



ISOLATION OF ANTICANCER AGENTS FROM *Tabernaemontana divaricata* (L.) R. Br. ex Roem. & Schult.

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Abstract

*Treatment of breast cancer is not easy because of the complexity in molecular physiology. Surgery, chemotherapy and radiation therapy includes different sources of treating breast cancer. The systemic chemotherapy faces lot of challenges, mainly adverse effects. So, there is a need of newer drugs to be developed against breast cancer. The present work tries to justify the folklore claims of *Tabernaemontana divaricata* (L.) R. Br. ex Roem. & Schult. through a sound scientific background using modern analytical tools. It was found that phytoconstituents of the plant showed good docking score against 3ERT protein. The petroleum ether extract and chloroform extract showed anticancer activity. Petroleum ether extract was found to have GI₅₀ value of 18.7µg/ml. Phytochemical Studies lead to the isolation of two compounds, 1 and 2. These compounds, isolated from the leaves were studied and characterized tentatively with the help of modern analytical tools like FTIR, ¹H NMR and MS. Based upon spectral studies, compound 1 was characterized as 2-(1,6a,6b,9,9,12a,14b-heptamethyl 1, 2, 4a, 5, 6, 6a, 6b, 7, 9, 10, 11, 12, 12a, 12b, 13, 14b-hexadecahydronicen-3-yl) propan-2-yl nonanoate. Compound 2 was found to be not pure enough to be characterized. The isolated compounds were also tested for their anti-proliferative activity against breast cancer.*

Keywords: Anticancer, *Tabernaemontana divaricata*, Isolation, Phytoconstituents

1. INTRODUCTION

The plant based drug design is one of the major sources of anticancer agents. Drugs like vincristine, vinblastine, etoposide, paclitaxel, camptothecin, topotecan and irinotecan are obtained from plants. Citarabine, aplidine and dolastatin are obtained from marine organisms. Dactinomycin, bleomycin and doxorubicin are obtained from micro-organisms. Agents like curcumin, resveratrol (red grapes, peanuts and berries), genistein (soybean), diallyl sulfide (allium), S-allyl cysteine (allium), allicin (garlic), lycopene (tomato), capsaicin (red chilli), diosgenin (fenugreek), 6-gingerol (ginger), ellagic acid (pomegranate), ursolic acid (apple, pears, prunes), silymarin (milkthistle), anethol (anise, camphor, and fennel), catechins (green tea), eugenol (cloves), indole-3-carbinol (cruciferous vegetables), limonene (citrus fruits), β-carotene (carrots) are different dietary sources that can be used as anticancer agents. The history of plants as a source of anticancer agents starts with the discovery of vinca alkaloids and isolation of podophyllotoxins. Vinca alkaloids are mainly used in the treatment of Hodgkin's disease and leukemia. Vincristine stops microtubule assembly. Homoharringtonine obtained from *Cephalotaxus harringtonia*. This acts by inhibiting the protein synthesis and blocking cell cycle replication. *Ipomoea batata* (Convolvulaceae) is source of 4-ipomeanol, which is specifically used for lung cancer (Bhanot et al., 2011).

Tabernaemontana is one of the genres used in Ayurveda, Chinese and Thai traditional medicine. The species of our interest is *Tabernaemontana divaricata*. Alkaloids, terpenoids, steroids, flavonoids, phenyl propanoids and enzymes has been isolated from different parts of



the plant. Most of the work has been carried out on the alkaloids. Around 66 different alkaloids have been isolated from different parts of the plants (Pratchayasakul et al., 2008).

2. MATERIAL AND METHODS

Collection of Plant Materials

The leaves of *Tabernaemontana divaricata* (Family: *Apocynaceae*) were collected from the campus of Birla Institute of Technology, Mesra, Ranchi in the month of September, 2013. The plant was identified and authenticated by Central National Herbarium of Botanical Survey of India, Kolkata as *Tabernaemontana divaricata* (CNH/11/2014/Tech. II/).

Extraction

The leaves of *Tabernaemontana divaricata* were cleaned thoroughly and dried. It was then powdered and subjected with extraction using Soxhlet apparatus. The plant material was defatted for 72 hours using petroleum ether (60-80⁰C) followed by successive extraction with chloroform, ethyl acetate and 80% v/v methanol. The solvents were removed under reduced pressure using rotary evaporator. They were further lyophilized.

Phytochemical Screening

The extracts which are having anticancer activity were further tested for various chemical constituents by following tests and tabulated in results. Phytochemical examinations were carried out for the extracts as per the standard methods (Raaman N., 2006).

Isolation & Characterization

In order to isolate the phytoconstituents, the leaves of *Tabernaemontana divaricata* were extracted several times with petroleum ether. Then it was evaporated under reduced pressure using Rota evaporator (Buchi). Then the extract was mixed with silica and sample was prepared which was further used for separation. The column was prepared using silica 100-200 column chromatography grade. Wet column was prepared. Silica was mixed with the non-polar solvents, petroleum ether and hexane. Later it was saturated with same solvent system, then with petroleum ether, n-hexane and toluene solvent system. Then the sample was loaded carefully, and separated using the solvent system developed using TLC and HPTLC. Isocratic elution was used to separate the phytoconstituents.

Anticancer assay using MCF-7 cell line

It is performed by **SRB assay** method, in which 0.4% (w/v) Sulforhodamine B (SRB) solution in 1% acetic acid was used to stain cell fixed by TCA for 30 minutes. Immediately after the staining, cells were washed with 1% acetic acid to remove the unbound dye. The remaining acetic acid solution was removed by striking the cell plate, the cell culture was then air dried to ensure that there was no moisture. To calculate the optical density (OD) bound dye was solubilized with 10mM Tris [tris (hydroxymethyl)aminomethane] base for 5 minutes on a gyratory shaker. The optical density was detected by using UV max microtiter plate reader or Beckman DU-70 spectrophotometer, best results were obtained at 564nm (Skehan et al., 1990).

3. RESULTS AND DISCUSSION

Phytochemical screening

The phytochemical screening of the petroleum ether extract of *Tabernaemontana divaricata* gave positive test for the presence of steroids and tannins. The chloroform extract gave positive results for steroids, glycosides. The ethyl acetate extract gave positive results for

alkaloids, flavonoids and phenol compounds. The 80% v/v methanol extract gave positive test for alkaloids, flavonoids and phenolic compounds.

Phytochemical screening results of different extracts obtained from leaves of *Tabernaemontana divaricata*

Phytoconstituents	Pet. ether	Chloroform	Ethyl Acetate	80% Methanol
Alkaloids	-	-	+	+
Flavonoids	-	-	+	+
Steroids & triterpenes	+	+	-	-
Carbohydrates	-	-	+	+
Saponins	-	-	-	-
Phenolic compounds	-	-	+	+
Glycosides	-	+	+	+
Tannins	-	-	+	+

Isolated Compound 1: Orange crystalline compound, m.p. 140-142 °C

The mass spectra: M+1 589

There is an ester

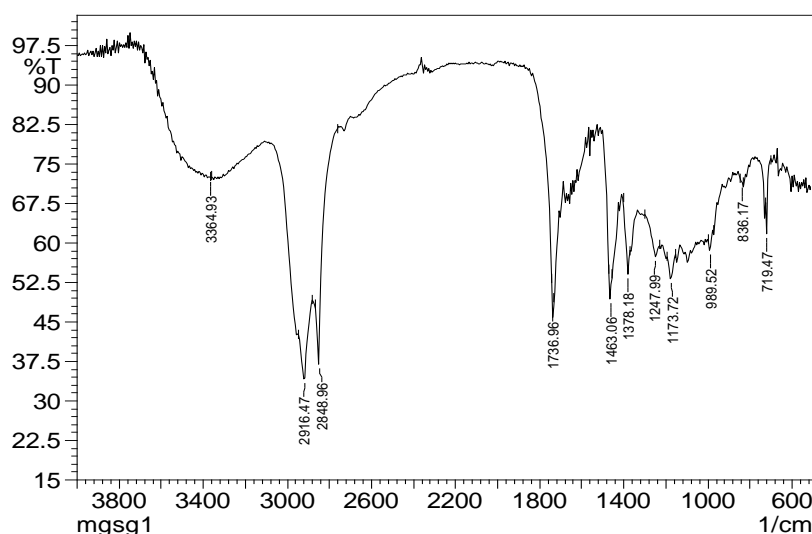
In the proton NMR there are no peaks corresponding to CH₂OCO- so the alcohol moiety must be a tertiary. The aliphatic part would support the idea of a fatty acid, the 2.30 peak would be alpha to CO.

UV: λ_{\max}^{MeOH} (nm): 446

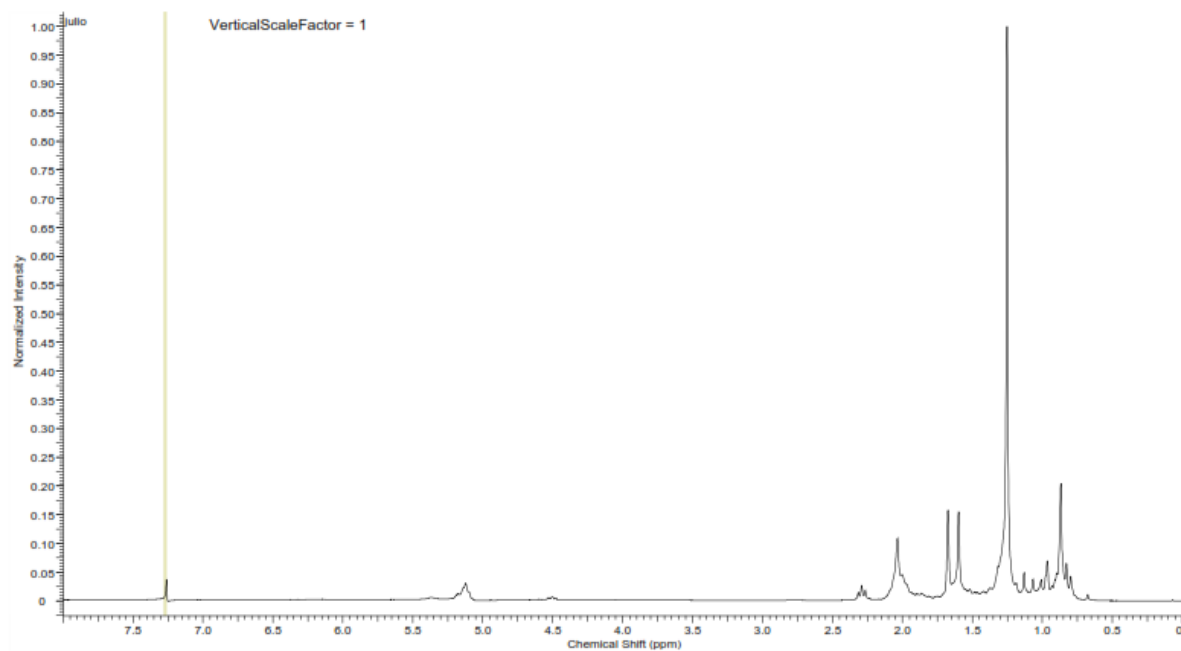
FTIR: ν_{\max}^{KBr} (cm⁻¹): 3364.93, 2916.47, 2848.96, 1736.96, 1463.06, 1378.18, 1247.99, 1173.72, 989.52, 836.17, 719.47

ESI-MS: m/z: 589

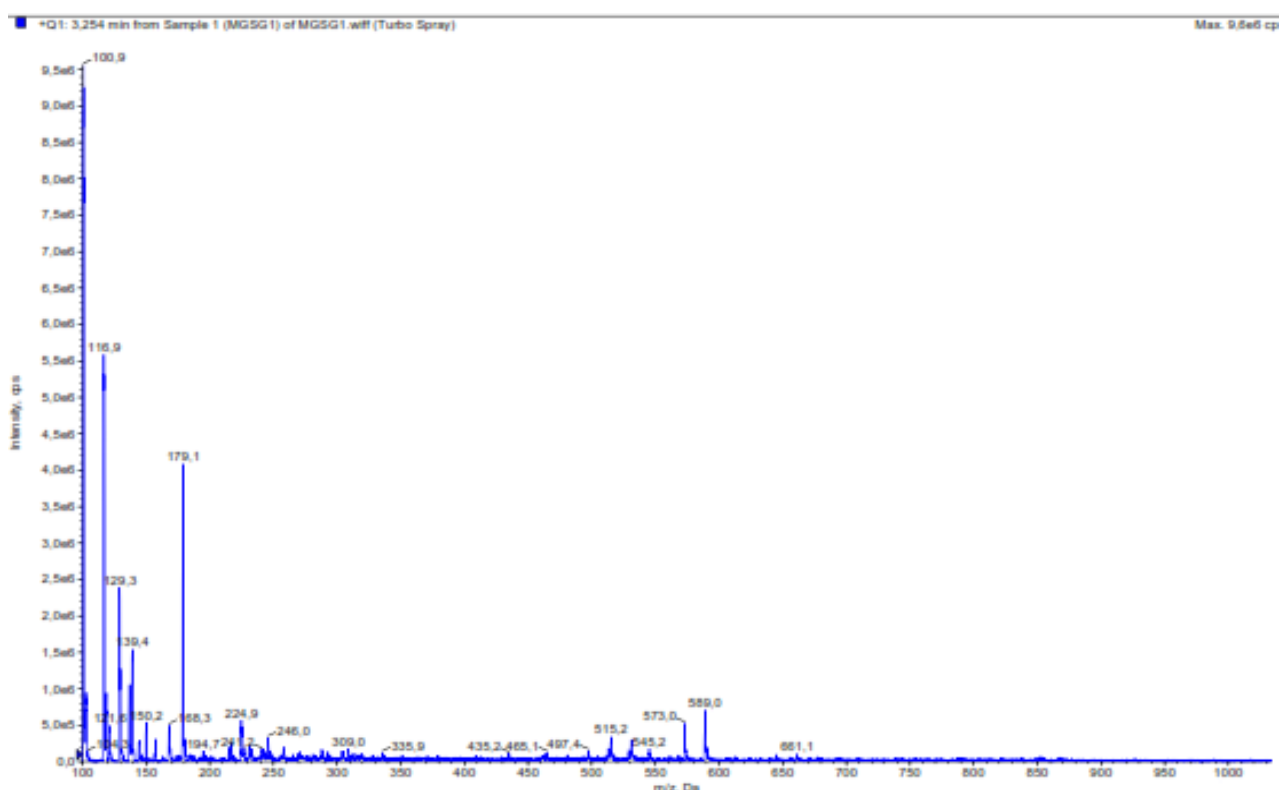
¹H NMR ^{CHCl₃} (δ): 0.68, 0.80, 0.87, 0.97, 1.0, 1.15, 1.3, 1.6, 1.68, 2.1, 2.3, 4.5, 5.15



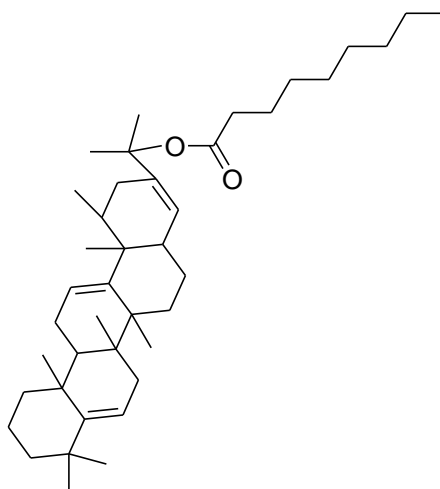
FT-IR of Compound 1



¹H NMR of Compound 1



Mass spectra of Compound 1



2-(1,6a,6b,9,9,12a,14b-heptamethyl 1, 2, 4a, 5, 6, 6a, 6b, 7, 9, 10, 11, 12, 12a, 12b, 13, 14b-hexadecahydropicen-3-yl) propan-2-yl nonanoate (Tentative)

In Pharmacological Studies, the extracts and the isolated compound were sent to Tata Memorial Centre for anticancer activity. The petroleum ether extract and chloroform extract showed anticancer activity. Petroleum ether extract was found to have GI₅₀ value of 18.7µg/ml. The isolated compounds were also tested for their anti-proliferative activity against breast cancer. The isolated compounds were found to have anti proliferative effect against breast cancer in synergistic way.

4. CONCLUSION

The present work tries to justify the folklore claims of *Tabernaemontana divaricata* (L.) R. Br. ex Roem. & Schult. through a sound scientific background using modern analytical tools. It was found that phytoconstituents of the plant *Tabernaemontana divaricata* showed good docking score against 3ERT protein.

Phytochemical Studies lead to the isolation of two compounds, 1 and 2. These compounds, isolated from the leaves of *Tabernaemontana divaricata* L. were studied and characterized tentatively with the help of modern analytical tools like FTIR, ¹H NMR and MS. Based upon spectral studies, compound 1 was characterized as 2-(1, 6a, 6b, 9, 9, 12a, 14b-heptamethyl 1, 2, 4a, 5, 6, 6a, 6b, 7, 9, 10, 11, 12, 12a, 12b, 13, 14b-hexadecahydropicen-3-yl) propan-2-yl nonanoate.

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