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EXTRACTION OF PHYTOCHEMICALS AND STUDY OF ANTIMICROBIAL PROPERTIES OF *AZADIRACHTA INDICA* (NEEM)

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ABSTRACT

The antimicrobial properties of plants have been investigated by a number of studies worldwide and many of them have been used as therapeutic alternatives because of their antimicrobial properties. Plants are the cheaper and safer alternative sources of antimicrobials. The study deal with the antibacterial activity of alcoholic extract of leaves of *Azadirachta indica* (NEEM) through agar well diffusion assay against. *E.coli, Staphylococcus aureus, Pseudomonas aeruginosa.*

KEYWORDS: *Azadirachta indica*, Antibacterial activity, Extraction, Diffusion Method

It is estimated that there are 250,000 to 500,000 species of plants on Earth (Borris, 1996). Many of these plants have been investigated scientifically for antimicrobial activity and a large number of plant products have been shown to inhibit growth of pathogenic bacteria. A number of these agents appear to have structures and modes of action that are distinct from those of the antibiotics in current use, suggesting that cross-resistance with agents already in use may be minimal (Soulsby, 2005). Medicinal plants may therefore be defined as a group of plants that possess some special properties or virtues that qualify them as articles of drugs and therapeutic agents, and are used for medicinal purposes. Plants have formed the basis of sophisticated traditional medicine (TM) practices that have been used for thousands of years by people in China, India, and many other countries. Some of the earliest records of the usage of plants as drugs are found in the Artharvaveda, which is the basis for Ayurvedic medicine in India. (Chopra and Doiphode, 2002)

An antimicrobial is an agent that kills microorganisms or inhibits their growth. Antibacterial, commonly known as antibiotics, are used against bacteria and antifungals are used against fungi. They can also be classed according to their function. (Cowan, 1999)

Azadirachta indica A. Juss (syn. *Melia azadirachta*) is well known in India and its neighboring countries for more than 2000 years as one of the most versatile medicinal plants having a wide spectrum of biological activity. The sanskrit name of the neem tree is 'Arishtha' meaning 'reliever of sickness' and hence is considered as 'Sarbaroganibarini'. The tree is still regarded as 'village dispensary' in India. The neem tree has been described Gramas *A. indica* as early as 1830 by De Jussieu (De Jussieu, A., 1830)

MATERIALS AND METHODS

Collection of Sample

The leaves of *A. indica* were collected from Bhilai C.G., India. It was ensured that the plant was healthy and uninfected. The leaves were washed under running tap water to eliminate dust and other foreign particles and to clean the leaves thoroughly and a particular amount of leaves dried under shadow

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Leaf extract

The completely shade dried material was coarsely powdered and allowed soxhlet for successive extraction with methanol and ethanol. The obtained liquid extracts were subjected to subjected to Rotary evaporator and subsequently concentrated under reduced pressure (in vaccum at 40°C) and evaporated to dryness and stored at 4°C in air tight bottle.

Methanol Extract

50g of dried leaf powder were taken in a separate container. To this 250ml of methanol was added and kept for 24 h with periodic shaking then filtered and the filtrate was collected. The procedure was repeated three times with fresh volume of methanol. The filtrates were pooled.

Ethanol Extract

50g of dried leaf powder of *Azadirachta indica* were taken in a separate container. To this 250 ml of ethanol was added and kept for 24 h with periodic shaking. Filtered and the filtrate was collected. The procedure was repeated three times. The collected filtrates were pooled.

Phytochemical Components

Phytochemical analyses were carried out according to the methods described by Trease and Evans (Trease and Evans, 1989) of the crude powder of leaves for the identification of phytochemicals like tannins, alkaloid, steroids, saponin and flavonoids.

Microorganisms

The Pathogenic strains of *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were used.

Antimicrobial Activity of Extracts

Well diffusion method

The agar well diffusion method technique (Bauer *et al*., 1966) was used to determine the antibacterial activity of the plant extracts. The test solution was prepared in Di methyl sulfoxide

(DMSO).

Procedure

Inoculate the different culture on Nutrient agar plate. A sterile 5mm cork borer was used to punch holes after solidification of media. The wells formed were filled with different concentrations of the extract which were labeled accordingly; 50mg/ml, 37.5mg/ml, 25mg/ml, 12.5mg/ml. The plates were then left on the bench for 1 hour for adequate diffusion of the extracts and incubated at 37°C for 48hours in upright condition. After incubation, the diameters of the zones of inhibition around each well were measured.

RESULTS AND DISSCUSSION

The medicinal values of the secondary metabolites are due to the presence of chemical substances that produce a definite physiological action on the human body. The most important of these substances include, alkaloids, glucosides, steroids, flavonoids, fatty oils, resins, mucilages, tannins, gums, phosphorus and calcium for cell growth, replacement and body building (Kubmarawa *et al*.). The phytochemical analysis of *A. indica* extract had earlier been reported by Kraus. In all extract highest zone of inhibition is shown against *E. coli* (16.63) and minimum against *P. aeruginosa* (8).

Table 1: Phytochemical analysis of Neem [*Azadirachta indica*]

Phytochemical constituents	Ethanol	Methanol
Alkaloids	+	+
Steriods	+	+
Saponin	+	+
Tanin	+	+
Flavonoids	+	+

Table 2: Zone of Inhibition of Ethanol Extract

Microbial Strains	12.5 mg/ml	25 mg/ml	37.5 mg/ml	50 mg/ml
Escherichia coli,	12.5±0.40	14.06±0.16	14.66±0.12	15.13±0.09
Pseudomonas aeruginosa	8±0.24	12.03±0.24	12±0.14	10.4±0.24
Staphylococcus aureus	10.33±0.94	9.96±0.16	8.9±0.28	11.13±0.12

Table 3: Zone of Inhibition of Methanol Extract

Microbial Strains	12.5 mg/ml	25 mg/ml	37.5 mg/ml	50 mg/ml
Escherichia coli,	12.43±0.30	11.63±0.04	10.3±0.21	16.63±0.16
Pseudomonas aeruginosa	7.43±0.41	9.66±0.04	13.03±0.28	10.4±0.24
Staphylococcus aureus	9.5±0.16	9.96±0.16	8.9±0.28	11.46±0.12

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