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ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES OF *EUPATORIUM ADENOPHORUM* SPRENGE

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INTRODUCTION

Medicinal plants are natural resource constituting one of the potential sources of bioactive chemical entities for drug development. Traditional medicinal uses of plants offer valuable indication to such drug development. It is estimated that about 60% of the world population and 80% of the population of developing countries rely on traditional medicine for their primary health care needs.[1]

Most of green plants act for a reservoir of effective chemo-therapeutants and can provide beneficial sources of natural drugs, natural pesticides and bio fertilizers. They have a long evolution of resistance against microbe and fungus which has lead to alternative directions in drug development. Extracts of the plants and secondary metabolites are getting more importance as potential sources for viral inhibitors during the recent decade. Plant extracts have significant potential as antimicrobial and antibacterial compounds against the various types of microorganisms. Thus, they can be used in the various treatments of infectious diseases caused by resistant microbes.[2]

Antimicrobial activity can be defined as a collective term for all active principles (agents) which inhibit the growth of bacteria, prevent the formation of microbial colonies and may destroy the microorganisms. In the field of antimicrobial finishes, many common terms are used including antibacterial, bactericidal, bacteriostatic, fungicidal, fungi static and biostatic.[2]

Medicinal plants are known to produce certain types of bioactive compounds and secondary metabolites which can inhibit the growth of different microorganisms. Extracts from of medicine plant have been known to possess antimicrobial effects and used for the purpose of food preservation and medicinal purposes. Considered as time tested and comparatively safe both for human used and for environment the herbal extracts have received much attention as a source of new antibacterial drugs.[3]

Every plant possess specific characteristic according to that it is used for the several treatments. In recent years, noble metal nanoparticles have been the subject of focused research due to their unique optical, electronic, mechanical, magnetic and chemical properties that are significantly different from those of bulk materials. Among them Silver nanoparticles have been well recognized For combating bacterial drug resistance problems, biogenic silver nanoparticles can acts as effective and alternative bacteriostatic agents.

The advantage of using plant materials in nanoparticles synthesis is it does not need any elaborate processes such as intracellular synthesis, compound purification steps and the maintenance of microbial cell cultures.[4]

In this study of plant we have concentrate preparation of nanoparticles of extracts of local variety of Eupatorium adenophorum Sprenge which have been tested for the antimicrobial activity on Gram-positive and Gram-negative bacteria. Infectious diseases are caused by pathogenic microorganisms, such as bacteria, viruses, parasites or fungi. Diseases can spread, directly or indirectly, from one person to another. Infectious diseases are the second leading cause of death worldwide. About one – fourth of all the medicines we use, come from rainforest plants. However, scientific have been conducted only to a limited extent with few medicinal plants.[5]

The present study was aimed to search for newer, safer and more potent antimicrobials components which may accomplish our present need. Herbal medicines have received much attention as a source of new antibacterial drugs since they are considered as time tested and comparatively safe for human. Medicinal plants are known to produce certain phytochemicals which react with other organisms in the environment, inhibiting bacterial or fungal growth.

Leaves and stems extracts of E. adenophorum reported to exhibit antibacterial effect towards Salmonella spp.,Bacillus subtilis, B. thurengiensis, Enterobacter aerogenes, Salmonella paratyphi, Staphylococcus aureus, Proteus mirabilis; and water solvent extract showed antibacterial effect towards Peudomonas aeruginosa, E. coli, S. aureus, Staphylococcus spp., Citrobacter frundii, Proteus spp., Bacillus subtilis, Enterobacter aerogenes, Salmonella spp., Salmonella paratyphi, Bacillus thurengensis.[7]

Drug Profile

Eupatorium adenophorum Sprenge commonly known as Crofton weed, Eupatory, Sticky snakeroot, White thoroughwort and Mexican devil is an erect perennial herbaceous shrub growing 1-2 m (3.3 or 6.6 ft) tall but occasionally reaching up to 3 m high.[9,10]

Introduction of Sample

Sample Name- *Eupatorium adenophorum Sprenge.*

Figure: *Eupatorium adenophorum Sprenge.*

Main Constituents - Leaves of *Eupatorium adenophorum Sprenge*

Uses

Fever
Cough
Cold

Scientific classification

Kingdom: Plantae
Clade: Tracheophytes
Clade: Angiosperms
Clade: Eudicots
Clade: Asterids
Order: Asterales
Family: Asteraceae
Genus: Ageratina
Species: *E. adenophorum*

Botanical name

Eupatorium adenophorum Spreng.

Synonyms

- *Ageratina adenophora (Spreng.)* King & H.Rob
- *Eupatorium glandulosum Michx.*
- *Eupatorium glandulosum Hort.* ex Kunth
- *Eupatorium pasadenense* Parish

Eupatorium adenophorum Spreng. Roots are yellow in color and when damaged or broken emit a distinct like a carrot smell. The leaves are oppositely arranged along the stems and are borne on stalks (petioles) that are 6 to10 cm (2.4 to 3.9 in) long by 3 to 6 cm (1.2 to 2.4 in) in width. The leaf are blades trowel-shaped, triangular, diamond-shaped (rhomboid) or with bluntly or sharply toothed margins and sharply pointed and mostly glabrous tips while their stalks are often glandular pubescent.[12] Seeds (12 mm long and 0.30.5 mm wide) are slender, reddish-brown or blackish-brown in colour, and slightly curved having 4-5 five slight ribs which run longitudinally. Their bodies are glabrous topped with a ring (pappus) of numerous whitish hairs (34 mm long), which are readily shed.[11] *Eupatorium adenophorum* sprenge grows as weed in many places of the world. It is a weed of railways, pastures, roadsides, disturbed sites, fence lines, waste areas and warmer temperate regions, riparian zones in subtropical and also found usually in urban moisture spaces, open woodlands, forest margins and rainforest clearings.[12,13]

Distribution

Eupatorium adenophorum Spenge is native to Costa Rica & Mexico. Central America but has naturalized in many other parts of the world as an introduced species such as Europe, Oceania and Asia.[14] Initially, it been has grown as an ornamental plant, but rapidly become invasive into farmland and bushland worldwide. It was first found introduced to Yunnan from the China-Burma border around 1940 and then spread towards North & East covering Tibet Provinces of China and Sichuan, Guizhou, Guangxi.[15,16] Its rapid spread is due in part to its allelopathic competition with other plant species.[17] It is also a weed in Australia, where it was introduced to Sydney in 1904. It has spread along the coastline of New South Wales and southern Queensland.[18] It has also spread in Hawaii and the mainland United States, where it is recognised as a weed in ten states of the Southwest. It is rated a Class 4 Noxious Weed under the NSW Noxious Weeds Act of 1993.[19] It is known as an invasive species in many tropical and subtropical countries and is almost naturalised in south-western USA, southern Europe, Australia, New Zealand, South Africa, Spain, India, Philippine, Malaysia, Singapore, Indonesia, Papua, New Guinea, Thailand, Burma, Vietnam, Nepal, Pakistan, China, Pacific Islands and the Canary Islands.[20,21]

Ethanomedicinal Significance

Different kinds of *Eupatorium* have been used in the traditional system of medicine all over the world. *Eupatorium adenophorum* sprenge has find therapeutic applications and accredited for diverse medicinal properties in traditional medicines as blood coagulant,

antimicrobial, analgesic, antipyretic, antiseptic and phenobarbitone induced sleep enhancer.[22,23,24] The whole plant's leaves and their shoots have traditionally been used as folklore medicines in different parts of the world. The juice of leaf is used to stop bleeding of cut and wounds, forming clots. Root juice is usually used to treat fever. The pure extracts (juice) of the leaf is poured in the eye to treat insomnia. A decoction of the plant has been recommended by the doctor to treat jaundice and ulcers.[12,13] Conventionally, decoction of leaves has been applied on cut wounds to stop bleeding and used against infection of gum and tooth ache.[25] A decoction of leaves is given to cure stomach-ache and mostly used by the tribal people of Meghalaya and Nagaland states of India.[26] Local populace of Kurseong and Darjeeling hill region in the Eastern Himalayas, use leaves of the plant for remedial purposes against oral and skin sores. Traditional users of Darjeeling Himalaya prescribe the shoots and young leaves of the plant against dysentery.[27] In Nainital of Kumaun region of Uttarakhand state in India, leaf juice is used in blood coagulation. In Garhwal region of the state, leaf aste is applied on cuts and wounds and paste mixed with mustard oil is useful for ulcer. In Nepal, the leaf juice is used as an antiseptic to treat cuts and wounds.[28] In Nigerian traditional medicine, is used to treat fever, diabetes, and inflammation.[29] In India, the leaf of the *Eupatorium adenophorum* are pharmacologically regarded as astringent, thermogenic & stimulant used in folk medicine for its antimicrobial, blood coagulating, analgesic, antipyretic and antiseptic properties.[30]

MATERIALS AND METHODS

Preparation of plant extract

Fresh leaves of *Epatorium adenophorum* sprenge were collected from Ukhimath, Rudraprayag (Uttarakhand) forest and Dehradun forest Uttarakhand, and washed several times with water to remove the dust particles and then air dried to remove the residual moisture. Then the air dried leaves cursed and was exhaustively extracted by hot percolation method (soxhalation) with different solvents of increasing order of polarity, starting with a highly nonpolar solvents viz, Petroleum ether followed by Pet ether, Chloroform, Ethanol (95%) and Water.[31,32]

Procedure for extraction

Initially about 100 gm of powder was extracted with 250 ml of solvent (Ethanol, chloroform and Pet. Ether). The extraction was continued until the solvent in the thimble became clear. After complete extraction, the extract was filtered and the solvent was distilled off using rotary vacuum flash evaporator. The obtained residue was dried in desiccators over anhydrous sodium sulphate. The average yield, colour, odour and constituency were recorded.

The left over mark was air dried at room temperature and was similarly extracted with Petroleum ether, Chloroform, Ethanol and Water respectively. All the extracts were stored in a refrigerator for preliminary phytochemical investigation and pharmacological screening.

Development of Standardization Parameters for Leaves of *Eupatorium adenophorum* Sprenge

Study of organoleptic characters-

- Colour
- Odour
- Taste

Study of Organoleptic Characters

The polyherbal formulation is studied for organoleptic characters like color, odour and taste using the sensory organs of our body.[33, 34]

Synthesis of Silver Nanoparticles

AgNO3 powder was dissolved in distilled water to prepare 10 mM AgNO3 stock solution from which a series of 1 mM, 2 mM, 3 mM and 4 mM AgNO3 solutions were prepared. The AgNO3 solutions were mixed with the aqueous extract of *Eupatorium adenophorum Sprenge* fresh leaves at a ratio of 1: 1 (v/v) to a volume of 50 mL in a flask. The flask was wrapped with an aluminium foil and was then heated in a water bath at 60°C for 5 hours. Furthermore, the mixture was stored in the refrigerator for the antibacterial activity test and further analysed by using UV-Vis spectrophotometer.

Characterisation of silver nanoparticles

UV-visible spectra analysis (Shimadzu dual beam UV 1800) was performed at room temperature using wavelength of 800-200nm. FTIR analysis performed using Shimadzu i (Range 400-4000cm-1) for the identification of the functional group present on surface of the siller nanoparticles. X-ray Diffraction (XRD) measurement was carried out using X-ray Diffractometer instrument.

Why Silver Nanoparticle?

The extremely small size of nanoparticles means they cxhibit enhanced or different properties when compared with the bulk material. The extremely small size of nanoparticles results in the particles having a large surface area relative to their volume. In the case of silver nanoparticles this allows them to easily interact with other particles and increases their antibacterial efficiency. This effect can be so great that one gram of silver nanoparticles is all that is required to give antibacterial properties to hundreds of square metres of substrate material. In order to understand how silver nanoparticles kill pathogens, an understanding of how bacteria, viruses live and grow is required.[35]

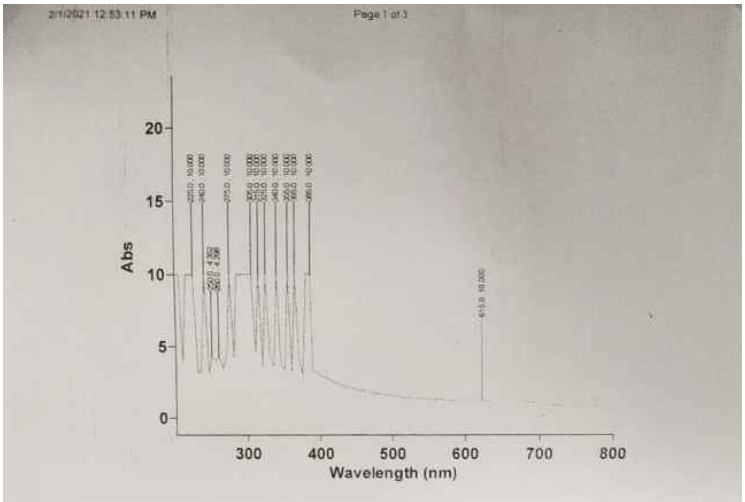

Figure: UV Spectra of Silver nanoparticles of Eupatorium adenphorum springe.

Bacteria

All bacteria use an enzyme as a form of chemical lung' in order to metabolise oxygen. Silver ions cripple the enzyme and stop the take up of oxygen. This effectively suffocates any bacteria, killing it within 6 minutes and leaving surrounding tissue or material unaffected.

Antimicrobial Activity

Agar well diffusion method

Agar diffusion refers to the movement of molecules through the agar matrix that is formed by the gelling of agar. The degree of the molecules movement related to the concentration of the molecules. This phenomenon forms the basis of the agar diffusion assay that is used to determine the susceptibility or resistance of a bacterial strain to an antibacterial agent. The antibiotic compound move from higher concentration to the surrounding lower concentration regions and form zone of inhibition by inhibiting microbial colonies. This diffusion was the basis of the agar diffusion assay.

A bacterial suspension is spread onto the surface of the agar. Then different concentration of antimicrobial compound is applied to a number of wells in the plate. Then incubate the plate for 24-48 hrs after incubation check the plate. If there is a clearing around the well, then the bacteria have been adversely affected by the compound. The size of the inhibition zone can be measured and related to standards, in order to determine whether the bacterial strain is sensitive to the antibiotic.[36]

RESULTS

Table 1: Physiochemical parameters values.

S.N.	Parameters	Value/ Inference
1.	**Organoleptic Characters**	
a)	Colour	Greenish
b)	Odour	Characteristic
c)	Taste	Astrigent
d)	Texture	Smooth

Table 2: Antimicrobial Activity of *Eupatorium adenophorum* Sprenge (leaf) in Alcoholic extracts.

S. No.	Bacteria strain	Diameter of Zone of Inhibition (mm) Alcoholic extract				
		a(20µl)	b(40µl)	c(60µl)	d(80µl)	Streptomycin(100µl)
1.	E.coli	10	10	15	20	50
2.	Bacillus	10	10	0	15	60
3.	S. Typhii	0	20	20	25	40

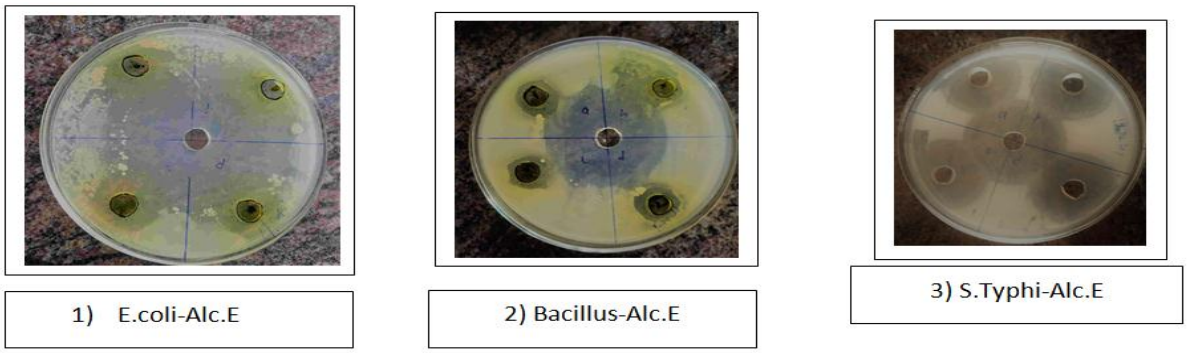

Figure: Antimicrobial potential of Alcoholic extracts of *Eupatorium adenophorum* Sprenge against three types of microbial organisms.

Table 3: Antimicrobial Activity of *Eupatorium adenophorum* Sprenge (leaf) in Pet. ether extracts.

S. No.	Bacteria strain	Diameter of Zone of Inhibition (mm) Pet. ether extract				
		a(20µl)	b(40µl)	c(60µl)	d(80µl)	Streptomycin(100µl)
1.	E.coli	15	20	20	30	60
2.	Bacillus	0	15	0	20	50
3.	S. Typhii	10	20	30	15	60

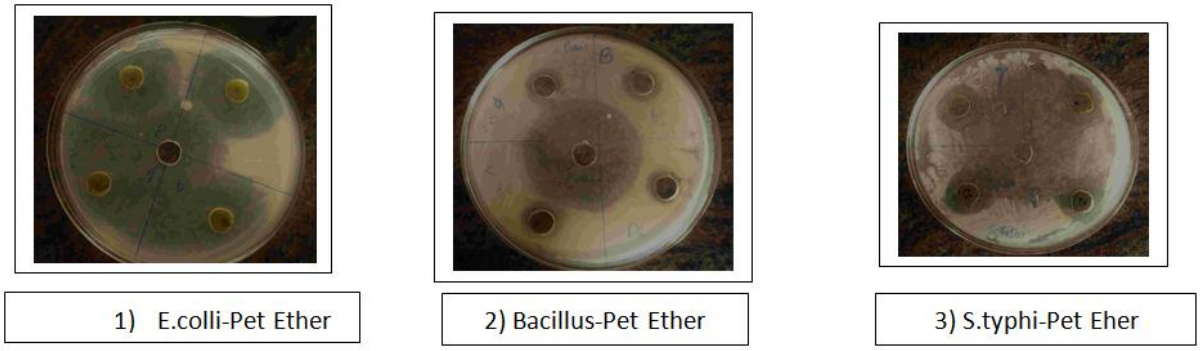

Figure: Antimicrobial potential of Pet.ether extracts of *Eupatorium adenophorum* Sprenge against three types of microbial organisms.

Table 4: Antimicrobial Activity *Eupatorium adenophorum* Sprenge (leaf) in Chloroform extracts.

S. No.	Bacteria strain	Diameter of Zone of Inhibition (mm) Chloroform extract				
		a(20µl)	b(40µl)	c(60µl)	d(80µl)	Streptomycin(100µl)
1.	E.coli	10	15	10	20	60
2.	Bacillus	10	20	15	20	70
3.	S. Typhii	10	20	0	30	50

Figure: Antimicrobial potential of Chloroform extracts of *Eupatorium adenophorum* Sprenge against three types of microbial organisms.

Table 5: Antimicrobial Activity of *Eupatorium adenophorum* Sprenge (leaf) in Aqueous extracts.

S. No.	Bacteria strain	Diameter of Zone of Inhibition (mm) Aqueous extract				
		a (20µl)	b (40µl)	c (60µl)	d (80µl)	Streptomycin(100µl)
1.	E.coli	0	25	20	0	50
2.	Bacillus	10	20	0	25	70
3.	S. Typhii	15	0	30	30	60

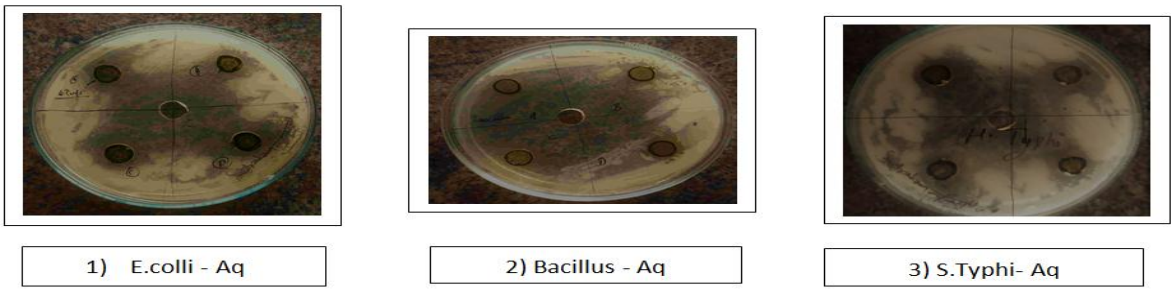

Figure: Antimicrobial potential of Aqueous extracts of *Eupatorium adenophorum* Sprenge against three types of microbial organisms.

Table 6: Antibacterial activity of Silver nanoparticles of *Eupatorium adenophorum* Sprenge in Aqueous extracts.

S. No.	Bacteria strain	Diameter of Zone of Inhibition (mm) Silver Nanoparticles				
		1mM	2mM	3mM	4mM	Streptomycin(100µl)
1.	E.coli	15	20	15	35	50
2.	Bacillus	0	30	35	0	60
3.	S. Typhii	15	30	0	40	70

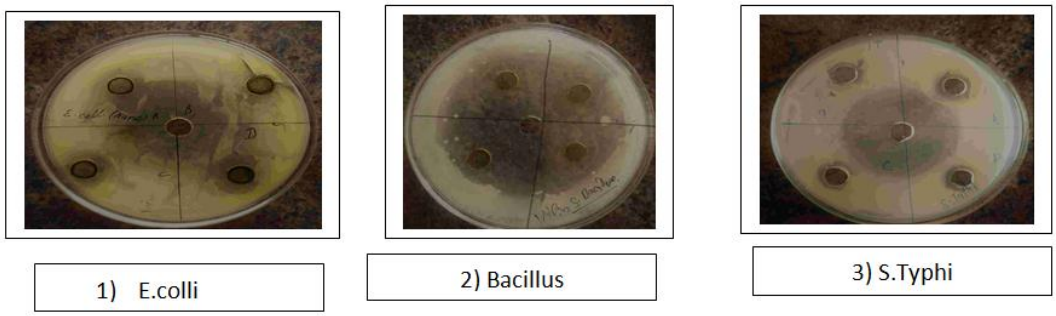

Figure: Antimicrobial potential of Silver nanoparticles of Aqueous extracts of Eupatorium adenophorum Sprenge against three types of microbial organisms.

DISCUSSIONS

The antibacterial studies were regulated on dried plant extracts of E. adenphorum Spreng using Bacillus subtilis, Escherichia coli, and S.typhi at concentration of 100µl/disc by agar well diffusion method. The significant antibacterial activity was determined by measuring the diameter of zone of inhibition and compared with the standard drug streptomycin. The Significant antimicrobial activity was determined by measuring the diameter of Zone of Inhibition and compared with the Standard drug Streptomycin. From the data of Table No. 2 to 6, It was found that all the crude extracts exhibited moderate to good antimicrobial activity against the bacterial pathogens tested herein.

It was found that all the crude extracts (80µgm) exhibited moderate to good antibacterial activity against the bacterial pathogens tested herein and the petroleum ether extract of E. adenophorum Spreng recorded largest zone of inhibition (15, 20, 25 mm in diameter) against B.subtilis, S.typhi and E. coli. Antimicrobial antibiotic streptomycin (100µgm/) was found to be active against all the bacteria tested herein. From the results of the presented in Table the petroleum ether extracts exhibited the lowest value against B. subtilis and the chloroform extracts of E. adenophorum Spreng exhibited the lowest value against E.coli.

CONCLUSION

In conclusion, The extracts (PE, Alc. & Aqueous) produced better inhibitory activities but less when compared to Standard drug against almost all three types of micro organisms. But best results showed Silver Nanoparticles of Aqueous extract of *Eupatorium* plant species. It provides some Scientific basis for some of the uses in Traditional medicine like treatment of boils and

scabies and as antiseptic. Therefore, It was suggestd the isolation and possible characterization of the active constituent (s) from the extracts of this plant species as possible Antimicrobial agents.

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