Int J Pharm Bio Sci 2014 Oct; 5(4): (P) 258 - 262

Research Article **Pharmacognosy**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

GC-MS ANALYSIS OF PHYTOCOMPONENTS IN THE METHANOLIC EXTRACT OF *AZADIRACHTA INDICA* (NEEM)

S. BALASUBRAMANIAN[1], D.GANESH [2] AND SURYA NARAYANA VVS[*3]

[1] Department of Life Sciences, Reva University, Bangalore, India.
[2]Department of Applied Genetics, Indian Academy Degree College, Centre for Research and Post Graduate Studies, Bangalore, India.
[3]Department of Molecular Virology, IVRI, Bangalore, India.

ABSTRACT

Azadirachta indica, is locally called as "Neem" belongs to the family *Meliaceae*. "Neem" is a medicinal herb traditionally used for the treatment of Diabetes, leprosy and Respiratory diseases. The present study was carried out to identify the phytocomponents present in the methanolic extract of the leaves of *Azadirachta indica* by GC-MS analysis. From the GC-MS results five compounds were identified as major constituents, they are Phytol, Linolenic acid, Homo-γ-linolenic acid, Palmitic acid and Tridecylic acid.

KEY WORDS: **GC-MS, *Azadirachta indica,* Leaves, Methanolic, Phytocomponents.**

SURYA NARAYANA VVS
Department of Molecular Virology, IVRI, Bangalore, India.

***Corresponding author**

INTRODUCTION

Azadirachta indica (neem) belonging to *Meliaceae* family is very important medicinal plant which is traditionally used to treat different diseases. Neem is a widely distributed in tropical and sub-tropical regions. The chemical constituents contain many biologically active compounds that can be extracted from neem, including alkaloids, triterpenoids, lavonoids, phenolic compounds, carotenoids, steroids and ketones. Traditionally, neem is most widely used in Indian Ayurvedic medicine system for the treatment of incurable diabetes [1-3]. Its crude extracts from bark and leaves have been used in folk medicine to control diseases such as leprosy, respiratory system and intestinal helminthiasis [4]. In addition, recent studies have shown that neem possesses anti-inflammatory, antipyretic, antiarthritic hypoglycemic, antibacterial, antigastric ulcer, antifungal, and antitumor activities [5-9].The present communication deals with the GC-MS analysis of phytocomponents in the methanolic extract of the leaves of *Azadirachta indica*.

MATERIALS AND METHODS

Collection of the plant material

The leaves of *Azadirachta indica were* collected from GKVK, University of Agricultural Sciences. Bangalore-65, India.

Extraction of Plant Material

Plant material (leaves, 20 Gms) was extracted with 250 mL of methanol at 60^{o}C for 8hrs in Soxhlet extractor. The methanolic extracts were filtered through Whatmann No. 1 filter paper. The filtrate was evaporated to dryness at 80^{o}C and stored until further analysis.

Preparation of stock solution

The extracts were reconstituted in methanol. Methanolic extracts (1 µl) were injected for GC-MS analysis.

Gas Chromatography-Mass Spectrometry analysis

The methanolic extract of the leaves of *Azadirachta indica* was subjected to GC-MS analysis on a GC- MS Clarus 500 Perkin Elmer system comprising a AOC- 20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: Restek Rtx^{R} – 5, (30 meter X 0.25 mm) (5% diphenyl / 95% dimethyl polysiloxane), running in electron impact mode at 70 eV; helium (99. 999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 1.0 µl was employed(split ratio of 10:1); injector temperature 280 ^{0}C. The oven temperature was programmed from 40°C (isothermal for 5 min.), with an increase of 6 ^{0}C / min to 280 ^{0}C, then ending with an isothermal for 15min at 280°C. Mass spectra were taken at 70 eV; a 0.5 seconds of scan interval and fragments from 40 to 550 Da. Total GC running time was 60 minutes.

Identification of Compounds

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute of Standard and technology (NIST).The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.

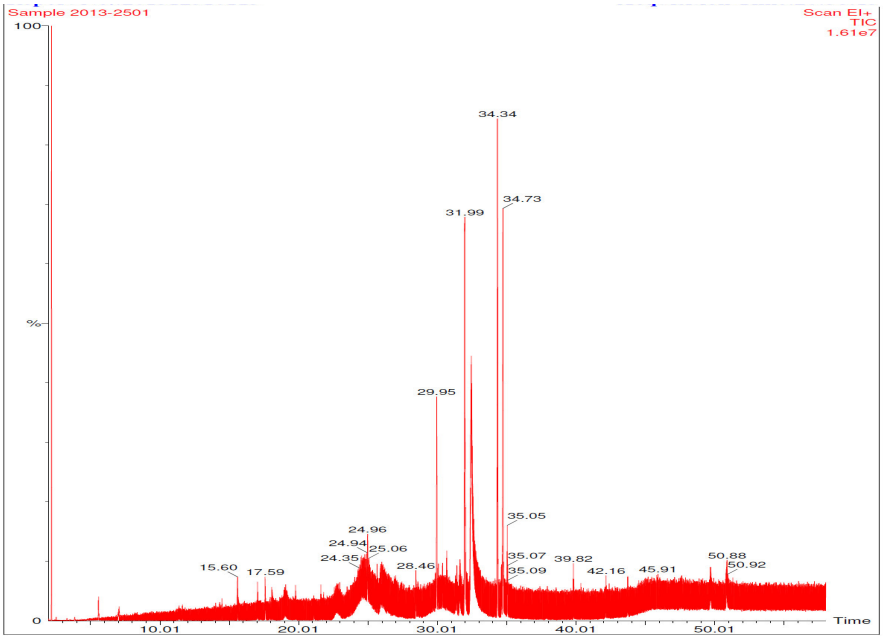

Figure 1
GC- MS chromatogram of the methanolic extract of the leaves of Azadirachta indica

RESULTS AND DISCUSSION

GC-MS analysis

GC-MS chromatogram of the methanolic extract of *Azadirachta indica* showed five major peaks (Figure-1) and have been identified after comparison of the mass spectra with NIST library (Table-1), indicating the presence of five phytocomponents. From the results, it was observed that presence of 3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol (synonym: Phytol), 9, 12, 15- Octadecatrienoic acid (synonym: Linolenic acid; α-Linolenic acid), 8, 11, 14-Eicosatrienoic acid (Synonym: Homo-γ-linolenic acid), N-Hexadecanoic acid (synonym: Palmitic acid) and Tridecanoic acid (synonym: Tridecylic acid) were the major components in the extract. The phytochemicals that contribute to the medicinal property of the plant leaves is listed in Table. 1. Phytol is reported to have antioxidant, antiallergic [10] antinociceptive and anti-inflammatory activities [11]. Recent studies have revealed that phytol is an excellent immunostimulant; it is superior to a number of commercial adjuvants in terms of long-term memory induction and activation of both innate and acquired immunity [12]. Phytol has also shown antimicrobial activity against *Mycobacterium tuberculosis* [13], [14] and *Staphylococcus aureus* [15]. Linolenic acid is known for its potential antibacterial, antifungal [16] anti arthritic and anti-inflammatory activities [17, 18]. Homo-γ-linolenic acid has gained importance due to its anti-inflammatory and anti-cancer action, and also it has been used in the treatment of rheumatoid arthritis [19]. The use of GLA as a benign, adjunctive therapy is used because rheumatoid arthritis patients develop gastrointestinal complications from routine non-steroidal anti-inflammatory drug and corticosteroid medications. The most recent studies indicate that dietary GLA reduces the average medial layer thickness of the vessel wall and reduces the size of atherosclerotic lesions in ApoE genetic knock-out mice [20]. Palmitic acid is reported to possess antibacterial and cholesterolaemic effects [21-25]. In addition, palmitic acid also possessed significant cytotoxicity against the MCF-7, WRL-68, CaCo2, Colo-320 DM cancer cell lines and hepatoprotection against galactosamine.

Table 1

Chemical constituents and its Activity of some of the phytocomponents identified in the methanolic extracts of the Leaves of Azadirachta indica by GC-MS.

Retention Time	Name of the Compounds	Molecular Formula	Molecular Weight	Activity**
34.34	3, 7, 11,15-tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296.53	Cancer-Preventive Antimicrobial anti-inflammatory anti-diuretic Antioxidant
34.73	9,12,15-Octadecatrienoic acid,(z,z,z)-	$C_{18}H_{30}O_2$	278.4296	Antibacterial Antifungal
34.73	8,11,14-Eicosatrienoic acid	$C_{20}H_{34}O_2$	306.482788	Astringent Anti-inflammatory Anticoagulant
31.99	N-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.424103	Antioxidant Nematicide 5-Alpha-Reductase-Inhibitor FLavor Hemolytic Hypercholesterolemic Pesticide Antialopecic Antiandrogenic Antifibrinolytic
31.99	Tridecanoic acid	$C_{13}H_{26}O_2$	214.344299	No activity reported

***Source: Dr. Duke's phytochemical and ethnobotanical database (online database)**

CONCLUSION

The presence of various bioactive compounds justifies the uses of the neem leaves for various ailments by local population. However, if individual phytochemical constituents are isolated from the plant and subjecting it to pharmacological activity will definitely give fruitful results.

ACKNOWLEDGEMENT

We wish to acknowledge Dr. P. Shridhar Reddy, Department of Life Sciences, REVA University and Skanda Life sciences Pvt. Limited, Bangalore for their support.

REFERENCES

1. MA Hossain, MR Nagooru., Biochemical profiling and total flavonoids contents of leaves crude extract of endemic medicinal plant *Corydyline terminalis* L. Kunth. Pharmacognosy Journal, 3: 25–30, (2011).
2. A Kar, A Dethi, A Ababa., Pharmacognosy and Phytochemistry, Nirali Prakashan, India, 238–239, (2007).
3. Z.Y. Ping, L. Yong, P.X. Ming., Global distribution and introduction cultivation state of *Azadirachta indica*, 792:33–42, (2002).
4. P. Prieto, M. Pineda, M. Aguilar., Activity of a standardized neem (*Azadirachta Indica*) seed extract on the rodent malaria parasite plasmodium berghei. Analytical Biochemistry, 269:337–341, (1999).
5. U Bandyopadhyay, K Biswas, A Sengupta et al., Clinical studies on the effect of Neem (*Azadirachta indica*) bark extract on gastric secretion and gastroduodenal ulcer. Life Sciences, 75(24):2867–2878,(2004).
6. B Sultana, F Anwar, R Przybylski., Antioxidant activity of phenolic components present in barks of *Azadirachta indica*, Terminalia arjuna, Acacia nilotica, and Eugenia jambolana Lam. Trees. Food Chemistry, 104 (3):1106–1114, (2007).
7. PE Ebong, IJ Atangwho, EU Eyong, GE Egbung, The antidiabetic efficacy of

combined extracts from two continental plants: *Azadirachta indica* (A. Juss) (Neem) and Vernonia amygdalina (Del.) (African Bitter Leaf). The American Journal of Biochemistry and Biotechnology,4(3):239–244,(2008).
8. S Mahapatra, CYF Young, M Kohli et al., Antiangiogenic effects and therapeutic targets of *Azadirachta indica* leaf extract in endothelial cells, Evidence-Based Complementary and Alternative Medicine, 14, ID 303019,(2012).
9. R Paul, M Prasad, NK Sah., Anticancer biology of *Azadirachta indica* L (neem): a mini review. Cancer Biology and Therapy, 12(6):467–476, (2011).
10. CCMP Santos, MS Salvadori, VG Mota, LM Costa, AACO Almeida, et al., Antinociceptive and antioxidant activities of phytol in vivo and in vitro models. Neurosci J Article, ID 949452, (2013).
11. KR Ryu, JY Choi, S Chung, DH Kim., Anti-scratching behavioral effect of the essential oil and phytol isolated from Artemisia princeps Pamp. In mice. Planta Med, 77: 22–26, (2011).
12. SY Lim, M Meyer, RA Kjonaas, SK Ghosh., Phytol-based novel adjuvants in vaccine formulation: 1. assessment of safety and efficacy during stimulation of humoral and cell-mediated immune responses. J Immune Based Ther Vaccines, 4:6, (2006).
13. MS Rajab, CL Cantrell, SG Franzblau, NH Fischer., Antimycobacterial activity of (E)-phytol and derivatives: a preliminary structure-activity study. Planta Med, 64: 2–4, (1998).
14. D Saikia, S Parihar, D Chanda, S Ojha, JK Kumar, et al., Antitubercular potential of some semisynthetic analogues of phytol. Bioorg Med Chem Lett, 2010, 20: 508–512, (2010).
15. Y Inoue, T Hada , A Shiraishi , K Hirose , H Hamashima , et al., Biphasic effects of geranylgeraniol, teprenone, and phytol on the growth of *Staphylococcus aureus*. Antimicrob Agents Chemother, 49: 1770–1774, (2005).
16. McGaw, LJ Jäger, AK Van J Staden., Isolation of antibacterial fatty acids from Schotia brachypetala. Fitoter, 73: 431-433, (2002).
17. S Lalitharani, VR Mohan, GS Regini, C Kalidass., GC-MS analysis of ethanolic extract of Pathos scandens L.leaf, J. Herb. Medi. Toxicology, 3: 159-160, (2009).
18. A Maruthupandian, VR Mohan., GC-MS analysis of ethanol extract of Wattakaka volubilis (l.f.) stapf. Leaf, Int. J. Phytomedicine, 3:59-62, (2011).
19. P DeLuca, D Rothman, RB Zurier., Marine and botanical lipids as immunomodulatory and therapeutic agents in the treatment of rheumatoid arthritis. Rheum. Dis. Clin. N. Am, 21:759–777, (1995).
20. YY Fan, KS Ramos, RS Chapkin., Combined dietary gamma-linolenic acid and n-3 polyunsaturated fatty acids retard atherosclerotic progression in ApoE knockout mice. In: Eicosanoids and Other Bioactive Lipids in Cancer, Inflammation and Related Diseases, Vol. 4 (Honn, K.□V., Nigam, S., Marnett, L.□J. & Dennis, E., eds.), (in press), (1998).
21. K Kiuchi, P Lowell., Reconstitution of the lipid depleted pyruvate oxidase system of *E.coli*, the palmitic effect. Archives of Biochemistry and Biophysics, 233:776-784, (1984).
22. Y Barbara, L Kerry, T Maureen, DG Erasmus., Pharmacological Screening of Pentanisia prunelloides and the isolation of the antibacterial compound palmitic acid. Journal of Ethanopharmacology, 79:101-107, (2002).
23. J Wang, D Wu, CS Wei., Structure activity relationship of reversibly lipidized peptides: studies of Fatty acid Desmopressin conjugates. Pharmaceutical Research, 19:609-614, (2002).
24. AM French, K Sundram, CM Thomas., Cholesterolaemic effect of palmitic acid in relation to other dietary fatty acid, Asia Pacific Journal of Clinical Nutrition, 11:S401-S407, (2002).
25. H Ohtani, K Hayashi, Y Hirata, K Nakashima, E Nishio, M Sacki, G Kajiyama., Effect of dietary cholesterol and fatty acid on plasma cholesterol level and hepatic lipo-protein metabolism. Journal of Lipid Research, 31:1413-1422, (1990).