Invitro Screening for Biological and Pharmacological Effects of Indigenous Medicinal Plants, *Mentha longifolia* and *Aloe vera*

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Summary: The crude ethanolic extracts of *Mentha longifolia* Linn. (leaves and stem) and *Aloe vera* (leaves) were screened for various *invitro* biological and pharmacological activities including antifungal, antibacterial, insecticidal, phytotoxic activities and brine-shrimp lethality. All the extracts exhibited remarkable (≥60%) phytotoxic activity in the highest tested concentration (500 ppm) against *Lemna minor* L. with *Aloe vera* extract showing complete inhibition of the studied plant. The *Mentha longifolia* Linn. (stem) and *Aloe vera* extracts were also explored to posses good (≥55%) antifungal activities against *Trichophyton longifusus*, (75% and 60%), and *Microsporum canis* (65% and 55%), respectively while *Mentha longifolia* Linn. (leaves) displayed only a weak (≤50%) activity against *Trichophyton longifusus* and *Fusarium solani* (20% each). These extracts were found to be devoid of any antibacterial, insecticidal activities and Brine shrimp lethality during this study.

Introduction

The genus *Mentha* belongs to the family Lamiaceae (Labiatae) consisting of about 25–30 species; most of them are found in temperate regions of Eurasia, Australia and South Africa. The most common species of the genus *Mentha* found in Pakistan are *M. pulegium, M. arevensis, M. spicata, M. longifolia, M. piperita* and *M. royleana* [1]. *Mentha longifolia* is distributed through out Eurasia and tropical Asia but uncommon in our region [2]. The *Mentha longifolia* is used in traditional medicines as antibacterial and against gastric problems [3]. The extracts of this plant also have shown insecticidal property [4]. Several essential oils [3,5] flavones and flavone-glycosides [2,3,6-7] have recently been obtained from this species.

Aloe vera (L.) (Liliaceae) is widely distributed in Asia, Africa, and in other tropical parts. It has been used in folk medicine for the treatment of skin complaints including wounds and burns, asthma, gastrointestinal disorders, in cosmetics and as an abortifacient [8-9]. Aloe and its bitter principles have a hypoglycemic effect [10]. *A. vera* gel has been used as a traditional medicine to induce wound healing, and as an anti-cancer, and anti-viral agent [11]. *Aloe vera* possesses curative or healing qualities, featured extensively in the area of dermatology. The medical applications of *Aloe vera* in digestive problems, as a virucidal, bactericidal, and a fungicidal agent and in gynaecological conditions are also extensive [12]. Vitamin C and *Aloe vera* gel extract supplementation were found to reduce the severity of chemical hepatocarcinogenesis [13]. *Aloe vera* treatment of wounds in diabetic rats enhances the process of wound healing [14]. *Aloe vera* has been used in the treatment of a variety of disorders including wounds and burns [15]. Aloe leaves contain a colorless and tasteless gel used in the treatment of skin diseases and a yellow exudate which is a well known purgative, the active principles of which have been identified as C-glucosylanthrone, aloin A, aloin B, homonataloin A and homonataloin B. *Aloe vera*, has been found to contain C- glucosylanthrones [16] and chromones [17].

The current studies were undertaken to explore the selected plants for various self-claimed pharmacological and biological activities to provide them scientific basis and also to explore some new activities of the said plants.

Results and Discussion

Mentha longifolia and *Aloe vera* grow in large quantities indigenously and these find various applications for the treatment of various ailments in traditional system of medicines [3,9,18]. The current study was designed with a view to explore the crude

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ethanolic extracts of these plants to confirm and provide scientific basis to the already claimed activities and also to explore for some new biological effects of these to be used in herbal remedies. The crude extracts of these plants were screened for various *invitro* biological activities including anti-fungal, antibacterial, insecticidal activities, phyto-toxicity, and brine shrimp lethality.

Antifungal activity of the crude extract of *Mentha longifolia* Linn. (Leaves and stem) and *Aloe vera* (Leaves) was tested against *Trichophyton longifusus, Candida albicans, Aspergilus flavus, Microsporum canis, Fusarium solani* and *Candida glaberata*. Growth in the medium containing the extracts was determined by measuring the linear growth in mm and the percent growth inhibition was calculated with reference to the negative control. The Table shows the *invitro* antifungal activities of crude extracts *Mentha longifolia* Linn. (Leaves and stem) and *Aloe vera*. The results indicated that the crude extracts of *Mentha longifolia* Linn. (Leaves and stem) exhibited a very good inhibitory activity (75% and 60% respectively) against *Trichophyton longifusus* while these displayed a reasonably good inhibition (65% and 55% respectively) against *Microsporum canis*. However, both these extracts were devoid of any antifungal activity against the rest of tested fungi. The crude extract of *Aloe vera* did not prove itself as a potent inhibitor of the tested fungi by just displaying a poor activity against Trichophyton longifusus (20%) and *Fusarium solani* (20%) with no activity against the rest of pathogens. *Aloe vera* has been claimed as a fungicidal agent [14], which the current study also confirms.

Results of the phytotoxic activity (Fig.) of the crude extracts were interpreted by analyzing the growth regulation in percentage calculated with reference to the negative control. Paraquat was used as standard inhibitor (0.902 ppm). The results (Fig.) showed that the crude extract of *Mentha longifolia* Linn. (Leaves & stem) have a reasonable phytotoxic activity against *Lemna minor* L. at highest

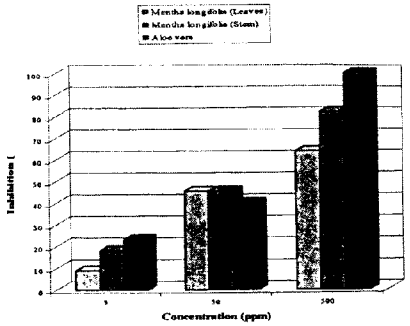

Fig: Phtotoxic activity of *Mentha longifolia* (Leaves and Stem) and *Aloe vera* (Leaves)

concentration (500 ppm) and caused and inhibition of the plant growth by 63.63% and 81.8% respectively. These also displayed some growth inhibition (45.45% and 45.45% respectively) at lower concentration (50 ppm) but were found to be quite inactive at the lowest tested concentration (05 ppm) and cause only 9.09% and 18.18% inhibition.

The *Aloe vera* extract showed an excellent inhibition (100%) of *Lemna minor* L. at highest tested concentration (500 ppm), however, it displayed a relatively week phytotoxic activity at lower concentrations (40% at 50 ppm) and (23.3% at 05 ppm).

The crude extracts of *Mentha longifolia* (leaves and stem) and *Aloe vera* were also screened for antibacterial and insecticidal activities and Brine Shrimp lethality studies but these extracts did not display any significant activity in these bioassays. The *Mentha longifolia* has been reported to be used in traditional medicines as antibacterial [3] but our results could not confirm any scientific value of this plant in this regard. Similarly, the extracts of this plant have shown insecticidal property previously [4] but our results did not display any such evidence, which might be due to the difference in flora or the season of collection.

Table. Antifungal Activities of Crude Extracts of *Metha longifolia* (Leaves and Stem) and *Aloe vera* (Leaves)

Name of Fungi	*Mentha longifolia* (Leaves)			*Mentha longifolia* (Stem)			*Aloe vera*			Standard Drugs	
	Linear Growth (mm)		Inhibition (%)	Linear Growth (mm)		Inhibition (%)	Linear Growth (mm)		Inhibition (%)	Name	MIC (µg/ml)
	Control	Sample		Control	Sample		Control	Sample			
Trichophyton longifusus	100	80	20	100	25	75	100	40	60	Miconazole	70
Candida albicans	100	100	0	100	100	0	100	100	0	Miconazole	110.8
Aspergilus flavus	100	95	05	100	100	0	100	100	0	Amphotericin-B	20
Microsporum canis	100	90	10	100	35	65	100	45	55	Miconazole	98.4
Fusarium solani	100	75	20	100	100	0	100	100	0	Miconazole	73.25
Candida glaberata	100	100	0	100	100	0	100	100	0	Miconazole	110.8

Experimental

Plant material

The plants were collected in the month of April from the Peshawar region (N.W.F.P, Pakistan). The identification was confirmed by Medicinal Botanic Center, PCSIR Laboratories Peshawar, Pakistan, where voucher specimens were deposited.

Extraction

The plants were oven dried (70°C) for 24 – 26 h, chopped into small pieces and finally pulverized into powder. The powdered plant material (*M. longifolia*, stem – 54 g, leaves – 42.5 g and *Aloe vera* – 50 g) was exhaustively extracted with ethanol. The solvent was evaporated at low temperature under reduced pressure in rotary evaporator to obtain crude extracts (*M. longifolia*, stem – 1.4 g, leaves – 1.7 g and *Aloe vera* – 2.2 g).

Antifungal activity

Antifungal activity of the extracts was evaluated by agar tube dilution method [19]. The extracts (24 mg) dissolved in sterile DMSO (1.0 ml), served as stock solution. Sabouraud dextrose agar (SDA) (4ml) was dispensed into screw cap tubes, which were autoclaved at 121°C for 15 minutes and then cooled to 50°C. The non-solidified SDA media was poisoned with stock solution (66.6µl), giving the final concentration of 400 µg of the extract/ml of SDA. Each tube was inoculated with a piece (4mm diameter) of inoculum removed from a seven days old culture of fungi. For non-mycelial growth, an agar surface streak was employed. Inhibition of fungal growth was observed after 7days of incubation at 28±1°C. A control experiment with test substance (medium supplemented with appropriate amount of DMSO) was carried out for verification of the fungal growth. Growth in the medium containing the extracts was determined by measuring the linear growth in mm and the percent growth inhibition was calculated with reference to the negative control. The following formula was used for calculation:

$$\text{Inhibition (\%)} = 100 - \frac{\text{Growth in sample (mm)}}{\text{Growth in control (mm)}} \times 100$$

Phytotoxic activity

Phytotoxic activity of the crude extracts was tested against the *minor* L. [20]. The medium was prepared by mixing various constituents in 100 ml distilled water and the pH was adjusted (5.5 - 6.5) by adding KOH solution. The medium was then autoclaved at 121°C for 15 minutes. The extracts dissolved in ethanol (20 mg/ml) serving as stock solution. Nine sterilized flasks, three for each concentration, were inoculated with 1000 µl, 100 µl and 10 µl of the stock solution for 500, 50, and 5 ppm respectively. The solvent was allowed to evaporate overnight under sterile conditions. To each flask, medium (20 ml) and plants (10), each containing a rosette of three fronds, of *Lemna minor* L., was added. One other flask supplemented with solvent and reference growth inhibitor (Paraquat), served as negative control. All flasks were plugged with cotton and kept in the growth cabinet for seven days. The number of fronds per flask were counted and recorded on day seven. The results were interpreted by analyzing the growth regulation in percentage calculated with reference to the negative control by the following formula:

$$\text{Growth regulation (\%)} = 100 - \frac{\text{No.of fronds in test sample}}{\text{No. of fronds in negative control}} \times 100$$

Antibacterial activity

The extracts were screened against various human pathogens including *Corynebacterium diphtheriae, Escherichia coli, Klebsiella pneumoniae, Proteus morganni, Pseudomonas aeruginosa, Salmonella typhi, Shigella boydii, Staphylococcus aureus,* and *Streptococcus pyogenes* by agar well diffusion method [21]. Nutrient agar plates were swabbed with a 2 – 8 h broth culture of respective bacteria. Wells (6 mm diameter) were dugged in the media in each of these plates using a sterile metallic borer with centers at least 24 mm apart. Samples [1 ml (3 mg/ml of DMSO)] were then added in their respective wells using sterilized dropping pipettes. Other wells supplemented with DMSO and reference antibacterial drug (Imipenum, 10 µg/disc) serving as negative and positive controls, respectively. The plates were immediately incubated at 37 °C for 14 – 19 h. Activity was determined by measuring the diameter of zones showing complete inhibition (mm). Growth inhibition was calculated with reference to positive control.

Brine-shrimp cytotoxicity

Artemia salina (brine-shrimp eggs) were used to determine the cytotoxic activity of the various

samples [22]. Three different concentrations (10, 100 and 1000 µg/ml) of test samples were prepared by dissolving in DMSO as the solvent. Seawater was prepared by dissolving commercially available sea salt (3.8 g) into tap water (1:l). Brine shrimps hatched in seawater media at 27°C for 48 h. Ten shrimps; seawater (5 ml) and different amounts of each test sample were put in a vial. Two other vials were supplemented with solvent and reference cytotoxic drug serving as negative and positive controls respectively. Etoposide (LD_{50} = 7.465 µg/ml) was used as the standard reference cytotoxic drug. All vials were incubated at 25 – 27°C for 24 h and the survived brine shrimps were counted. The data was analyzed with Finney computer program to determine LD_{50} values with 95% confidence interval.

Insecticidal activity

Tribolium castaneum, *Sitophylus oxyzae*, *Rhyzopartha dominica*, and *Trogoderma granarum* were used to determine the insecticidal activity of the samples [18]. The insecticidal activity of plant extracts was determined by direct contact application using filter paper. 1 ml of the samples was applied by micropipette to filter papers (90 mm diameter), which gave 1571.33 µg/cm^2 concentration. After drying under a fume hood for 2 min, each filter paper was placed in the petri dish and then 10 adults of each of *Tribolium castaneum*, *Sitophylus oxyzae*, *Rhyzopartha dominica*, and *Trogoderma granarum* were placed in each petri dish which was covered with a lid. A check batch was treated with solvent for determination of solvent effect. A control batch was kept for the determination of environmental effects. Another batch was supplemented with reference insecticide (permethrin, 235.71µg/cm^2). All these were kept without food throughout 24 h exposure period. Mortality count was done 24 h after treatment.

References

1. S.I. Ali and Y.J. Nasir, *Flora of Pakistan* No. **192**, 256 (1990).
2. M.A. Ali, M. Saleem, W. Ahmad, M. Parvez, and R.Yamdagni, *Phytochemistry*, **59**, 889 (2002).
3. S. Ghoulami, A. Idrissi, and S. Fkih-Tetouani, *Fitoterapia* **72**, 596 (2001).
4. M. J. Pascual-Villalobos and A. Robledo, *Ind. Crops Prod.*, **8**, 183 (1998).
5. D. Nori-Shargh, H. Norouzi Arasi, S. Mohammad, M. Mirza, and K. Jaimand, *J. Essent. Oil Res.* **12**, 111 (2000).
6. M.I. Abu-Al-Futuh, O.H. Abdelmageed, R.M. Jamil, and P. Avato, *J. Essential Oil Res.* **12**, 530 (2000).
7. A. Kar, S. Panda and S. Bharti, *J. Ethnopharmacol.*, **81**, 281 (2000).
8. M. Sharaf, M.A. El-Ansari and N.A.M. Saleh, *Fitoterapia*, **70**, 478 (1999).
9. M.A. Ajabnoor, *J. Ethnopharmacol.* **28**, 215 (1990).
10. A. Paez, G.M. Gebre, M.E. Gonzalez and T.J. Tschaplinski, *Environ. Exp. Bot.*, **44**, 133 (2000).
11. A. Femenia, E.S. Sánchez, S. Simal and C. Rosselló, *Carbohyd. Polym.*, **39**, 109 (1999).
12. N.A. Shamaan, K. A. Kadir, A. Rahmat and W.Z.W. Ngah, *Nutrition*, **14**, pp. 846 (1999).
13. P. Chithra, G.B. Sajithlal and G. Chandrakasan, *J. Ethnopharmacol.*, **59**, 195 (1998).
14. G. Grindlay and T. Reynolds, *J. Ethnopharmacol.*, **16**, 117 (1980).
15. N. Okamura, N. Hine, S. Harada, T. Fujioka, K. Mihashi, N. Nishi, K. Miyahara and A. Yagi, *Phytochemistry*, **45**, 1519 (1997).
16. N. Okamura, N. Hine, S. Harada, T. Fujioka, K. Mihashi, N. Nishi and A. Yagi, *Phytochemistry*, **43**, 495 (1996).
17. Atta-ur-Rehman, M. Ashraf, M.I. Choudhary, Habib-ur-Rehman and M.H. Kazmi, *Phytochemistry*, **40**, 427 (1995).
18. D. Nath, N. Sethi, R.K. Singh and A.K. Jain, *J. Ethnopharmacol.*, **36**, 147 (1992).
19. J.L. McLaughlin, C.J. Chang and D.L. Smith, "Bench-Top" Bioassays for the Discovery of Bioactive Natural Products an update. *In:* Atta-ur-Rahman (Ed.) *Studies in Natural Products Chemistry*, Elseveir Science Publishers B.V., Amsterdam, **Vol. 9**, (1991).
20. Atta-ur-Rahman, M.I. Choudhary and J.W. Thomsen, "Manual of Bioassay Techniques for Natural Product Research", Harward Academic Press, Amsterdam, (1991).
21. B.N. Meyer, N.R. Ferrigni, J.T. Putnam, L.B. Jacobsen, D.E. Nichols and J.L. McLaughlin, *Planta Med.*, **45**, 31 (1982).
22. S.N.H. Naqvi, and F. Parveen, *Pak. J. Entomol.*, **6**, 35 (1991).