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Antimicrobial activity of *Eupatorium adenophorum* (Crofton weed) extract on *Staphylococcus aureus* and *Escherichia coli* isolates from wound infection

Saroj Rai[1]* and Ruma Jas[2]

[1]ICAR-National Dairy Research Institute, ERS, Kalyani, West Bengal, India, [2]West Bengal University of Animal and Fishery Sciences, Belgachia, Kolkata, India

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Abstract

Eupatorium adenophorum plant collected from Darjeeling Himalayan region was selected to analyse its antimicrobial potential against *Staphylococcus aureus* and *E. coli* bacteria. *Eupatorium adenophorum* had a better antimicrobial effect on *Staphylococcus aureus* (ZOI- 26.00±2.70) with MIC of 12.5 mg/ml as compared to *E coli*. Preliminary qualitative tests were also highly positive for steroids, alkaloids, glycosides, phenolic compounds, tannins, flavonoids, diterpenes, triterpenes and saponins. The antibacterial potency of *Eupatorium adenophorum* could be exploited for treatment of wounds in animals.

Keywords: *Ethnobotany, Eupatorium adenophorum, Staphylococcus aureus*, *wound infection*

The use of Himalayan herbs for animal treatment is seen as a holistic approach and a readily available quick fix by the indigenous folklore living in far-flung areas from towns and districts of Sikkim and Darjeeling Hills. The region is listed as the world's 25th biodiversity hotspot (Myers *et al*., 2000) situated between 87°59' and 88°53' East longitude and, 26°31' and 28°10' North latitude in India, respectively (Chhetri *et al*., 2005). Different groups of people inhabit this region, including the Nepali, Bhutia and Lepcha community (O'Malley, 1907). The community dependence on medicinal plants have evolved over time through experience and has been proven effective for treatment of wound infections, reproductive problems, digestive problems, snake bites, fractures, haemorrhage etc. *Eupatorium adenophorum* or Crofton weed or Banmara in local language meaning the forest killer grows abundantly on roadsides or forest areas of Sikkim and Darjeeling hills is known to have potential uses on cuts and wounds (Kumar, 2002), with analgesic (Mandal *et al*., 2005) and antipyretic (Prajapati, 2003) activity. *E. adenophorum* is a herbaceous shrub barely of 2m height (Fig.1). Leaves are dull green and serrated with reddish purple branches. Flowers have white, cylindrical florets about 3.5 mm long (Muniappan *et al*., 2009) with slender angular brownish black seeds (Wolff, 1999). The plant is used by simply crushing the leaves and placing them over cuts and wounds and bandaging for a day or two. The bioactive constituents are 5-O-trans-o-coumaroylquinic acid methyl ester, macranthoin G, macranthoin F, and chlorogenic acid methyl (Zhang *et al.,* 2013).

**Corresponding author: drsaroj.rai@gmail.com, Ph. +91- 9674895039*

Fig. 1. E. adenophorum

Therefore, the study is intended to explore the antimicrobial potential of Crofton weed claimed to have a profound effect in curing wounds of animals caused by *Staphylococcus aureus* and *E. coli* bacteria.

Materials and methods

The work was conducted in ICAR- NDRI, ERS Kalyani, WB, India. *Eupatorium adenophorum* plant was collected from Darjeeling Himalayan region. Darjeeling lies between 26°31' 05" - 27° 13' 10" N latitude and 87° 59' 30" - 88° 53' E longitude, and is the northern most district of the Indian state of West Bengal. The predominant microorganisms from joint ill of calves *S. aureus,* and *E. coli* were isolated after growing them in Baird parker egg yolk tellurite agar and Mac Conkey agar respectively. Positive control of these bacteria were derived using ATCC strains available commercially. After rinsing them in clean water, the plant was dried in hot air oven at 60 °C for 24 to 30 hrs and ground to a fine powder, soaked for extraction in aqueous solution at 1:10 ratio. It was evaporated by rotary evaporator and the extract was dissolved in DMSO at a concentration of 100mg/mL (Nagappan, 2012). The extracts were tested for various qualitative tests. Qualitative phytochemical analysis of the extract was tested for the presence of bioactive compounds using methods described by Harborne (1998).

Tests for detection of steroids (Salkowski's test)

Fifty milligrams of the extract were dissolved in 3 mL of chloroform. Few drops of concentrated sulphuric acid were added and the solution was allowed to stand. Formation of red colour directed the presence of steroids

Tests for detection of alkaloids

One gram of the extract was mixed with 5 mL of ammonia and then extracted with an equal volume of chloroform. To this extract,5 mL of dilute hydrochloric acid was added. The acid layer obtained was further tested with the following reagents for the presence of alkaloids.

Tests for detection of glycosides (Benedict's test)

Approximately 50 mg of the extract was mixed with 1 mL of water and then 5 mL of Benedict's reagent was added to it. Formation of brown or red precipitate indicated the presence of reducing sugars.

Test for detection of phenolic compounds (Ferric chloride test)

Five milligrams of the extract was dissolved in 1 mL of water and five drops of 10 per cent ferric chloride were added to it. Development of bluish black colour specified the presence of phenols.

Tests for detection of tannins (Ferric chloride test)

Treated two milligrams of the extract with 3 mL of one per cent ferric chloride solution. Development of a blue-black or a brownish green colouration showed the presence of tannins.

Tests for detection of flavonoids (Ferric chloride test)

Treated 2 mL of the methanol extract (0.5 g extract in 10 mL methanol) with four drops of neutral ferric chloride solution. The formation of green colour indicated the presence of flavonoids.

Test for detection of diterpenes

About five milligrams extract was mixed with 3 mL of five per cent copper acetate solution. The formation of green colour showed the existence of diterpenes.

Tests for detection of triterpenes (Salkowski test)

Mixed 3 mL of chloroform to three milligrams of extract and it was shaken with 3 mL concentrated sulphuric acid. The development of yellow colour in the lower layer on standing indicated the presence of triterpenes.

Tests for detection of saponins (Froth test)

Approximately 200 mg of the extract was shaken with 5 mL of water. The continuation of foam production for ten minutes designated the occurrence of saponins.

Screen herb extracts on bacterial spp.

The minimum inhibitory concentration (MIC) of the *Eupatorium adenophorum* extract was determined according to the method described by Kang *et al.* (2011). Different concentrations (w/v) of the extract were prepared with DMSO at the range of 75, 50, 25, 12.5, 6.25 and 3.13 per cent. The antimicrobial activity was also determined by agar well diffusion method at different concentrations. The MIC was determined by the microplate method against the bacteria. Bacterial cultures used for both assays were grown overnight and adjusted to McFarland standard 0.5, equivalent to 10^8cfu/ml. The extracts were dissolved in DMSO to a concentration of 10mg/mL and 100 µL was added to the first well of the microtitre plate and serially diluted with water. Bacterial cultures of 100 µL were added to each well. Gentamicin was used as a positive control and DMSO as a solvent control. As an indicator of growth, 40µL of 0.2mg/mL p-iodonitrotetrazolium violet (INT) (Sigma) was added to the microplate wells and incubated at 37° for 2 hrs. This dye, an electron acceptor, was reduced by the viable microorganism to a coloured product. Therefore, the yellow tetrazolium dye was reduced by viable microorganisms to a pink/purple colour.

Results and discussion

During preliminary qualitative screening of *Eupatorium adenophorum*, there was the presence of steroids, alkaloids, glycosides, phenolic compounds, tannins, flavonoids, diterpenes, triterpenes and saponins (Fig. 2 & Table 1).

Table 1. Phytochemical analysis of plant extracts of *E. adenophorum*

Phytochemical test	Result
Steroids	+
Alkaloids	+
Glycosides	+
Phenolic compounds	+
Tannins	+
Diterpenes	+
Triterpenes	+
Saponins	+

Present +; Absent -

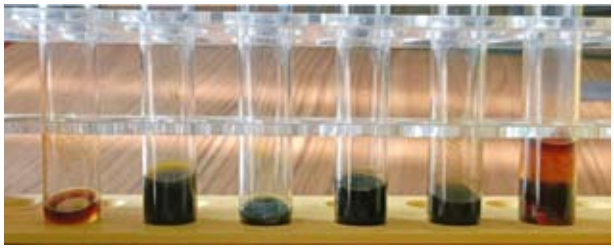

Fig. 2. Qualitative screening of *E. adenophorum*

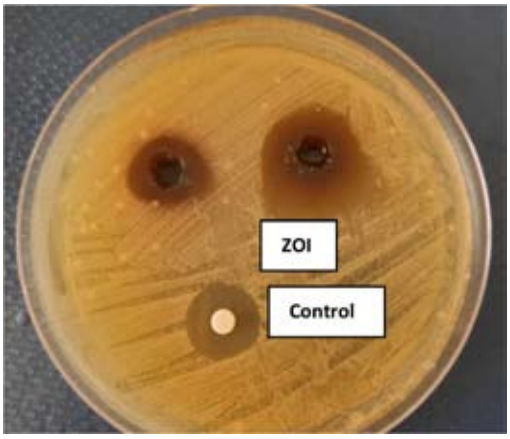

Fig. 3. Antibacterial effect of *E. adenophorum* against *S. aureus*

The leaf extracts exhibited the zone of inhibition (Table 2) ranging from 26.00±2.70 mm (range 30mm to 18mm) to *S. aureus* and was most sensitive at 500 µg/mL (Fig. 3). MIC was estimated to be at 50 mg/mL and 12.5 mg/mL for *S. aureus* and *E. coli*, respectively.

The importance of the plants lies on its bioactive components and its ability to inhibit the most important bacterial species. These are the foundation of its usability and popularity as medicinal herbs. There is a constant need for cost-effective therapies treating wound infections and promoting plant products as therapies. Antibiotic resistance being the major concern worldwide and new agents from plants could benefit as alternatives which can be used safely and effectively. But *Eupatorium adenophorum*, on ingestion cause severe pathological changes of the intestine in rats (Sun *et al*., 2019). The essential oils of

Table 2. Zone of inhibition and minimum inhibitory concentration

	Concentration	*Staphylococcus aureus*	*E coli*
Average ZOI	500 µg/mL	+++	-
	250 µg/mL	+	-
	125 µg/mL	-	-
	62.5 µg/mL	-	-
	32.6 µg/mL	-	-
	Control (Gentamycin)	++	++
MIC (mg/mL)		12.5	50

Average ZOI (mm): Non sensitive (<8 mm, -), Sensitive (Between 9 - 14 mm, +), Very sensitive (Between 15 - 19 mm, ++), Extremely sensitive (Larger than 19 mm, +++) (Watanya *et al.* 2016)

Eupatorium adenophorum showed antibacterial and antifungal activity though the root essential oil exhibited maximum inhibition and MIC of 0.05 µL mL^{-1} against *S. aureus* (Ahluwalia, *et al*., 2014). Also, Manandhar *et al*. (2019) reported antibacterial action of its methanolic extract against Methicillin-resistant *S. aureus* with the MIC of 12.5 mg/mL. Traditionally, *E. adenophorum* is used on cuts and wound infections in Darjeeling areas, which have been known to have a proven wound healing effect. Sharma *et al*. (2018) have also found the plant to possess significant ($p<0.01$) wound healing properties through use of gel formulation made from plant extract applied on the wound model during 13 days study.

Conclusion

The present study indicates that *E. adenophorum* has potential antibacterial on infections caused by *S. aureus* though its activity on *E. coli* is negligible.

Conflict of interest

The authors claim no conflict of interest

References

Ahluwalia, V., Sisodia, R., Walia, S., Sati, O.P., Kumar, J. and Kundu, A. 2014. Chemical analysis of essential oils of *Eupatorium adenophorum* and their antimicrobial, antioxidant and phytotoxic properties. *J. Pest Sci.,* **87**: 341-349.

Chhetri, D.R., Parajuli, P. and Subba, G.C. 2005. Antidiabetic plants used by Sikkim and Darjeeling Himalayan tribes, India. *J. Ethnopharmacol.* **99**: 199–202.

Harborne, J.B. 1998. Phytochemical methods: A guide to modern techniques of plant analysis. (3rd Ed.). Chapman and Hall, London. 320 hotspots for conservation priorities. *Nature.* **40**: 853-85.

Kumar, S. 2002. *The Medicinal plants of North East India*, Scientific Publishers (India), Jodhpur, 203p.

Manandhar, S., Luitel, S. and Dahal, R.K. 2019. *In vitro* antimicrobial activity of some medicinal plants against human pathogenic bacteria. *J. Trop. Med.* **2:** 2019:1895340. doi: 10.1155/2019/1895340. PMID: 31065287; PMCID: PMC6466868.

Mandal, S.K., Boominathan, R., Parimaladevi, B., Dewanjee, S. and Mandal, S.C. 2005. Analgesic activity of methanol extract of *Eupatorium adenophorum* Speng leaves. *Indian J. Exp. Biol.* **43** (7): 662-663.

Muniappan, R., Reddy, G.V. and Raman, A. 2009. *Biological control of tropical weeds using arthropods,* Cambridge University Press, First Edition, Publisher: Cambridge University Press, Cambridge, UK, 481p.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., da-Fonseca, and Kent, J. 2000. Biodiversity hotspots for conservation priorities. *Nature.* **403**(6772):853-458. doi: 10.1038/35002501. PMID: 10706275

Nagappan, R. 2012. Evaluation of aqueous and ethanol extract of bioactive medicinal plant, *Cassia didymobotrya* (Fresenius) Irwin and Barneby against immature stages of filarial vector, *Culex quinquefasciatus* Say (Diptera: Culicidae). *Asian Pacific J. Trop. Biomed.* **2** (9): 707–711.

O'Malley, L.S.S. 1907. *Darjeeling district gazetteer*. Gyan Publishing House, New Delhi, 248p.

Prajapati, P. and Sharma, A. 2003. *Handbook of Medicinal Plants- A Complete Source Book:* Agrobios Publication, 345p.

Sharma, T., Bhide, B. and Acharya, R. 2018. Ethnomedicinal claims on wound healing activity of certain leaf drugs a review. *Int. J. Ayurvedic Med*. **9**: 42-78.

Sun, W., Zeng, C., Yue, D., Liu, S., Ren, Z., Zuo, Z., Deng, J., Peng, G. and Hu, Y. 2019. *Ageratina adenophora* causes spleen toxicity by inducing oxidative stress and pyroptosis in mice. *R. Soc. Open Sci.* **6**: 190-127

Watanya, J., Prapansak C. and Nipa C.2016. Antimicrobial activity of thai-herbal plants against foodborne pathogens *E. coli*, *S. aureus* and *C. Jejuni*. Agric. *Agric. Sci. Procedia.* **11**: 20 – 24.

Wolff, M. 1999. Winning the War on Weeds: The Essential Gardener's Guide to Weed Identification and Control**,** Kangaroo Press, 112p.

Zhang, M., Liu, W.X., Zheng, M.F., Xu, Q.L., Wan, F.H., Wang, J., Lei, T., Zhou, Z.Y. and Tan, J.W. 2013 Bioactive quinic acid derivatives from *Ageratina adenophora*. *Mol*ecules. **18** (11):14096-104. doi: 10.3390/molecules181114096. PMID: 24241153; PMCID: PMC6269784. ■