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Antimicrobial activity of volatile oil of *Morina longifolia* Wall.ex.Dc. from Uttarakhand

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Abstract: The chemical profile of the hydro distilled volatile oil obtained from the aerial parts of *Morina longifolia* Wall.ex.Dc. from Kumaun Himalaya was analyzed by capillary GC-FID and GC-MS. The objective of the present study was to evaluate the antimicrobial activity of leaf essential oil of *Morina longifolia.* The in vitro antibacterial activities of the essential oils were evaluated against a total of six bacteria,viz, *Salmonella typhi, Klebsiellap neumoniae, E.coli, Staphylococcus aureus, Streptococcus mutans, Bacillus subtilis* and antifungal activity tested against *Candida albicans* and *Candida glabrata.*
Key words: Dipsaceae, volatile oil, GC-MS, antimicrobial activity.

Introduction

Morina longifolia Wall. of family Dipsaceae is found at the height of 3000-3500 m. It has spiny margined leaves and long interrupted spike of flowers, but flowers white to rose pink and paired bracts subtending whorls with an enlarged ovate base and fused below. Flowers with long slender corolla tube to 2.5 cm, somewhat two lipped the lips to 6 mm, hairy. Leaves strap-shaped with shallow 3-spined lobes and long pointed spiny apex[1]. It is commonly known as "Whorl flower". Its stem leaves and flowers are used in Tibetan medicine. They are said to have a sweet and astringent taste with a heating potency. They are digestive, emetic and stomachic and are used in the treatment of stomach disorders[2]. The plant possesses strong aromatic properties, used as incense and in the preparation of dhup, agarbatties, etc[3]. The root paste has been applied externally on wounds and the aroma of the flowers has been used for unconsciousness in Indian traditional medicine[4]. The plant used in treatment of maggot wounds[5].

Previous reports on *Morina* species showed that five new phenylpropanol derivatives, called morinins, as well as two known compounds as 3,4-dimethoxycinnamylalcohol methyl ether, and *p*-methoxy cinnamaldehyde, reported from the methanol extracts of the roots of the medicinal Chinese plant, *M. chinensis*[6]. A novel acylated flavonol glycoside, quercetin 3-O-[2' "-O-(E)-caffeoyl]-alpha-L-arabinopyranosyl-(1-6)-beta-D-galactopyranoside was isolated from whole plant of *Morina nepalensis*[7]. Seven new phenylpropanol derivatives, named morinins A-G (1-7), along with five known compounds, 4-*O*-methylcinnamyl alcohol, 4-*O*-methylcinnamyl methyl ether, 4-*O*-methylcinnamyl acetate, *p*-methoxybenzaldehyde, and 4-*O*-methyl-(*E*)-

coniferyl alcohol, have been isolated from the roots of the medicinal Chinese plant, *Morina chinensis*[8]. In general, wild plants have been regarded as a natural reservoir of novel and more exotic fragrances however even after the assessment of *Morina longifolia* properties and its use in traditional medicine; the attention has been limited because of the lack of information about its chemical studies[9]. In the present investigation an attempt was made to carry out the antimicrobial study of volatile oils of *Morina longifolia* collected from Western Himayan region of Uttrakhand.

Materials and methods

Plant Material:

The fresh aerial parts of *M. longifolia* were collected from Milam glacier of Kumaun Himalaya at the altitude of 3600 m. Plant herbaria were identified in Botanical Survey of India and Forest Research Institute, Dehradun. The voucher specimen (NO.CHEM/DST/06/03) has been deposited in the Phytochemistry Research Laboratory, Kumaun University, Nainital.

Oil Isolation:

The fresh plant materials (1.5 kg each) were subjected to steam distillation using a copper electric still, fitted with spiral glass condensers the yields 0.32%. The distillates were saturated with NaCl and extracted with *n*-hexane and dichloromethane. The organic phase was dried over anhydrous Na_2SO_4 and the solvent was distilled off in a rotary vacuum evaporator at 30^o C.

Antimicrobial activity:

The *in vitro* antibacterial activities of the essential oils were evaluated against a total of six bacteria, *viz, Salmonella typhi,* (Clinical isolated) *Klebsiella pneumoniae,* (MTCC-109), *E. coli* (MTCC-1610), *Staphylococcus aureus* (MTCC-96) *Streptococcus mutans* (MTCC-890), *Bacillus subtilis* (MTCC-121). The antifungal activity of the oils was performed against *Candida albicans* (MTCC-1637) and *Candida glabrata* (MTCC-3019). The test strains were purchased from the Institute of Microbial Technology (IMTECH), Chandigarh. MTCC (Microbial Technology Culture Collection) numbers represents the standard strain numbers assigned to these microorganisms. The cultures of bacteria and fungi were maintained on their appropriate agar slants at 4^o C throughout and used as stock cultures.

Determination of zone of inhibition (ZOI)

The antimicrobial activity of the essential oils was investigated by the disc diffusion method using 24–48 h grown strains reseeded on Nutrient Broth (bacterial strains) and Potato Dextrose Agar (PDA, fungal strains).[10] The cultures were adjusted to 5 × 106 CFU/mL with sterile water. 100 μL of the suspensions were spread over Nutrient agar and PDA plates to obtain uniform microbial growth. Filter paper discs (6.0 mm in diameter) were impregnated with 20 μL of the oils and then placed onto the agar plates which had previously been inoculated with the test microorganism. The petri dishes were kept at 4^o C for 2 h. The plates were incubated at 37^o C (24 h) and at 30^o C (4 h) for bacterial and fungal strains, respectively. The diameter of the inhibition zones (mean values) were measured in millimeter and considered as the zone of inhibition (ZOI). All experiments were performed in triplicate.

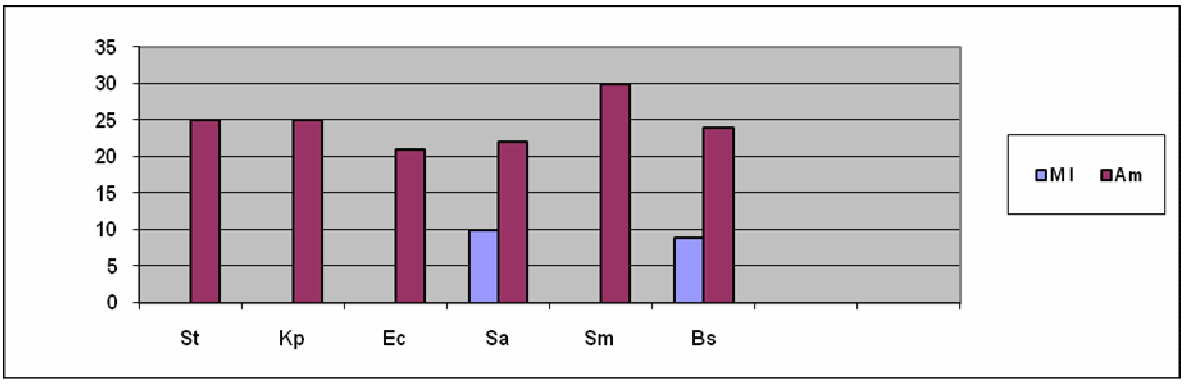
Determination of the minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) values were determined using a modified agar-well diffusion method.[10] In the agar-well diffusion technique, two-fold serial dilutions of the essential oils were prepared by diluting oil with hexane to achieve a decreasing concentration range from 50 to 2.70 μL/mL (for the fungi) and 50 μL/mL to 4.47 μL/mL (for the bacteria), using 100 μL of a suspension containing 5 × 106 CFU/mL of bacteria spread on nutrient agar plates, whereas the fungal strains were reseeded on PDA. The wells were filled with 20 μl of essential oil solutions in the inoculated Nutrient PDA agar plates. The bacterial cultures were incubated at 37° C for 24 h, while fungal cultures were incubated at 30° C for 48 h. The least concentration of each essential oil showing a clear zone of inhibition was taken as the MIC. n-Hexane was used as the negative control. Chloramphenicol and amphotericin B were used as positive controls for bacteria and

fungi, respectively. Antimicrobial (antibacterial and antifungal) activity of *M .longifolia* leaf oil by disc diffusion assay (10µl of oil/disc) against different microorganisms shown in the table 1 and 2 respectively.

Table 1: Antibacterial activity of leaf volatile oil of *M. longifolia* by disc diffusion assay (10µl of oil/disc) (Zone of inhibition and MIC)

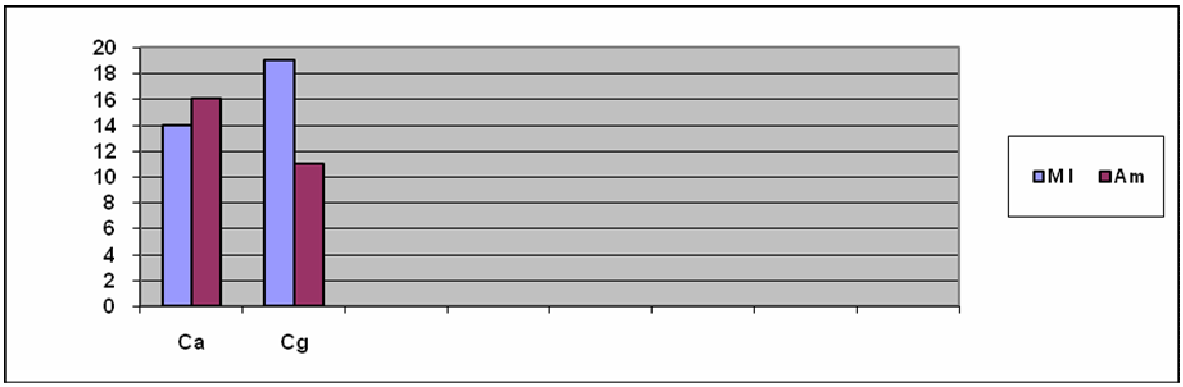
Oil/antibiotic	***S. typhi***	***K. pneumoniae***	***E. coli***	***S. aureus***	***S. mutans***	***B. subtilis***
M. longifolia	Na	Na	Na	10 mm (6.20)	Na	9 mm (4.74)
Cp.(10µg/disc)	25 mm	25 mm	21mm	22 mm	30 mm	24 mm



Antibacterial activity of leaf volatile oil of *M. longifolia* (Ml= *Morina longifolia,*
Cp= Chloramphenical) Bacteria: St, *Salmonella typhi* ; Kp, *Klebsiella pneumoniae* ; Ec, *Escherichia coli* ; Sa, *Staphylococcus aureus*; Sm, *Streptococcus mutans* ; Bs *Bacillus subtilis* No inhibition zone, Na= not active, Chloramphenicol (10 µg/disc).

Table 2: Antifungal activity of leaf volatile oil of *M. longifolia* by well diffusion assay (40µl of oil/well)

Oil /antifungal	*Candida albicans*	*Candida glabrata*
M. longifolia	14 mm (3.20)	19 mm (2.70)
Amphotericin B (20µg)	16 mm	11 mm



Antifungal activity of leaf volatile oil of *M. longifolia* (Ml = *Morina longifolia* ,
Am= Amphotericin B) Fungal strains: Ca, *Candida albicans* ; Cg, *Candida glabrata* ;
No inhibition zone, Amphotericin B 20µg

Result and discussion

From the table 1 and 2 given below we can see that the antibacterial activity against *Staphylcoccus aures*, ZOI 10 mm, and *Bacillus subtilis* ZOI 9 mm of volatile oil of *M. longifolia* with respect to standard, *viz* chloramphenicol, ZOI 22 mm and 24 mm showed not so good activity. The volatile oil is not active against rest bacterial strains. But in case of antifungal activity showed by volatile oil of *M. longifolia* against *Candida albicans* and *Candida glabrata*, exhibit largest ZOI 14 mm and 19 mm with respect to standards viz amphotericin B (20 μg), ZOI 16 mm and 11 mm respectively. The GC and GC-MS analysis of leaf oil of *Morina longifolia*, showed the major constituents in leaf oil were germacrene D (10.75 %) α-pinene (4.84 %), bicyclogermacrene (4.26 %), α-cadinol (4.26 %), (*E*)-citronellyl tiglate (4.20 %) β-phellandrene (3.24 %)[11]. *Morina* species showed that, a new aromatic glycoside characterized as 2,6-dihydroxy-5-methoxy-(3-C-glucopyranosyl) benzoic acid was isolated along with four known compounds from the aerial parts of *Morina longifolia*[12].

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