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NEEM – AN INVALUABLE BIORESOURCE

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ABSTRACT

Neem *Azadirachta indica* is known for its various medicinal applications and has great significance in Indian traditional medicine. In the present study, aqueous and acetone extracts of neem were analyzed for inherent components, anti-microbial activities against common pathogens and compared with standard antibiotics. Aqueous extract was effective against four Gram positive, three Gram-negative pathogens and acetone extract against three Gram positive and one Gram negative pathogens isolate. Qualitative and HPTLC analysis showed the presence of glucose, proteins, alkaloids, flavonoids, terpenes, saponins, caffeic acid, xantho-eriodictyl, silychristin, taxifolin and iso-silybin. Considering the pharmacological, broad spectrum anti-microbial activities and potential for controlling both microbial and insect pests, deliberations can be made about use of *A. indica* as an antimicrobial agent, dietary supplement and a prebiotic.

KEY WORDS: ***Azadirachta indica;*** **Aqueous and Acetone extract; Antimicrobial activity; HPTLC Finger printing; Flavonoids; Prebiotic.**

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INTRODUCTION

Neem (family Meliaceae), is also known as Indian lilac, limba, margosa, nim, nimba and vempu. Neem or "nimba" is considered as a "bestower of good health". Uses of neem are so varied that the tree is called the "village pharmacy" of South Asia. Every part of the tree finds application in indigenous herbal use. Dried and ground *A. indica* leaves are used as dietary supplements in human beings and for treatment of a variety of human ailments[1]. Neem parts elaborate more than 140 chemically diverse and structurally complex, biologically active compounds. All parts, leaves, flowers, seeds, fruits, roots and bark have been used traditionally for treating inflammation, infections, fever, skin diseases and dental disorders. Neem leaf constituents exhibit immunomodulatory, anti-inflammatory, antihyperglycaemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic and anticarcinogenic properties[2]. Its lactic secretion has an anti-poison effect, used against tuberculosis, leprosy, treatment of eye- problems, inflammation of liver, diseases of the skin and uterus, tumors, piles and tooth-ache. Its seed-oil is used as a hair-tonic, anti-lice agent and against headache[3]. Leaf extract is used as an insecticide and anti-feedant. Parenterally, seed extract is used as a laxative, anti-emetic, anti-helmenthic, spermicide, used to combat snake-bite, anti-viral and insect repellant. It contains compounds with anti-bacterial and anti-fungal properties[4]. Bark decoction is used to treat heart-burn, fever and cough. The young fruit is used as an astringent, tonic and purgative. Seed oil is used locally as a stimulant, anti-septic, for dressing wounds and chronic forms of skin diseases like eczema, ring-worms, scabies, erysipelas and sloughing ulcers with chaulmoogra oil[5]. Aqueous extracts of green leaves were found to be more biotoxic, showing an inhibitory effect at all concentrations, while tender twigs, neem seeds, dry leaves, were inhibitory at 75- 100% [6]. Anti-acne activity of leaf extracts of *A. indica* was observed[7]. *A. indica* was found to have anti-bacterial activity against standard cultures of *S. aureus* (NCTC-3750), *E. coli* (ATCC-1948), *Proteus mirabilis* (NCIM-2087), *B. subtilis* (NCIM-2063), *Bacillus stereothermophilus* (NCIM –2328), clinical isolates of *Klebsiella aerogenes*, *Klebsiella pneumoniae*, NAG *Vibrio*, *Salmonella typhimurium*, *Salmonella paratyphi* and *Shigella flexneri*[8]. The present investigation was carried out to study the antimicrobial activity of *A. indica* against some bacterial pathogens causing infections, compared with that of standard antibiotics, analyzed for inherent components and Minimum Inhibitory Concentration.

MATERIALS AND METHODS

The present study was carried out in the Department of Microbiology, Bharati Vidyapeeth's, Dr. Patangrao Kadam Mahavidyalaya, Sangli, Maharashtra, India.

Preparation of aqueous and acetone extracts

Known weight of fresh leaves of *A. indica* were collected, washed with distilled water, suspended in 50 ml sterile distilled water and crushed in a grinder. Extraction was repeated and extract filtered through muslin cloth and shadow dried. For acetone extract, same procedure was repeated with acetone[9, 10].

Phyto-chemical investigation

Aqueous extract of *A. indica* was subjected to qualitative chemical investigation using standard tests and reagents to check the presence of active groups; Glucose, Proteins, Alkaloids[11], Fats[12], Sterols, Triterpenoids, Flavonoids, Tannins[13], Glycosides[4].

Qualitative analysis of the aqueous extract by HPTLC[14]: General analysis

Mobile Phase: - Toluene : Ethyl acetate : Formic acid (100%) :: 7 : 3 : 0.2; Developing reagent / Derivatizing agent : 5 % Methanolic sulphuric acid.

Flavonoid analysis

Mobile phase -: Chloroform: Acetone : Formic acid :: 7.5: 1.7 : 0.4; Developing reagent /

Derivatizing agent: Natural products / Polyethylene Glycol 28.

Microorganisms used

Gram positive :- *Bacillus subtilis, Staphylococcus aureus, Enterococcus fecalis.* **Gram negative :-** *Escherichia coli, Salmonella typhi, Shigella flexneri* and *Proteus vulgaris.* **Yeast** : *Candida albicans*, **Fungus:** *Aspergillus niger.*

Standard antibiotics

Common and recently prescribed standard antibiotics were used as a control, to compare with the response of pathogenic isolates.

***Agar-cup diffusion assay method*[15]**

0.1 ml. of each isolate was spread on a sterile Nutrient agar plate (20 ml.) separately. 0.1 ml. of the aqueous and acetone extracts of *A. indica* were aseptically added to cups, made with sterile cork borers, on the plate. Diameters of zones of inhibition were measured, after incubation at 37^0c. for 24 hrs. The procedure was repeated for sensitivity of pathogens to standard antibiotics.

***Dilution method for Minimum Inhibitory Concentration (MIC)*[16]**

The cultures were adjusted to 0.5 McFarland turbidity (5 x 10^5 CFU / ml). Controls with 0.5 ml of culture medium were used. The tubes were filled with 100 µl of sterile H_2O and 100 µl of the plant extracts. Each well was inoculated with 100 µl of bacterial suspension. The tubes were incubated at 37°C for 24 hrs. The lowest concentration of plant extract that exhibited no growth of the organism was considered as MIC. The most sensitive culture to aqueous extract was studied for MIC.

OBSERVATIONS AND RESULTS

Qualitative chemical investigation of the plant extract showed the presence of Glucose (1.5-2 %), Proteins, Alkaloids, Flavonoids and Glycosideswhile analysis by HPTLC depicted peaks corresponding to Terpenes, Saponins and Caffeic acid. Analysis of Flavonoids by HPTLC showed peaks corresponding to Xantho-eriodictyl, Sily-Christin, Taxifolin and Iso-silybin [Table 1]. Aqueous extract of *A. indica* inhibited the growth of *B. subtilis, S. aureus, Strep. sp. E. coli, S. typhi, Sh. flex*neri, *P. vulgaris.* Acetone extract inhibited the growth of *B. subtilis, S. aureus, E. fecalis, E. coli* as well as *C. albicans* and *Asp. niger*. *Shigella flexneri* was found to be most sensitive to the aqueous extract while *Staphylococcus aureus* to acetone extract [Table 2] The standard antibiotics, Lomefloxacin (LO) inhibited ten of the bacterial pathogens, Gentamicin (G), Cephotaxime (CX), Netillin (NT), Ofloxacin (OF), Pefloxacin (PF) inhibited nine, Ceftazidime (CT), Nitrofurantoin (NF) inhibited six, Ciprofloxacin (CP), Doxicycline (DO) inhibited five, Amikacin (AM) inhibited three, Augmentin (AU) inhibited two while Cefuroxime (CO) and Cefoperazone (CF) inhibited one each [Table 3].

Table 1

Results of general and flavonoid analysis of aqueous extract of A. indica by HPTLC

General analysis (200 nm), (325 V)				
Peak	Max Rf	Area	Comparative % area	Identified component
1.	0.02	2341.9	10.22	**Terpenes**
3.	0.08	62.51983.5	0.27	**Terpenes**
4.	0.14	1983.5	8.65	**Saponins**
Flavonoid analysis (366 nm, 199v)				
1.	0.31	2300.3	7.66	**Xantho -Eriodictyol**
2.	0.36	3782.6	12.60	**Silychristin**
3.	0.43	8035.0	26.76	**Taxifolin**
4.	0.63	12301.4	40.97	**Iso- Silybin**

Table 2
Antimicrobial activity of aqueous and acetone extracts of neem

Name of the pathogen	Aqueous extract (0.0037gm./0.1 ml.) [Mean ± S.D.]	Acetone extract (0.0014gm./0.1 ml.) [Mean ± S.D.]	t Test	Significance
B. subtilis	8.5 ± 1.04	7.83 ± 1.47	0.91	NS
S. aureus	10.83 ±1.16	17.66 ± 1..966	7.32	***
E. fecalis	10.33 ± 0.816	16.66 ± 1..966	7.28	***
E. coli	8 ± 1.37	16.16 ± 2.71	6.58	***
S. typhi	7.5 ± 1.04	-	-	
S. flexneri	16 ± 1.67	-	-	-
P. vulgaris	14.5 ± 1.87	-	-	-
C. albicans	-	17.66 ± 1.21	-	-
Asp. niger	-	19.16 ± 1.47	-	-

Table 3
Antibiotic sensitivity of the pathogenic isolates to standard antibiotics by disc diffusion method

Sr. No	A' Biotic	µg	*B.s.*	*S.a.*	*E.f.*	*S.l.*	*E.c.*	*K.p.*	*S.t.*	*S.p B.*	*S.f.*	*P.a.*	*P.v.*	*S.m.*	*C.a.*	*A.n.*
1	AM	30	-	-	-	-	19.83 ± 1.16	18.83 ± 0.75	-	-	-	-	27.16 ± 1.83	-	-	-
2	AU	30	17.66 ± 1.21	-	-	-	-	-	-	-	-	16.83 ± 1.16	-	-	-	-
3	CX	30	14.33 ± 1.75	17.5 ± 1.87	19 ± 1.41	19.83 ± 1.16	19.5 ±1.04	-	-	20.83 ± 1.16	22.5 ± 1.04	21.33 ± 1.63	28.16 ± 0.98	-	-	-
4	CP	5	-	-	-	-	-	31.33 ± 1.36	32.16 ± 1.16	-	-	26.83 ± 1.16	37.66 ± 1.86	25 ± 1.41	-	-
5	CO	30	-	-	-	-	-	-	-	-	-	-	25.16 ± 1.16	-	-	-
6	CF	75	-	-	-	-	-	-	-	-	-	-	16.33 ± 1.03	-	-	-
7	CT	30	29.66 ± 1.86	24.66 ± 1.86	23.5 ± 1.04	-	17.33 ± 1.21	-	18.33 ± 1.03	-	22.16 ± 1.16	-	-	-	-	-
8	G	10	-	29.16 ± 1.47	26.66 ± 1.21	25.66 ± 1.966	13.66 ± 2.16	27 ± 1.41	27 ± 2.60	31.16 ± 2.56	-	26.66 ± 1.03	23 ± 1.26	21.16 ± 1.722	-	-
9	LO	30	-	20 ± 1.41	19.5 ± 1.04	21 ± 1.41	18.33 ± 0.81	14.66 ± 0.81	17.5 ± 1.04	21.5 ± 1.04	21.83 ± 0.75	-	28.66 ± 1.032	19.5 ± 1.04	-	-
10	NT	30	18.83 ± 1.83	19.5 ± 1.04	22.66 ± 1.63	23.83 ± 1.94	-	-	18.33 ± 0.81	19.83 ± 1.16	18.5 ± 1.04	-	23.66 ± 1.966	19.33 ± 1.63	-	-
11	OF	5	26.33 ± 4.22	20.16 ± 1.16	21.5 ± 1.04	24.16 ± 1.16	26.83 ± 1.16	13 ± 0.89	19.5 ± 1.37	-	21.5 ± 1.04	-	-	20.16 ± 1.47	-	-
12	PF	5	-	18.5 ± 1.04	17.83 ± 1.16	21.16 ± 0.75	19.83 ± 1.16	-	19.5 ± 1.04	19.5 ± 1.04	18.5 ± 1.04	-	31.66 ± 0.51	29.33 ± 1.03	-	-
13	NX	10	19.83 ± 1.16	33 ± 2.366	-	21.5 ± 1.04	-	41.5 ± 1.04	32.5 ± 2.25	40.5 ± 1.51	-	39.33 ± 1.21	-	19.5 ± 1.04	-	-
14	DOXI	10	-	19.16 ± 1.4719	-	-	15.5 ± 1.04	28.33 ± 2.87	20.66 ± 0.816	22 ± 1.41	-	-	-	-	-	-

Key : * = P < 0.05 (0.05 – 2.23) –significant, ** = P < 0.01 (0.01 – 3.17)-highly significant
****** = P < 0.001 (0.001 – 4.59)- very highly significant, NS = Not Significant***
"-" refers to no antibacterial effect of medicinal plant on the mentioned bacterial strain at mentioned dose, "+" refers to antibacterial effect of medicinal plant on the mentioned bacterial strain at mentioned dose, Figures indicate size of zone of inhibition in mm.
AM-Amikacin, AU-Augmentin, CX-Cephotaxime, CP-Ciprofloxacin, CO-Cefuroxime, CF-Cefoperazone, CT-Ceftazidime, G-Gentamicin, LO-Lomefloxacin, NT-Netilmicin,Netillin, OF-Ofloxacin, PF-Pefloxacin, NX-Norfloxacin, DOXI- Doxicycline.

g) MIC of aqueous extract of *A. indica* for *Shigella flexneri* was found to be 2.59 X 10^2 µg / ml.

DISCUSSION

Present study showed the presence of glucose, proteins, alkaloids, flavonoids and glycosides in the aqueous extract of neem. HPTLC indicated the presence of terpenes, saponins, caffeic acid and flavonoids like xantho-eriodictyl, sily-christin, taxifolin and iso-silybin. Neem, a unique source of diverse chemical compounds, can be used for developing modern drugs after study of its bioactivity, mechanism of action,

pharmacotherapeutics, toxicity, standardization and clinical trials[17]. Flavonoids, natural products of high pharmacological potency have been reported to show multiple biological effects, like anti-microbial effect[18]. Alkaloids, plant secondary metabolites, have shown anti-microbial activity[19]. The extract is found to contain Taxifolin, a plant flavonoid, powerful natural antioxidant and anti-inflammatory agent[20], Caffeic acid, a lipophilic alkylamide, considered an immunostimulant, responsible for anti-inflammatory, anti-bacterial and anti-viral effects of the plants[21]. Present investigation showed the antimicrobial activity of extract of *A indica* extracts. Aqueous extract was found to be antimicrobially more effective, comparable with previous study[8,6]. NIM-76 preparation of *A. indica* leaves inhibited growth of *Escherichia coli* and *Kleibsiella pneumonia, Candida albicans* and *Polio virus* replication[22]. Chewing sticks (Miswak) of *A. indica* used as oral hygiene tools showed anti-plaque, anti caries and antibacterial effects at 50% concentration on *Streptococcus mutans* and *Streptococcus faecalis*[23]. Herbal products and their extracts like neem have shown significant advantages over chemical mouthwashes, which if formulated, can be safely by people and may lead to improvement in the general dental health[24]. 50% acetone extract of root, bark and leaves of *A. indica* showed anti-inflammatory activity in carrageenan induced edema in rats[1]. Increasing concentration of ethanol extract of Neem leaves exhibited increase in antibacterial activity against *Staphylococcus aureus* and MRSA[25]. Ethanolic and dichloromethane leaf extracts exhibited significant anti-bacterial activity against Gram positive and Gram negative bacterial species[26]. Oil from leaves showed better antimicrobial activities against many infectious microorganisms due to high level of protein and carbohydrate contents[27]. Chloroform leaf extract showed antimicrobial activity against human pathogens causing dental caries, *Micrococcus albus, Staphylococcus aureus, Proteus vulgaris* and *Pseudomonas aeruginosa*[28]. Methanol and ethanol leaf extracts showed anti bacterial activity against *E.coli, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhimurium, Bacillus pumilus,* compared with gentamycin 200 mg and gentamycin10 mg.[29]. Fungistatic effect of neem against commercially important obligate pathogen *Sphaerotheca fuliginea* (powdery mildew) indicated potential for controlling microbial and insect pests[30]. Antifungal activity of neem extract, compared with copper, mercury, molybdenum and zinc ferrocyanides for *A. niger* showed high activity for natural antifungals with metal ferrocyanides complexes, in comparison to them individually and highest for mercury ferrocyanide with neem and may be used for skin infection[31]. Neem oil showed inhibitory effects on Fusarium oxysporum f. sp. medicagenis and Fusarium subglutinans, may be used for management of host plants[32]. Immunopotentiating effects (antibody titres) in birds given neem fruit supplemented diet, were significantly higher than those fed with basal diet and antibiotics, against Newcastle virus[33]. Neem leaf preparations might be potential immune adjuvants for inducing active immunity tumor antigens[34]. Neem oil causes an elevation of immunoreactive and bioactive TNF-alpha and gamma-interferon in serum[35]. Neem treated mice had higher IgM and IgG levels[36]. Substitution of antibiotic by the neem diets resulted in significantly higher humoral immune responses in broiler chicks[37]. Neem seed oil has even shown dose-dependent analgesic activity[38].

CONCLUSION

The extracts of neem leaves are effective anti-microbial agents, against common pathogens causing infections. Neem is an important bioresource, which can be utilized for the benefit of mankind. The changing global scenario, towards the use of nontoxic plant products, advocates development of modern drugs from neem for the control of various diseases and its use as a prebiotic (nondigestible feed ingredients that stimulate the activity and growth of beneficial native bacteria in the GI tract, eliminating the pathogenic ones). Using natural products as therapeutic agents will probably prevent development of resistance in microorganisms and prove veritable and cheaper substitutes for conventional drugs.

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