Chapter-7

Isolation, Identification and standardization of effective phytochemical from hydro-methanol (60:40) mixed solvent extract of *Andrographis paniculata* Nees

Contents

7.0 Introduction

Andrographis paniculata (*A. paniculata*) Nees, acanthaceae family, is found largely in adjoining areas of tropical regions of Asia (Hossain et al, 2014). It also grows across South India with a wide range of diversity. In many countries of Asia, it is commonly considered as a very beneficial medicinal plant, used as traditional home remedies and even as few purified extract forms in modern Ayurveda too. The constituent substance andrographolide, is very bitter, colourless, crystalline and biterpenoid lactone compound (Sermkaew et al, 2013). Ethanol or methanol extracted active compounds from the whole aerial part of the plant, leaf and stem of *A. Paniculata* constituted more than 20 diterpenoids and 10 flavonoids and sterol compounds. The main bioactive diterpenoid in *Andrographis paniculata* is 'Andrographolide' having molecular formula ($C_{20}H_{30}O_5$) and structure shown in (Fig-1). It is found about 4% from whole plant extract, stem extract contain 0.8–1.2%, and 0.5–6% in leaf extract. It has been reported about broad pharmacobiological actions of andrographolide, such as analgesic, anti-inflammatory, anti-pyretic, hepatoprotective, anti-viral, antimalarial, antidiabetic, anti-hyperlipidemic, antithrombotic and antineoplastic (Nugroho et al, 2012; Nugroho et al, 2014).

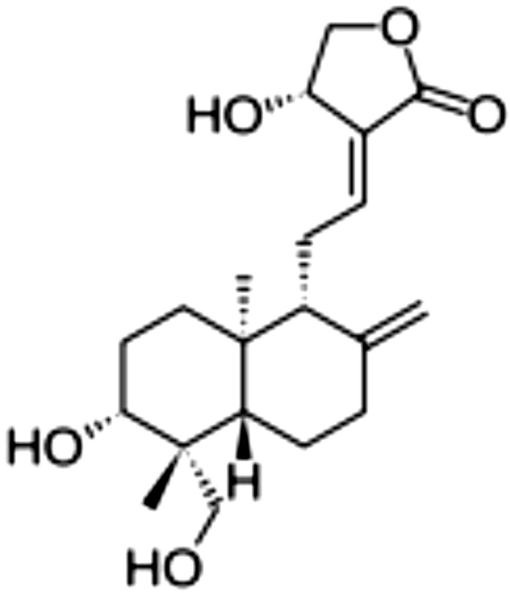

Figure 1: Molecular structure of 'Andrographolide'

In both developing and developed countries herbal elixir including pharmaceuticals, health products, cosmetics, and food supplement have a great demand since natural products are known for their non-toxicity, minor side effects, availability and cost-effectiveness. In India, recent upsurge in the demand of the herbal medicine is noticed because they have now proven health benefits, good remedial actions, safer than synthetic chemical drugs, low cost and affordability.

Besides this, production of high-purity and most potent bioactive compounds from medicinal plants, the isolation and purification of the most active compound from a multi-component mixture are needed.

Hence, the aim of the present research is isolation, identification and quantification of andrographolide separated from hydro-methanol (60:40) mixed solvent extract of *Andrographis paniculata* Ness by using HPTLC, FTIR and HPLC to get andrographolide (ANDRO).

7.1 Methodology

7.1.1 Chemicals

Procurement of necessary chemicals, reagents and other articles was described earlier in chapter -II

7.1.2 Collection, Identification and Preservation of Plant material

Plant material Collection, Identification and Preservation were described in Chapter-IV.

7.1.3 Preparation of aqueous extract

Aqueous extract preparation was described in chapter-IV.

7.1.4 Preparation of methanol extract:

Methanol extract preparation was described in chapter-IV.

7.1.5 Preparation of Hydro-Methanol (60:40) Mixed solvent extract

Mixed solvent extract (60:40) preparation was described in Chapter-V.

7.1.6 Identification of andrographolide (ANDRO) was carried out by HPTLC

HPTLC methods were carried out for the quantitative estimation of the active biological substance, diterpenoid andrographolide (ANDRO) in *Andrographis paniculata* Nees. The present research work was focused mainly on the development and validation of HPTLC process for detection of isolated principal constituent in extracts of *A. paniculata.* HPTLC method is not only a very sensitive method with advantage of lower limit of detection (LOD), but also precise in detection even at very low concentration of isolated major compound in different samples extract of

Andrographis paniculata. Methanol is a better solvent than water and most appropriate for the extraction of andrographolide derivatives (Rajani et al, 2000). It has been confirmed in our laboratory during the period of experiments by using methanol as a solvent.

7.1.7 Identification of andrographolide (ANDRO) was detected by FT-IR Spectroscopy

Both the residue and standard ANDRO were scanned for absorption maxima (λ max) by the help of UV-Vis Spectrophotometer (methanol as blank) within 200 to 400 nm. The functional groups of ANDRO of the characteristic absorption bands were ascertained through FT-IR analyses (KBr disc) within 400 to 4000 cm-1 and determined the molecular weight by LCMS/MS.

7.1.8 Quantification of Andrographolide (ANDRO) by HPLC

Quantification of andrographolide was undertaken with HPLC system (The Agilent, Germany) consisting of standard auto-sampler-model G1329A,thermostat column-model G1316A, vacuum degasser -model G1322A, quaternary pump-model G1314B and variable wavelength detector. The separation was done on a stainless steel silica based Zorbax Eclipse XDB–C18 column (ϕ4.6 mm×150 mm, 5 μm). The column temperature was kept at 30ºC constantly. ANDRO was eluted by utilising mobile phase having methanol and 0.1% v/v H3PO4 (70:30) at adjusted flow rate of 1 ml/min. The eluent was then monitored at 223 nm. To analyse the known concentration of ANDRO, the standard curve was taken and plotted between the concentration of ANDRO and the area under the curve. This plotting was utilised to determine the concentration of andrographolide (ANDRO) in the unknown solution. Samples were prepared in the

methanol of 10 mg/l concentration, and filtered through 0.22 µm filter. 10 µl of filtrated sample was injected in the column which one kept constant. All the solvents were first filtered through 0.22 µm filter and sonicated for 10 minute to remove any dissolved gases and suspended particles.

7.2 Results

A. Identification of isolated ANDRO was done by HPTLC method

The most prominent band parallel to the reference band was eluted out and taken for further analysis.

Figure-2 (a): HPTLC of isolated and standard ANDRO with 5% methanol sulphuric acid after derivatisation.

Figure-2 (b): HPTLC of isolated ANDRO compared with the standard before derivatisation.

Détails of Samples (a) and (b): T1, T3 and T12—ANDRO: 5µl; T2—FGP-AQ: 5 µl; T4— ANDRO (STD): 2 µl; T6— ANDRO (STD): 4 µl; T7—FGP-AQ: 10µl; T8—ANDRO (STD): 6µl; T10— ANDRO (STD): 8µl; T11— ANDRO (STD): 10 µl.

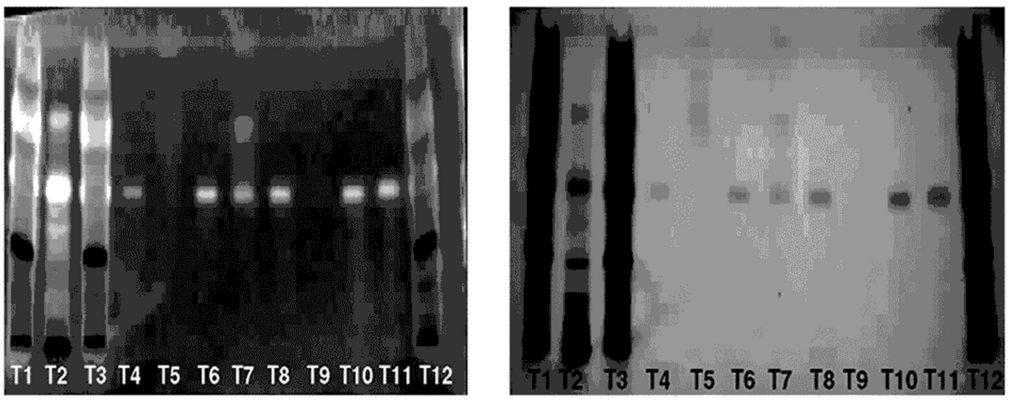

Figure 2: Identification of andrographolide (ANDRO) by HPTLC.

B. Identification of andrographolide was detected by FT-IR

The FT-IR spectra of mixed hydro-methanol (60:40) solvent extract of *Andrographis paniculata* showed that between 4000 and 1200 cm−1 spectral region few intense bands are prominently seen(Figure-3: a3: b) and three notable features are noted: (i) a broad band near 3400 cm−1 due to (–OH) stretching, (ii) a strong band near 1700 cm−1 due to *ν*(–COOR, ester) stretching frequency, (iii) one intense band near 1670 due to *ν*(C-C) stretching frequency. To confirm the origin of several bands which is mentioned above, the IR spectrum of blank KBr pellet was recorded. It was found that the blank KBr pellet did not show any band in the spectral region between 4000 and 1200 cm−1 ;thus it was inferred that the observed band of the above mentioned wave frequency numbers were developed certainly in presence of hydroxyl group (–OH), ester group (–COOR) and carbon–carbon double bond (C = C). As the spectrum of isolated ANDRO clearly matched with standard ANDRO, it was confirmed the presence of ANDRO (andrographolide) in the mixed hydro-methanol (60:40) solvent extract of *Andrographis paniculata.*

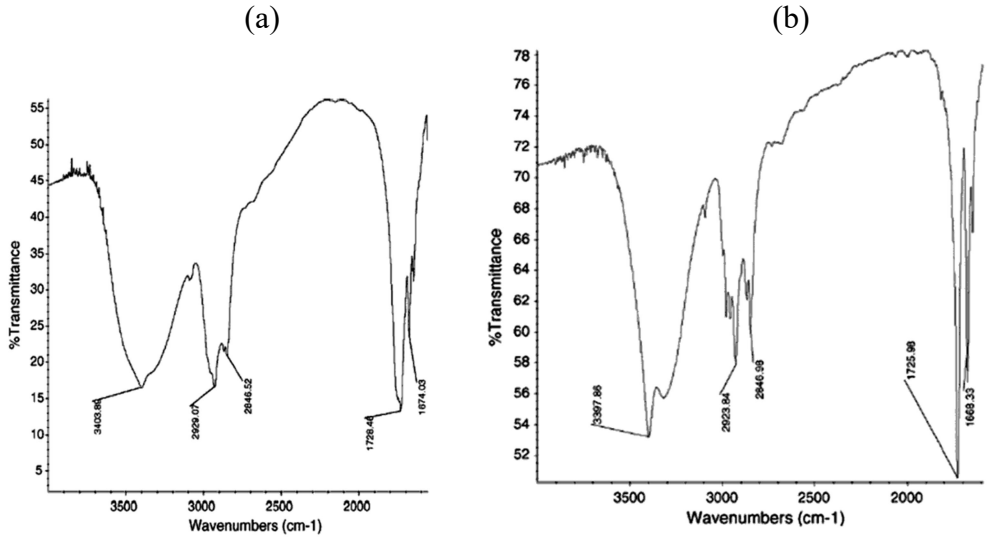

Figure 3: Identification of ANDRO detected by FT-IR; a) isolated ANDRO, (b) pure standard ANDRO.

C. Quantification of andrographolide by HPLC

The quantification of ANDRO was performed by HPLC using standard ANDRO (Sigma) and the concentration of ANDRO that was isolated from mixed hydro-methanol (60:40) solvent extract of *Andrographis paniculata*. Blue line showed isolated andrographolide; black line indicated standard andrographolide (ANDRO) from Sigma laboratory (Figure-4).

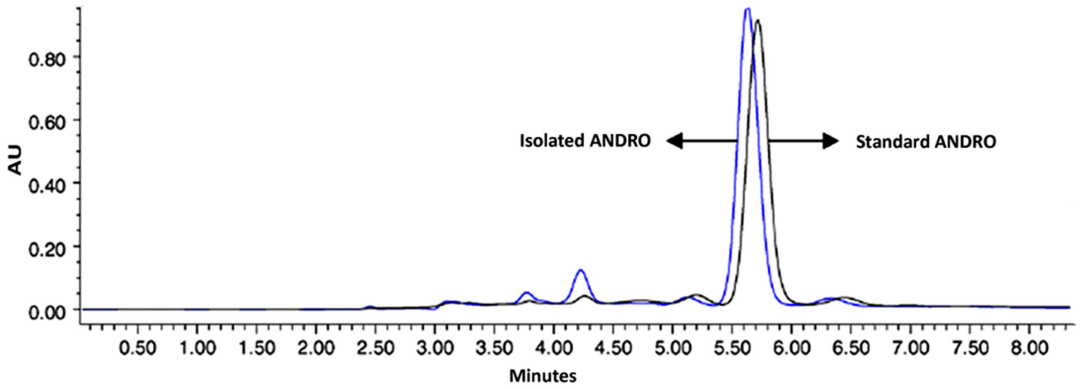

Figure 4: The quantification of isolated ANDRO (blue) was performed with standard pure ANDRO (black).

7.3 Discussion

We have observed significant protective and preventive role of hydro-methanol (60:40) mixed solvent extract of *Andrographis paniculata* Nees from previous studies and it may be used as potential therapeutic natural herbal product for amelioration of chromium (VI) mediated organ damage. In the current study, effective compounds are identified by HPTLC and FT-IR comparing with pure standard andrographolide purchased from Sigma chemical laboratory Ltd (Figure 2&3). It has been confirmed

that identified compound from hydro-methanol (60:40) mixed solvent extract of *Andrographis paniculata* is similar to standard andrographolide. On the other hand, most effective compound has been quantified by HPLC by using standard andrographolide (Figure-4). It has been proved that andrographolide is present in this particular mixed hydro-methanol (60:40) solvent extract of *Andrographis paniculata*.

7.4 Conclusion

Most potent compound andrographolide (ANDRO) has been identified and quantified from the mixed hydro-methanol (60:40) solvent extract of *Andrographis paniculata* by using the standard methods of HPTLC, FT-IR and HPLC. From this point of view**,** we can conclude that significant ameliorative role of particular hydro-methanol (60:40) mixed solvent extract of *Andrographis paniculata* is mostly owing to presence of active diterpenoid compound 'andrographolide'.