Characterization of Azadirachtin from ethanolic extract of leaves of *Azadirachta indica*


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Received on:20-09-2011; Revised on: 15-10-2011; Accepted on:10-12-2011

**ABSTRACT**

The neem tree has long been recognized for its unique properties both against insects and in improving human health. It is grown in most tropical and subtropical areas of the world for shade, reforestation and for the production of raw material for natural insecticides and medicines. Azadirachta, a complex tetra-nor triterpenoid limonoid from various parts of the neem, is the main component responsible for the toxic effects in insects. The compound is extremely active as an antifeedant and growth disruptant in a large range of insect species, whilst retaining very low mammalian toxicity. In the present work we have investigated the qualitative and quantitative determination of Azadirachtin isolated from the *A.indica*. Qualitative estimation was carried out by thin layer chromatographic (TLC) method. The simultaneous determination of the Azadirachtin was carried out by HPLC techniques. HPLC analysis revealed that the % Azadirachtin content in the ethanolic extract of leaves was found to be 73.62.

**Key words:** Azadirachtin, *Azadirachta indica*, HPLC, TLC & isolation

**INTRODUCTION**

Herbal drugs play an important role in health care programs especially in developing countries. Ancient Indian literature incorporates a remarkably broad definition of medicinal plants to be potential sources of medicinal substances [1]. For centuries, plant and plant products have been used for treating various illnesses. Today, several medicinal plants and their products are still in use, being employed as home remedies, over the counter drugs as well as raw materials for the pharmaceutical industry and they represent a substantial proportion of the global drug market [2]. *Azadirachta indica* A. Juss, known as neem in vernacular, belongs to the family melliaceae and is widely distributed in Asia, Africa and other tropical parts of the world [3]. Neem also known as Chinaberry or Persian lilac tree. Neem is a versatile medicinal plant, almost every part of which is being used in folklore and traditional systems of medicine for the treatment of a variety of human ailments. Neem is chemically rich. There are over 300 plant secondary compounds [4]. Most of the active compounds are terpenoids, found in the leaves, fruit, seeds, stem, and root bark. Of most prominence and of most commercial use is the tetrnortriterpenoid azadirachta (a limonoid), the main ingredient in many commercial products, including insecticides. Azadirachta, a major compound of the neem has potent antifeedant, growth and reproductive regulating properties. Likewise, nimbin, a limonoid from neem, is also involved in improving pesticide properties [5]. The Neem tree is also considered as a natural insecticide/pesticide plant and the quality of pesticide and pharmacological products depend upon the contents of Azadirachta and nimbin in the plant.

Azadirachtin, is a complex tetrnortriterpenoid limonoid from the various parts of the neem. Of all of the limonoid in neem, azadirachtin and its about 25 natural analogues are the most biologically active. Of these, azadirachtin-A (Aza A) is the most plentiful and biologically active one [6] and it is generally Aza A that is used for commercial insecticides. Aza A is about 80% of the azadirachtin found in neem, while up to 15% of the azadirachtin may be Aza B (3-tigloylazadirachtol). The chemical name for Azadirachtin A is: dimethyl[2aR,2aaR,3bR,4bR,5aR,6aR,7aR]-7a-methyl-2,7-dihydroxy-4-methyl-8-[(2-methyl-1-oxo-2-butenyl)oxy]-4(3a,6a,7,7a)-tetrahydro-6a-hydroxy-7a-methyl-2,7-dimethanofuro[2,3-b]oxireno[35,16]-dicarboxylate. The Empirical formula of azadirachtin A is C₃₅H₄₄O₁₆ and the relative molecular mass of azadirachtin A 720.7. [8]

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**Figure 1:** A.indica(Leaves)

**Figure 2:** Azadirachtin A

**Figure 3:** Azadirachtin B

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PLANT MATERIAL
The Leaves of A. indica were obtained from the medicinal garden of L.N.C.P. Bhopal (M.P.) in the month of October 2011. The plant was authenticated and a voucher specimen was deposited.

PREPARATION OF EXTRACT
About 70 gm of leaves powder was extracted with ethanol by soxhletation and the solvent was recovered by distillation. The extract was concentrated under reduced pressure and air dried[9].

QUALITATIVE ANALYSIS BY THIN LAYER CHROMATOGRAPHY
For thin layer chromatographic studies of azadirachtin, precoated Silica gel F254 aluminum plates (20 X 20cm) were used. The azadirachtin was separated using different solvent systems. The colour and Rf values were recorded by observed the plates on UV light[10].

QUANTITATIVE ESTIMATION OF AZADIRACHTIN BY HPLC
The HPLC analysis was performed using a LC-100, CyberlabTM, Salo Torrace, Millbury, MAO 1527, USA with LC-UV-100 UV detector. A CAPCELL (C-18) HPLC-packed column (4.6 mm I.D.X 250 mm), type MG 5 µm, number AKAD/05245 was used for the chromatographic separations. The mobile phase consisted of solvent A:B[30:70]. Solvent A is acetonitrile and solvent B is water. The separation was performed using isocratic elution (0-10 min) with a flow rate of 1.0 ml/min and a column temperature of 25°C. The injection volume was 25µl, and UV detection was effected at 219 nm. HPLC grade solvents were obtained from Shyam brothers, 27- sindhi market, Bhopal (M.P.). Azadirachtin pure marker was procured from Natural Remedies, Vijaywada, Bangalore.

Preparation of sample solution
Accurately 1mg sample was dissolved in 7ml acetonitrile. Transferred this solution in 10 ml volumetric flask and the volume was made up with acetonitrile. Then 1ml of above solution was pipetted out in 100ml volumetric flask and the volume was made up with acetonitrile.

Preparation of standard solution
Accurately 1mg standard Azadirachtin of known purity was dissolved in 5ml of acetonitrile by shaking. Transferred this solution in 10 ml volumetric flask and the volume was made up with acetonitrile. Then 1ml of above solution was pipetted out in 100ml volumetric flask and the volume was made up with acetonitrile.

Azadirachtin content % = \( \frac{A_1 \times W_1 \times P}{A_2 \times W_2} \)

Where, \( A_1 \) = Peak area of Azadirachtin in sample solution
\( A_2 \) = Peak area of Azadirachtin in standard solution
\( W_1 \) = Weight in g of sample
\( W_2 \) = weight in g of standard
\( P \) = Purity of standard Azadirachtin

RESULT AND DISCUSSION
A. indica leaves have been reported various medicinal uses. Keeping in view of the ethno-pharmacological importance of leaves A. indica the extraction of important phytoconstituents Azadirachtin was undertaken for standardization. Evaluation of dried leaves extract (Table 1) showed the following characters: colour- greenish brown with acrid bitter taste. The solubility study of sample was determined (Table 1). The extracted sample was subjected for qualitative estimation by TLC analysis (Table 2). The construction of chromatographic fingerprints plays an important role in the quality control of complex herbal medicines. Chemical fingerprints obtained by chromatographic techniques are strongly recommended for the purpose of quality control of herbal medicines. HPLC separation of isolated sample with reference to standard was performed on a Cyber Lab C-18 column (250 x 4.0 mm, 5μ). Thus chromatographic fingerprint should be considered to evaluate the quality of herbal medicines globally considering multiple constituents present in the herbal medicines[11]. HPLC analysis revealed that the % Azadirachtin content in the ethanolic extract of leaves was found to be 73.62. The RT of standard and sample were found to be 3.6 & 2.9 respectively.
CONCLUSION

Neem is a useful and beneficial plant in a number of ways. It has outstanding pesticide properties and in many human medicinal studies, has tremendous potential. Products of the neem tree have a wide variety of uses including the provision of medicines, pesticides, fuel wood, timber and food. Azadirachtin plays an important role in quality of the neem product. The Azadirachtin was extracted from leaves of A.indica and identified by TLC and quantified by HPLC methods. Azadirachtin from neem effects insects in a variety of different ways: as an antifeedant, insect growth regulator and sterilant. An accurate and convenient method of quantization of the well-known biopesticide azadirachtin by high-performance liquid chromatography has been developed.

REFERENCE


Source of support: Nil, Conflict of interest: None Declared