

Antibacterial and Antifungal Effect of *Eupatorium adenophorum* Spreng against Bacterial and Fungal Isolates

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Abstract

A research work on antimicrobial effect of water solvent and organic solvent extracts of different concentration of *Eupatorium adenophorum* (Spreng) was conducted at Nepal Academy of Science and Technology (NAST) during August 2008. Fifteen strains of bacteria, six strains of fungi and two concentrations, 50% and 100% of plant extracts were taken for the study. Among the 15 strains of bacteria, most of them were inhibited with *E. adenophorum* extracts and only three species, *Klebsiella oxytoca*, *K. pneumoniae* and *Shigella dysenteriae* did not show antibacterial activity with the same extract. Extracts obtained from the organic solvent and water solvent showed different antimicrobial properties with the same bacterial strains. Those bacterial strains whose growth was inhibited by water solvent could not be inhibited by organic solvent extracts. Organic solvent extract showed antibacterial effect towards *Proteus* spp., *Salmonella* spp., *Staphylococcus* spp., *Bacillus subtilis*, *B. thurengiensis*, *B. cereus*, *Enterobacter aerogenes*, *Salmonella paratyphi*, *Staphylococcus aureus*, *Proteus mirabilis* and water solvent extract showed antibacterial effect towards *Pseudomonas aeruginosa*, *E. coli*, *Staphylococcus aureus*, *Staphylococcus* spp., *Citrobacter freundii*, *Proteus* spp., *B. subtilis*, *B. thurengiensis*, *Enterobacter aerogenes*, *Salmonella* spp. and *S. paratyphi*. Altogether 12 species out of 15 were inhibited by *E. adenophorum* extracts. Both solvent extracts showed high antibacterial effect towards *Proteus* spp., *Staphylococcus* spp., *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Among the six species of the fungi, *Fusarium* spp. were inhibited by plant extract while the *Aspergillus* spp. and *Stenophyllum botryosum* did not show any effect of the extracts. The plant extracts showed selective effect with different strains of bacteria and fungi, which indicated that they were confined to cure the same bacterial diseases and fungal diseases.

Key words: plant extract, bacteria, fungi, antibacterial, antifungal, *Eupatorium adenophorum*

Introduction

Pharmacological industries have produced large number of new antibiotics throughout of the world on one hand but on the other hand resistance to these antibiotics by microorganisms has increased. Microorganisms have the genetic ability to transmit and acquire resistance towards drugs. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for potential antimicrobial activity.

Most of green plants represent a reservoir of effective chemo-therapeutants and can provide valuable sources of natural drugs, natural pesticides and biofertilizers. They have a long evolution of resistance against microbial agents which has led to alternative

directions in drug development. Therefore, extracts of plants and phytochemicals are getting more importance as potential sources for viral inhibitors during the recent decade. Plant extracts have great potential as antimicrobial compounds against microorganisms. Thus, they can be used in the treatment of infectious diseases caused by resistant microbes. The use of traditional medicine is widespread in Nepal and much of the population still rely on it. In Nepal each and every plant has its own value such as antibacterial, antiviral, antifungal and biofertilizer properties. The use of plant preparations in this tradition has been well documented (Manandhar, 1985, 1986, 1987, 1989a,b,c, 1990a,b, Bhattarai, 1993, Shrestha and Joshi, 1993, Shrestha Vaidya *et al.* 2009) although only a few

species have been screened for biological activity (Bhakuni *et al.* 1969).

Eupatorium adenophorum Spreng is a perennial shrub, exotic to Nepal, due to its adverse growth it is also called Banmara. Systematic screening of such type of unwanted plants may result in the discovery of novel effective compounds. In Nepal its extracts and dried powder are widely used to check plant diseases as well as biofertilizer to increase fertility of the soil. It is also used as fuel wood by biobroking process and used in biogas plant. The aim of this work is to study the effect of the plant extract on bacterial and fungal organisms.

Materials and Methods

Generally plant extracts are extracted with different kinds of solvents such as organic and water solvents. For extraction, fresh leaves and stems of the *E. adenophorum* collected and dried on shade condition and grinded in powder form. For water solvent extraction, 500 g of shade dried *E. adenophorum* plant powder was soaked in 2.5 l of distilled water for 3 days then squeezed and filtered with the help of cotton cloth. Water content of this filtrate was evaporated till the solution reduced to semisolid form. This solution was poured in petridish and kept in a dessicator which contained silica gel for residual water absorption from the extract. From this dried extract, 50% (0.5g/ml) and 100% (g/ml) concentrated solution were made by using distilled water. Finally, zone of inhibition of the extract against different bacteria was measured.

Fifteen identified bacterial strain were collected from National Institute of Science and Technology (NIST). They were cultured into nutrient broth solution and incubated for 3-4 h for their maximum growth. The bacterial broth solution compared with the turbidity of 0.005% solution of Barium chloride (BaCl₂). The turbidity of the bacterial growth solution was not more than the turbidity of BaCl₂ solution and these bacterial solutions were swabbed on the MHA (Muller Hinton Agar) media finely with the help of cotton swab. After completion, bored the swapped media with the help of sterile borer and formed well on the media. Fifty microlitre (50µl) extract and control solution were poured with the help of the micro pipette on each well, then left for sometime for diffusion. All the plates were incubated at 37 °C for 24- 48 h. A control well was made on each plate by applying distilled water for water solvent and ethanol for organic solvent extract (Shrestha & Piya 2002, Shrestha Vaidya *et al.* 2005, 2008).

For organic solvent extract, 25 g shade dried *E. adenophorum* powder was taken, placed in Soxhlet glass tube, poured ethanol and Soxhlet apparatus was run till colorless. This mixture of extract was evaporated and dried with the help of rotary apparatus and placed in a dessicator for residual absorption of water from the extract. Generally all the extracts were made on same solution which were used to extract the plant extract. So in this research ethanol was used. From this dried extract, 50% (0.5g/ml) and 100% (g/ml) concentrated solution were made in ethanol.

Different species of fungi obtained from Nepal Agricultural Research Council (NARC) were cultured on potato dextrose agar (PDA) media for pure culture and incubated at 27 °C for their growth. Calculated volume of 100% extract solution was poured in the PDA media before sterilization. Different volumes of 100% extract solution were used for increasing concentration on PDA media. In this way, different concentrated PDA media of the extract (5%-20%) were made. Fungal discs were cut out with the help of the borer and placed on the PDA media which contained plant extract and incubated at 27 °C for their growth. Finally, the growth of fungi was observed from the fungal disc.

Results and Discussion

All concentrations of *E. adenophorum* extract showed selective effect towards the bacterial strains. Among all tested bacterial and fungal strains, this plant extracts showed antibacterial and antifungal effect towards many bacterial and fungal strains and no effect towards few bacterial and fungal strains.

Among the 15 species of bacteria, most of them had inhibited growth with *E. adenophorum* extracts and only 3 species *Klebsiella oxytoca*, *K. pneumoniae* and *Shigella dysenteriae* were not affected with same extract. Organic solvent extract showed antibacterial effect towards *Proteus* spp., *Salmonella* spp., *Staphylococcus* spp., *Bacillus subtilis*, *B. thurengiensis*, *Enterobacter aerogenes*, *Salmonella paratyphi*, *Staphylococcus aureus*, *B. cereus*, *Proteus mirabilis*; and water solvent extract showed antibacterial effect towards *Pseudomonas aeruginosa*, *E. coli*, *S. aureus*, *Staphylococcus* spp., *Citrobacter freundii*, *Proteus* spp., *Bacillus subtilis*, *Enterobacter aerogenes*, *Salmonella* spp., *Salmonella paratyphi*, *Bacillus thurengiensis*. Altogether 12 species out of 15 had inhibited growth with *E. adenophorum* extracts. Both solvent extracts showed high antibacterial effect

towards *Proteus* spp., *Staphylococcus* spp., *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Among the fungal strains *Fusarium moniliformae*, *F. eroliferum*, *F. proliferatum* and *F. oxysporum* were inhibited by this plant extract while the *Aspergillus niger* and *Stenophyllum botryosum* did not show which is similar to the work of the Tian *et al.* 2007 found that the volatile oil extracted from *E. adenophorum* inhibited four types of fungal pathogens.

E. adenophorum showed antimicrobial properties against different microorganisms. The preliminary results obtained from the crude water and organic solvent extracts indicate that further investigation and screening is worthwhile. From this result extracts obtained from the organic solvent and water solvent showed different antimicrobial properties with the same bacterial strains. Those bacterial strains which inhibited their growth by water solvent could not be inhibited by organic solvent extracts. This antibacterial effect depends on solvents which are used to extract the plant extract. Those

bacterial strains which were inhibited with organic solvent extract could not be inhibited by water solvent extract. This depends on the presence of polar and non-polar bioactive or inhibitory compounds on the extract. Organic solvent extracted more concentration of non-polar bioactive compounds from the plant powder and water solvent extracted more concentration of polar bioactive compounds. So, presence of different concentration of polar and non-polar compounds on the extract showed different inhibitory effect towards same bacterial strains. Water solvent, extracted more amount of polar compounds and less amount of non-polar compounds, inhibited the antibacterial effect of non-polar compounds which are present in same extract. Organic solvent, extracted more amount of non-polar compounds and less amount of polar compounds, inhibited the antibacterial effect of polar compounds which are present in same extract.

Higher the concentration of the extract showed higher inhibition zone while the lower concentration showed lower inhibition zone or no effect.

All outputs of this work are presented in Fig. 1 and 2.

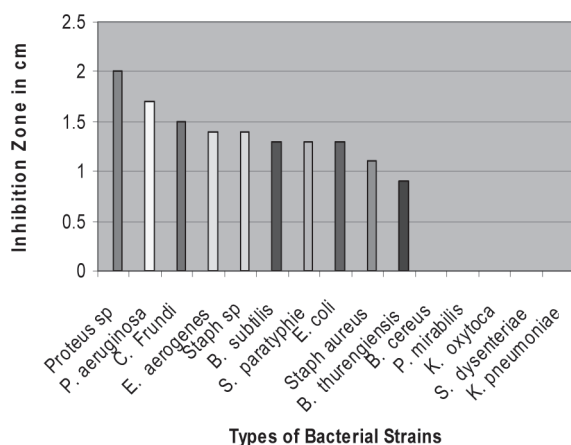


Fig. 1. Effect of 100% *E. adenophorum* water solvent extract towards bacterial strains growth.

The above bar diagrams show the inhibited bacterial strains and their area of inhibition zone by using of *E. adenophorum* plant extracts. From these diagrams, it was found that 10 species of bacteria were inhibited by 100% water solvent extract and also 10 species were inhibited by 100% organic solvent extract out of 15 species. These bacterial species were also tested with 50% concentration of same extract solution that inhibited only few species. It was showed that

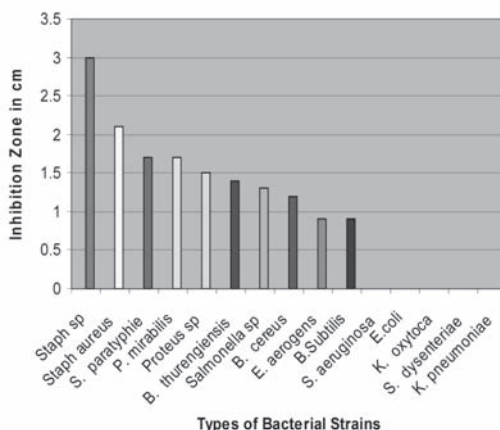


Fig. 2. Effect of 100% *E. adenophorum* organic solvent extract towards bacterial strains growth

higher the concentration higher as the inhibited number of species and lower the concentration was the lower inhibition number of species.

Salmonella sp. causes typhoid and paratyphoid fever on mammals. *Staphylococcus* spp. causes urinary track infection, wound infection i.e. post operative, food poisoning, boils, abscesses, endocarditis, toxic shock syndrome diseases on human and mastitis on cattle.

Escherihia coli causes gastroenteritis. *E. coli* and *Proteus* spp. are opportunistic human pathogens. They attack at the time of low immunity power and cause UTI. *Citrobactor frundii* and *Enterobacter aerogenes* cause diseases at the time of low immunity power. *B. thureingiensis* is pathogenic to the insects. *Pseudomonas aeruginosa* is also opportunistic human pathogen causes infecting wound, burn and UTI on humans. All the above bacterial strains were inhibited by *E. adenophorum* extracts (Fig. 4 and 5). In present study, *S. aureus* was inhibited this plant extract which is analogous to the work of Tomoko *et al.* (2002) they found that *S. aureus* inhibited their growth by plants extracts of tropical and sub-tropical plants.

Different types of *Fusarium* spp. are the causal organisms of soil born disease of plants and sometimes they may cause seed born disease also. They cause



Fig. 3. *Eupatorium adenophorum* in natural habitat

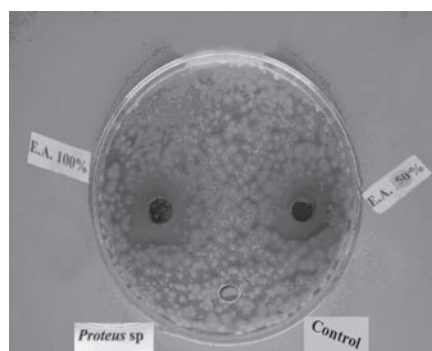


Fig. 5. Inhibitory zone of bacteria

many wilting diseases in several crops. *E. adenophorum* extract inhibited the growth of *F. moniliformae* (Fig. 6) which automatically inhibits the growth of foot rot disease of rice, pith canker of *Pine*, disease of potato tuber and maize seedlings. *F. oxysporum* also causes the wilting disease of lentil, tomato and banana. This plant extract also inhibited the growth of *F. oxysporum* (Fig. 6). So this plant extract can be used to control the wilting disease of such representative plants. Similarly this plant extract inhibited the growth of *F. proliferatum* and *F. eroliferum* which automatically inhibits the growth of diseases caused by these organisms.

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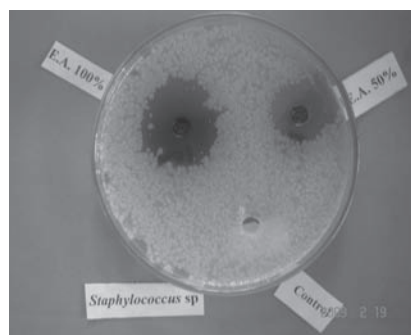


Fig. 4. Inhibitory zone of bacteria



Fig. 6. Total inhibition of fungal growth

References

Bhakuni, D.S., M.L. Dhar, B.N. Dhawan and B.N. Mehrotra. 1969. Screening of Indian plants for biological activity, part II. *Indian Journal of Experimental Biology* 7: 250-262.

Bhattarai, N.K. 1993. Folk medicinal use of plants for respiratory complaints in central Nepal. *Fitoterapia* 64: 163-169.

Manandhar, N.P. 1985. Ethnobotanical notes on certain medicinal plants used by Tharus of Dang-Deokhuri district, Nepal. *International Journal of Crude Drug Research* 23 (4): 153-159.

- Manandhar, N.P. 1986. Ethnobotany of Jumla district, Nepal. *International Journal of Crude Drug Research* **24** (2): 81-89.
- Manandhar, N.P. 1987. Traditional medicinal plants used by tribals of Lamjung district, Nepal. *International Journal of Crude Drug Research* **25** (4): 236-240.
- Manandhar, N.P. 1989a. Medicinal plants used by Chepang tribes of Makawanpur district, Nepal. *Fitoterapia* **60** (1): 61-68.
- Manandhar, N.P. 1989b. Useful wild plants of Nepal. Franz Steiner Verlag Wiesbaden, GmbH, Stuttgart, Germany.
- Manandhar, N.P. 1989c. Folk-lore medicine of Chitwan district, Nepal. *Ethnobotany* **2**: 31-38.
- Manandhar, N.P. 1990a. Traditional phytotherapy of Danuwar tribes of Kamlakhonj in Sindhuli District, Nepal. *Fitoterapia* **61**(4): 325-331.
- Manandhar, N.P. 1990b. Medicobotany of Gorkha district, Nepal. An elucidation of medicinal plants. *International Journal of Crude Drug Research* **28** (1): 17-25.
- Shrestha, I. and N. Joshi. 1993. Medicinal plants of the Lele village of Latipur District, Nepal. *International Journal of Pharmacognosy* **31**(2): 130-134.
- Shrestha Vaidya, G. and Piya S. 2002. Study of antagonistic action of two ectomycorrhizal fungi isolated from pine forest of Dadeldhura. In: *Proc. of international seminar on mountains*, (Eds. F.P. Neupane & K.M. Bhattacharya) Kathmandu pp. 571-577.
- Shrestha Vaidya, G., K. Shrestha and H. Wallander. 2005. Antagonistic study of ectomycorrhizal fungi isolated from Baluwa forest (Central Nepal) against with pathogenic Fungi and Bacteria. *Scientific Worlds* **3** (3): 49-52.
- Shrestha Vaidya, G., K. Shrestha, B.R. Khadgi, N.C. Johnson and H. Wallander. 2008. Organic matter stimulates arbuscular mycorrhizal fungi in *Bauhinia purpurea* and *Leucaena diversifolia* plantations on eroded slopes in Nepal. *Restoration Ecology* **16** (1): 79-87.
- Shrestha Vaidya, G., K. Shrestha and H. Wallander. 2009. Evaluation of antimicrobial activity of *Lantana camara* Linn. against pathogenic fungi and bacteria. *Journal of Ethnopharmacology* **60**: 163-172.
- Tian, Yu, Hou Jing, Wu Jian-Ping, He Lan, Cao Ao-Cheng. 2007. Study on the volatile components from *Eupatorium adenophorum* Spreng and its antifungal activity. *Chinese Journal of Pesticide Science* **2**.
- Tomoko, N., A. Takashi, T. Hiromu, I. Yuku, M. Hiroko, I. Munekazu, A. Fujio and W. Kazuhito. 2002. Antibacterial activity of extracts prepared from tropical and sub-tropical plants on methicillin resistant staphylococcus aureus, *Journal of Health Science* **48**: 273-276.

