Asian Australas. J. Biosci. Biotechnol. 2018, 3 (2), 170-173

Asian-Australasian Journal of Bioscience and Biotechnology
ISSN 2414-1283 (Print) 2414-6293 (Online)
www.ebupress.com/journal/aajbb

Short Communication

Identification of certain bioactive compounds with anthelmintic properties in *Azadirachta indica* and *Clerodendrum viscosum*

Md. Nuruzzaman Munsi[1], Md Ershaduzzaman[2], Sonia Akther[1*], Md. Zakir Hassan[3,] Md. Mamunur Rahman[4] and Milon Mondal[5]

[1]Goat and Sheep Production Research Division, BLRI, Savar, Dhaka, Bangladesh
[2]Farming System Research Division, BLRI, Savar, Dhaka, Bangladesh
[3]Animal Health Research Division, BLRI, Savar, Dhaka, Bangladesh
[4]Sheep Development Project, BLRI, Savar, Dhaka, Bangladesh
[5]Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh

[*]Corresponding author: Sonia Akther, Goat and Sheep Production Research Division, BLRI, Savar, Dhaka, Bangladesh. E-mail: soniahossain77@gmail.com

Received: 07 August 2018/Accepted: 26 August 2018/ Published: 30 August 2018

Abstract: This study was undertaken to detect certain bioactive compounds with anthelmintic properties in *Azadirachta indica* and *Clerodendrum viscosum* by using high performance liquid chromatography (HPLC). From the HPLC analysis *A. indica* showed peak retention time which was similar to standard phenolic compounds including tannic acid (*A. indica* retention time 3.270 min, STD retention time 3.271 min) and pyrogallol (*A. indica* retention time 3.948 min, STD retention time 3.795 min). Benzoic acid (*C. Viscosum* retention time 6.092 min, STD retention time 6.067 min), tannic acid (*C. Viscosum* retention time 3.322 min, STD retention time 3.271 min) and quercetin (*C. Viscosum* retention time 4.967 min, STD retention time 4.222 min) was detected in leaf part of *C. Viscosum.* Most of these ingredients have well-known anthelmintic roles. Thus, it can be concluded that *Azadirachta indica* and *Clerodendrum viscosum* leaves contain bioactive compounds with anthelmintic properties.

Keywords: bioactive compounds; *Azadirachta indica*; *Clerodendrum viscosum*; HPLC analysis

1. Introduction

For many years use of herbal drugs has become popular due to their easy availability, therapeutic potential, least side effects and minimum cost. At present nearly 80% of the world population rely on plant based drugs for their health care need (Sermakkani and Thangapandian, 2012). Different bioactive amines are playing important role for development of novel compounds, which might be crucial for maintaining a healthy society. The human civilization has been maintaining an intimate relationship with the plants from time immemorial. They depend on plants and other natural sources for their well-being and survival (Shil *et al*., 2014). Various plants still available in the nature are yet to be explored for their medicinal potential (De *et al.*, 2013). Continuous resistance of plants give the importance for the development new semi-synthetic and synthetic compounds. The novel molecules from plant sources have been instrumental in development of structurally modified compounds, which assist a lot in the development of modern therapeutic system. The screening of plant extracts is an innovative strategy to find therapeutically active compounds in many plant species (Zhang *et al.*, 2013). Hence, High Performance Liquid Chromatography (HPLC) associated with particular detection techniques have become a sophisticated means for analysis of various compounds. *Clerodendrum viscosum* Linn. (Family: Verbenaceae), is a shrub having quadrangular stem, large leaves of ovate shape, acuminate apex, entire or denticulate margin, cylindrical petiole and hairy leaves (Vinodh *et al*., 2013). The plant is of 0.9-2.4 meter in height and flowers are whitish-pink in color with long pubescent pedicels in stalked cymes and the fruits are

four lobed drupe of 8 mm in diameter. This plant is common throughout India, Bangladesh, Myanmar, Thailand and Indonesia. The leaf and root have been used in Indian traditional medicine for the treatment of asthma, fever, bronchitis, skin diseases, epilepsy, inflammation, tumors, worm infestation and snake bite (Kirtikar, 1971). The fresh leaf juice is used as vermifuge, bitter tonic, febrifuge in malaria fever, especially in children (Bhattacharjee *et al.,* 2011; Prakash *et al.,* 2011). The various parts of the plant are reported to have many biological activities like, antimicrobial (Kirtikar *et al*., 1971), cytotoxic (Oly *et al.*, 2011), anthelmintic antioxidant (Rahman *et al.*, 2011) and antinociceptive. Neem has been extensively used in Ayurveda, Unani and Homoeopathic medicine and has become a cynosure of modern medicine. Neem elaborates a vast array of biologically active compounds that are chemically diverse and structurally complex. More than 140 compounds have been isolated from different parts of neem. All parts of the neem tree- leaves, flowers, seeds, fruits, roots and bark have been used traditionally for the treatment of inflammation, infections, fever, skin diseases and dental disorders. The medicinal utilities have been described especially for neem leaf. Neem leaf and its constituents have been demonstrated to exhibit immunomodulatory, anti-inflammatory, antihyperglycaemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic and anticarcinogenic properties. Literature survey revealed that till date, no work has been reported on HPLC analysis of methanol extract of *Clerodendrum viscosum and Azadirachta indica* leaves. Therefore, in our present study, it was thought worthwhile to isolate and characterize the bioactive phytochemical compounds from methanol extract of the plant with the help High Performance Liquid Chromatography (HPLC) technique.

2. Materials and Methods

2.1. Collection of plants

The plant of *A. indica and Clerodendrum viscosum* were collected from different areas of Savar upzilla, Bangladesh during the month of November-December 2016.

2.2. Extraction of the plant

The collected plant leaves were sun dried. Then they were heated through Oven to be fully dried at below 40ºC for two days. The fully dried leaves were then ground to make them powder by the help of a suitable grinder. The whole powders were extracted by cold extraction with solvent methanol and kept for a period of three (03) days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then these were filtered through Whatman filter paper. The filtrate (methanol extract) obtained was evaporated by Rotary evaporator (Bibby RE-200, Sterilin Ltd., UK) at 5 to 6 rpm and at 68ºC temperature. It rendered a gummy concentrate of greenish black color. The gummy concentrate was designated as crude extract. Then the crude extract was dried by freeze drier and preserved at 4ºC (Shobana *et al.,* 2009).

2.3. Phytochemical analysis

All the extracts were subjected to qualitative phytochemical screening to identify the presence of alkaloids, flavonoids, carbohydrates, gum, reducing sugars, saponins, steroids, tannins and terpenoids using the established methods of HPLC. Plants powder (5 g) was dissolved in 50 mL acidified deionized water (pH 2, achieved by the addition of 0.2 M HCl). The solution was passed through preconditioned C18 cartridges (3 mL 3 500 mg) purchased from Agilent Technologies. The cartridges were preconditioned by sequentially passing through 3 mL each of methanol and acidified water (pH 2) at a drop-wise flow rate. The aqueous extract solution (10 mL) was then applied to the preconditioned cartridges at a drop-wise flow rate to ensure the efficient adsorption of phenolic compounds. The adsorbed phenolics were then eluted from the cartridges with 1.5 mL of 90% v/v methanol/ water solution at a drop-wise flow rate. The entire extraction procedure was repeated three times. The eluent was collected and stored at 22^0C before HPLC analysis using an HPLC system

3. Results and Discussion

From the present study methanol extracts were made from the leaves of *A. indica and Clerodendrum viscosum* in which various bioactive compounds were found by HPLC (Table 1). *A. indica and Clerodendrum viscosum* contain tannic acid, pyrogallol, benzoic acid, quercetin. From the HPLC analysis certain bioactive compounds were found in *Azadirachta indica* and *Clerodendrum viscosum* leaves which were detected by HPLC, whereas *Azadirachta indica* (neem) leaves contained phenolic compounds like tannic acid (*A. indica* retention time 3.270 min, STD retention time 3.271 min) and pyrogallol (*A. indica* retention time 3.948 min, STD retention time 3.795 min) (Figure 1). On the other hand, *Clerodendrum viscosum* leaves contained benzoic acid (*C. Viscosum* retention time 6.092 min, STD retention time 6.067 min), tannic acid (*C. Viscosum* retention time 3.322 min,

STD retention time 3.271 min) and quercetin (*C. Viscosum* retention time 4.967 min, STD retention time 4.222 min) (Figure 2).The tannic acid, pyrogellol and quercetin have some anthelmintic properties that act against earthworms (*Pheretima posthuma*), tapeworms (*Raillietina spiralis*) and roundworms (*Ascaridia galli*) (Haque Rabiu and Mondal Subhasish, 2011). The preliminary phytochemical screening of *Clerodendrum viscosum* using generally accepted laboratory technique for qualitative determinantion showed the presence of steroids, saponins, phenolic compounds and tannins (Chandrashekar and Rao, 2018) which were almost similar to our study.

Table 1. Bioactive compounds of *Azadirachta indica and Clerodendrum viscosum* of methanol extract.

Name of plants	Compounds	RT(min)	STD RT(min)
Azadirachta indica	Tannic acid	3.270	3.271
	Pyrogallol	3.948	3.795
C. Viscosum	Benzoic acid	6.092	6.067
	Tannic acid	3.322	3.271
	Quercetin	4.967	4.222

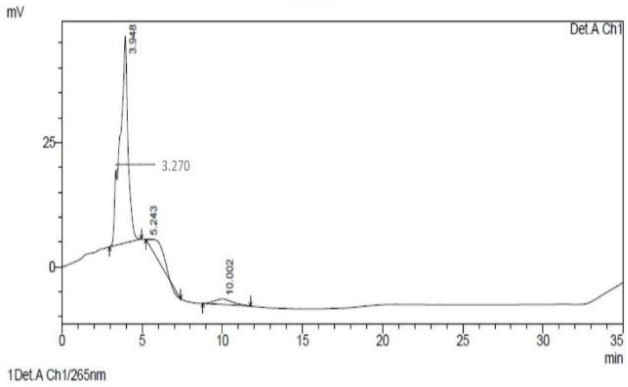

Figure 1. HPLC chromatograph of methanolic extract of *Azadirachta indica.*

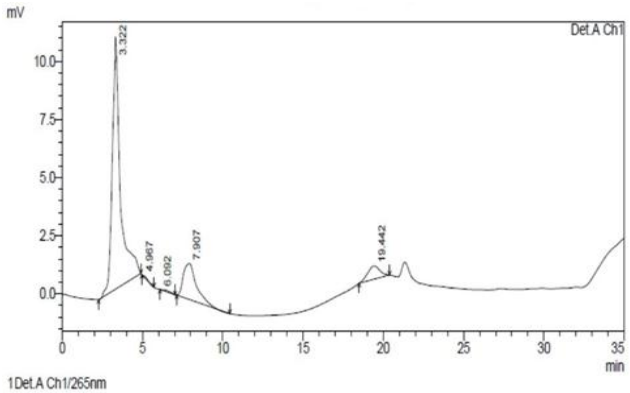

Figure 2. HPLC chromatograph of methanolic extract of *Clerodendrum viscosum.*

4. Conclusions

It can be concluded that *Azadirachta indica* and *Clerodendrum viscosum* leaves possess bioactive compounds with anthelmintic properties which have been detected accurately by HPLC.

Acknowledgements

The authors express their special thanks to Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh.

Conflict of interest

None to declare.

References

Bhattacharjee D, A Das, SK Das and GS Chakraborthy, 2011. *Clerodendrum Infortunatum* Linn: A Review. J Adv Pharm Healthcare Res., 1: 82–85.

Chandrashekar R and SN Rao, 2018. Phytochemical analysis of ethanolic extract of leaves of *Clerodendrum viscosum* (EELCV). World Journal of Pharmacy and Pharmaceutical Sciences.

De S, A Dey, AM Babu and S Aneela, 2013. Phytochemical and GC-MS analysis of bioactive compounds of *Sphaeranthus amaranthoides* Burm. Pharmacogn J., 5: 265–268.

Haque Rabiu and Mondal Subhasish, 2011. Investigation of in Vitro Anthelmintic activity of Azadirachta Indica Leaves. Int. J. Drug Dev. & Res., 3: 94-100.

Kirtikar KR and BD Basu, 1971. 2nd ed. Dehradun: International Book Distributors. Indian Medicinal Plants, p. 1950.

Oly WT, W Islam, P Hasan and S Parween, 2011. Antimicrobial activity of *Clerodendrum viscosum* vent.(Verbenaceae). Int. J. Agric. Biol., 13: 222–226.

Prakash G, V Rajalakshmi, N Thirumoorthy, P Ramasamy and S Selvaraj, 2011. Antioxidant activity of ethanolic extracts of *Clerodendrum viscosum vent* and *Biophytum condolleanum wight*. Der Pharmacia Lettre, 3: 248–251.

Sermakkani M and V Thangapandian, 2012. GC-MS analysis of *Cassia Italica* leaf methanol extract. Asian J. Pharm. Clin. Res., 5: 90–94.

Shil S, M Dutta Choudhury and S Das, 2014. Indigenous knowledge of medicinal plants used by the Reang tribe of Tripura state of India. J. Ethnopharmacol., 152: 135–141.

Shobana S, VG Vidhya and M Ramya, 2009. Antibacterial activity of garlic varieties (Ophioscordon and Sativum) on enteric pathogens. Curr. Res. J. Biol. Sci., 1: 123–126.

Subapriya R and S Nagini, 2005. Medicinal properties of neem leaves: a review. Curr. Med. Chem. Anticancer Agents, 5: 149-6.

Vinodh KS, A Natarajan, K Devi and B Senthilkumar, 2013. Chemical composition of aqueous leaf extract of Murraya Koenigii. Int. J. Pharm. Biol. Archiv., 4: 493–497.

Zhang A, H Sun and X Wang, 2013. Recent advances in natural products from plants for treatment of liver diseases. Eur. J. Med. Chem., 63:570–577.