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Activity guided fractionation and identification of fatty acid derivative from alcoholic extract of *Wrightia tomentosa*

Kandasamy Nagarajan[1], Dilip K. Singh[2], *Ira Sharma[1], Ramesh B. Bodla[3].

[1]Department of Pharmaceutical Chemistry, KIET School of Pharmacy, Ghaziabad, India
[2]IIMT College of Medical Sciences, Meerut, India
[3]Pharmaceutical Chemistry, Delhi Institute of Pharmaceutical Sciences and Research (DIPSAR), New Delhi, India

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ABSTRACT

Aim of the study was to identify and characterize the bioactive principle from the roots of Wrightia tomentosa. For isolation the compound, the dried root powder of Wrightia tomentosa was subjected to hot extraction with 70% ethanol to chromatography. One compound ($WTEF_{24}$) was isolated and purified by ethanol: water (2.5: 7.5). Preliminary phytochemical screening reveals the presence of flavonoids, alkaloids, saponins and fatty acids. TLC analysis for the isolated fraction $WTEF_{24}$ showed the presence of fatty acids with the corresponding Rf value: 0.89 using chloroform: glacial acetic acid: methanol: water (64: 32: 12: 8) with U.V chamber visualization. The mass spectrum of $WTEF_{24}$ showed the characteristic parent molecular ion protonated peak $(M+1)^+$ at m/z 335, which corresponds to the fatty acid derivative, methyl 6,7-dithia stearate.

Key words: *Wrightia tomentosa*, 70% ethanol, roots, methyl 6, 7-dithia sterarate.

INTRODUCTION

Natural products have been one single most successful source of medicines. Each plant is like a chemical factory capable of synthesizing unlimited number of highly complex and unusual chemical substances whose structures could otherwise escape the imagination forever[1].

Wrightia tomentosa Roem & Schult, family: Apocynaceae, is widely distributed at an altitude of 600m in the Himalayas. A novel isoflavone, Wrightiadione isolated from the plant possess cytotoxic activity against murine P_{388} lymphocytic leukemia cell line [2]. The root barks are found to be useful in Snake bite and Scorpion-stings [3]. The ethanolic bark and leaf extract of *Wrightia tomentosa* possesses significant anti-allodynic effects [4] with no observable signs of toxicity [5] and antihyperglycemic activity [6] in streptozotocin induced diabetic rats. The alcoholic extract of *Wrightia tomentosa* dried bark was reported to exhibit markedly high antioxidant potency (IC_{50} value of 75.0 µg/ml from DPPH radical Scavenging assay), suitable for prevention of human disease [7]. The butanol extract of the plant was shown to have anti-

microbial activity against both gram positive and gram negative organisms [8]. The leaf extract of *Wrightia tomentosa* has proved to be extremely useful against non-tuberculous mycobacterium (NTM) infections [9], which are becoming a major concern for hospitals and medical clinics.

Our objective is to study a design of isolation and characterization of bioactive pure component from the roots of *Wrightia tomentosa.*

MATERIALS AND METHODS

Plant material

The roots of *Wrightia tomentosa* were collected from the hills of Yercaud forest, Salem district of Tamilnadu and identified[10], authenticated by an acknowledged Botanist, Professor M.B. Viswanathan, Co-ordinator, Centre for Herbal Drug Discovery and Development of the Research Department of Bharathidasan University, Tiruchirappalli, Tamilnadu, India and the voucher specimen was deposited thereafter at Bharathidasan University (BDUT/545).

Extraction and isolation

The roots of *Wrightia tomentosa* were dried at room temperature and reduced to a coarse powder. The powder material was subjected to qualitative tests [11] for the identification of various phytoconsitituents like flavonoids, alkaloids, saponins and fatty acids. Then the powder (180 gm) was subjected to Soxhlet extraction with 70% ethanol separately for 72 hours at a temperature of 80-90^{0}C. The extracts were concentrated and the solvent was completely removed by Rotary vacuum evaporator (Buchi). Green waxy residue was obtained.

The concentrated hydro alcoholic root extract (0.75gm) were taken in a china dish separately and heated continuously on a water bath by gradually adding dichloromethane in a small portion with constant stirring till desired consistency was obtained. Silica gel (for column chromatography, 200 mesh size) was then added (weighed quantity 20gm for root extract) slowly with continuous mixing with steel spatula till desired consistency of the mixture was obtained. It was air dried and larger lumps were broken to get a smooth free flowing mixture.

A column of 5.0 ft. length and 16 mm of internal diameter was taken and dried. The lower end of the column was plugged with absorbent cotton. The column was clamped and fitted in vertical position on a stand. The column was then half-filled with *n*-hexane. Silica gel was then poured in small portions and allowed to settle gently until the necessary length of the column was obtained. The dried silica gel slurry containing the hydro alcoholic extract of root was poured in the column separately and then eluted successively with different solvents, in the order of *n*-hexane, ethyl acetate: *n*-hexane (5: 95), ethyl acetate: *n*-hexane (15: 85), ethyl acetate: *n*-hexane (25: 75), ethyl acetate: *n*-hexane (40: 60), ethyl acetate: *n*-hexane (55: 45), ethyl acetate: *n*-hexane (70: 30), ethyl acetate: *n*-hexane (85: 15), ethyl acetate, ethyl acetate: chloroform (75: 25), ethyl acetate: chloroform (50: 50), ethyl acetate: chloroform (25: 75), chloroform, chloroform: acetone (75: 25), chloroform: acetone (50: 50), chloroform: acetone (25: 75), acetone, acetone: ethanol (75: 25), acetone: ethanol (50: 50), acetone: ethanol (25: 75), ethanol, ethanol: water (75: 25), ethanol: water (50: 50), ethanol: water (25: 75), water and *n*-butanol: glacial acetic acid: water (4: 1: 5). Twenty six fractions were collected in a conical flask and marked. The marked fractions were subjected to TLC to check homogeneity of various fractions [12]. Chromatographically identical various fractions (having same Rf values) were combined together and concentrated. They were then crystallized with suitable solvent systems.

Physico-chemical characterization of pure isolate

One pure bioactive lead was isolated and the isolated pure fraction was tentatively identified by qualitative chemical analysis. Further identification and characterization are done by TLC and Mass spectral analysis.

TLC analysis

Initially TLC analysis was performed with universal solvent system ethyl acetate: *n*-hexane (6: 4) for various phytoconstituent identification and later on, individual phytoconstituents are identified by performing specific TLC analysis using specific solvent systems as mobile phase for each category of phytoconstituents, which has been already known with qualitative chemical analysis.

Mass spectral analysis

The electro spray mass spectrum for the isolated one test compound was recorded (figure.1) on Thermo Finnigan LCQ advantage max ion trap mass spectrometer at SAIF, CDRI, Lucknow. The 10µl samples (dissolved in solvent such as methanol/ acetonitrile/ water) were introduced into the ESI source through Finnigan Surveyor auto sampler. The mobile phase (90: 10 MeOH/ ACN: H_2O) flowed at the rate of 250µl/min by MS pump. Ion spray voltage was set at 5.3 KV and capillary voltage 34 V. The MS scan run up to 2.5 min and the spectra print outs are averaged for over 10 scan at peak top in TIC. The mass spectra give information on various types of peaks and determining the molecular formula for the isolated compound after successful interpretation [13].

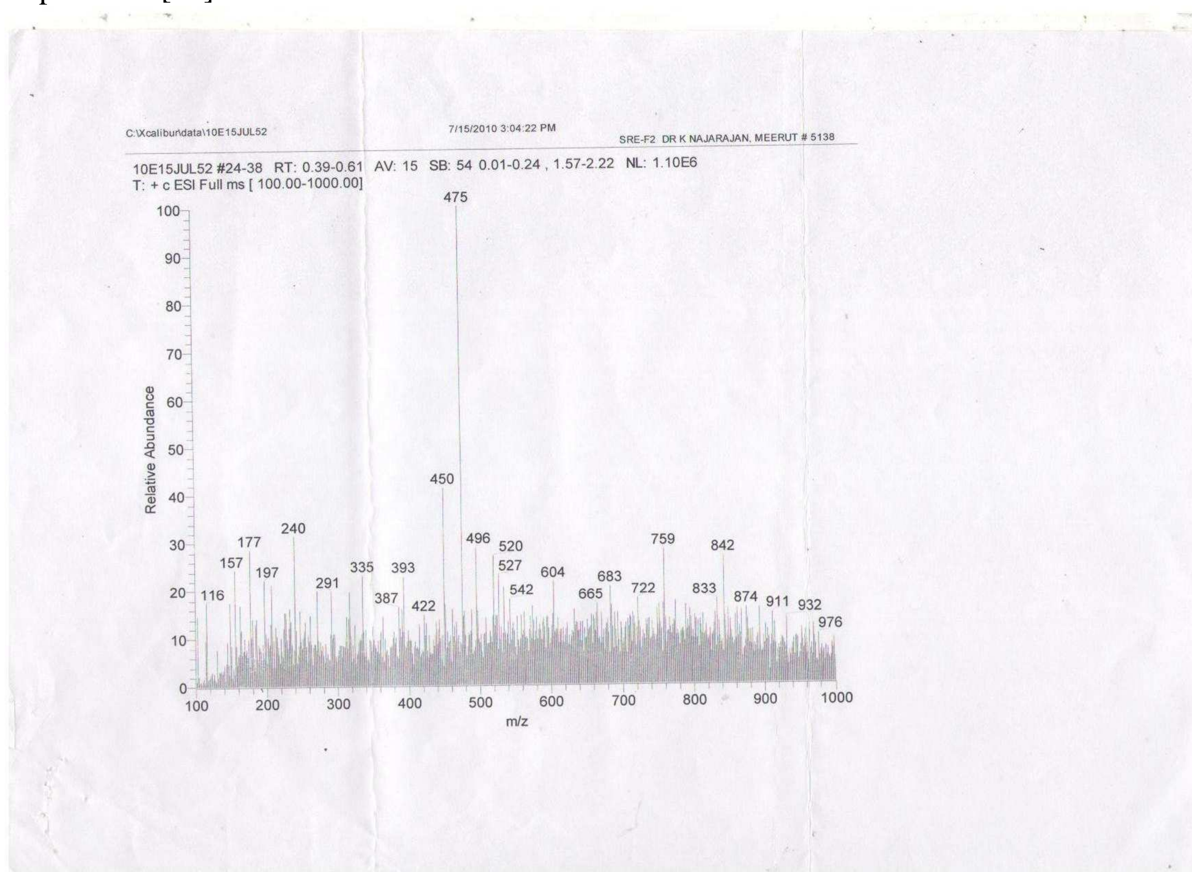

Fig .1: Mass spectrum of a fatty acid derivative $WTEF_{24}$ of *Wrightia tomentosa*.

RESULTS AND DISCUSSION

1. Preliminary phytochemical screening

The results of phytochemical screening showed the presence of fatty acid for $WTEF_{24}$ as major active constituent in addition to the presence of flavonoids, alkaloids and saponins.

2. Isolation and preliminary identification of bioactive lead from *Wrightia tomentosa* roots

Elution of root drug in column with 25% ethanol-water, i.e. (Fraction 24) yielded dark brown residue, Rf: 0.89 (chloroform: glacial acetic acid: methanol: water, 64: 32: 12: 8) and positive with lipid test for ethanol as it turns milky white for fatty acid and was designated as $WTEF_{24}$. The bioactive lead $WTEF_{24}$ was identified as fatty acid by TLC analysis and qualitative chemical analysis.

3. Physico-chemical characterization of bioactive lead from *Wrightia tomentosa*

The isolated bioactive lead, $WTEF_{24}$ was characterized by Mass spectral datas. Preliminary Phytochemical screening and TLC results also revealed that the pure component ($WTEF_{24}$) was basically a fatty acid derivative.

Test compound 1 ($WTEF_{24}$)

Mass spectral analysis also indicates the evidence of methyl 6, 7-dithia stearate as the tentative final structure of isolated fatty acid derivative with the corresponding molecular $(M+1)^+$ protonated ion peak at m/z 335.. All the above data suggests that the fatty acid root isolate ($WTEF_{24}$) eluted from column was found to be methyl 6, 7-dithia stearate (fig.2).

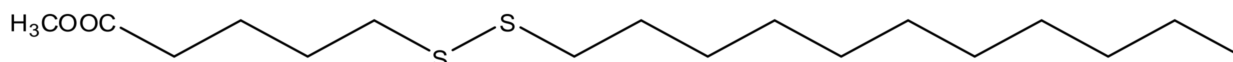

Fig.2: Chemical structure of methyl 6, 7-dithia stearate

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