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Phytochemical studies and screening of leaf extracts of *Azadirachta indica* for its anti-microbial activity against dental pathogens

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Abstract

The present study was carried out to evaluate the antimicrobial properties of Azadirachta indica leaves against certain bacterial strains causing dental carries using cup plate method and disc diffusion method. The leaf extracts of Azadirachta indica were prepared using different solvents like methanol, chloroform and petroleum ether and are screened for its anti-microbial activity. The best suitable extract was further optimized from the results of anti-microbial studies. The phytochemical screening of the leaf extracts was performed. The strains of four human pathogenic bacteria causing dental caries are Micrococcus albus, Staphylococcus aureus, Proteus vulgaris and Pseudomonas aerogenosa. The anti-microbial activities of petroleum ether extract, chloroform extract, methanol extract of neem leaves are checked by disc diffusion method. All the leaf extracts exhibited significant inhibition.Comparative study of the results obtained from both the methods indicates that the Chloroform Extract shows better anti-microbial activity against desired strains. All the extracts showed concentration dependent activity comparable with the reference Drug Streptomycin.

Key Words: *Azadirachta indica*, Anti-microbial activity, Streptomycin.

INTRODUCTION

Herbal medicine represents one of the most important fields of traditional medicine. WHO recognized that medicinal plants played an important role in the health care of about 80% of the world population in developing countries and depend largely on traditional medicine [1-2]. It is estimated that about 75% of the 120 biologically active plant derived compounds, presently in the use worldwide, have been derived through follow up researches to verify the authenticity of the data from folk and ethnomedicinal uses. So, there is a great scope for new drug discoveries based on traditional plant uses [3-5]. Neem (*Azadirachta indica*) is perhaps the most useful traditional medicinal plant in India. Each part of the neem tree has some medicinal property and is thus commercially exploitable. During the last five decades, apart from the chemistry of the neem compounds, considerable progress has been achieved regarding the biological activity and medicinal applications of neem. 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 [6-7]. It is now considered as a valuable source of unique natural products for development of medicines against various diseases and also for the development of industrial products.

Now a days, the most common problem faced is dental carries which is due to indulging in different variety of diets and improper oral hygiene. The pathogens causing dental carries mainly include *Micrococcus albus*, *Staphylococcus aureus*, *Proteus vulgaris* and *Pseudomonas aerogenosa*. In the present work, different extracts of leaves of neem were prepared using the solvents like methanol, chloroform and petroleum ether. The prepared extracts were screened for their anti-microbial activity against pathogens causing dental carries by using disc diffusion method. The best suitable extract was further optimized from the results of anti-microbial studies.

MATERIALS AND METHODS

Plant Collection

The leaves of *Azadirachta indica* were collected from local market and dried in shade for a week.

Chemicals

All the solvents used in this study were purchased from Merck Chemicals, India, of analytical grade.

Preparation of different extracts of Azadirachta indica

Mature fresh leaves of *Azadirachta indica* were washed first in sterilized distilled water, followed by washing in mercuric chloride solution (0.1 %) and again washed in sterilized distilled water. Leaf material was weighed and transferred on to a sterile mortar and pestle to make a crude crushing of the material, following which it was transferred on to a sterile homogenizer and finely crushed. About 100 grams of coarsely powdered plant material was exhaustively extracted for 2 h with 200 ml of different solvents individually at their boiling point temperature in soxhlet apparatus [8]. The solvents used for extraction are methanol, chloroform and petroleum ether. The extracts obtained were filtered and evaporated under reduced pressure using Rota-vapour. The extracts were dissolved in dimethyl-sulphoxide to make the final concentrations which were kept in refrigerator till used.

Extractive value of different extract of Azadirachta indica

In present study to investigate the antimicrobial activity of the *Azadirachta indica*; three different extract of the *Azadirachta indica* were prepared using different solvents. The extractive values of the different extracts were calculated [9] and reported in Table 1 with their description. According to the extractive values of the different extracts of the *Azadirachta indica* the highest compounds were solubilised and extracted with chloroform.

Preliminary Phytochemical Analysis

Phytochemical screening of plant extracts was done following the standard procedure by Santaram (1983), Chhabra et al (1984) and Harbone (1984). All the prepared plant extracts were subjected to preliminary phytochemical screening for the presence of alkaloids, quinines, resins, tannins, fixed oils, flavanoids, fats, saponins, phenolic compounds, Proteins and carboxylic acids [10-11]. The results were shown in table 2.

Disc Diffusion Method

a) Preparation of Discs

From the plant extracts, 50 mg and 100 mg of crude extracts were dissolved in 1 ml of 4 % dimethyl sulphoxide (DMSO) and 0.2 ml of the prepared extracts were loaded on to the filter paper discs (Sterilized Whatmann No. 1 filter paper discs of 6 mm diameter) to get 20 mg / disc concentration and allowed to dry at room temperature in laminar air flow chamber [13-16].

b) Micro organisms used

The screening of the anti microbial activity of crude extracted *Azadirachta indica* were carried out individually on active cultures of, *Staphylococcus aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Micrococus albus.*

c) Preparation of media

Muller Hinton Agar (MH, Hi media) was used. The formula (gm/litre) Beef 2g, casein acid hydrolysate 17.5g, starch 1.5 g and agar 17g; pH 7.4 ± 0.2.

About 38g of MH agar was weighed and dissolved in 1000 ml of distilled water and adjusted to pH 7.4 ± 0.2, sterilized by autoclaving at 121 ^{0}C for 15 minutes at 15 psi pressure and was used for sensitivity tests [13-16].

d) Antimicrobial activity

The antimicrobial activity of the extracts was evaluated by disc diffusion method [17](4a). Previously prepared paper discs containing different extracts were placed individually on the surface of the petriplates, containing 20 mL of respective media seeded with 0.1 ml of previously prepared microbial suspensions individually (10 CFU/mL). Standard antibiotic Streptomycin (20 µg/disc) obtained from Hi-media, Mumbai, was used as positive controls. The discs containing petroleum ether, chloroform and methanol served as negative controls. The assessment of antimicrobial activity was based on measurement of inhibition zones formed around the discs. The plates were incubated for 24 h at 37°C and the diameter of the inhibition zones was recorded.

RESULTS AND DISCUSSION

The leaf extracts of *Azadirachta indica* on phytochemical screening showed the presence of triterpenes, glycosides and fatty acids as chemical constituents. The results are shown in table 1.The anti-microbial activity of various Neem extracts like petroleum ether extract, chloroform extract, methanolic extract are evaluated and compared using disc diffusion method. All the test extracts of *Azadirachta indica* possess significant antimicrobial activity against the dental pathogens. Among the three extracts, the chloroform extract showed a higher activity than other extracts (Table-3).

Table 1 Extractive values of leaf extracts of *Azadirachta indica*

S. No.	Type of Extract	Extractive Value (in gm)
1	Petroleum ether extract	4.2
2	Chloroform extract	2.5
3	Methanol extract	1.2

This may be due to the solvent extract containing different constituents having antimicrobial activity. Chloroform was proved as the most effective solvent for extracting broad spectrum of antimicrobial compounds from plants.

Table 2 Phytochemical analysis of leaf extracts of *Azadirachta indica*

S. No.	Plant constituents	Petroleum Ether Extract	Chloroform Extract	Methanol Extract
1.	Test for Alkaloids	–	–	–
2.	Test for Volatile oils	–	–	–
3.	Test for carboxylic acids	–	–	–
4.	Test for Fixed oils	–	–	–
5.	Test for Phenols	–	–	─
6.	Test for Quinones	–	–	–
7.	Test for Resins	–	–	–
8.	Test for Saponins	–	–	–
9.	Test for Tannins	─	–	–
10.	Test for Xantho proteins	–	–	–
11.	Test for Glycosides	+	+	+
12.	Test for Coumarins	–	–	–
13.	Test for Emodins	–	–	–
14.	Test for Fatty acids	+	+	+
15.	Test for Triterpenes	+	+	+
16.	Test for Cardinolides	–	–	–

+ indicates the presence of the constituent.
─ indicates the absence of the constituent.

Table 3 Antimicrobial screening of leaf extracts of *Azadirachta indica*

Dental Pathogen	Leaf Extracts of *Azadirachta indica*(500µg/ml)			
	Petroleum Ether Extract (mm)	Chloroform Extract (mm)	Methanol Extract (mm)	Streptomycin (mm)
Staphylococcus Aureus	14	18	10	22
Proteus vulgaris	16	20	08	28
Pseudomonas aeruginosa	08	10	8.5	12
Micrococcus albus	10	14	7.5	20

CONCLUSION

It may be concluded from this study that *Azadirachta indica* leaf extract has antimicrobial activity against dental pathogens. It is expected that using natural products as therapeutic agents will probably not elicit resistance in microorganisms. This can explain the rationale for the use of the plant in treating infections in traditional medicine. The plant could be a veritable and cheaper substitute for conventional drugs since the plant is easily obtainable and the extract can easily be made via a simple process of maceration or infusion. It is essential that research should continue to isolate and purify the active components of this natural herb and use in experimental animals.

REFERENCES

[1] Fransworth, N.R., O. Akerele, A.S. Bingel, D.D. Soejarto and Z.G. Guo, 1985. Medicinal plants in therapy. Bulletin of the World Health Organisation, **2004**, 63, 965-981.
[2] National Research Council. Neem: A tree for solving global problems. National Academy Press, Washington, DC. **1992**.

[3] Pushpangadan, P. and B. Kumar, 2005. Ethnobotany, CBD, WTO and the Biodiversity Act of India. Ethnobotany, **2005**, 17, 2-12.
[4] Chopra RN, Glossary of Indian Medicinal Plants, 1st edition. CSIR, **1956**, 113-117.
[5] Yoganarasimhan SN. Medicinal Plants of India, **2000**, 2, 194-198.
[6] R. Subapriya; S. Nagini, *Medicinal properties of neem leaves: a review*, Pharmacognosy Review, June **2004**.
[7] Varma, G. S., *Miracles of Neem Tree*, Rasayan Pharmacy, New Delhi, **1976**.
[8] Pushp K Jain, "Neem – The bitter gem" - "Karwa amrit" Pharmacognosy review, July **2006**.
[9] Kokate, C.K.: Practical Pharmacognosy, Vallabh Prakashan, New Delhi, **2005**.
[10] Bakshu, L.Md, Ethnomedicobotanical and Phytochemical evaluation of certain rare, endemic and endangered medicinal plants from Eastern Ghats. Andhra Pradesh, India. Thesis, S.K.University, **2002**.
[11] Harbone, J.B.: Phytochemical Methods- A Guide to Modern Techniques of Plant Analysis, Chapman and Hall London, **1998**.
[12] Bauer AW, Kirby WM, Sherris JC, Turch M. Antibiotic susceptibility testing by standardised single disc method. Amer. J. Clin. Pathology, **1966**, 45, 493-6.
[13] Agnese AM, Perez C, Cabrera JL. Adesmia aegiceras: antimicrobial activity and chemical study. Phytomedicine **2001**, 8, 389-394.
[14] Acar JF and Goldstein FW, Disc susceptibility test: Antibiotics in laboratory medicine, 4th edition, edited by L. victor, Williams and Wilkins publishers, **1998**.
[15] Bauer AW, Kirby WMM, Sherris JC and Turck M, Antibiotic susceptibility testing, by a standardized single disc method, Am J Clinical pathology, **1966**, 45, 493-496.
[16] National Committee for clinical laboratory standards, performance standards for anti microbial disc susceptibility testing, twelfth information supplement (M100-S12), Wayne, PA: NCCLS, **2002**.