Biological Forum – An International Journal **15(1): 101-106(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Andrographolide from the Methanol Fraction of *Andrographis paniculata* Leaves: Isolation and Characterization

Anand Shriram Baley[1*] and Tarkeshwar Prasad Shukla[2]
[1]Research Scholar, Oriental University, Indore (Madhya Pradesh), India.
[2]Ph.D. Supervisor, Oriental University, Indore (Madhya Pradesh), India.

(Corresponding author: Anand Shriram Baley)*
(Received: 26 November 2022; Revised: 18 December2022; Accepted: 27 December, 2022; Published: 07 January, 2023)
(Published by Research Trend)

ABSTRACT: The medicinal plant *Andrographis paniculata,* often referred to as kalmegh, is used in many different cultures for pain relieving of stomach, symptoms of fever, gallbladder problems, intestinal worms, treatment of liver problems functioning, weakness. The leaf is a component of several patented Indian herbal proprietary medicines used to treat liver problems, such as Kalmeghasava and Kalmeghnamay Haub. Diterpenoids, flavonoids, and polyphenols make up its main chemical components. According to reports, the plant's andrographolide, neoandrographolide, and kalmeghnin constituents are its active ingredients. Andrographanin, andrograpanoside, 14-deoxy-12-methoxyandrographolide, and deoxyandrographolide are a few of the other chemical components. Extraction of pure andrographolide from methanol fraction was the main challenge of the research study. This study's goal was to separate andrographolide from *A. paniculata* leaves and analyze the bioactive components of the methanol fraction. Using column chromatography and gradient elution with several mobile phases, the isolation was carried out. The isolated chemical was analyzed using spectroscopy. Spectral analysis was used as the foundation for the structure elucidation process (UV, IR, 1H NMR, and MASS). The extracted substance from the methanol fraction of leaves was identified as andrographolide based on its spectral properties. This is the first such account of an andrographolide chemical found in the methanol fraction of *A. paniculata* leaves that has potential as a treatment for a number of ailments.

Keywords: *Andrographis paniculata*, Isolation, Andrographolide, Acanthaceae, Spectral analysis.

INTRODUCTION

In nations like China (Chang and But 1986) and India, where traditional medicine has been practiced for hundreds of years, plants have moulded the fundamental principles (Kapoor, 1990). It is well known that many other civilizations use plants in their traditional medicine. Basic and fundamental health related issues in 80% of the world's population are dependent on traditional regular medicines as estimated by the World Health Organization. Remaining 20% population in developed countries, plant products related medicinal products are used as a big component of majority of health care infrastructure. Medicinal products developed from plant origin have important role in wellbeing and disease control (Farnsworth *et al.*, 1985). *A. paniculata* (Acanthaceae, Chuanxinlian), a medicinal plant native to Taiwan, Mainland China, and India, is used to treat liver disorders, different types of infections (Kub *et al*., 2020), children's gastrointestinal complaints, virus related gastroenteritis (Lovelin and Praveen 2021), gastrointestinal discomfort, the common symptoms of cold, and infections of respiratory track (Negi *et al*., 2008; Roxas and Jurenka 2007; Kligler *et al*., 2006). In Chinese medicine, the aerial portion of *A. paniculata* is frequently employed. Chinese medical philosophy states that *A. paniculata* is utilized for detoxication and cools and soothes interior heat, inflammation (Huang and Wu 2002); Chao *et al.*, 2009; Mandal *et al.*, 2001). Diterpenoids, flavonoids, and polyphenols are the herb's main bioactive ingredients (Rao *et al.*, 2004; Xu *et al*., 2010). Along with certain additional substances as 14-deoxy-11, 12-dehydroandrographolide, 14-deoxyandrographolide, and kalameghinetc, it primarily comprises the diterpene lactone andrographolide (Dhiman *et al.*, 2012; Kulyal *et al.*, 2010). Andrographolide (Fig. 1) is the major compound to which most of the pharmacological activities of the herb are attributed. Several different types of processes are used for isolation of andrographolide (Mohan *et al*., 2013), employed a microwave assisted method using water and MeOH as solvents. Rajani *et al*. (2000), employed cold

maceration with a mixture of dichloromethane (DCM): MeOH (1:1). The extract was concentrated to a syrupy mass and repeatedly washed with toluene to remove green color. Repeated crystallization was done in MeOH to obtain pure andrographolide. (Borooah *et al*., 2011) used MeOH extract of *A. paniculata* which was chromatographed on silica gel column and eluted with a gradient of chloroform ($CHCl_3$) and MeOH. (Majee *et al*., 2011) fractionated MeOH extract of A. *paniculata* leaves in ethyl acetate (EtOAc) and water. EtOAc extract was partitioned with n-butanol which gave a precipitate of crude andrographolide; it was further purified by column chromatography on silica gel using $CHCl_3$ and MeOH. (Chao and Lin 2010) extracted the whole plant powder of *A. paniculata* in 95% ethanol, fractionated into EtOAc soluble fraction by liquid - liquid extraction and from it, andrographolide was separated by column chromatography of silica gel. (Kumoro and Hasan 2007) reported soxhlet extraction of the herb with MeOH or extraction with super critical carbon dioxide to be the best choice for isolation of pure andrographolide. There are reports where whole plant material of *A. paniculata* was extracted by refluxing with MeOH and quantity of andrographolide in the extract was determined by HPLC. Meenu *et al*. (2012); Vijaykumar *et al*. (2007); Song *et al*. (2013) extracted the herb with 70% ethanol coupled with sonication for HPLC quantification of andrographolide. But the above methods involve a number of steps and are time consuming. In the present study, we made an attempt to isolate andrographolide in a crystalline form by simpler methods which may be commercially feasible for scale up.

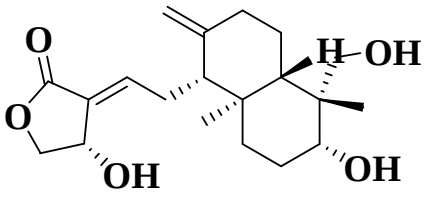

Fig. 1. Andrographolide.

MATERIALS AND METHODS

Plant materials. *A. paniculata* leaves were gathered in Bhopal, Madhya Pradesh. The plant's leaves were cleaned, air dried, and then ground into powder. The specimen was submitted for authentication at the Saifia Science College in Bhopal. The entire plant was validated in the lab of Saifia Science College, Bhopal, by the scientist Dr. Saha Naaz, and the voucher number is 139/Saif./Sci./Clg./Bpl.

Chemical reagents. SD Fine-Chem. Ltd. (Mumbai, India),The Hi Media Laboratories Pvt. Ltd. and SRL Pvt. Ltd.(Mumbai, India), provided the required chemicals for research. The solvent and compounds used for practical work were all of analytical grade.

Extraction by soxhlation process. Defatted *A. paniculata* leaves powder weighing 250mg was poured into a Soxhlet apparatus thimble. Methanol was used for the extraction for 8–10 hours, and the heating mantle temperature was set to 40–60°C. The extract was subjected for evaporation above their boiling temperatures after completion of extraction process. Dried crude concentrated extract was weighed to calculated the extraction yield. It was then conveyed to glass vials (6 2 cm) and kept in a refrigerator (4°C) till it was required for further analytical process (Dutta *et al*., 2020).

Isolation of compounds from methanol fraction of *A. paniculata.* For the purpose of isolating the bioactive components, a methanolic extract of *A. paniculata* was submitted to silica gel column chromatography. Chromatography was performed using a vertical glass column constructed of borosilicate material. Before packing, the column was thoroughly dried after being cleaned with acetone. Silica gel (# 60-120) was used as the adsorbent during the wet packing procedure used to pack the column. Hexane was used to make the slurry, which was then put into the column. After combining them with a small bit of silica gel on top of the column, 1gm of extract was added. The technique used for column chromatography was gradient elusion. The column was eluted with toluene (100%), toluene: ethyl acetate (90:10), toluene: ethyl acetate (80:20), toluene: ethyl acetate (70:30), toluene: ethyl acetate (60:40), toluene: ethyl acetate: formic acid (50: To determine the existence of a certain drug, TLC analysis was done on the concentrated fractions/elutes that had been collected. Using TLC, several phytocompounds were searched for in the fractions/elutes of the *A. paniculata* methanolic extract produced from silica gel column chromatography. The phytocompounds with identical Rf values were gathered into one fraction.

Thin layer chromatography. Thin layer chromatography was performed using different solvent systems on TLC plates of silica gel 60 F254 pre-coated with layer thickness of 0.2 mm, including toluene: ethyl acetate: formic acid (5:4.5:0.5), toluene: methanol (9:1), and n-hexane: ethyl acetate (8:2). Spots were manually applied using a capillary tube, plates were air dried by use of an air blower, and TLC chambers were produced with the appropriate solvent solutions at room temperature. Sulphuric acid solution spraying and UV light imaging were used to see spots on TLC plates. Values for Rf were computed.

Spectroscopic characterization. The isolated compounds' UV spectra were captured in methanol spanning a 200-800 nm scanning range, and the compounds' maximum absorbances were calculated. A Shimadzu 1700 twin beam UV-VIS spectrophotometer was used to record the spectra. At SAIF, Chandigarh, a Waters Micromass Q-Tof Micro mass spectrometer was used to record EIMS (electron impact mass spectrum)

in the positive mode. A pellet was formed using the isolate and 200 mg of FT-IR grade KBr. The sample pellet was put into the sample holder, and SAIF, Chandigarh, used a Model RZX (Perkin Elmer) FT-IR spectrometer to record spectra in the range of 375-7500 cm^{-1}. At SAIF, Chandigarh, India, 1H NMR spectra were captured using an FT-NMR Cryomagnet Spectrometer 400 MHz (Bruker) with TMS as an internal standard. Methanol and DMSO were the solvents employed. Chemical shifts are displayed with TMS serving as an internal reference in ppm levels. Silica gel 60 was used for column chromatography. Prior to use, chromatography solvents were distilled. Using TLC plates, thin layer chromatography (TLC) was carried out (Silica Gel G-60).

RESULTS

TLC of the methanolic extract of *A. paniculata* was carried out using several solvent systems (solvent system was selected on the basis of literature survey). The highest number of spots that were clearly visible were found via TLC using the solvent system 5:4.5:0.5 for toluene, ethyl acetate, and formic acid. Image 1. Using the solvent mixture of toluene, ethyl acetate, and formic acid (50:45:5), it was discovered that the yield of the crude isolated chemical was roughly the same (75 mg). The main component of the extracts, according to a preliminary TLC analysis, was andrographolide. The isolated fractions underwent TLC, and the Rf value was assessed against the corresponding standards. The standard of terpenoids was determined to have the same Rf as the F6 fraction Fig. 2. Compound andrographolide was found to have a melting point of 235°C and a UV lambda maximum value of 224nm (Fig. 3). A very strongly broad peak at 3397.48 cm^{-1} for the O-H bond vibrations of the hydroxyl group was seen in the infrared spectra of the isolated molecule (Fig. 4). ^{1}H-NMR spectrum indicated δ6.91 (d, J = 5.5, 1H), 6.16 (m, 1H), 5.19 (d, J = 8, 1H), 4.64 (s, 1H), 3.41 (d, J=4.5, 1H) 2.50 (d, J=6.4, 1H), 2.34 (s, 1H), 2.18 (s, 1H), 1.94 (s, 1H), 1.68 (d, J=4.4, 1H), 1.36 (s, 1H), 0.94 (s, 1H). Intensively visible peak was observed at 6.91 which correspond to the peak of the proton at alcohol group. The peak was observed at 6.61 which are of the proton present at the 4 position of the oxolane ring. The peak observed at 5.19 and 4.64 are corresponds to the proton present at 6 and 7 position of the bicyclic ring. Rest all the peaks observed between the ranges of 3.5 to 1.0 is of the aliphatic protons present in the cicyclic ring Fig. 5. The isolated fraction (F6 molecular)'s ion peak M+ was discovered at m/e 351.2167, which is consistent with its molecular formula of $C_{20}H_{30}O_5$, and is seen in Fig. 6. Based on the aforementioned observations, andrographolide was identified as the isolated chemical.

DISCUSSION

A white crystalline solid substance with a melting point of 2350°C and a UV lambda maximum value of 244nm was identified as the isolated phytochemical. A noticeable phenolic alcohol (-OH) peak was discovered at 3398.29 cm^{-1}, which is in the range of 3550–3200, in the infrared spectra of an isolated chemical. Ester (C=O) stretching and (C-O) stretching peak were found to be at 1728.06 cm^{-1} and 1221.36 that comes within the standard value of1750-1735. Alkane (C-H) stretching and alkene (=C-H) bending peaks were found to be at 2929.73cm^{-1} and 1675.82 cm^{-1}. It confirms that the main functional group alcohol, aromatic and ester are present in andrographolide. ^{1}H-NMR spectrum indicated δ6.91 (d, J = 5.5, 1H), 6.16 (m, 1H), 5.19 (d, J = 8, 1H), 4.64 (s, 1H), 3.41 (d, J=4.5, 1H) 2.50 (d, J=6.4, 1H), 2.34 (s, 1H), 2.18 (s, 1H), 1.94 (s, 1H), 1.68 (d, J=4.4, 1H), 1.36 (s, 1H), 0.94 (s, 1H). Intensively visible peak was observed at 6.91 which correspond to the peak of the proton at alcohol group. The peak was observed at 6.61 which are of the proton present at the 4 position of the oxolane ring. The peak observed at 5.19 and 4.64 are corresponds to the proton present at 6 and 7 position of the bicyclic ring. Rest all the peaks observed between the ranges of 3.5 to 1.0 is of the aliphatic protons present in the cyclic ring. The molecular ion peak M+ of the isolated fraction (F6) was observed at m/e 351.2167 which corresponds to its molecular formula $C_{20}H_{30}O_5$and is shown in the Fig. 6. Major fragmentation peaks appeared at m/z 351, 301, 275, 203, and 181 in the compounds isolated by chromatographic method.

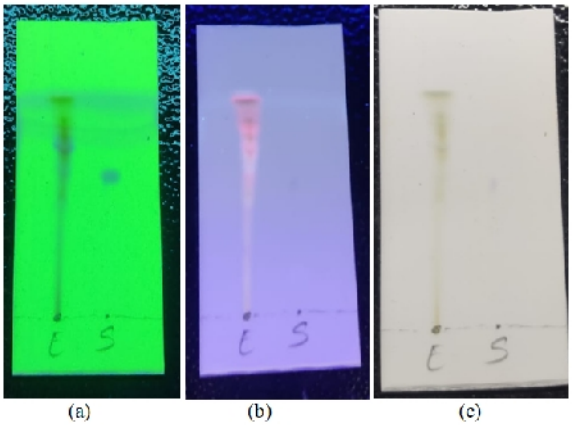

Fig. 1. Images of TLC of *A. paniculata* methanolic extract in Toluene: ethyl acetate: formic acid (5:4.5:0.5) in a) Short UV light, b) Long UV light, and c) visible light.

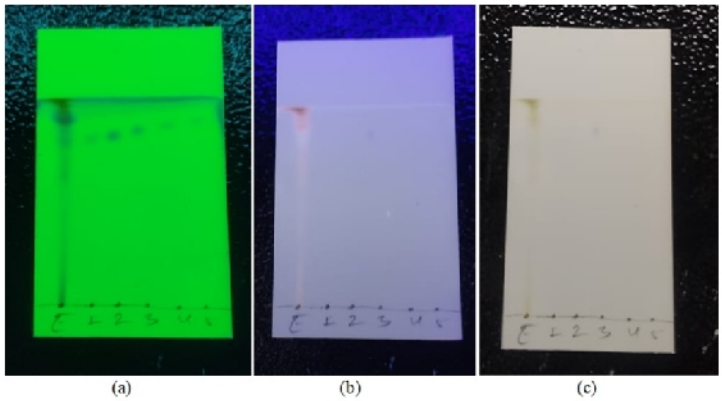

Fig. 2. Images of TLC of isolated fraction (F6) in Toluene: Ethyl acetate: formic acid (50:45:5) in a) Short UV light, b) Long UV light, and c) visible light.

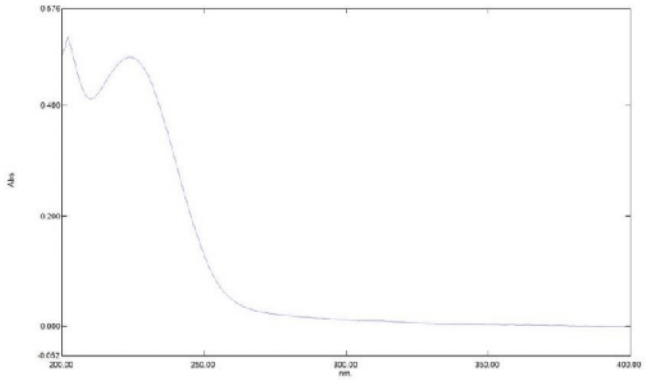

Fig. 3. Lambda maxofandrographolide.

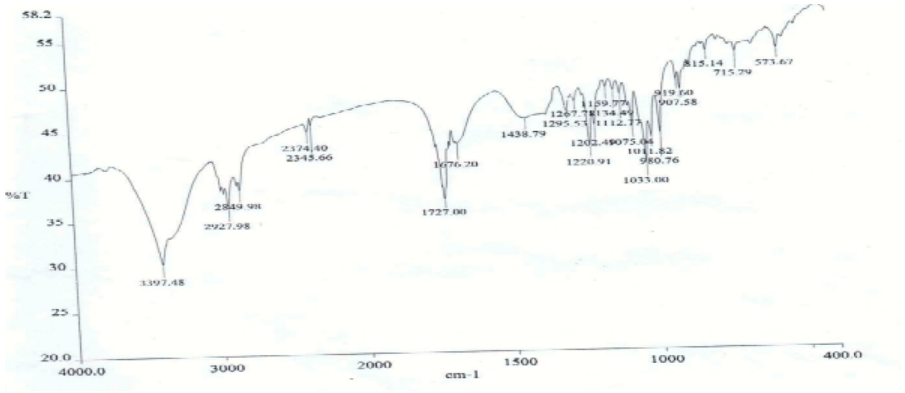

Fig. 4. IR spectra of the isolated lead compound.

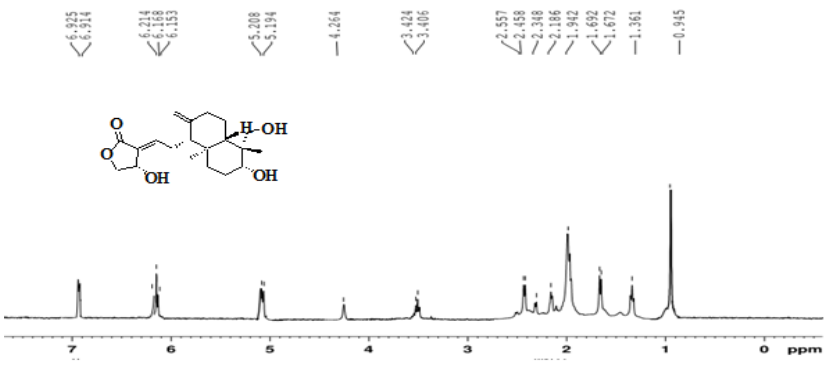

Fig. 5. ^{1}H-NMR spectra of the isolated lead compound.

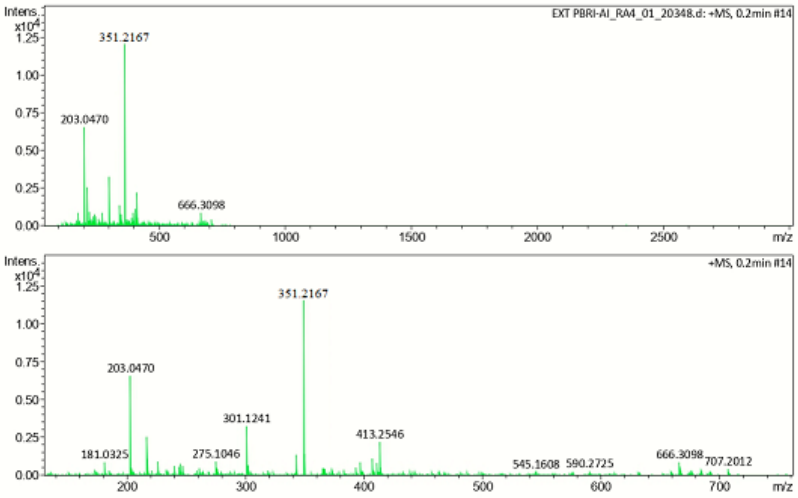

Fig. 6. Mass spectra of the isolated lead compound.

CONCLUSION AND FUTURE SCOPE

The methanol fraction of the leaves of the acanthaceae family plant *A. paniculata* was successfully used for the phytochemical analysis. Physical, chemical, and spectral data allowed for the compound's identification as andrographolide. The biological activity displayed by the plant's methanolic fraction must be attributed to the andrographolide extracted from this fraction. As a result, it is now up to the pharmacologists and biologists to conduct individual bioactivity tests on the andrographolide to learn more about the plant. Therefore, the current research will encourage scientific communities to conduct additional research on this significant medicinal plant in the near future.

Conflict of Interest. None.

REFERENCES

Borooah, D. D. and Borua, P. K. (2011). Column chromatography for isolation of andrographolide from in vitro grown medicinal plant *Andrographis paniculata* Wall. ex Nees. *Int J ApplBiol Pharm.*, *2*, 571-575.

Chang, H. M. and But, P. P. H. (1986). *Pharmacology and Applications of Chinese Materia Medica: (Volume I)*.

Chao, W. W., Kuo, Y. H., Li, W. C. and Lin, B. F. (2009). The production of nitric oxide and prostaglandin E2 in peritoneal macrophages is inhibited by *Andrographis paniculata, Angelica sinensis* and *Morus alba* ethyl acetate fractions. *Journal of Ethnopharmacology*, *122*(1), 68-75.

Chao, W. W. and Lin, B. F. (2010). Isolation and identification of bioactive compounds in *Andrographis paniculata* (Chuanxinlian). *Chinese medicine*, *5*(1), 1-15.

Dhiman, A., Goyal, J., Sharma, K., Nanda, A. and Dhiman, S. (2012). A review on medicinal prospectives of *Andrographis paniculata* Nees. *Journal of Pharmaceutical and Scientific Innovation (JPSI)*, *1*(1).

Dutta, R., Sharma, M. K. and Jha, M. (2020). Pharmacological evaluation of antiasthmatic activity of *Fumaria officinalis* extracts. *Plant Archives*, *20*(2), 4308-4315.

Farnsworth, N. R., Akerele, O., Bingel, A. S., Soejarto, D. D. and Guo, Z. (1985). Medicinal plants in therapy. *Bulletin of the world health organization*, *63*(6), 965.

Huang, C. J. and Wu, M. C. (2002). Differential effects of foods traditionally regarded as 'heating' and 'cooling' on prostaglandin E2 production by a macrophage cell line. *Journal of biomedical science*, *9*(6), 596-606.

Kapoor, L. D. (1990). *Butea monosperma*. In *CRC handbook of Ayurvedic medicinal plants* (p. 86). CRC Press Boca Raton.

Kligler, B., Ulbricht, C., Basch, E., Kirkwood, C. D., Abrams, T. R., Miranda, M. and Woods, J. (2006). *Andrographis paniculata* for the treatment of upper respiratory infection: a systematic review by the natural standard research collaboration. *Explore: The Journal of Science and Healing*, *1*(2), 25-29.

Kub, T. N. T., Ahmad, N. N., Salmuna, Z. N., Harun, A. and Asma, S. (2020). Evaluation of Herbal Formulation using *Andrographis paniculata* Gel as an Antifungal Agent against Superficial Mycoses onto Animal Model. *International Journal on Emerging Technologies*, *11*(5), 330-338.

Kulyal, P., Tiwari, U. K., Shukla, A. and Gaur, A. K. (2010). Chemical constituents isolated from *Andrographis paniculata*.

Kumoro, A. C. and Hasan, M. (2007). Supercritical carbon dioxide extraction of andrographolide from *Andrographis paniculata*: effect of the solvent flow rate, pressure, and temperature. *Chinese Journal of Chemical Engineering*, *15*(6), 877-883.

Lovelin Shweta X. and Praveen K. (2021), Synergistic Efficacy of *Centella asiatica* and *Andrographis paniculata* in the Treatment of Gastroenteritis in Parvo Viral Infection with Special Reference to Oxidative Stress. *Biological Forum – An International Journal*, *13*(3b), 167-172

Majee, C., Gupta, B. K., Mazumder, R. and Chakraborthy, G. S. (2011). HPLC method development and characterization of bio-active molecule isolated from *Andrographis paniculata*. *International Journal of PharmTech Research*, *3*(3), 1586-1592.

Mandal, S. C., Dhara, A. K. and Maiti, B. C. (2001). Studies on psychopharmacological activity of *Andrographis paniculata* extract. *Phytotherapy Research: An*

International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, *15*(3), 253-256.

Meenu, S., Aakanksha, S. and Sandeep, T. (2012). Quantitative HPLC analysis of Andrographolide in *Andrographis paniculata* at two different stages of life cycle of plant. *Acta Chimica and Pharmaceutica Indica*, *2*(1), 1-7.

Mohan, M., Khanam, S. and Shivananda, B. G. (2013). Optimization of microwave assisted extraction of andrographolide from *Andrographis paniculata* and its comparison with refluxation extraction method. *Journal of Pharmacognosy and Phytochemistry*, *2*(1).

Negi, A. S., Kumar, J. K., Luqman, S., Shanker, K., Gupta, M. M. and Khanuja, S. P. S. (2008). CORRIGENDUM: Recent Advances in Plant Hepatoprotectives: A Chemical and Biological Pro¢ le of Some Important Leads. *Medicinal Research, Reviews*, *28*(5), 821.

Rajani, M., Shrivastava, N. and Ravishankara, M. N. (2000). A rapid method for isolation of andrographolide from *Andrographis paniculata* Nees (Kalmegh). *Pharmaceutical Biology*, *38*(3), 204-209.

Rao, Y. K., Vimalamma, G., Rao, C. V. and Tzeng, Y. M. (2004). Flavonoids and andrographolides from *Andrographis paniculata*. *Phytochemistry*, *65*(16), 2317-2321.

Roxas, M. and Jurenka, J. (2007). Colds and influenza: a review of diagnosis and conventional, botanical, and nutritional considerations. *Alternative Medicine Review*, *12*(1).

Song, Y. X., Liu, S. P., Jin, Z., Qin, J. F. and Jiang, Z. Y. (2013). Qualitative and quantitative analysis of *Andrographis paniculata* by rapid resolution liquid chromatography/time-of-flight mass spectrometry. *Molecules*, *18*(10), 12192-12207.

Vijaykumar, K., Murthy, P. B., Kannababu, S., Syamasundar, B. and Subbaraju, G. V. (2007). Estimation of Adrographolide in *Andrographis paniculata* herb, extracts and dosage forms. *International Journal of Applied Science and Engineering*, *5*(1), 27-39.

Xu, C., Chou, G. X. and Wang, Z. T. (2010). A new diterpene from the leaves of *Andrographis paniculata* Nees. *Fitoterapia*, *81*(6), 610-613.

How to cite this article: Anand Shriram Baley and Tarkeshwar Prasad Shukla (2023). Andrographolide from the Methanol Fraction of *Andrographis Paniculata* Leaves: Isolation and Characterization. *Biological Forum – An International Journal, 15*(1): 101-106.