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Effect of medicinal plants on *Moraxella cattarhalis*

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ABSTRACT

Objective: To determine the antimoraxella activity of Ethiopian medicinal plants extracts. **Methods:** Two clinical isolates of *Moraxella cattarhalis (M. cattarhalis)* with different antibiotic sensitivity pattern were tested to determine their susceptibility to garlic [*Allium sativum (A. sativum)*], bark of cinnamon [*Cinnamomum zeylanicum (C. zeylanicum)]*, clove [*Syzygium aromaticum (S. aromaticum)*], and leaves of avocado [*Persea americana (P. americana)*], rosemary [*Rosmarinus officinalis (R. officinalis)*] and prickly poppy [*Argemone mexicana (A. mexicana)*]. Disk diffusion assay and broth dilution method were used to measure zone of inhibition, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of plant extracts against *M. cattarhalis*. **Results:** Both the strains of *M. cattarhalis* exhibited similar sensitivities to the extracts of medicinal plants. Antimoraxella activity was exhibited only by garlic, avocado leaves and cinnamon. Garlic was found to be more antagonistic to *M. cattarhalis* than cinnamon and avocado. Garlic and avocado leaves have shown similar MIC (30 mg/mL) where as their zone of inhibition (15 and 11 mm, respectively) were different. **Conclusions:** Garlic, cinnamon and avocado leaves extracts represents alternative source of natural antimicrobial substances for use in clinical practice for the treatment of cases of *M. cattarhalis*. Further research on the effects of these extracts on *M. cattarhalis* can be rewarding to pursue in the search for new broad spectrum antimicrobial agents.

1. Introduction

Moraxella catarrhalis (M. catarrhalis) is an important cause of lower respiratory tract infection, and in immunocompromised host, the bacterium can cause a variety of severe infections. Because *M. catarrhalis*, has long been considered a harmless commensal, relatively little is known about this bacterium. The emergence of *M. catarrhalis* as a pathogen in the last decade, together, with increasing prevalence of β – lactamase producing strains, has renewed interest in these bacterial species[1]. Resistance to conventional antimicrobial agents is rising worldwide, it is necessary to have alternative agents available[2].

The search for newer sources of antibiotics is a global challenge, since many infectious agents are becoming resistant to synthetic drugs. Plants have the major advantage of still being the most effective and cheaper alternative sources of drugs. The local use of natural plants as primary health remedies, due to their pharmacological properties, are quite common in Asia, Latin America and Africa[3].

The objective of the present investigation was to determine the inhibitory activity of Ethiopian medicinal plants and herbal extracts against *M. cattarhalis* using disk-diffusion and broth dilution methods.

2. Materials and methods

The plant material used in this study collected from Harar, an eastern city in Ethiopia, located on a hilltop about five hundred kilometres from Addis Ababa with an altitude of 1 885 meters, latitude of 42°7′0″ and longitude of 9°19′0″, during spring (mid-March to mid-April 2010). Plants were identified by the Plant Science Department of Haramaya University of Ethiopia.

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2.1. Medicinal plants extraction procedures

2.1.1. Garlic

Garlic [*Allium sativum (A. sativum)*] extract was prepared according to method described by Bari and Douglas[4]. Briefly, the peeled fresh garlic (80 g) was chopped and homogenized in 100 mL sterile distilled water, centrifuged, filtered through Whatman No.1 filter paper and then sterilized by filtration (0.45 mm). By subtracting the weight of insoluble material from the weight of original cloves, the final concentration of garlic extract in solution was determined to be 60% (w/v).

2.1.2. Cinnamon and clove[5]

The cinnamon [*Cinnamomum zeylanicum (C. zeylanicum)*] bark and clove [*Syzygium aromaticum (S. aromaticum)*] was grounded in a grinding machine separately (Philips Mixer Grinder) in order to obtain a fine dry powder. The powder was weighed, using a single pan analytical electronic weighing balance (Ohaus), and the cinnamon/clove extract was obtained by means of a maceration process. The cinnamon/clove powder was soaked in 50% ethanol (1 g of powder per 5 mL of solvent) in a 250 mL Erlenmeyer flask for a period of 48 h at room temperature with frequent shaking. The flasks were closed with a cotton plug and aluminium foil. The mixture was then centrifuged (MSE Mistral 1000) at 3 500 × *g* for 20 min and finally filtered through Whitman filter paper No.1. The filtrate was collected and concentrated under reduced pressure in a rotary vacuum evaporator (Buchan) until a semi-solid substance was obtained, which was then dried in a crucible under a controlled temperature (45 ℃) to obtain a solid powder. The powder was weighed, reconstituted in dimethyl sulfoxide (DMSO), sterilized by filter (0.45 mm) and stored at -20 ℃.

2.1.3. Leaves of rosemary [Rosmarinus officinalis (R. officinalis)], avocado [Persea americana (P. americana)] and prickly poppy [Argemone mexicana (A. mexicana)][6]

Leaves were washed, dried and macerated. To produce methanol extracts, 5 g of leaves powder were allowed to stand in 100% methanol (80 mL) for 24 h at room temperature; solution was then dried in a rotary evaporator and stored at 6 ℃ until use. Methanol extracts were then diluted to 1 mg/mL in sterile media. Products were filtrated through 0.22 μm pore size diameter filters (Whatman filters), and stored at -20 ℃.

2.2. Antibacterial assay

2.2.1. Disk diffusion assay[7]

Four-five isolated colonies of the test strain inoculated into a tube containing 9 mL of Mueller-Hinton broth (Himedia, Mumbai, India). The inoculated tube was incubated at 37 ℃ for 5 to 6 h to match with 0.5 McFarland. A sterile cotton swab was dipped into the standardized bacterial test suspension and used to evenly inoculate the entire surface of Mueller Hinton Agar (Himedia) plates enriched with 5% sheep blood.

Discs were placed on the surface of inoculated plates and incubated at 37 ℃. Negative controls were prepared using the same solvent without the plant extract. A reference antibiotic, Chloramphenicol (Oxoid), was used as a positive control. The inoculated plates containing the impregnated discs were incubated in an upright position at 37 ℃ for 24 to 48 h. The results were expressed as the zone of inhibition around the paper disk in mm.

Sterilized discs (6 mm) soaked in 1mL of aqueous decoctions of black pepper, garlic and for others 10 mg of extract dissolved in 1 mL of DMSO. Whatman filter number 5. The concentration for each disc was 10 μL (100 discs/mL of aqueous decoction of each spice).

2.2.2. Broth dilution method

Briefly, each isolate of *M. cattarhalis* was grown in Mueller-Hinton broth, according to National Committee for Clinical Laboratory Standards (NCCLS), now called as Clinical Laboratory Standards (CLS) guide lines[8]. Bacterial cell suspension with turbidity equivalent to McFarland 0.5 was used.

Plant extract concentration ranged from 240 to 2.5 mg/mL, and 25 mL of the bacterial cell suspension was added to the serially diluted extracts. All incubations were at 37 ℃ for 24 h and the highest dilution where there was no growth was recorded as the minimum inhibitory concentration (MIC) of a particular extract. Minimum bactericidal concentration (MBC) was defined as the lowest concentration yielding negative subcultures.

2.2.3. Effect of temperature on the antibacterial activity of garlic and cinnamon extracts

The effect of temperature on the antibacterial activity of extracts was determined by the methods as described by Lee *et al*[9] Extract solutions were incubated at room temperature and 100℃ in a water bath for 15 min. Then, the extracts were cooled down and stored at 4 ℃ until use. Extracts were deep freezed at -10 ℃ for one month.

Two clinical isolates of *M. cattarhalis* (isolated from sputum of cases of lower respiratory tract infection) with different antibiotic sensitivity pattern were tested to find out their susceptibility to different Ethiopian medicinal plants. Their antibiotic sensitivity pattern was done by the disc diffusion method and broth macrodilution method as per the NCCLS instructions mentioned in manual of clinical microbiology[10].

3. Results

Antibiogram sensitivity pattern of two test strains of *M. cattarhalis*: Strain 1, Sensitive (ceftriaxone, erythromycin, chloramphenicol); Intermediate (methicillin, amoxyclave, cefotaxime, co-trimoxazole); Resistant (gentamicin, ciprofloxacin, ofloxacin). Strain 2 was sensitive to all the

above antibiotics except the co-trimoxazole.

Even though the two strains of *M. cattarhalis* used in this study have different antibiotic sensitivity pattern but their sensitivities to the extracts of medicinal plants are almost similar, as shown in the Table 1.

Table 1
Antibacterial activity of medicinal plants against *M. cattarhalis*.

Medicinal plants	*M. cattarhalis* 1	*M. cattarhalis* 2
	ZI/MIC/MBC	ZI/MIC/MBC
Garlic	15/30/>240	14/30/>240
Cinnamon	11/120/240	11/120/240
Avocado leaves	11/30/120	12/30/120
R. officinalis	NZI	NZI
Clove	NZI	NZI
A. mexicana	NZI	NZI
Control	NZI	NZI
Chloramphenicol	21	22

ZI: Zone of inhibition (mm), MIC: Minimum inhibitory concentration (mg/mL), MBC: Minimum bactericidal concentration (mg/mL), NZI: No zone of inhibition.

No difference was found in the size of zone of inhibition after 24 and 48 h of incubation for all extracts. MIC and MBC were performed only for those extracts which exhibited zone of inhibition.

Garlic was found to be more antagonistic to *M. cattarhalis* than cinnamon and avocado leaves. The zone of inhibition of heated extract of cinnamon at 100 ℃ for 15 minutes was increased to 13.5 mm for both strains of *M. cattarhalis* compare to the extract of room temperature which was only 11 mm for both the strains of *M. cattarhalis*. But MICs were same for both the extracts.

4. Discussion

Various studies has been done to determine the antibacterial activities of medicinal plants and herbs against commonest gram positive and gram negative bacterial pathogens but none of the study is done on *M. cattarhalis*.

In our study, garlic MBC has not reported till 240 mg/mL of media, it could be more than this value. There is a difference of 1 mm in the zone of inhibition of two strains but their MIC (30 mg/mL) is similar. There might be a little difference even in their MIC that could be in between 15 to 30 mg/mL.

Garlic (*A. sativum*) typical odor and antibacterial activity depends on allicin produced by enzymatic activity of allinase (a cysteine sulfoxide lyase) on alliin after crushing or cutting garlic clove. Allicin and other thiosulfinates are believed to be responsible for the range of therapeutic effects reported for garlic. Garlic extract has been reported to inhibit growth of various gram-positive and gram-negative bacteria. It is also active against multidrug resistant organisms such as *Pseudomonas aeruginosa (P. aeruginosa)*, *Klebsiella pneumoniae (K. pneumoniae)* and *Mycobacterium tuberculosis (M. tuberculosis)*. The antifungal and antiviral activity of garlic extract has also been reported[11].

The pH of crude garlic preparation at different concentration was measured and it was found to be from pH 7.0 to 7.3, indicating that pH might not be a factor for the antibacterial activity of garlic in the present study, as tested organisms able to grow in this pH range.

The antibacterial activity of heated crude preparation of garlic at 100 ℃ for 15 minutes was not revealed up to the concentration of 60 mg/mL of media. This could be due to high volatility nature of the allicin. Studies demonstrate that heating has a negative influence on beneficial effects of garlic and loses majority of the allicin potential[12]. But deep freezed garlic at -10 ℃ for one month showed antibacterial activity similar to garlic that was not deep freezes.

Avocado *(P. americana)* leaves have been reported to possess anti-inflammatory and antifungal activities; and also high antimycobacterial activity[6]. Garlic and avocado leaves has similar MIC where as their zone of inhibition are different. This could be due to the difference in their diffusion ability. On the contrary zone of inhibition of cinnamon and avocado leaves extract are same but their MIC are different, again it could be due to the low diffusing property of avocado leaves extract.

The activity of cinnamon is due to the presence of cinnamaldehyde, an aromatic aldehyde that inhibits amino acid decarboxylase activity, and has been proven to be active against many pathogenic bacteria. Cinnamaldehyde is highly electro-negative, which interfere in biological processes involving electron transfer and react with nitrogen-containing components, *e.g.* proteins and nucleic acids, and therefore inhibit the growth of the microorganisms.

Cinnamon extract was found to be effective against gram positive and gram negative bacteria, gram positive were found to be more sensitive than gram negative. Gupta *et al* reported that cinnamon oil inhibited the growth of all the test bacteria including those which were resistant by cinnamon extract[5].

The zone of inhibition of heated extract of cinnamon was greater than the non-heated extract. MICs were same for both the extracts (heated at 100 ℃ and non-heated), for heated extract MIC could be less than 120 mg/mL and more than 60 mg/mL. Thus the antibacterial activities of cinnamon extract were found to increase with increasing temperature. This might be due to the partial destruction of interfering components present in the extraction of cinnamon.

Cloves [*Syzygium aromaticum (S. aromaticum)*] are used in Ayurveda, Chinese medicine and Western herbalism. The cloves are antimutagenic, anti-inflammatory, antioxidant, antiulcerogenic, antithrombotic and antiparasitic. The aqueous infusion and decoction of clove exhibited maximum activity against *P. aeruginosa*. *Klebsiella ozaenae*, *K. pneumoniae*, *Serratia marcescens*, *Salmonella typhi*, *Shigella dysentriae* and *Vibrio cholerae* were found resistant

to aqueous infusion and decoction while essential oil showed strong antibacterial activity against all bacterial isolates tested[13-15]. Cinnamon and clove oil is not used in our study otherwise even clove might have exhibited some antimoraxella activity. Commercial oil of Ethiopian cinnamon/clove is not available and there is no facility to extract oil in our laboratory.

Rosemary (*R. officinalis*) is used as a spice in the preparation of non-vegetarian dishes and it is not used as a medicinal plant in Ethiopia. This plant has not been evaluated before in Ethiopia for antibacterial activity. Several studies have been done worldwide to check its antimicrobial activity against different bacteria but it has not been tested against *M. cattarhalis*. *R. officinalis* has antimicrobial activity against gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), and fungi (*Candida albicans* and *Aspergillus niger*)[16]. But our study revealed that it has no activity against *M. cattarhalis*.

Prickly poppy (*A. mexicana*) is used as a medicinal plant in several countries including Ethiopia. The fresh seed extract contains protein-dissolving substances, effective in the treatment of warts, cold sores, cutaneous infections, skin diseases, itches, and also dropsy and jaundice. Its leaves and seeds have activity against gram positive and gram negative pathogenic multi-drug resistant bacteria[17]. But in our study this plant found ineffective against *M. cattarhalis*.

In conclusion, garlic, cinnamon and avocado extracts were found to be antagonistic for *M. cattarhalis*. Hence, they represents alternative source of natural antimicrobial substances for use in clinical practice for the treatment of cases of *M. cattarhalis*. The study also shows that further research on the effects of these extracts on *M. cattarhalis* can be rewarding to pursue in the search for new broad spectrum antimicrobial agents.

Conflict of interest statement

We declare that we have no conflict of interest.

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