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Antimicrobial effect of *Artemisia vulgaris* essential oil

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ABSTRACT

Antimicrobial effect *Artemisia vulgaris* essential oil (AVEO) was evaluated on 616 strains of 84 different pathogenic, potentially pathogenic and environmental microbial species belonging to 29 different genera using disc diffusion method on Mueller Hinton agar. A clear zone (>8 mm) of inhibition around a 5mm disc containing 50 µg essential oil indicated its antimicrobial activity. Only 20.9% strains were sensitive to AVEO, however all strains of *Candida albicans, Kluyvera cryocrescens, Leminorella ghirmontii,* and *Micrococcus agilis* and majority (>75%) of *Bacillus* spp. were sensitive to the oil. On the other hand majority of the enterobacteria including *Escherichia coli, Salmonella enterica, Klebsiella* spp, *Edwardsiella* spp. strains and gram positive cocci (staphylococci, streptococci) were resistant to AVEO. All the five strains of *Aspergillus niger* but none of the six strains of *A. flavus* were sensitive to AVEO. The minimum inhibitory concentration (MIC) of AVEO was determined through agar dilutiom method ranged between ≤ 1µg to 32µg/ ml for sensitive strains while none of the resistant strain could be inhibited to grow at the level below 128 µg/ml. Detailed analysis of the results revealed that AVEO may contain considerable antifungal and antibacillary activity which may be exploited for enhancing its therapeutic value in its already known medicinal uses.

KEYWORDS

Antibacterial;
Antifungal;
Antimicrobial;
Artemisia vulgaris;
Aspergillus;
Candida;
Escherichia coli;
Klebsiella;
Salmonella.

INTRODUCTION

Artemisia vulgaris (commonly known as mugwort plant in English) contains several active ingredients including essential oils (cineole and thujone), flavonoids, triterpenees and coumarin[1]. There are several theories for the origin word 'Mugwort', one tale is that the word has come from the word "mug" as it has been used in flavoring drinks since ages[2], others say is has come from the old Norse muggi, meaning "marsh", and Germanic "wuertz", meaning "root", having moth repelling properties[3]. In Ukrain, the plant is named chornobylnik after Chornobyl= Chernobyl= the place where mugwort grows. However, it is *nagadamni* in Sanskrit for its utility in cardiac complaints as well as feelings of unease, unwellness and general malaise[4]. In traditional medicine, it is known to thin the blood and to have hallucigenic properties, its juice is applied for stopping bleeding, as febrifuge and purgative and its decoctum is taken to relieve colds and coughs. In Japanese and

Chinese Medicine, *Artemisia vulgaris* is used for moxibustion, i.e., the herb is placed on the skin, attached to acupuncture needles, or rolled into sticks and waved gently over the area to be treated. *Artemisia vulgaris* moxibustion had has been claimed to heal radiation poisoning, as an effective therapy to achieve cephalic positioning of breeched fetuses before the gynaecological intervention because the herb induces not only uterine movement but also regulate fetal heart rate and movement[5,6]. Despite of its common use and occurrence all over the globe its antimicrobial activity is little explored[6].

As far as its antimicrobial effects are concerned little is evident, however *Artemisia vulgaris* has shown its efficacy against trichinellosis in rats[7] and amoebiasis[8]. Antimicrobial activity of *Artemisi vulgaris* is highly disputed due to contradicting reports, some observations reported only feeble activity against different serotypes of *Escherichia coli*[9] and other bacteria[10] while other group could not detect any inhibition of *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Candida albicans*[11]. Although, other species of *Artemisia* has been shown to posses wide spectrum activity against many of the pathogenic bacteria but MIC for methnolic extract of *A. nilagricia* was determined to be ≤ 32 mcg/ ml for most of the bacteria but *Bacillus subtillis*[8], [12-15]. Therefore, the present study was conducted to evaluate antifungal and antibacterial activity in essential oil of *A. vulgaris*, a common species prevalent in India to evaluate the various claims and the spectrum of activity against a wide range of microbes.

MATERIALS AND METHODS

Artemisia vulgaris essential oil (AVEO)*:* Volatile oil was extracted from fresh leaves and twigs by hydro-distillation using Clevenger's apparatus. For this, during the month of June (rainy season) leaves and twigs of *Artemisia vulgaris* with vegetative growth were collected from the wastelands in and around Tadong, East district, Sikkim at an elevation of 5500feet asl. Leaves and twigs (250 g) 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. The AVEO was yellow in colour with a yield of 0.375% (v/w) on fresh weight basis.

Fungal and bacterial strains

Five *Aspergillus niger*, six *A. flavus*, three *Penicillium* spp., seven *Candida albicans* strains and 595 bacterial strains of 26 genera isolated and maintained at Microbiology Laboratory of ICAR Research Complex for NEH Region, Nagaland Centre, Jharnapani, Nagaland, India were revived and checked for purity as per standard procedure[16]. Besides, reference strain, *E. coli* (E3376), sensitive to all antibacterial substances and another *E. coli* (E382) resistant to all antibacterial agents were used in the study to determine the MIC of the essential oil of *A. vulgaris*.

Determination of Antimicrobial activity of *Artemisia vulgaris* essential oil

The antibacterial activity was determined by disk diffusion method and minimum inhibitory concentration (MIC) test[17-19]. For disk diffusion test, sterile disks of five mm diameter were soaked in methanolic solution of essential oil and dried at room temperature to contain 50µg of essential oil. Mueller Hinton agar (MHA; Hi-Media, Mumbai) plates were swabbed with 6-8 hour growth of test bacteria or overnight Sabrauds' broth (Hi-Media Mumbai) growth of yeast and fungal strains, allowed to dry. *Artemisia vulgaris* discs with standard positive control disc (50µg mercuric chloride) and negative control disc (disc soaked in methanol and dried) was placed on the MHA plate. Plates were incubated overnight at 37°C for bacteria and for 48-72 hours at 22°C for mold/fungi and yeasts before reading the inhibition zone measured in mm.

For determination of MIC, two reference strains of *E. coli* (E382 and E3376), one resistant (E3376) one sensitive (E382) to all available antimicrobials, and two test strains (one sensitive and one resistant, if available) each of *Candida albicans, Bacillus coaggulans, Aeromonas hydrophila, Edwardsiella tarda, Salmonella enterica ssp. indica* and *Klebsiella pneumoniae*, agar dilution susceptibility test was performed based on modified method of

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TABLE 1 : Effect of *A. vulgaris* essential oil (AVEO) on strains of different microbial groups

Microbial group (Number species tested)	No. strains tested	Resistant	% Sensitive
Budvicia aquatica	3	3	0.0
Citrobacter spp. (3)	17	17	0.0
Hafnea alvei	1	1	0.0
Lactobacillus acidophilus	1	1	0.0
Leclercia adecarboxylata	1	1	0.0
Morganella morganii	3	3	0.0
Proteus spp. (4)	12	12	0.0
Pragia fontium	8	8	0.0
Providencia spp. (2)	1	1	0.0
Serratia spp. (5)	5	5	0.0
Xenorhabdus luminescens	1	1	0.0
Streptococcus spp. (8)	32	31	3.1
Salmonella enterica ssp. *indica*	19	18	5.3
Escherichia spp. (4)	50	47	6.0
Pseudomonas spp. (3)	16	15	6.3
Staphylococcus spp. (5)	34	31	8.8
Enterococcus spp. (15)	153	138	9.8
Ervinia ananas	9	8	11.1
Klebsiella spp. (3)	51	44	13.7
Edwardsiella spp. (2)	17	14	17.6
Enterobacter spp. (9)	13	10	23.1
Aeromonas spp. (8)	72	52	27.8
Bacillus spp. (15)	73	18	75.3
Kluyvera cryocrescens	1	0	100.0
Leminorella ghirmontii	1	0	100.0
Micrococcus agilis	1	0	100.0
Penicillium spp. (1)	3	2	33.3
Aspergillus spp. (2)	11	6	45.5
Candida albicans	7	0	100.0
Total	616	487	20.9

NCCLS and CLSI[17-19]. Briefly, essential oil dissolved in sterilized dimethyl-sulphoxide (DMSO; 1024 µg /ml) was taken as standard and two fold dilutions were made to achieve 512, 256, 128, 64, 32, 16, 8, 4, 2 and 1 µg/ml concentration of essential oil in molten (at 45^{0}C) MHA. Plates were poured and after solidification, the plates were spot inoculated with loop-full (2 µl) of overnight grown bacterial/ yeast cultures. The test was carried out in triplicates and plates were incubated overnight at 37°C for bacteria and 22°C for yeast. After 18 to 24 hours, the MIC was determined.

RESULTS AND DISCUSSION

All the 616 strains under study were sensitive to mercuric chloride discs (50 µg) but only 20.9% microbial cultures were sensitive to AVEO. Similar results of mercuric chloride sensitivity have been reported earlier for other microbes[20]. Observations revealed that all strains of *Candida albicans* (7), *Kluyvera cryocrescens* (1), *Leminorella ghirmontii* (1) and *Micrococcus agilis* (1) were sensitive to AVEO but the small number of strains tested may not be used to make general statement. However, of the 73 strains

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TABLE 2 : Antimicrobial effect of *A. vulgaris* essential oil on strains of different microbes

Microbes tested	Strains tested	Resistant	Sensitive	% sensitive
Aeromonas caviae	6	5	1	16.7
Aeromonas eucranophila	11	8	3	27.3
Aeromonas hydrophila	15	9	6	40.0
Aeromonas media	9	6	3	33.3
Aeromonas salmonicida ssp. achromogenes	1	1	0	0.0
Aeromonas salmonicida ssp. salmonicida	4	2	2	50.0
Aeromonas salmonicida ssp. smithia	2	1	1	50.0
Aeromonas schubertii	7	4	3	42.9
Aeromonas sobria	3	3	0	0.0
Aeromonas veronii	14	13	1	7.1
Aspergillus flavus	6	6	0	0.0
Aspergillus niger	5	0	5	100.0
Bacillus anthracoides	3	0	3	100.0
Bacillus badius	7	2	5	71.4
Bacillus brevis	4	1	3	75.0
Bacillus circulans	4	0	4	100.0
Bacillus coaggulans	27	8	19	70.4
Bacillus lentus	7	4	3	42.9
Bacillus marcerans	4	2	2	50.0
Bacillus pentothenticus	16	1	15	93.8
Bacillus subtilis	1	0	1	100.0
Budvicia aquatica	3	3	0	0.0
Citrobacter amalonaticus	1	1	0	0.0
Citrobacter diversus	2	2	0	0.0
Citrobacter freundii	14	14	0	0.0
Candida albicans	7	0	7	100.0
Enterococcus avium	1	1	0	0.0
Enterococcus caecorum	21	16	5	23.8
Enterococcus casseliflavus	21	20	1	4.8
Enterococcus dispar	24	22	2	8.3
Enterococcus durans	2	1	1	50.0
Enterococcus faecalis	13	12	1	7.7
Enterococcus faecium	11	11	0	0.0
Enterococcus gallinarum	12	12	0	0.0
Enterococcus hirae	37	32	5	13.5
Enterococcus mundatii	4	4	0	0.0
Enterococcus solitarius	1	1	0	0.0
Enterococcus bovis	6	6	0	0.0
Edwardsiella hoshiniae	1	1	0	0.0
Edwardsiella tarda	16	13	3	18.8
Enterobacter agglomerans	7	4	3	42.9
Enterobacter amnigenus II	1	1	0	0.0
Enterobacter cloacae	1	1	0	0.0
Enterobacter gregoviae	3	3	0	0.0

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Microbes tested	Strains tested	Resistant	Sensitive	% sensitive
Enterobacter amnigenus I	1	1	0	0.0
Ervinia ananas	9	8	1	11.1
Escherichia blattae	6	6	0	0.0
Escherichia coli	38	36	2	5.3
Escherichia furgusonii	6	5	1	16.7
Hafnea alvei	1	1	0	0.0
Klebsiella oxytoca	2	1	1	50.0
Klebsiella pnumoniae ssp. pneumoniae	48	42	6	12.5
Klebsiella terrigena	1	1	0	0.0
Kluyvera cryocrescens	1	0	1	100.0
Lactobacillus acidophilus	1	1	0	0.0
Leclercia adecarboxylata	1	1	0	0.0
Leminorella ghirmontii	1	0	1	100.0
Micrococcus agilis	1	0	1	100.0
Morganella morganii	3	3	0	0.0
Proteus mirabilis	5	5	0	0.0
Proteus penneri	4	4	0	0.0
Proteus vulgaris	3	3	0	0.0
Penicillium spp.	3	2	1	33.3
Pragia fontium	8	8	0	0.0
Providencia heimbachae	1	1	0	0.0
Pseudomonas aeruginosa	15	14	1	6.7
Pseudomonas fluorescens	1	1	0	0.0
S. enterica ssp. indica	19	18	1	5.3
Serratia marcescens	2	2	0	0.0
Serratia rubidiae	3	3	0	0.0
Staphylococcus aureus	13	13	0	0.0
Staphylococcus epidermidis	2	1	1	50.0
Staphylococcus sciuri	14	12	2	14.3
Staphylococcus xylosus	2	2	0	0.0
Staphylococcus spp.	3	3	0	0.0
Streptococcus gallinarum	2	2	0	0.0
Streptococcus milleri	3	3	0	0.0
Streptococcus agalactiae	1	1	0	0.0
Streptococcus alactolyticus	1	1	0	0.0
Streptococcus caseolyticus	1	0	1	100.0
Streptococcus mobilis	21	21	0	0.0
Streptococcus spp.	3	3	0	0.0
Xenorhabdus luminescens	1	1	0	0.0
Total	616	487	129	20.9

tested for *Bacillus* spp, >75% were sensitive to AVEO (TABLE. 1) indicating that AVEO may be of immense value in controlling aerobic spoilage of food often caused by *Bacillus* spp. strains. Resistance of most of the bacterial strains to AVEO (TABLE 1) indicated that the herb may be of little antimicrobial potential except for few groups of bacteria as reported earlier[11]. In the same genus of microbes AVEO had altogether different

effect on strains of different species as all *A. niger* were inhibited to grow by AVEO but it had no effect on *A. flavus*, a toxigenic group, similar observation were evident for strains of various species of aeromonads (TABLE 2). Most of the environmental aeromonad (*Aeromonas salmonicia*) were sensitive to AVEO while those aeromonads often reported to be associated with infection including *A. caviae, A. veronii*[21-22] were resistant to AVEO, however many (40%) strains of the most common aeromonad (*A. hydrophila*) often associated with infections in mammals[23] were sensitive to AVEO. Similar pattern of variation in sensitivity as observed for aeromonads was evident with strains of different species of *Enterobacter* and *Enterococcus* (TABLE 2). Thus on we can conclude that study on small number of strains can not be revealing and may not be of much value while predicting antimicrobial action of any antimicrobial substance specifically of herbal origin. The variation in observation from earlier reports[9-11] might be either due to the fact that earlier workers used only a few strains, though in higher concentration. Low concentration of AVEO (50µg per disc) in discs in the present study was selected to find viability of the utility of AVEO as drug, in earlier observation use of almost 1 mg extract per disc[9-11] is practically unachievable concentration in biological system without causing any toxicity, at that high concentration one may find antimicrobial activity but of little value unless MIC is estimated further.

Although all the strains showing sensitivity to AVEO discs (50µg) had MIC 〉32 µg/ ml but it varied, minimum being just 〈1 µg / ml for *Bacillus coaggulans* strain and as much as 32 µg / ml for *Klebsiella pneumonia* strains. Similarly among the resistant strains, minimum MIC was 128 µg/ ml for most of the strains tested but 512 µg/ ml for *Klebsiella pneumonia* strain (TABLE 3). The wide variation in MIC even for the strains of a group and of different genera explains the contradiction in earlier observations[9-11] in respect to antimicrobial activity of *A. vulgaris*. The important reason for contradiction might be due to use of a few selected strains, it hardly make any difference that strains were either reference or of field origin as a reference sensitive strain of *E. coli* (E382) was resistant to AVEO (MIC 128 µg/ ml) while one reference resistant *E. coli* (E3376) was sensitive to AVEO (MIC 2 µg/ ml), therefore to test the sensitivity of herbal drugs the known references may not be of similar value as they are designed for evaluation of antibiotics rather than herbal drugs.

TABLE 3 : MIC of *A. vulgaris* essential oil (AVEO) for different bacteria and yeasts

Microbial strains	Zone of inhibition around 50mcg disc in mm	Sensitive/ resistant in Disc diffusion assay	MIC in µg /ml
E. coli (E382)	0	Resistant	128
E. coli (E3376)	16	Sensitive	2
Candida albicans	15	Sensitive	2
Bacillus coaggulans	14	Sensitive	≤1
Bacillus coaggulans	0	Resistant	128
Aeromonas hydrophila	15	Sensitive	2
Aeromonas hydrophila	0	Resistant	128
Edwardsiella tarda	8	Sensitive	16
Edwardsiella tarda	0	Resistant	128
Salmonella enterica ssp. *indica*	10	Sensitive	8
Salmonella enterica ssp. *indica*	0	Resistant	256
Klebsiella pneumonia	11	Sensitive	32
Klebsiella pneumonia	0	Resistant	512

Therefore, the present study might be much more informative methodologically and also provide a wider view of antimicrobial potential of AVEO because of the feasible methodology used for testing antimicrobial activity of the herbal drugs and evaluation of effect of AVEO on large number (616) of microbial strains of wide diversity (84 species of 29 genera).

CONCLUSION

The study on antimicrobial activity of *Artemisia vulgaris* essential oil on 616 microbial strains revealed that it inhibited growth of 20.9% microbes but it is not equally active against yeast, mold, Gram positive and Gram negative bacteria but varies with the strain of pathogen. It appears to be more active against yeast,

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mold and *Bacillus* strains rather than to known pathogens like *Staphylococcus aureus*, *Streptococcus* spp., *E. coli*, *Salmonella* and *Klebsiella pneumoniae*, *A. hydrophila*, *E. tarda* strains.

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