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Study of phytochemical screening of neem (*Azadirachta indica*)

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Abstract
The medicinal plants have been used for years in daily life to treat diseases all over the world. *Azadirachta indica* is a very useful traditional medicinal plant in the sub-continent. Each part of the tree has some medicinal properties. Phytochemical analysis gave positive results for steroids, phenolic compounds, alkaloids, glycosides, terpenoids, flavonoids and tannins. Leaf extract of *Azadirachta indica* contains pharmacologically bioactive constituents that may responsible for its activity against test organisms.

Keywords: phytochemical, *Azadirachta indica*, bioactive compounds

Introduction
Azadirachta indica (Neem) is an evergreen tree, cultivated in various parts of subcontinent. Every part of the tree has been used as traditional medicine for household remedy against various oilments from antiquity. Neem has been extensively used as Ayuveda, Urani and Homeopathic medicine. The Sanskrit name of Neem tree is Arishtha, meaning reliever of sickness (Kausik *et al*., 2002) [7] Chemical investigation on the products of Neem tree was extensively undertaken in the middle of the 20th century. The Neem Tree is an incredible plant that has been declared the Tree of the 21st century by the United Nations (Puri, 1999) [14]. In India, it is commonly known as 'Divine Tree', 'Life giving tree', 'Nature's Drugstore', 'Village Pharmacy' and 'Panacea for all diseases' (Shoforowa, 1993) [18]. Extracts from the Neem tree (*A.indica*) also called 'Dogonyaro' in Nigeria are most consistently recommended in ancient medical texts for gastrointestinal upsets, diarrhoea and intestinal infections, skin ulcers and malaria (Schmuttere, 1995) [17]. Its leave can be used as drug for diabetes, eczema and reduce fever. Barks of Neem can be used to make toothbrush and the roots has an ability to heal diseases and against insects. The seed of Neem tree has a high concentration of oil. Neem oil is widely used as insecticides, lubricant, drugs for variety of diseases such as diabetes and tuberculosis (Puri., 1999; Elvin- Lewis., 1980; Kumar., *et al*; 2009) [14, 8]. In Africa, extracts from Neem leaves have provided various medicinal preparations.
The Neem (*Azadirachta indica*) is an evergreen robust tree, belongs to the family Meliaceae. It is mostly found in tropics and sub-tropical areas of the world (Africa and Asia). Occur in medium to large size and have brown to dark grey bark and a dense rounded pinnate leaves (Ogbuewu, 2008) [12]. It has an extensive deep root system which is responsible for their survival in arid and semi-arid area of the world. The Chemical constituents contain many biologically active compounds that can be extracted from neem, including alkaloids, flavonoids, triterpenoids, phenolic compounds, Carotenoids, steroids and ketones. Azadirachtin is actually a mixture of seven isomeric compounds labeled as azadirachtin A-G and azadirachtin E is more effective (Verkerk *et al*., 1993) [20]. Other compounds that have a biological activity are salannin, volatile oils, meliantriol and nimbin (Jacobson, 1990) [6].
Antibiotic resistance is a major concern and development of new agents from plants could be useful in meeting the demand for new antimicrobial agents with improved safety and efficacy (Srivastra *et al.*, 2002). In this study, we have shown that streptomycin in combination with *A.indica* leaf extract shows increase in bactericidal effect and can be used to treat the infection caused by these bacteria. The preliminary phytochemical tests were performed by qualitative type and from the phytochemical investigations it was observed that alkaloids, tannins and phenolic compounds, flavonoids, terpenoids and steroids, saponins, Glycoside and reducing sugar were present in the extracts. *Azadirachta indica* leaves possessed good anti-bacterial activity confirms the presence of bioactive compounds and is useful for rationalizing the use of this plant in primary health care (Saradha Jyothi Koona and Subbarao Budida 2011) [16]. The presence of these secondary metabolites in plants, produce some biological activity in man and animals and it is responsible for their use as herbs. These compounds also serve to protect the plant against infection by microorganisms, predation by insects and herbivores, while some plants give their odors and or flavors and some still are responsible for their pigments (Mahmood *et al*., 2008) [9].
In some cases, the activity has been associated with specific compounds or classes of compounds. These active constituents can be used to search for bioactive lead compounds that could be used in the partial synthesis of more useful drugs (Mahmood *et al*., 2008 and Ogbonnia *et al*., 2008) [9, 11].

Materials and Methods
Collection of plant material
The leaves *Azadirachta indica* were collected from Cheyyar, Thiruvannamalai District, Tamil Nadu, India. The species were identified and authenticated at the Department of Botany, Arignar Anna Government Arts College, Cheyyar, Tamil Nadu. The leaves were shade-dried, cut into small

pieces and coarsely powdered. The coarse powder was used for extraction with various solvents.

Preparation of plant extracts

Thousand grams of dry powders leaves were taken in individual aspirator bottle; 2.5 liters of solvents (water and ethanol) were used and the mixtures were shaken occasionally for 72 hours. Then the extract was filtered. This procedure was repeated three times and all extracts were decanted and pooled. The extracts were filtered before drying using Whatman filter paper no.1 on a Buchner funnel and the solvent was removed by vacuum distillation in a rotary evaporator at 40˚C; the extracts were placed in pre-weighed flasks before drying. Finally the aqueous and ethanol extracts of *A.indica* leaves were used for the preliminary physiochemical screening.

Phytochemical Analysis

The phytochemical tests were conducted using standard procedures to identify the constituents as described by Edeoga *et al.*, (2005) [1] and Harborne (1973). Tests for the presence of the alkaloids, saponins, tannins, terpenoids, flavonoids, glycosides, reducing sugar polysaccharides, phytosteroles and phenols were carried out (Marinova *et al*., 2005) [10].

Phytochemical tests were done to find the presence of the active chemical constituents such as alkaloid, flavonoids, glycosides, triterpenoids, steroids, tannin and phenols, reducing sugar, carbohydrates and protein and amino acids by the following procedure. (Kokate., 2000, Harbone., 1999, Prashanth Tiwari *et.al.*; 2011) [4].

Tests for alkaloids

To the extract, dilute hydrochloric acid was added, shaken well and filtered. With the filtrate, the following tests were performed.

Mayer's reagent test

To 3 ml of filtrate, few drops of Mayer's reagent were added along sides of tube. Formation of creamy precipitate indicates the presence of alkaloids.

Tests for carbohydrates

Molisch Test

2 ml of aqueous extract was treated with 2 drops of alcoholic α-naphthol solution in a test tube and then 1 ml of concentrated sulphuric acid was added carefully along the sides of the test tube. Formation of violet ring at the junction indicates the presence of carbohydrates.

Tests for reducing sugars

Benedict's Test

Equal volume of Benedict's reagent and extract were mixed in a test tube and heated on a water bath for 5-10 minutes. Solution appears green, yellow or red depending on the amount of reducing sugar present in the test solution which indicates the presence of reducing sugar.

Tests for Flavonoids

Alkaline reagent test

The extract was treated with few drops of sodium hydroxide solution separately in a test tube. Formation of intense yellow color, which becomes colorless on addition of few drops of dilute acid indicates the presence of flavonoids.

Tests for Glycosides

Borntrager's test

To 3 ml of test solution, dilute sulphuric acid was added, boiled for 5 minutes and filtered. To the cold filtrate, equal volume of benzene or chloroform was added and it was shaken well. The organic solvent layer was separated and ammonia was added to it. Formation of pink to red color in ammonical layer indicates the presence of anthraquinone glycosides.

Tests for tannin and phenolic compounds ferric chloride test

A small amount of extract was dissolved in distilled water. To this solution 2 ml of 5% ferric chloride solution was added. Formation of blue, green or violet color indicates presence of phenolic compounds.

Test for saponin

Froth test

The extract was diluted with distilled water and shaken in a graduated cylinder for 15 minutes. The formation of layer of foam indicates the presence of saponins.

Tests for protein and amino acids ninhydrin test

3 ml of the test solution was heated with 3 drops of 5% Ninhydrin solution on a water bath for 10 minutes. Formation of blue color indicates the presence of amino acids.

Tests for triterpenoids and steroids

Salkowski's test

The extract was treated with chloroform and filtered. The filtrate was added with few drops of concentrated sulphuric acid, shaken and allowed to stand. If the lower layer turns red, sterol is present. Presence of golden yellow layer at the bottom indicates the presence of triterpenes.

Result and Discussion

The phytochemical analysis of plant extract using Aqueous, Ethonal, and Chloroform was showed in Table 1. From phytochemical analysis, redusing sugar were found in *Azadirchta indica* in the solvent such as choloroform, ethanol, and aqueous. The ethanol extract of *Azardirchta indica* showed the presence of alkaloids, glycosisdes, flavonoids, soponines, tanins phenolic compounds and reducing sugar. Alkaloids, flavonoids, glycosides, reducing sugar, Tannins, Soponins polysaccharides and phenolics were observed in chloroform extract of *Azadirachta inidica* but there was absence of terepenoides and phytosterols. Aqueous extract of *Azadirchta indica* showed the presence of alkaloids, flavanoides, glycoside reducing sugar, polysaccharides and phenols but there was absence of tannins, spononines and trepenoides.

Antibiotics resistance is a major concern and developments of new agents from plants could be useful in making the demand for new antimicrobial agents with improved safety and efficacy (Srivastra and Stiukla kumar., 2000) [19]. The

preliminary phytochemical investigations it was observed that alkaloids tanninnns, phenols, flavanoides, terpenoides, sterols, saponins, glycosides and reducing sugar were present in the extract. The presence of these secondary metabolites in plants produces some biological activity in man and animals and it is responsible for their uses as herbs in primary health care (Saradha Jyothi Koona and Subbarao Budida 2011) [16]. These compounds also serve to protect the plant against infections by microorganisms, predations by insects and herbivores, while their odor and flavor are responsible for their pigments (Mahmood *et al*., 2008) [9].

The extracts of *A. indica* showed the presence of glycosides, alkaloids, tannins, flavonoids, terpenoids, saponins, reducing sugar, polysaccharides and phenols were found in presence of chloroform extracts. The Saponin were observed in the chloroform extract of *A. indica*. The Chloroform extracts of *A. indica* showed the absences of terpenoids and phytosterols. The free radical scavenging capacity of the crude extracts of the medicinal plants was determined using DPPH as described by Reena *et al* 2012 [15].

Hill (1985) reported that the medical value of the plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plant are alkaloids, tannins, flavonoids and phenolic compounds. Presently many scientist and organizations are search of traditional remedies as alternative medicines. It has been estimated that about 25% of all prescribed medicines today are substances derived from plants (Puri, 1999) [14]. Plants have estimated more than 7000 different compounds in use today antibiotics, decongestants and analysis compounds. The World Health Organization (WHO) estimates that up 80% of the world's people rely on plants for their primary health care, Since western pharmaceuticals are other expensive in accessibly or unsuitable and are always accompanied with various side effects (Puri., 1999) [14].

Table 1: Phytochemicals screening of leaf extract *A. indica* in different solvents

Tests	Plant extracts		
	Aqueous	**Ethanol**	**Chloroform**
Alkaloids	+	+	+
Flavanoids	+	+	+
Glycosides	+	+	+
Reducing Sugar	+	+	+
Tannins	-	+	+
Saponins	-	+	+
Terpenoids	-	-	-
Polysaccharides	+	+	+
Phytosterols	+	-	-
Phenols	+	+	+

+: Present; -: Absent

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