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Full Length Research Paper

Isolation of mangiferin and amyloid β-protein from n-hexane extract of roots of *Wrightia tomentosa*

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The aim of this study is to identify and characterize the bioactive principles from the roots of *Wrightia tomentosa.* For isolation, the compound, the dried root powder of *W. tomentosa* was subjected to hot extraction with n-hexane to chromatography. Two compounds ($WTRHF_4$ and $WTRHF_7$) were isolated and purified by chloroform: toluene (7.5: 2.5) and chloroform: ethyl acetate (6: 4), respectively. The infra-red (I.R) spectra of $WTRHF_4$ showed specific absorption bands for proteins, *viz.,* 3689 to 3629 cm^{-1} for COOH stretching; 2375 cm^{-1} for NH_3^+ stretching; 1726 cm^{-1} for COO^- stretching; and the Mass spectrum of $WTRHF_4$ showed the parent molecular ion $(M)^+$ cum base peak at m/z 663.5. In addition, the I.R spectra of $WTRHF_7$ showed specific absorption bands for flavonoids, *viz*., 3780 cm^{-1} for stretching alcohol and phenol; 1098 to 1048 cm^{-1} for C-O-C stretching; 1445 to 1375 cm^{-1} for O-H δ ip phenol; 1739 cm^{-1} for C=O stretching and the mass spectra of $WTRHF_7$ showed a parent molecular ion $(M+1)^+$ peak at m/z 423.4 which corresponds to the molecular formula $C_{19}H_{18}O_{11}$. From the physical, chemical and spectral characteristics, $WTRHF_4$ and $WTRHF_7$ were concluded as amyloid β-protein ($A\beta_{17-28}$) and mangiferin, respectively.

Key words: *Wrightia tomentosa*, n-hexane, roots, mangiferin, amyloid β-protein.

INTRODUCTION

Natural products have been one of the most successful sources of medicines. Each plants 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 (Shinde and Dhalwal, 2007)

Wrightia tomentosa Roem and 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 leukaemia cell line (Lee et al., 1992). The root barks are found to be useful in snake bite and scorpion-stings (Kirtikar and Basu, 1980). However, the ethanolic bark and leaf extract of *W. tomentosa* possesses significant anti-allodynic effects with no observable signs of toxicity and antihyperglycemic activity in streptozotocin induced diabetic rats. The alcoholic extract of *W. tomentosa* dried bark was reported to exhibit markedly high antioxidant potency (IC_{50} value of 75.0 µg/ml from DPPH radicals scavenging assay), suitable for prevention of human disease. The butanol extract of the plant was shown to have anti-microbial activity against both gram positive and gram negative organisms. The leaf extract of *W. tomentosa* has proved to be extremely useful against non-tuberculoses mycobacterium (NTM) infections (Nagarajan, 2008), which are becoming a major concern for hospitals and medical clinics. *W. tomentosa* leaf and fruit showed the best antibacterial activity among the various extract used, methanol extract followed by ethyl acetate extracts showed better antibacterial activity (Kaneria et al., 2009).

The methanol (dichloromethane) extract of the leaves of *W. tomentosa* was tested for anti-inflammatory activity

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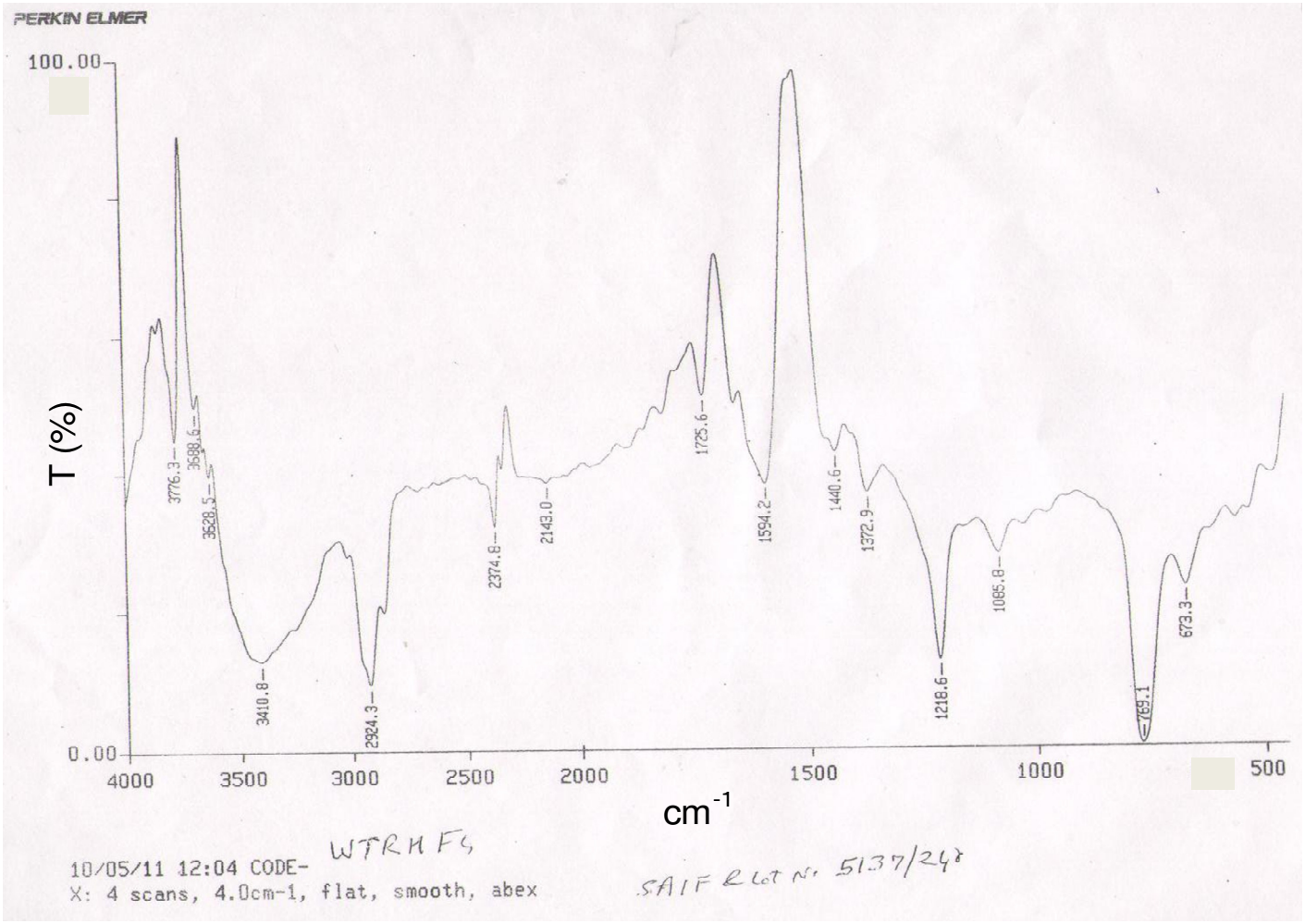

Figure 1. Slice through the graph representing I.R spectra of bioactive leads $WTRHF_4$ from *W. tomentosa.*

by dextran induced paw oedema rat model and it was found that the extract significantly ameliorate the dextran induced oedma (David, 2010).

Since the root portion has not been extracted and isolated for its active constituents in solvent like ethanol, this is the first attempt that the authors planned to carry out on this. Our objective is to study a design of isolation and characterization of bioactive pure components from the roots of *W. tomentosa.*

MATERIALS AND METHODS

Plant materials

The roots of *W. tomentosa* were collected from the hills of yercavd forest, Salem district of Tamilnadu and identified by the method of Mattehew (1982), and 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 thereafter deposited at Bharathidasan University (BDUT/545).

Extraction and isolation

The roots of *W. tomentosa* were dried at room temperature and reduced to a coarse powder. The powder material was subjected to qualitative tests (Harborne, 1998) for the identification of various phytoconsitituents like alkaloids, saponins and proteins. Then the powder (180 g) was subjected to Soxhlet extraction with n-hexane separately for 72 h at a temperature of 69°C. The extracts were concentrated and the solvent was completely removed by rotary vacuum evaporator (Buchi) then light green residue was obtained.

The concentrated n-hexane root extract (1 g) were taken in a china dish separately and heated continuously on a water bath by gradually adding n-hexane 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 20 g for root extract) slowly with continuous mixing with steel spatula till desired consistency of the mixture was obtain. It was air dried and larger lumps were broken to get a smooth free flowing mixture.

A column of 5.0 feet length and 16 mm of internal diameter was taken and dried. The lower end of the column was plugged with

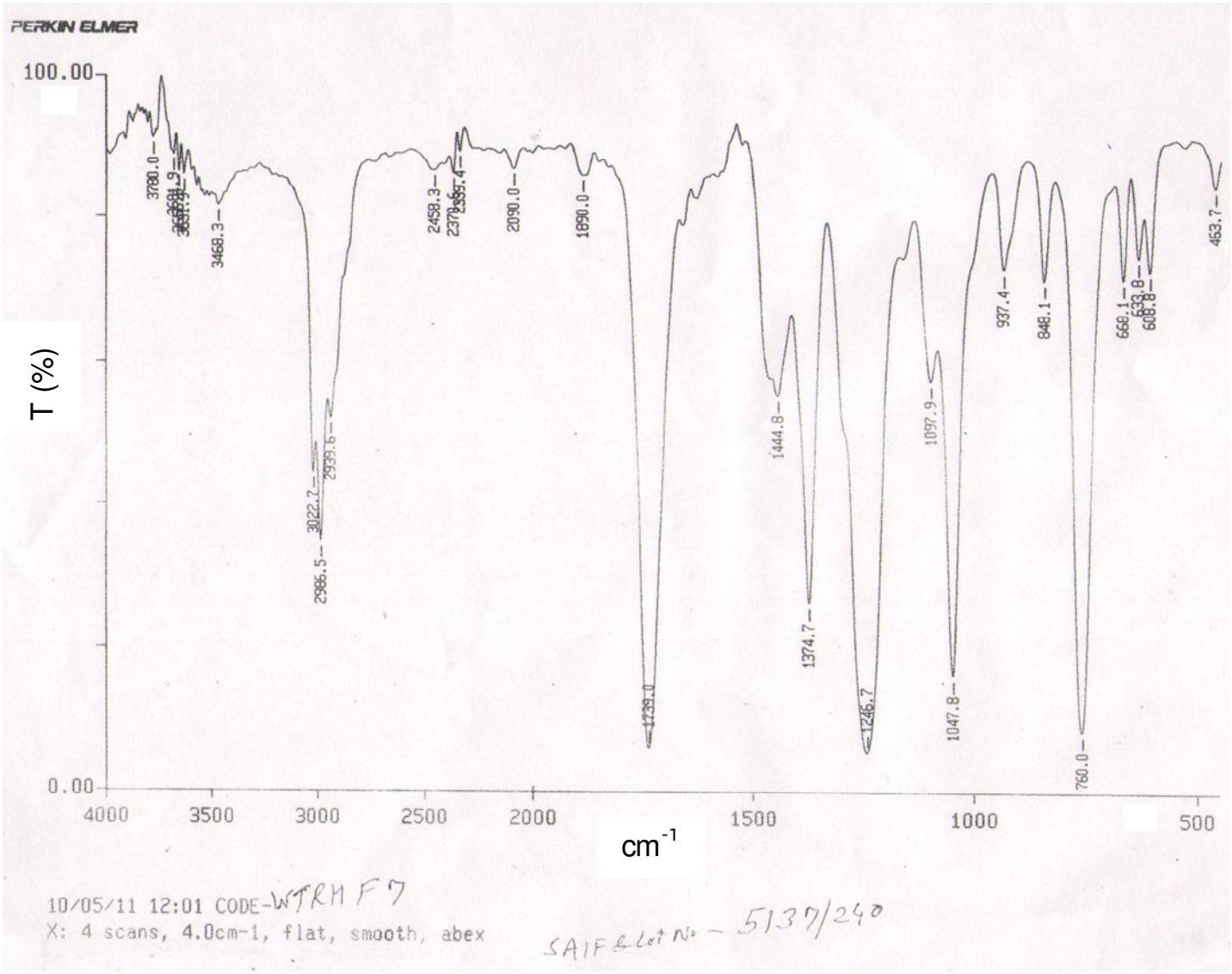

Figure 2. Slice through the graph representing I.R spectra of bioactive leads $WTRHF_7$ from *W. tomentosa*.

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 n-hexane extract of root was poured in the column separately and then eluted successively with different solvents in the order of toluene [toluene: chloroform (7.5: 2.5), toluene: chloroform (5: 5), toluene: chloroform (2.5: 7.5)]; chloroform [chloroform: ethyl acetate (8: 2), chloroform: ethyl acetate (6: 4), chloroform: ethyl acetate (4: 6), chloroform: ethyl acetate (2: 8)]; ethyl acetate [ethyl acetate: ethanol: water (100: 13.5: 10), ethyl acetate: ethanol (7.5: 2.5), ethyl acetate: ethanol (5: 5), ethyl acetate: ethanol (2.5: 7.5)]; ethanol [chloroform: ethanol: glacial acetic acid (9: 9: 1)]; n-butanol [n-butanol: water(6: 4), n-butanol: water (2: 8), and n-butanol: acetic acid: water (4: 4: 1)]. Twenty fractions were collected in a conical flask and marked. The marked fractions were subjected to TLC to check homogeneity of various fractions (Stahl, 1969). 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

Two pure bioactive leads were isolated and the isolated pure fractions were tentatively identified by qualitative chemical analysis. Further identification and characterization was done using I.R and mass spectra analysis.

Infra red (I.R) spectra analysis

The I.R spectra were recorded on Perkin Elmer RX1 at Sophisticated Analytical Instrument Facility (SAIF) Central Drug Research Institute, Lucknow. The isolated test compounds were subjected to I.R using KBr/chloroform with the study of absorption of infra-red radiation functional groups clearly (Silverstein and

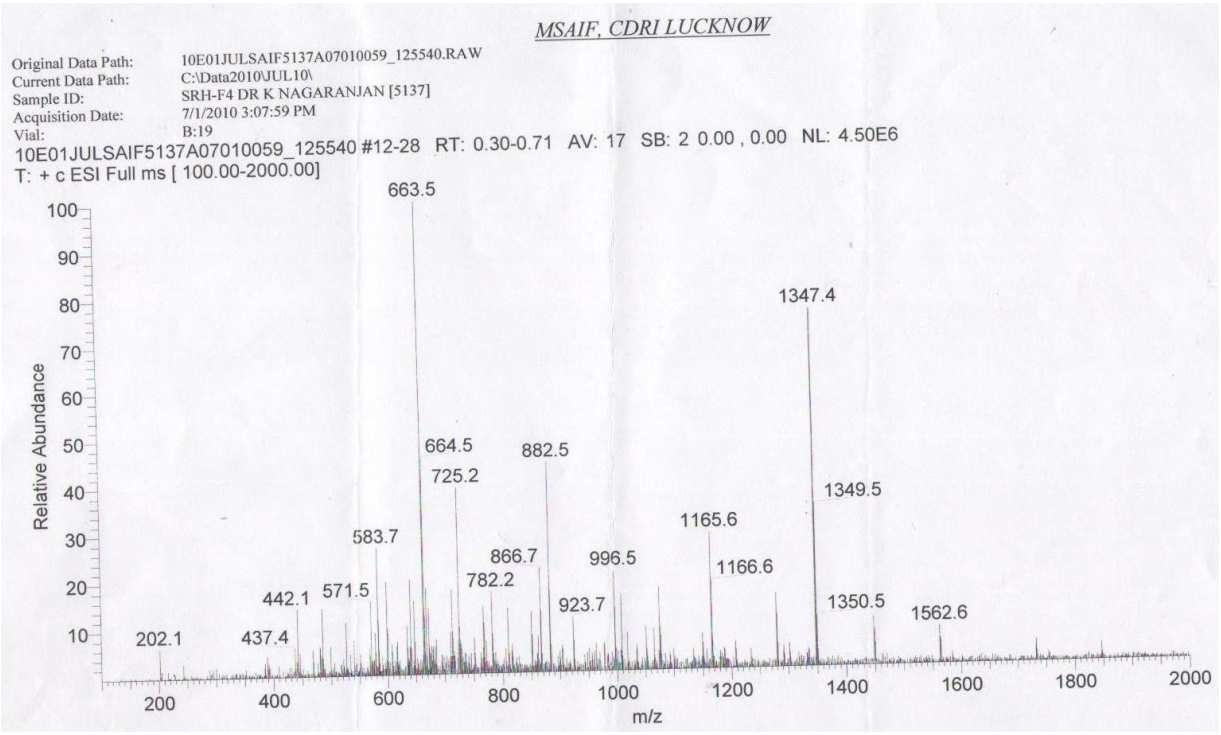

Figure 3. Mass spectrum of a protein component $WTRHF_4$ of *W. tomentosa.*

Webster, 2005). The I.R results in the two isolated leads are expressed subsequently in Figure 1 and Figure 2 with the work.

Mass spectra analysis

The electrospray mass spectral for the isolated two test compounds were recorded as indicated in Figures 3 and 4 on Thermo Finnigan LCQ advantage max ion trap mass spectrometer at SAIF, CDRI, and Lucknow. The 10 µl samples (dissolved in solvent such as methanol/acetonitrile/water) were introduced into the ESI source through Finnigan Surveyor Autosampler. The mobile phase (90: 10 MeOH/ACN: H_2O) was selected based on the preliminary investigation such as solubility and it was maintained at the flow rate of 250 µl/min by MS pump. Ion spray voltage was set at 5.3 KV and capillary voltage 34 V (Gross, 2004). The MS scan run up to 2.5 min and the spectra print outs was arranged for over 10 scan at peak top in TLC. The mass spectra give information on various types of peaks and determining the molecular formula for the isolated compounds after successful interpretation.

The mass spectral data of $WTRHF_7$ as from Figure 5, showed various significant peaks in the spectrum. When analyzing the spectrum of narrow region 200 to 600 m/z, we found that the characteristic peak was obtained at 423.4 m/z which was confirmed as (M+1) peak for the component magniferin, as further observed with preliminary data results, through I.R and C,H,N analysis.

RESULTS AND DISCUSSION

Preliminary phytochemical screening

The results of phytochemical screening showed the presence of protein for $WTRHF_4$ and flavonoids for $WTRHF_7$ as major active constituents in addition to the presence of alkaloids, saponins, steroids, and glycosides.

Isolation and preliminary identification of bioactive leads from *W. tomentosa* roots

Elution of root drug in column with 25% toluene-chloroform, that is, fraction 4 yielded dark green amorphous powder, Rf: 0.90 (ethyl acetate: n-hexane; 6: 4) and positive with Biuret reagent for proteins and was designated as $WTRHF_4$.

Similarly, the elution of root drug in column with 60% chloroform- ethyl acetate, that is, fraction 7, yielded brown colour powder Rf : 0.83 (ethyl acetate: n-hexane; 6: 4) and positive with Shinoda test for flavonoid and was subsequently designated as $WTRHF_7$. The two bioactive leads ($WTRHF_4$ and $WTRHF_7$) were identified as protein and flavonoids by TLC analysis and qualitative chemical analysis.

Physico-chemical characterization of bioactive leads from *W. tomentosa*

The isolated bioactive leads, $WTRHF_4$ and $WTRHF_7$ were characterized by I.R and mass spectral data. Preliminary phytochemical screening and TLC results revealed that the pure components ($WTRHF_4$ and $WTRHF_7$) were

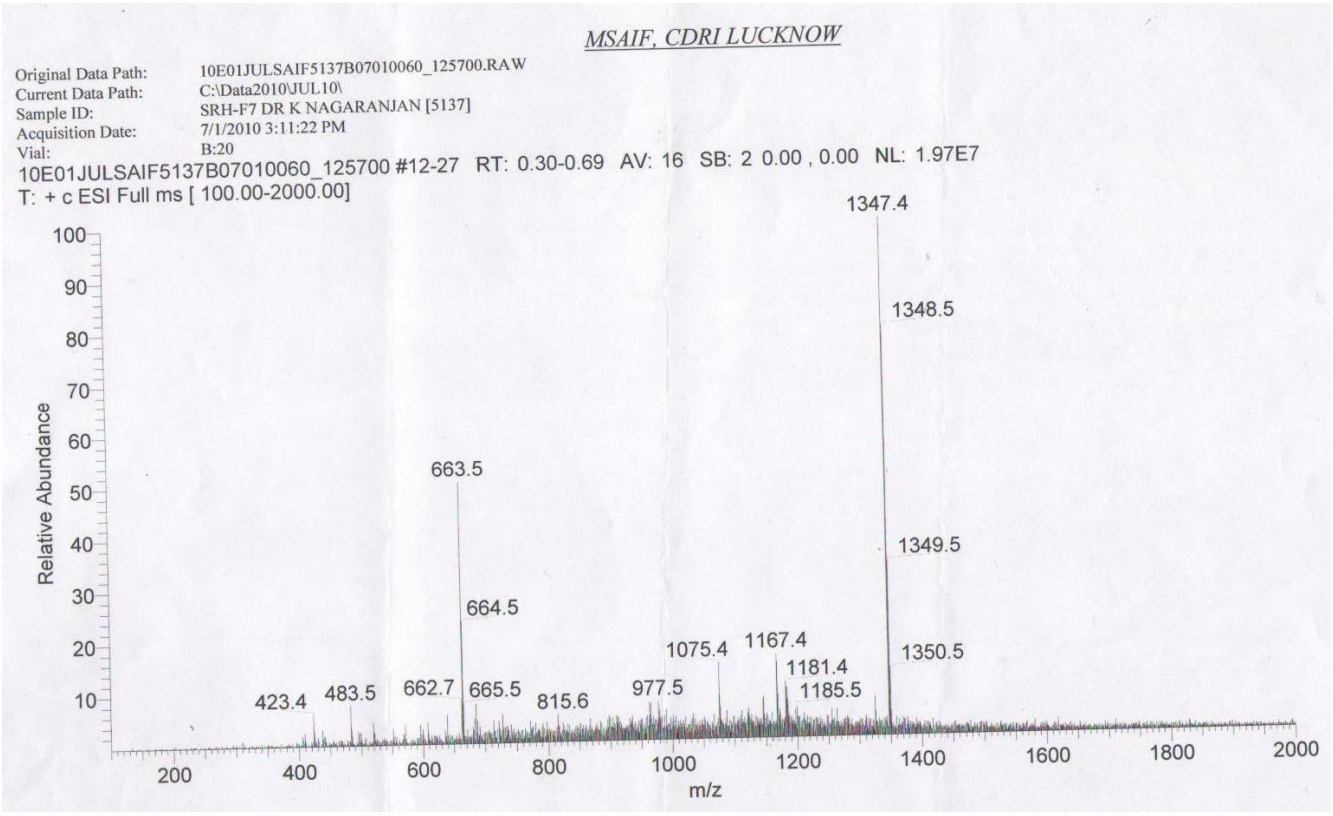

Figure 4. Mass spectrum of isoflavonoid component WTRHF$_7$ of *W. tomentosa*.

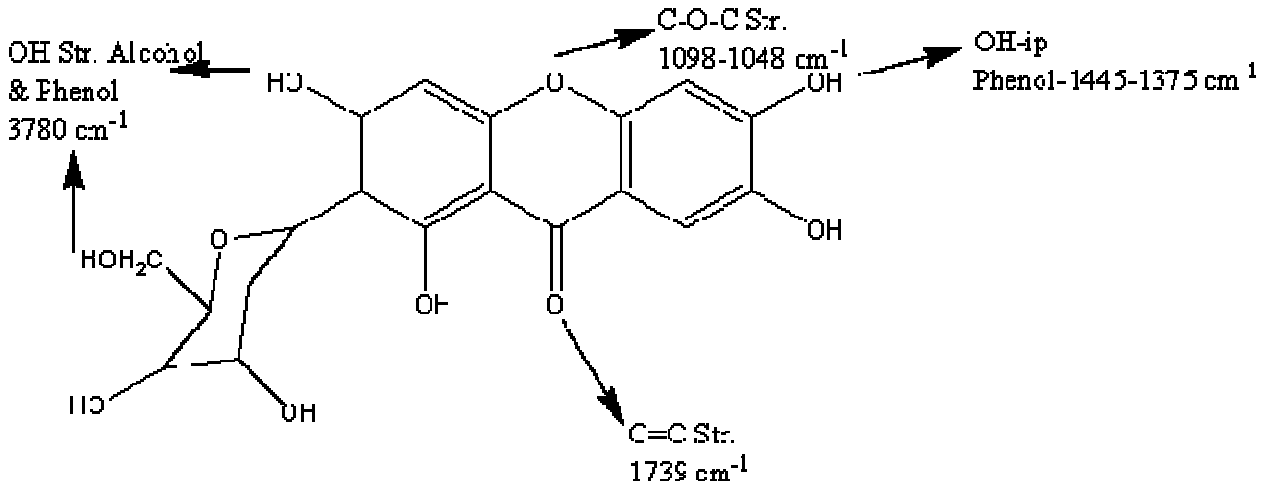

Figure 5. Chemical structure of mangiferin.

basically protein and flavonoids class compound.

Test compound (WTRHF$_4$)

The I.R spectra shows characteristic functional group bands for the presence of amyloid β-protein at 3689 to 3629 cm^{-1} (COO-H stretching carboxylic acid, O-H stretching alcohols and phenols); 3411 cm^{-1}(N-H stretching pyrrole); 2924 cm^{-1} (NH stretching H bended Pyrrole); 2375cm^{-1} (NH^+_3 stretching primary ammonium/ NH^+_2 stretching primary amine/ NH^+ stretching secondary

amine); 2153 cm^{-1} (-NΞC- isonitrile / $-N^{+}ΞN$ stretching diazo compounds); 1726 cm^{-1} (COO^{-} stretching C=O stretching aldehyde/ ketones/α- keto ester/ amide carbamate); 1594 to 1441 cm^{-1} (C-H stretching five ring heteroaromatic ring skeleton); 1373 cm^{-1} (O-H δ ip alcohol/phenol); 1219 cm^{-1} (aromatic C-H and aromatic hydrocarbon); 1085 cm^{-1} (C-O-C stretching furan); 769 cm^{-1} (COO^{-} δ formates); and 673 cm^{-1} (COO^{-} δ benzoate). Mass spectral analysis also indicates the evidence of amyloid β-protein ($Aβ_{17-28}$) as the final structure of isolated protein with the corresponding molecular (M^{+}) ion cum base peak at 663.5 m/z. All these spectral data, suggests that the protein root isolate ($WTRHF_4$) eluted from column was found to be amyloid β-protein ($Aβ_{17-28}$).

Test compound ($WTRHF_7$)

The I.R spectra shows characteristic functional groups bands for the presence of mangiferin at 3780 cm^{-1} (OH stretching alcohol and phenol); 3022cm^{-1} (Aromatic C-H stretching aromatic hydrocarbon); 2987 to 2940 cm^{-1} (C-H stretching alkanes); 2090 to 1890 cm^{-1} (overtones of heteroaromatic compounds); 1739 cm^{-1} (C=C stretching cyclic alkenes; C=O stretching aldehyde); 1445 to 1375 cm^{-1} (O-H δ ip alcohols and phenols =CH δ ip alkenes); 1098 to 1048 cm^{-1} (C-O-C stretching); and 937 to 848 cm^{-1} (CH δ), Mass spectral analysis also indicates the evidence of mangiferin as the final structure of isolated flavonoid class with the corresponding $(M+1)^{+}$ ion peak at m/z 423.4 which corresponds to the molecular formula $C_{19}H_{18}O_{11}$ as observed from Figure 5. All these spectral data suggests that the flavonoid root isolate $WTRHF_7$ eluted from the column was found to be mangiferin.

Hence, from the physical, chemical and spectral characteristics, $WTRHF_4$ and $WTRHF_7$ were concluded as amyloid β-protein ($Aβ_{17-28}$) and mangiferin, respectively.

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