humans it is widely used as an inexpensive antioxidant in cosmetics and foods, vitamin E is good for the skin and is a common ingredient of skin creams and lotions that encourages skin healing and reduces scarring after injuries such as burns[14,15]. Hence presence of α-tocopherol (vitamin E) in *Memecylon* species validates the traditional use of *Memecylon* species in the treatment of skin diseases.

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Conflicts of Interest:

The authors declare no conflicts of interest.

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