ISSN : 0974 - 7508 Volume 8 Issue 6

Trade Science Inc.

Natural Products

An Indian Journal

Full Paper

NPAIJ, 8(6), 2012 [233-237]

Antimicrobial activity on common pathogens in essential oil of aerial parts of *Selinum wallichianum*

Bhoj Raj Singh[1,4]*, Vidya Singh[2], Raj Karan Singh[3], Saroj Toppo[3], Nazrul Haque[2], Ngullie Ebibeni[1]

[1]ICAR Research Complex for NEH Region, Jharnapani - 797 106, Nagaland, (INDIA)

[2]NRC on Mithun, Jharnapani-797 106, Nagaland, (INDIA)

[3]ICAR Research Complex for NEH Region, Sikkim Centre, Tadong - 737 102, Gangtok, (INDIA)

[4]Central Disease Diagnosis Laboratory, Centre for Animal Disease Research and Diagnosis, Indian Veterinary Research Institute, Izatnagar - 243 122, (INDIA)

E-mail: : brs1762@yahoo.co.in

Received: 16th April, 2012 ; Accepted: 16th May, 2012

ABSTRACT

Selinum wallichianum is a perennial herb, grows in temperate Himalayan forests is traditionally used as incense and therapeutics in common ailments like, cough, cold, fever, wounds, stomachache and toothache etc. The present study was designed to evaluate the antimicrobial activity and minimum inhibitory concentration (MIC) of Selinum wallichianum essential oil (SWEO) extracted from leaves and tender stem branches on two reference strains of E. coli, (E3376 and E3382) and 94 bacterial strains of 10 genera isolated from clinical cases, environment and food items. Both the reference strains were sensitive to the oil while among the test strains, none of the Bacillus coaggulans strains, one isolate each of E. coli, Aeromonas hydrophila and Lactobacillus acidophilus, three strains of Klebsiella pneumoniae and majority of Enterococcus strains were resistant to SWEO. The MIC of sensitive and resistant strains was ≤32 µg/ml and >32 µg/ml, respectively. The findings justified the ethnomedical use of S. wallichianum extract to cure pyrexia, gastrointestinal infection, wound infection and food popisoning etc. Although antibacterial activity has been reported in ethanolic extract of roots of S. wallichianum earlier, it appears to be the first report of the potent antimicrobial activity in essential oil of aerial parts of S. wallichianum.

KEYWORDS

Antibacterial;
E.coli;
Aeromonas hydrophila;
Klebsiella pneumoniae;
Streptococcus pyogenes.

INTRODUCTION

Selinum wallichianum (DC, synonyms, *Selinum tenuifolium, Peucedanum wallichianum*) a tall glabrous perennial herb, has two to three pinnatisect leaves with rhombic to ovate-lanceolate terminal leaf lobes, teethed on margins. It is commonly known as Milk Parsley in English and Bhutkesh (hair of demon) in Hindi, Gurang and Nepali and Tanak in Amchi. This herb grows luxuriantly in Himalayan forests at an altitude of 2600 - 4200 m. It has tap-root and large umbels of

Full Paper

white flowers blooming in summer and autumn. Grow in moderately fertile, moist but well-drained soil in full sun or partial shade[1].

Selinum wallichianum powdered root is used as incense in magico-religious rites in the Kumaon hill region in India and powdered leaves and flowers are taken with hot water to cure stomachache, cough, cold, fever, while the paste made of leaves and flowers is applied on cuts and wounds for rapid cure[2-5]. *Roots of similar herbs growing at the similar altitude namely, Selinum candollei* and *Selinum vaginatum* (also recognized as Bhootkesh), are used to treat toothache[6].

Other species of *Selinum* viz., *S. candollii* (also known as Bhutkeshi) has also been used as tonic, *S. tenuifolium* (Matosal) as insecticide and nervine sedative, and *S. varigatum* (Matoila) as nervine sedative in Manali region in India[7]. Roots of *Nardostachys grandiflora* often used to adulterate *Selinum candollii* and *S. wallichianum are* valued for their antispasmodic and stimulant properties and is therefore useful in the treatment of fits and heart palpitations, to treat constipation and regulate urination, menstruation and digestion both in the Unani and Ayurvedic systems of medicine[8,9]. An ethanolic extract of *N. grandiflora* rhizomes has been found to protect against liver damage induced by thioacetamide in rats while its rhizome is used as brain or uterine tonics, stimulants, external pain killers, as an antiseptic, for the treatment of epilepsy, hysteria, convulsions, heart palpitations, high blood pressure, fever, anxiety, insomnia, asthma and other bronchial problems and acidity in Himalayan regions, while in Tibetan medicine it is valued for treating complaints including epilepsy, wounds, coughs, colds and high blood pressure[9].

Although all parts of *S. wallichianum* are useful to treat one or more ailments in ethnic medicine, little is scientifically understood about their biological activity. Recently, root extract of *S. wallichianum* has been reported to inhibit growth of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* strains in disc diffusion assay[2]. From reviewing the ethnomedicinal uses of *S. wallichianum*, it is evident that its aerial parts are more useful to treat cuts and wounds[3-5,10] but little is known about its antibacterial activity. Therefore, the study was conducted to evaluate the antimicrobial activity of essential oil of *S. wallichianum* (extracted from its leaves, tender braches and stem) on commonly occurring pathogens of zoonotic significance isolated from clinical, environmental and food samples.

MATERIAL AND METHODS

Selinum wallichianum essential oil (SWEO)

Volatile oil was extracted from fresh leaves and twigs by hydro-distillation using Clevenger's apparatus. For this, during the month of June, leaves and twigs of *Selinum wallichianum* with vegetative growth were collected from alpine pastureland of Gnathang region, East district, Sikkim at an elevation of 14000 feet asl. Fresh leaves and twigs (400 g) with were chaffed and mixed with distilled water (1 litre) in a round bottom flask (2 litres) and boiled for 3 h. The oil (lighter than water) was collected from the nozzle of the condenser and stored at 4°C.

Bacterial strains

Ninety four bacterial strains of 10 genera isolated from pig (27) and cattle (21) rectal swabs (from diarrhoeic animals), mithun (*Bos frontalis*) vaginal (of infertile cows) swabs (16) and meat (1), duck (6) cloacal swabs (healthy birds), water tank (1), and Axone, a Nagaland food (22) were revived from stocks maintained at Microbiology Laboratory of ICAR Research Complex for NEH Region (TABLE 1), Nagaland Centre, Jharnapani, Nagaland, India. All revived strains were checked for purity as per standard procedure[11]. Besides, reference strain, *E. coli* (E3376), sensitive to all antibacterial substances and another *E. coli* (E3382) resistant to common antibacterial agents were used in the study to determine the MIC of the essential oil of *Selinum wallichianum*.

Determination of Antimicrobial activity of SWEO

The antibacterial activity was determined by disk diffusion method and minimum inhibitory concentration (MIC) test[12,14]. For disk diffusion test, sterile disks of five mm diameter were soaked in methanolic solution of SWEO and dried

TABLE 1 : Sensitivity pattern of for bacteria of public health significance to *Selinum wallichianum* essential oil (SWEO).

Bacteria tested	Source of isolation	No. of Strains tested	No. (%) of strains resistant
Aeromonas hydrophila	Duck, CS	2	1 (50.0)
Aeromonas hydrophila	Pig RS	6	0 (0.0)
Bacillus coaggulans	Pig RS	4	0 (0.0)
Bacillus coaggulans	Cattle RS	9	0 (0.0)
Bacillus coaggulans	Tank Water	1	0 (0.0)
Bacillus coaggulans	Axone, Food	3	0 (0.0)
Bacillus coagulans	Mithun, VS	1	0 (0.0)
Citrobacter freundii	Mithun meat	1	0 (0.0)
Enterococcus caecorum	Axone, Food	17	10 (58.8)
Enterococcus casseliflavus	Axone, Food	1	1 (100.0)
Enterococcus faecalis	Mithun, VS	5	2 (40.0)
Edwardsiella tarda	Pig RS	13	0 (0.0)
Enterobacter agglomerans	Mithun VS	1	0 (0.0)
Escherichia coli	Reference E3376	1	0 (0.0)
Escherichia coli	Reference E3382	1	0 (0.0)
Escherichia coli	Cattle RS	7	0 (0.0)
Escherichia coli	Mithun VS	6	1 (16.7)
Escherichia coli	Pig RS	3	0 (0.0)
Klebsiella pnumoniae ssp. pneumoniae	Duck CS	4	1 (25.0)
Klebsiella pnumoniae ssp. pneumoniae	Cattle RS	5	0 (0.0)
Klebsiella pnumoniae ssp. pneumoniae	Mithun VS	2	1 (50.0)
Klebsiella pnumoniae ssp. pneumoniae	Pig RS	1	1 (100.0)
Lactobacillus acidophilus	Axone, Food	1	1 (100.0)
Streptococcus pyogenes	Mithun VS	1	0 (0.0)
Total		96	19 (19.8)

CS, cloacal swab; RS, rectal swab; VS, vaginal swab

at room temperature, each disc contained 50µg of SWEO. Mueller Hinton agar (MHA; Hi-Media, Mumbai) plates were swabbed with 6-8 hour nutrient broth (Hi-Media Mumbai) growth of test bacteria, allowed to dry. *SWEO* discs with standard positive control disc (50µg mercuric chloride) and negative control disc (disc soaked in methanol and dried) were placed on the MHA plate. Plates were incubated overnight at 37°C before reading the inhibition zone measured in mm.

For determination of minimum inhibitory concentration (MIC) of SWEO for two reference strains of *E. coli* (E3382 and E3376), and all the 94 test strains, agar dilution susceptibility test was performed based on modified method of NCCLS [12] and CLSI[13,14]. Briefly, essential oil dissolved in sterilized dimethyl-sulphoxide (DMSO; 1024 mg /ml) was taken as standard and it was added in molten (at 45^0C) MHA after suitable two fold dilutions to achieve 512, 256, 128, 64, 32, 16, 8, 4, 2 and 1 µg/ml concentration of essential oil in MHA. Plates were poured and after solidification, the plates were spot inoculated with loop-full (2 µl) of overnight grown bacterial. The test was carried out in triplicates and plates were incubated overnight at 37°C. After 18 to 24 hours, the MIC results were read.

RESULTS AND DISCUSSION

Fresh leaves and had 35% dry matter content. The oil (SWEO) was lighter than water and light yellow in colour. Recovery of SWEO was 1.07 % on dry matter basis.

Results of disc diffusion assay for sensitivity of bacterial (TABLE 1) revealed that both the reference strains of *E. coli* (E3376, E3382) were sensitive to SWEO irrespective of their antimicrobial drug resistant pattern, similar to other 15 clinical isolates of *E. coli*. Only one isolate *E. coli* resistant to SWEO was from Mithun (*Bos frontalis*) vaginal swab. All *Bacillus coaggulans* strains irrespective of their origin were sensitive to SWEO, however of the two strains of *Lactobacillus acidophilus* isolated from Axone one was resistant to SWEO. None of the *Aeromonas hydrophila* of pig origin was resistant to SWEO but one of the two isolates of duck origin was resistant. None of the 13 *Edwardsiella tarda* but majority of enterococci (13 of 23) strains was resistant to SWEO irrespective to the species they belonged. Only 25% of *Klebsiella pneumoniae* were resistant to SWEO.

Results of MIC determination (TABLE 2) closely corroborated with the results obtained with disk diffusion method and all those which were sensitive with disk diffusion method had MIC =32 µg of SWEO per ml of medium while those resistant had SWEO MIC >32 µg per ml of medium. In general, all the bacillus and *E. coli* strains had SWEO MIC between 1µg/ ml to 16 µg/ ml. One of the common pyogenic organism, *Streptococcus pyogenes*, often causing wound infection[15,18], had SWEO MIC just 4 µg/ ml.

Ethnomedical use of paste of leaves of *Selinum wallichianum* for rapid cure of wounds[2-5] appears

Full Paper

TABLE 2 : Estimated minimum inhibitory concentration (MIC) of *Selinum wallichianum* essential oil (SWEO) for bacteria of public health significance

Bacteria tested	MIC for sensitive (by disc diffusion test) strains (no.)	MIC for resistant (by disc diffusion test) strains (no. and source)
Aeromonas hydrophila	1-32 µg/ ml (7)	256 µg/ ml (1, from Duck)
Bacillus coaggulans	1-16 µg/ ml (18)	No strain was resistant
Citrobacter freundii	16 µg/ ml (1)	No strain was resistant
Enterococcus caecorum	16-32 µg/ ml (7)	128 to >512 µg/ ml (10, all from Axone)
Enterococcus casseliflavus	16 µg/ ml (1)	512 µg/ ml (1, from Axone)
Enterococcus faecalis	16-32 µg/ ml (3)	128 to 256 µg/ ml (2, from Mithun)
Edwardsiella tarda	1-32 µg/ ml (13)	No strain was resistant
Enterobacter agglomerans	32 µg/ ml (1)	No strain was resistant
Escherichia coli E-3382	1 µg/ ml (1)	No strain was resistant
Escherichia coli E-3376	1 µg/ ml (1)	No strain was resistant
Escherichia coli	1-16 µg/ ml (15)	64 µg/ ml (1, from Mithun)
Klebsiella pnumoniae ssp. pneumoniae	1-32 µg/ ml (9)	64-512 µg/ ml (3, one each from duck, pig and Mithun)
Lactobacillus acidophilus	8 µg/ ml (1)	64 µg/ ml (1, from Axone)
Streptococcus pyogenes	4 µg/ ml (18)	No strain was resistant

to be an effective remedy as the essential oil from leaves of *Selinum wallichianum* might effectively inhibit growth of most of the bacteria causing wound infection including *S. pyogenes, Enterobacter* spp. *Enterococcus* spp., *E. coli* and *K. pneumoniae* in concentration (=32 µg/ ml) quite lower than it may occur in paste of fresh plant (0.37% of wet weight). Streptococci, *Escherichia coli, Enterobacter* spp. and enterococci are leading causes of wound infection all over the globe[15,18] while klebsiellae are one of the most common emerging cause of burn wound infection[19]. Our observations also justified the ethnomedical use *Selinum wallichianum* extract to cure stomach ache and digestive disorders[8,9] as SWEO inhibited growth of many bacteria often associated with gastrointestinal infection associated with food poisoning including *E. coli, E. tarda, K. pneumoniae* and *A. hydrophila*[20-23]. Similarly, use of *Selinum wallichianum* extract to cure fever and other systemic disorders[2,4,8,9] often associated with infections with bacteria that might be inhibited due to potent antibacterial activity detected in aerial parts of *S. wallichianum*. Although antibacterial activity has been reported in ethanolic extract of *S. wallichianum* earlier[2], it appears to be the first report of the potent antimicrobial activity in essential oil of aerial parts of *S. wallichianum* often used by people to cure different ailments[2,5,8,10]. In earlier studies no indication could be made about the MIC of *S. wallichianum* extract[2], determination of MIC of SWEO in this study indicated that SWEO and the herb itself may be quite valuable as an herbal antimicrobial. Effectiveness of SWEO on several potentially pathogenic bacteria isolated from sick as well as healthy animals and birds indicated that it may be an important component of future veterinary formulation for external and internal use; however, further explorations are necessary on larger number of strains from more cases of disease.

ACKNOWLEDGEMENTS

Authors are thankful to Director and Joint Director ICAR Research Complex for NEH Region, Barapani, and Nagland Centre, respectively for extending facilities to conduct this piece of work; help rendered by Moa, supporting staff at Nagaland Centre was of immense value in conducting the laboratory work.

REFERENCES

[1] Selinum Wallichianum; Royal Horticulture Society, URL: http://apps.rhs.org.uk/plantselector/plant?plantid=1822 **(2011)**.
[2] S.Bhattarai, R.P.Chaudhary, R.S.L.Taylor, S.K.Ghimire; Nepal J.Sci.Technol., **10**, 83 **(2009)**.
[3] M.B.Gewali; Aspects of Traditional Medicine

Aspects of Traditional Medicine in Nepal. Institute of Natural Medicine, University of Toyama 2630 Sugitani, Toyama 930-0194, Japan, http://sureshawale.blogspot.com **(2008)**.

[4] Y.C.Lama, S.K.Ghimire, Y.A.Thomos; Medicinal Plants of Dolpo, Amchis Knowledge and conservation. WWF Nepal Program, Kathmandu, Nepal **(2011)**.

[5] N.P.Manandhar; Plants and People of Nepal. Timber Press, Inc.Portland, Oregon, USA **(2000)**.

[6] G.Singh, G.S.Rawat; Indian J.Fundamental Appl.Life Sci., **1**, 35 **(2011)**.

[7] M.S.Rana, S.S.Samant; Indian J.Traditional Knowledge., **10**, 439 **(2011)**.

[8] S.K.Jain; Medicinal Plants. National Book Trust, New Delhi, India, **(1996)**.

[9] T.Mulliken, P.Crofton; Bundesamt für Naturschutz (BfN), Federal Agency for Nature Conservation Konstantinstrasse 110 53179 Bonn, Germany, **(2008)**.

[10] S.Bhattarai, R.P.Chaudhary, R.S.L.Taylor; J.Ethnobiol.Ethnomed., **2**, 41 **(2005)**.

[11] B.R.Singh; Labtop for Microbiology Laboratory. Lambert Academic Publishing, AG and Co. KG, Koln, Germany, **(2009)**.

[12] National Committee for Clinical Laboratory Standards; Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. Approved Standard - NCCLS Document M7-A6, Wayne **(2003)**.

[13] Clinical and Laboratory Standards Institute; Performance Standards for Antimicrobial Susceptibility Testing. Eighteenth Informational Supplement CLSI Document M100-S18, Wayne **(2008)**.

[14] Clinical and Laboratory Standards Institute; Method for Dilution Antimicrobial Susceptibility Tests for Bacterial that Grow Aerobically; Approved Standard - Eighth Edition. CLSI document M07-A8, Wayne **(2009)**.

[15] A.L.Bisno, D.L.Stevens; New England J.Med., **334**, 240 **(1996)**.

[16] M.Collier; Recognition and management of wound infections. http://www.worldwidewounds.com/2004/january/Collier/Management-of-Wound-infections. html. Retrieved on November 12 **(2011)**.

[17] A.Giacometti, O.Cirioni, A.M.Schimizzi, M.S.Del Prete, F.Barchiesi, M.M.D`Errico, E.Petrelli, G.Scalise; J.Clin.Microbiol., **38**, 918 **(2000)**.

[18] J.M.Janda, S.L.Abbott, R.A.Brenden; Eur.J.Clin.Microbiol.Infect.Dis., **16**, 189 **(1997)**.

[19] M.Mehta, P.Dutta, V.Gupta; Indian J.Plastic Surgery., **40**, 25 **(2007)**.

[20] B.R.Singh, K.N.Kapoor, R.S.Rathore, R.K.Agarwal, A.Kumar, K.N.Bhilegaonkar; J.Food Sci.Technol., **33**, 251 **(1996)**.

[21] B.R.Singh, K.N.Kapoor, R.S.Rathore, A.S.Yadav, R.K.Agarwal, K.N.Bhilegaonkar, A.Kumar; Bev.Food World., **23**, 51 **(1996)**.

[22] B.R.Singh, A.K.Tiwari; Indian J.Exp.Biol., **34**, 1254 **(1996)**.

[23] A.S.Yadav, B.R.Singh, K.N.Kapoor, S.S.Verma; Indian J.Vet.Res., **5**, 45 **(1996)**.