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Antimicrobial Activity of Aqueous Neem Leaves Extracts

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Abstract **– The aqueous extraction of neem leaves were carried out at 60 ˚*C under acidic (0.5 N HCL), neutral (D/W) & alkaline (0.5 N NaOH) conditions.* Moisture, ash, total protein, soluble protein, carbohydrate and lipid content were determined from fresh neem leaves. The carbohydrate & soluble protein content as well as antimicrobial activities were measured from dry neem leaves. After extraction, the plant material was pretreated with n-Hexane to remove lipids and oils. *Soluble protein, carbohydrate as well as antimicrobial effect of aqueous extracts were estimated. All of native extracts at concentration of 20 µ gm/ml had various inhibition activities against the Gram* positive bacteria (Staphylococcus aureus), the Gram-negative bacteria (Escherichiacoli, Pseudomonus aeruginos) yeast (Saccharemyce cerevisiaethe) and fungi (Aspergillus niger and Penicillium cryusogenus). In the light of these results we concluded that level of antimicrobial activities of the various extracts depends on both the protein and carbohydrate contents. Generally, the high level of protein and carbohydrate contents of extract had better antimicrobial activities.**

Keywords **– Neem Tree, Aqueous Extracts, Antimicrobial Activity, Gram Positive Bacteria, Gram Negative Bacteria, Yeast, Fungi.**

I. INTRODUCTION

Neem tree (Azadirachta indica) of family meliaceae is evergreen tree found in most tropical countries. Almost every part of the tree has been in use since ancient times to treat a number of human ailments and also as a household pesticide (Vennugopal, 1994).

Neem has been extensively used in ayuruvede, unani and homeopathic medicine and has become a cynosure of the neem tree is “Arishtha” (nimba) meaning” reliever of sickness” (Schmutterer et al., 1995). Neem Leaf is a virtual living pharmacy and is a powerful antibacterial and antifungal. Its quercetin content (polyphenolic flavonoid) helps to combat infections of certain fungi.

Polysaccharide such as arabino-fucoglucanes and fucogalacto-glucorabinanes has also antimicrobial activities. (Fujiwara et al., 1984) Flavonoids, flavonoglycosides, dihydrochalocones, tannins and others are also important constituents of bark, leaves, fruits and flowers of neem. (Devakumar and SukhDev, 1996).

More than 140 compounds have been isolated from different parts of neem. Its strong garlic odour (alliaceous) and its medicinal properties have been attributed to the presence of sulphur containing compounds (Nadkarni and Nadkarni, 1954; Koul; 2004) and number of primary amines and secondary amines were detected (Atawodi and Spiegelhalder, 1994). Neem is used in treatment of various skin diseases (Dhawan and Ratnaik, 1993) and it has antibiotic properties (Sharma, 1993).

Dental gel containing neem extract significantly reduces the plaque index and bacterial count. Neem extracts acts on four organisms causing dental caries i.e. *Streptococcus mutans, Streptococcus salivavius, Streptococcus mitis,* and *Streptococcus sanguis*. (Prashant et al., 2007).

Biswas et al., (2002) have also shown that different types of extracts from various parts of neem tree (bark, s-

-eed, leaf) have anti-inflammatory, antipyretic, analgesic, immunostimulant, hypoglycemic, anti-ulcer, anti-fertility, anti malarial, antibacterial, antifungal, anti viral, anticarcinogenic, antioxidant, hepato protective effects.

II. MATERIALS AND METHODS

The chemical properties i.e. moisture, ash, total protein, soluble protein, carbohydrate, and lipids were determined from fresh neem leaves. Soluble protein, carbohydrate & antimicrobial activities were measured from different aqueous extracted dry neem leaves.

Collection of Plant Material:

The neem leaves used in this work were collected from the campus of the Anand People's Medicare Society. The moisture, fat and ash from fresh neem leaves were determined according to AOAC (1982) method. Total nitrogen of the investigated sample (0.3gm) was determined according to the usual micro-Kjeldahl method (AOAC, 1980). The crude protein was calculated by multiplied the total nitrogen by 6.25. The total carbohydrates were determined according to Trevelyan et al., (1952) method.

Sample Preparation:

The fresh neem leaves were shadow dried to remove moisture. Extraction of neem leaves was singly carried out with 0.5N HCL, O.5N NaOH or distilled water. 5gm dry leaves were extracted in 100 ml of different solvent i.e. 0.5N HCL, O.5N NaOH or water. Extracted samples were incubated at 60°C in water bath for 3 hours. (Wafaa et al., 2007)

The sample was filtrated. After filtration sample was treated with Hexane to remove fat. The fat free sample used for protein, carbohydrate, and antimicrobial activity estimation. Soluble protein was done by Lowry method. The total carbohydrates were determined according to Trevelyan et al., (1952).

Antimicrobial Activities:

Antimicrobial activity of crude extracts was tested against the Gram-positive bacteria i.e. *Staphylococcus aureus*, the Gram-negative bacteria i.e. *Escherichia coli* and *Pseudomonus aeruginos*, the fungi i.e. *Aspergillus niger* and *Penicillium citrinum,* and the yeast i.e. *saccharemyce cerevisiae*. The stock cultures of bacteria were maintained on nutrient agar slants, and yeasts & fungi were maintained on potato dextrose slants at 4°C.

Antimicrobial Screening (Agar Diffusion Method):

For screening the antibacterial activity the disc diffusion assay (Lunette, 1985) technique was used. The agar diffusion method was used to evaluate the antibacterial effect of the isolated extracts as well as to test the antifungal activity. Inoculums of each of the bacterial strain were suspended in 2 ml of nutrient broth and were divided in to four parts & cup borer was used to make four cup in each sector. Each cup was filled up with different extracting solution i.e. Streptomycin, 0.5N HCL, O.5N NaOH or D/W. Plates were incubated in refrigerator for 1 hour as to allow diffusion of antimicrobial agent. Plates were incubated in upright position at 37°C in the dark. Zones of inhibition were examined after 24hr.

Fungi were grown at 30°C and maintained at on potato dextrose (PD) medium. Inoculums of each strain were prepared from an overnight. The culture was diluted in potato dextrose medium (1:200). . Zones of inhibition of

fungi were measured after 48-72 hours incubation at 30°C. For positive controls, streptomycin as antimicrobial agent was used. In addition, for negative controls, dried discs that had been soaked in sterile water served as carrier control. The inhibition zone was measured around the disc.

All values expressed as mean ± SEM, where SEM represents the standard error of mean. Students' unpaired 't' test was performed to evaluate the statistical differences. The p value of 0.05 or less was regarded as significant.

III. Results and Discussion

Chemical Analysis:

The present study was carried out with a broad objective of assessing the moisture, ash, total protein, soluble protein, carbohydrate, and lipids from fresh neem leaves as well as carbohydrate, soluble protein, and antimicrobial activities from dry neem leaves.

The ash, protein, carbohydrates, and lipids content of fresh neem leaves & were calculated on wet weight (g/100 g wet weight) basis. Chemical composition of fresh neem leaves was characterized by ash (2.75 gm %), moisture (49.66 gm %) & fat (1.096 gm%). The carbohydrate content was 16.15 gm % & 16.23 gm % in fresh neem & in dry neem leaves, respectively. There was not much variation & no significant differences were observed. (Table 1)

Total protein & soluble protein of fresh neem leaves were 6.97 gm%, 12.25 gm %, respectively while it was less in dry neem leaves. The higher value of soluble protein in fresh neem leaves (12.52 gm %) was observed then the dry neem leaves (10.57 gm %). The 14 % soluble protein was loss during the shadow drying process. (Table 2)

After the extraction, the plant material was pretreated with n-Hexane to remove lipids and oils. The highest total carbohydrate i.e.19.983 gm% was observed in 0.5N HCL extracted samples & lowest was found in 0.5 N NaOH extracted sample. The highest soluble protein value was recorded in 0.5 N NaOH extracts (16.87 gm%) & lowest in 0.5 N HCL extracts (8.314 gm%) & D/W extract (10.5 gm%). (Table 3)

Table 1. Ash, Moisture & fat content of fresh leaves.

Parameters	gm%
Ash	2.75
Moisture	49.67
Fat	1.09

Table 2. Total protein, soluble protein & carbohydrate content of fresh & dry neem leaves.

Parameters	Neem Leaves (gm%)	
	Fresh	Dry
Total Protein	6.98	5.76
Soluble Protein	12.25	10.57

Table 3. Soluble protein & total carbohydrate content of dry neem leaves extracted in different aqueous solution.

Extracts	Soluble Protein (gm%)	Total CHO (gm%)
D/W	10.57	16.23
0.5N HCl	8.31	19.98
0.5N NaOH	16.87	13.62

Antimicrobial Activities:

The antibacterial activity of the aqueous extract of neem leaves in acidic, neutral & alkaline solution were screened against six clinical strains Gram-positive bacteria i.e. *Staphylococcus aureus*, the Gram-negative bacteria i.e. *Escherichia coli* and *Pseudomonus aeruginos*, the fungi i.e. *Aspergillus niger* and *Penicillium citrinum*, yeast i.e. *Saccharemyce cerevisiaee.* (Table 4)

E. Coli:

The highest zone of inhibition was observed in 0.5 N HCL extract (10.5 mm) & lower zone of inhibition in 0.5N NaOH extract (4 mm). The highest zone of inhibition in different extracts & in control against *Escherichia coli* was observed in 0.5 N HCL followed by D/W extract (6 mm), control (5.2 mm) and 0.5 N NaOH (4 mm) extract.

Table 4. Inhibition response of native extracts on tested microbes in comparison with standard antimicrobial substances.

Microbes	Zone of Inhibition			
	Standard Streptomycin	Extraction		
		D/W	0.5N HCl	0.5N NaOH
E.C	5.25	6.00	10.50	4.00
P.A	5.50	5.75	9.50	6.00
S.A	6.75	5.00	10.50	8.50
A.N	5.50	5.00	8.50	9.00
P.C	6.75	6.75	8.50	7.75
S.C	6.00	7.50	9.00	8.75

E.C = *E Coli* S.A = *Staphylococcus aureus*, P.A = *Pseudomonus* aeruginos, A.N = *Aspergillus niger*, P.C = *Penicillium chrysogenum*, S.C = *Saccharemyce cerevisi*.

Pseudomonas Aeruginos:

The highest zone of inhibition observed in 0.5 N HCL extract (9.5 mm) & lower zone of inhibition in 0.5N NaOH extract (6 mm). The highest zone of inhibition of different extract & control against *Pseudomonas aeruginosa* was observed in 0.5 N HCL followed by 0.5 N NaOH (6 mm) extract, D/W extract (5.7 mm) and control (5.5 mm).

Staphylococcus Aureus:

The highest zone of inhibition observed in 0.5 N HCL extract (10.5 mm) & lower zone of inhibition in D/W extract (5 mm). The highest zone of inhibition of different extract & control against Staphylococcus aureusa was

observed in 0.5 N HCL followed by 0.5 N NaOH (8.5 mm), extract control (6.7 mm) and D/W extract (5 mm).

Aspergillus Niger:

The lower zone of inhibition observed in 0.5 N HCL extract (8.5 mm) & highest zone of inhibition in 0.5N NaOH extract (9 mm). The highest zone of inhibition of different extract & control against Aspergillus niger was observed in 0.5N NaOH (9 mm), followed by extract 0.5 N HCL (8.5 mm), control (5.5 mm) and D/W extract (5 mm).

Penicillium Chrysogenum:

The highest zone of inhibition observed in 0.5 N HCL extract (8.5mm) & lower zone of inhibition in 0.5N NaOH extract (7.7 mm). The highest zone of inhibition of different extract & control against P. chrysogenum was observed in 0.5 N HCL followed by 0.5 N NaOH (7.7 mm), extract control (6.7 mm), and D/W extract (6.7mm).

Saccharomyces Cerevisiae:

The highest zone of inhibition observed in 0.5 N HCL extract (9mm) & lower zone of inhibition in D/W extract (7.5mm). The highest zone of inhibition of different extract & control against *Saccharomyces cerevisiae* was observed in 0.5 N HCL followed by 0.5 N NaOH (8.7 mm) extract, D/W extract (7.5 mm), and control (6 mm).

In 0.5 N HCL extract had more activity on the organisms than the other extracts. In O.5 N HCL extract had high activity against the *E Coli* & *Staphylococcus aureus.* While lower activity against the *Aspergillus niger* & *Penicillium chrysogenum.* In 0.5 N NaOH extract shows the high activity against the *Aspergillus niger* & lower activity against the *E coli.* D/W extract had lower antimicrobial activity than 0.5 N NaOH extract & 0.5 N HCL extract. The D/W extract showed the high activity against the *Saccharemyce cerevisiae* & lower activity against the *Staphylococcus aureus* & *Aspergillus niger.*

In the light of these results conclude that level of antimicrobial activities of the various extracts was compared with the chemical composition of each extract to determine the chemical composition: activity relationship of each extract. Generally, the high-level protein and carbohydrate content of extract had better antimicrobial activities.

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