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Bioprospecting of Neem for Antimicrobial Activity against Soil Microbes

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Abstract:- The Present studies was carried to evaluate the antimicrobial activity against soil microbes which causes corrosion on buried metal in acidic mediated soil by agar well diffusion using aqueous and ethanol extract of Neem leaves. These plants were subjected to solvent extraction with water and ethanol on increasing the polarity to identify and isolate the antimicrobial active materials. Crude aqueous extract of Neem is significantly inhibited the important microbes which causing corrosion such as S. aureus, Streptococcus, B. subitilis, Lactobacillus, Proteus, Cornybacterium, Pseudomonas, A.niger, Mucor and Desulphovibro sp. Results of the present studies investigation suggests that Neem are important effective plant for further work on isolating and characterizing of antimicrobial active materials.

Keywords:- Bioprospecting, Neem, S.aureus, Streptococcus, B. subitilis, Lactobacillus, Proteus, Cornybacterium, Pseudomonas, A.niger, Mucor and Desulphovibro sp, Agar well diffusion.

I. INTRODUCTION

Bioprospecting is the exploration of biodiversity for commercial valuable genetic and biochemical resources has been seen as a potentially powerful tool for conservation. In biodiversity, plants may dominate because of their self sustaining ability, whereas animals depend upon Producers for survival. Plants prepare primary and secondary metabolites which are protective against biotic and abiotic factors. Plants are sources of naturally occurring compounds with complex molecular structures and having different physical, chemical and biological activities.

In present studies, Neem leaves extract used to control the corrosion which is caused by microbes present in soil and also in acidic medium. Plants which have property of corrosion inhibition called as "Green inhibitors" that produces some phytochemical substance to prevent corrosion. Soil as a corrosive environment is probably of greater complexity than other environment. The corrosion process of buried metal structures is extremely valuable and can vary negligibly. i.e. Pipes or tube or tube wells under soil can be perforated within 3 months – 6 months, leads to uniform corrosion attack which is caused by microbes present in soil.

The role of microbes in soil is not only causing diseases to Plants and Animals and also it leads to cause corrosion of metals due to chemical activities associated with their metabolites, growth and reproduction. The chemistry of soil can be complex and much soil contains strong buffering agents, which resist the ability of the soil pH to change. The presence of microbes, especially sulphate and chloride reducing bacteria and also other microbes can also make the conditions corrosion leading to MIC. Most of the microbes are anaerobic in condition, with pH 6.8 that contain sulphate and chloride, organic substances and minerals which are live in poorly drained out at 20°C - 30°C. During metabolism of microbes, the extracted oxygen from sulphates or chlorides converts the soluble to insoluble substances which easily attack the metal surfaces.

The use of plant extract not only for medicinal uses but also for inhibitory property of corrosion is the most important. Hence the present study was conducted to express the inhibitory activities of selected plants against corrosion caused by soil microbes, when extraction of leaves of aqueous and ethanol solvents.

II. MATERIALS AND METHODS

A. SOIL SAMPLE COLLECTIONS

The soil samples used for this work were collected from 3 different locations near Krishna rajapura area in Bangalore. The samples were labeled according to the site of collection as Garden soil samples, Sewage soil samples and Household soil samples. The samples were transported in polyethylene bags in ice pack to the laboratory. When samples could not process immediately, they were stored at 4^0 C for no longer than 18 to 24 h.

B. MICROBIOLOGICAL ANALYSES

The soil sample was mixed, and a suspension of 1 g (dry weight equivalent) in 10 ml of sterile water was prepared. 1 ml of the soil suspension was then diluted serially (ten-fold) and used in the estimation of aerobic heterotrophic bacterial and fungal populations by standard spread-plate dilution method described by Seeley and VanDemark (1981), in triplicate. Nutrient agar containing 0.015% (w/v) nystatin (to inhibit fungi growth) was used for bacteria isolation and incubation was at 35°C for five days. Potato dextrose agar to which 0.05% (w/v) chloramphenicol has been added (to inhibit bacteria growth) was used for fungal isolation, and incubation was at ambient temperature for seven days. Pure isolates of representative communities were maintained on agar slant at 4°C. Identification of isolates was based on cultural, microscopic, and biochemical characteristics with reference to Bergey's manual of determinative bacteriology (1989) for bacteria, and Talbot (1978) for fungi.

C. DETERMINATION OF SOIL PH

Soil pH was determined according to the procedure described by Akpor et al. (2006) using Horiba make D-51 pH meter - Measuring object & amp.

D.BIOLOGICAL SAMPLE CLLECTIONS

Leaves of the *Azadirachta indica* tree were collected from in the Krishnarajapura area, Bangalore, Karnataka. It was ensure 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 them thoroughly and a particular amount of leaves dried under shadow and some fresh leaves kept.

E.PREPARATION OF AQUEOUS EXTRACT OF PLANT SAMPLES

10 grams of air dried powder N*eem* leaves were placed in different distilled water bath and boiled for 6 h. At intervals of 2 h it was filtered through 8 layers of muslin cloth and centrifuged at 5000 x g for 15 min. The supernatant of different samples was collected. After 6 h, the supernatant of different samples was concentrated to make the final volume one-fourth of the original volume. Finally 10 g of each material was extracted in 25 ml of distilled water giving a concentration of 40 mg/0.1 ml. It was then autoclaved at 121 °C and 15 lbs pressure and stored at 4 °C.

F.PREPARATION OF ETHANOL EXTRACT OF PLANT SAMPLES

10 grams of air dried powder *Neem* leaves were placed in 100 ml of organic solvent (ethanol) in a conical flask, plugged with cotton and then kept on a rotary shaker at 190-220 rpm for 24 h. After 24 h, it was filtered through 8 layers of muslin cloth and centrifuged at 5000 x g for 15 min. The supernatant of different samples was collected and the solvent was evaporated to make the final volume one-fourth of the original volume, giving a concentration of 40 mg/0.1 ml. It was stored at 4 °C in airtight bottles for further studies.

G.ASSAY OF ANTIMICROBIAL ACTIVITY USING AGAR WELL DIFFUSION METHOD

The 20 ml of sterilized Muller Hinton Agar for bacteria and Potato dextrose agar for fungal was poured into sterile petriplates, after solidification, 100µl of fresh culture of microbes were swabbed on the respective plates. The wells were punched over the agar plates using sterile gel puncher at concentration 100µl of each plant extract were added to the wells. The plates were incubated for 24 hours at 37°C. After incubation the diameter of inhibitory zones formed around each discs were measured in mm and recorded.

III. RESULT

The antimicrobial activities of Neem leaves were carried out. Most of the extract shows an antibacterial and antifungal activity against the human pathogens and also corrosion such as *S. Aureus, Streptococcus, B. subtitles, Lactobacillus, Proteus, Cornybacterium, Pseudomonas, A.niger, Mucor* and *Desulphovibro* sp.. Both gram-positive and gram-negative bacteria were sensitive to the extracts.

IV. SOIL pH

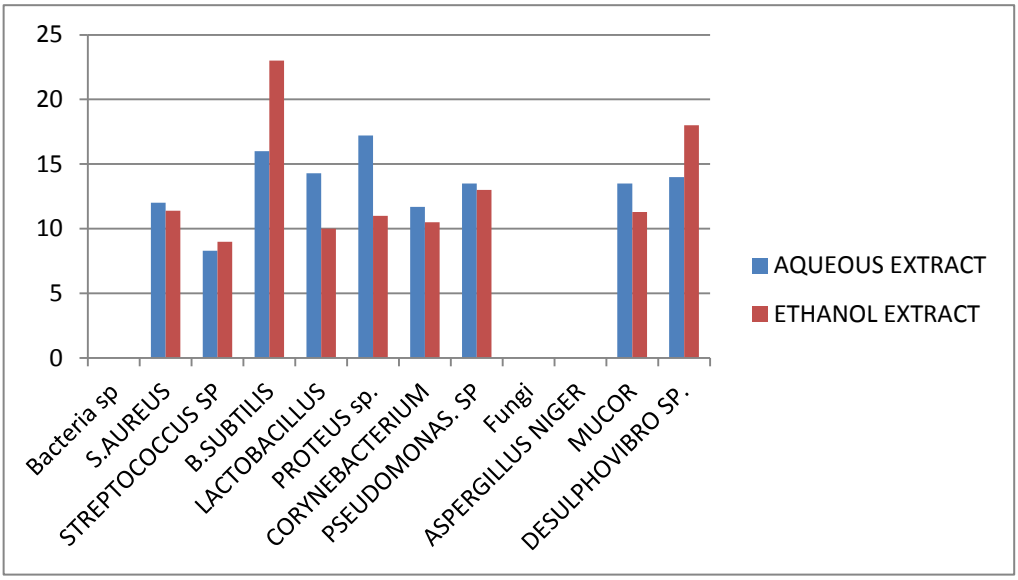
The pH values ranged from 6.3 – 7.3. The soil pH in Sewage soil was higher than household soil and garden soil. However, differences in the soil pH values of the different sampling locations were not observed to be statistically significant.

MICROBIAL ANALYSIS OF SOIL

The occurrence of aerobic and anaerobic heterotrophic microbes presents in the different soil samples.

MICROBES	GARDEN SOIL	HOUSEHOLD SOIL	SEWAGE SOIL
AEROBIC BACTERIA			
Lactobacillus sp.	Present	Present	Absent
Bacillus sp.	Present	Present	Present
Proteus	Present	Absent	Absent
Pseudomonas	Present	Present	Present
Micrococcus	Present	Absent	Absent
Streptococcus	Absent	Present	Present
Staphylococcus	Present	Present	Present
Corynebacterium	Present	Present	Present
ANAEROBIC BACTERIA			
Desulphovibrio	Present	Present	Present
D. Africans	Present	Present	Present
FUNGI			
Asperigillus niger	Present	Present	Present
Penicillium	Present	Present	Present
Mucor	Present	Present	Present
Trichophyton	Present	Present	Present
Microsporium	Present	Present	Present

The crude extracts containing multiple organic components including flavonoids, tannins, alkaloids, triterpenoids, all of which are known to have antibacterial affects. Neem leaves extract contain phenolics compounds like tannins that are very good antimicrobial agent. Thus it may be summarized that the class of natural compounds must exhibit the antimicrobial activity. The metabolites have been shown to be responsible for various therapeutic activities of medicinal plants. Flavonoids especially are known to be effective antimicrobial agent against a wide array of microorganisms. The activity is attributed to their ability to complex with extra cellular and soluble proteins and with bacterial cell wall.



ANTIMICROBIAL ACTIVITY OF AQUEOUS EXTRACT OF *NEEM* IN AGAR WELL DIFFUSION METHOD (MM).

Disc and well diameter = 6 mm, Zone of inhibition (claustro et.al)

Interpretative ranges	range (mm)	Descriptive
zone of inhibition	< 10	Resistant
zone of inhibition	10 to 13	Intermediate
zone of inhibition	14 to 19	Sensitive
zone of inhibition	> 19	very sensitive
no zone	-----	------

Thus the plant extracts can be used as an important antibiotic to cure disorders caused by the different strains of microbes. The present studies conclude these extract could inhibit human pathogens growth and also corrosion. The results are encouraging but precise assessment is utterly necessary before being situate in practice as well as the most active extracts can be subjected to isolation of the therapeutic antimicrobials and undergo secondary pharmacological evaluation.

The results obtained shows that different strains responded differently to both aqueous and ethanol fresh neem leaves extracts. On these strains, aqueous and ethanol extracts of fresh neem leaves used shows the zone of inhibition in mm formed. The zone of inhibition for aqueous and ethanol extract decreases as the dilution factor increases. According to graph, ethanol extract is more efficient than aqueous extract of fresh neem leaves. Ethanol extract is more efficient against B.subtilis and Desulphovibrio species than aqueous extract. Aqueous extract is more efficient against Proteus species than ethanol extract. Both aqueous and ethanol extract have equal efficient against the remailing of the strains. B.subtilis and Desulphovibrio are sulphate and chloride reducing microbes which causes the corrosion on buried metal in acidic mediated soil.

Ethanol extract of fresh neem leaves were more effective. The plant extract contains some phytochemical substances which are secondary metabolites. The active constituents which are present in the leaves are slightly soluble in both aqueous and ethanol solvents such as Azardirachtin, Terepenoids, tannins, alkaloids, etc. Extract of aqueous and ethanol of fresh neem leaves is compared to consider that ethanol extract is more effective than aqueous. All extracts used during this study, the microbial activity increased with the increase of extract concentration, this means that the zone of inhibition was higher on plates that contain extract with low dilution factor, observed in report done by Esimone et.al. (1998) which says that the plant extracts inhibit the growth of various microbes at different concentration. Like this, similar results recorded obtained where the increase in the concentration of extracts corresponds to the increase of diameter of zone of inhibition. In this study, the active constituents may be present in insufficient quantities in the plant extracts to show activity with the different concentration.

CONCLUSION

Ethanol extracts were more efficient in all cases of fresh neem leaves. This study reveals about antimicrobial effect of neem will make it easier for dosage determination and chemotherapeutic index of the extract. This study informs that antimicrobial effect of

neem can change depending on neem plant extract, used, the solvent used, moreover the extract concentration of the fresh neem leaves because each extract has its minimum inhibition concentration (MIC) which is the highest dilution of a plant extract that still retain an inhibitory effect against the microbes growth which causes corrosion. These plant extract inhibits the growth of corrosion causing microbes occur in soil.

