

Effect of Metal Ions and Sugars on Antimicrobial Properties of *Acacia Nilotica* (L.).

Abstract

Bioactive compounds extracted by Soxhlet extraction method were screened for possible antimicrobial activities against various bacterial pathogens such as *E. coli*, *P. aeruginosa* and *S. aureus*. Effect of various elicitors such as various sugars and metal ions were studied against bacterial pathogens. For almost all the plant extract, very good antimicrobial activity was recorded against almost all the pathogens selected in the study.

Keywords : *Acacia Nilotica*, Antimicrobial Activity, Soxhlet Extract.

Introduction

Acacia nilotica (L.) Del. syn. *Acacia arabica* (Lam.) Wild. (Mimosaceae) is an imperative multipurpose plant (Kaur et al., 2005; Baravker et al., 2008). *A. nilotica* is a pan tropical and subtropical genus with species abundant throughout Asia, Australia, Africa, America, Australia-Pacific region, throughout the south of Asia, Africa and in North and South America. The genus *Acacia* occupies vast areas of these domains and is to be found in a wide range of different habitats, from coastal to subalpine regions, and from high rainfall to arid in land areas, growing in tropical, sub-tropical and warm temperate regions (Maslin and McDonald, 2004). Nevertheless, most species are distributed primarily in the dry tropics, and several Australian *Acacia* species have become highly invasive weed surround the world (Blakesley et al. 2002), including southern Europe.

A number of secondary metabolites have been reported from various *Acacia* species including amines and alkaloids, cyanogenic glycosides, cyclitols, fatty acids and seed oils, fluoro acetate, gums, non-protein amino acids, terpenes, hydrolysable tannins, flavonoids and condensed tannins (Seigler, 2003; Malviya, et. al., 2011). However, the secondary metabolites of only a small proportion of *Acacia* species have been examined in detail.

The plant is considered to be antispasmodic and anti-dysenteric. Pods and tender leaves are reported to treat diarrhea. The plant has been shown to exhibit antibacterial, anti-inflammatory, antiplatelet aggregatory activity, cestocidal activity, antibacterial effects, spasmogenic, vasoconstrictor actions, antihypertensive, antispasmodic activities, inhibitory effect against hepatitis C virus, cytotoxic activity and antioxidant activity. Leaves and pods are an excellent fodder with anti-inflammatory properties, rich in protein. The pods have molluscicidal and algicidal properties. Bark used in the treatment of hemorrhages, cold, diarrhea, tuberculosis and leprosy. Root used as an aphrodisiac and the flowers for treating syphilis lesions. Gum obtained from the tree is pharmaceutically used as suspending and emulsifying agent and in preparation of many formulations.

Materials and Methods

Leaves and bark of *Acacia nilotica* were obtained from actively growing tree near MRD LifeSciences, Gombi Nagar, Lucknow. The leaves and bark were dried in the hot air oven at 50°C and grinded to get powder. The powder was stored at the room temperature throughout the study. Bioactive components were extracted by Soxhlet extraction using methanol as solvent (Mishra, et. al., 2011).

Bacterial cultures were collected from IMTECH, Chandigarh. The strains of bacteria selected to assess susceptibility pattern against the extracts prepared in the study included one positive gram bacteria (*S. aureus*) and two gram negative bacteria (*P. aeruginosa* and *E. coli*).

The antibacterial activity of *Acacia nilotica* peel powder was determined by agar well diffusion method against *S. aureus*, *P. aeruginosa* and *E. coli* (Mahida and Mohan 2007; Abubakar 2009; Droby, et. al., 1987; Kumpoun et. al. 2007; Mishra, et. al., 2011).

Vinita Srivastava

Assistant Professor,
Deptt. of Chemistry,
D. A. V. P. G. College,
Kanpur

M.K.Sinha

Assistant Professor,
Deptt. of Zoology,
D. A. V. P. G. College,
Kanpur

Minimum Inhibitory Concentration (MIC) is the minimum concentration of a compound to kill or to check the organism's growth. A low MIC value shows that extract has high antibacterial activity so active extracts obtained by agar well diffusion assay were further subjected to determine the Minimum Inhibitory Concentration (MIC) required for the bacterio-static effects by standard micro-dilution agar double layer methodology. MIC was calculated as per methods described earlier (Mishra, et. al., 2012).

Various elicitors such as different sugars and metal ions were supplemented with plant extracts to observe possible enhanced activity of plant metabolites against bacterial pathogens. Different concentration of sugars and metal ions were selected while observing antimicrobial properties, data shown in results (Mishra, et. al., 2011).

Results

Different plant parts after successful extraction procedures gave different amount and color of plant metabolites as mentioned in table below.

Samples	Soxhlet Extract	Yield (%)	Compound color
Leaves	3 days	24.4	Dark greenish black
Bark	3 days	10.0	Brown

Table 1: Yield of plant extract from different parts for different extraction procedure.

All these bioactive phytochemicals were recovered after removing respective solvents and were stored as crystalline form at room temperature until use.

Antimicrobial Properties of Phytochemicals Against Various Pathogens

The plant extracts were analyzed against three pathogenic bacteria, their activities were recorded and are tabulated below in table 2 & 3. All the experiments were performed in triplicate and the data mentioned here is the average of zone of inhibition recorded.

Pathogens Extract	E. coli	S. aureus	P. aeruginosa
Soxhlet leaves	21.0mm	20.0mm	20.0mm
Soxhlet bark	00.0mm	18.0mm	00.0mm

Table 2: Antimicrobial activity of plant extract against pathogens, shows zone of inhibition (mm) at 24 hrs.

Pathogens Extract	E. coli	S. aureus	P. aeruginosa
Soxhlet leaves	26.0mm	26.0mm	20.0mm
Soxhlet bark	00.0mm	18.0mm	00.0mm

Table 3: Antimicrobial activity of plant extract against pathogens, shows zone of inhibition (mm) at 48 hrs.

Effect of Different Elicitors on Potency of Phytochemicals

Different metal ions and sugars were supplemented with plant extracts for the improved antibacterial activities of plant extracts. The data shown here indicates the improvement of activity (in percentage; at Y axis) for all of the pathogens

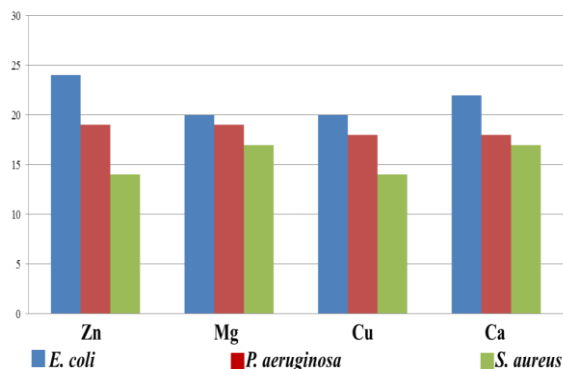


Fig. 1: Photograph showing effect of metal ions on zones of inhibition against bacterial pathogens for plant extracts.

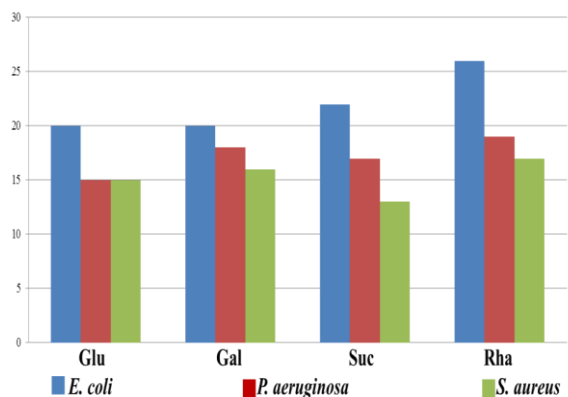


Fig. 2: Photograph showing effect of sugars on zones of inhibition against bacterial pathogens for plant extracts.

Discussions

Since the time of evolution, a simultaneous competition for growth, development and reproduction among living organisms is seen for maximum utilization of available resources. This kind of phenomenon could not be completely eliminated from the biome as it plays a vital role in species improvements after several generations or sometimes suddenly in next generation, resulting emergence of new strains, cultivars, varieties and species. This kind of competition also had evolved another kind of interaction among living organisms like parasitism, mutualism in which either one or both organism is benefited by supporting or destroying the first one. This led to a defence system among the organisms for their protection by parasite either eating it from outside or infected inside of the organism. Some plants have external modified body parts like hooks, spines, latex, and many other modifications including chemical strength within the plant. Plants have several specialized biomolecules, not used routinely by its own metabolism, could be synthesized and preserved into the plant body that could be used on demand at the time of infection by other organisms as a self defence system. Metabolites are the intermediates compounds and products of metabolism. The term metabolite is usually restricted to small molecule. Metabolites have various functions, including fuels, structure, signalling, stimulatory and inhibitory effects on enzymes, catalytic activity on their

own (usually as a cofactor to an enzyme), and defence. A primary metabolite is a kind of metabolite that is directly involved in normal growth, development and reproduction. Alcohol is an example of primary metabolite produced in large-scale by industrial microbiology. Secondary metabolites are important mediators of ecological interactions between plant and their environment. Investigations into the chemistry of living organisms have reached a stage where one can say with reasonable certainty those pathways of vitamins, minerals, amino acids and practically all components that comprise a living system are known. Thus, with identity to a known compound being a criterion, it is conceivable that there are scientific principles that will allow one to distinguish dietary ingredients that are in accord with the statutory meaning from those that fall outside that definition.

One interaction that they play a central role, is defence against herbivores. This is a particularly true in the expanding young leaves of tropical forest trees. Unlike mature leaves, which following full expansion becomes highly toughened by structural tissues, young leaves are soft and relatively nutritious. In the absence of toughness, secondary chemistry is commonly the front line of defence against herbivores. Young leaves, therefore, are likely to be focal point of selection of defence chemistry.

Our experimental data suggests that leaves contain a large amount and diversity of secondary metabolites in comparison to other plant parts selected in the study. A positive result shows that *A. nilotica* is having strong antibacterial properties. Out of various elicitors, almost all sugars and metal ions selected in this study, improved the antibacterial properties of the plant against pathogens.

Acknowledgement

The author is thankful to the principal, D. A. V. P. G. College, Kanpur for providing laboratory facilities and to director, MRD Life Sciences for providing support of lab facilities to complete my work.

References

1. S. Malviya, S. Rawat, M Verma, A. Kharia 2011. Preliminary Phytochemical Investigations of *Acacia nilotica* Linn Plant. Curr. Pharm. Res. CPR 1(2): 91-100.
2. Seigler D.S. 2003. Phytochemistry of *Acacia-sensulato*. Biochem. Syst. Ecology., 31(8): 845-873.
3. Abubakar, M. E. 2009. Antibacterial efficacy of stem bark extracts of *Mangifera indica* against some bacteria associated with respiratory tract infections. Sci. Res. & Essay 4 (10): 1031-1037.
4. Droby, S.; Prusky, D.; Jacoby, B. & Goldman, A. 1987. Induction of antifungal resorcinols in flesh of unripe mango fruits and its relation to latent infection by *Alternaria alternate*. Physiol. Mol. Pl. Path. 30(2): 285-292 .
5. Kumpoun, W.; Uthaiyura, J. & Boonyakiat, D. 2007. Antifungal compound content in mango latex at different maturity ISHS Acta Horticulturæ Europe-Asia Symposium on Quality Management in Postharvest Systems - Eurasia :804
6. Mahida, Y. & Mohan, J. S. S. 2007. Screening of plants and their potential antibac activity against

Staphylococcus and *Salmonella* spc. Natural Prod. Radiance. 6(4): 301-305.

7. Mishra, R. P. & Siddique, L. 2011. Antimicrobial activities of different parts of *Anthocephalus cadamba* and its phytochemical quantification. Asian J. Pl. Sci. Res. 1(2): 1-7.
8. Mishra, R. P.; Deepak; Singh, S. P. & Anwar, R. 2011. Study of culture conditions and antimicrobial properties of *Acinetobacter baumannii*. J. Pharma. Biomed. Sci. 12(16): 1-3.
9. Mishra, R. P.; Yadav, S. & Anjali. 2012. Study of antimicrobial activities of *Citrus limetta*. J. Pharma. Biomed. Sci. 19(15): 1-4.