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Antimicrobial Studies of in-vitro Propagated Three *Mentha* Species on Novel Media

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Abstract

Three species of *Mentha* were propagated on MS medium and novel KFA medium for assessing comparative antimicrobial activity against some of the common pathogens. KFA propagated *M. spicata, M. arvensis* and *M. citrata* showed significant antimicrobial activity against *Escherichia coli* and *Candida albicans* as compared to plants propagated in MS medium. 70% ethanolic leaf extract of KFA propagated *M. citrata* showed highest zone of inhibition and *M. spicata* showed lowest zone of inhibition against *Escherichia coli.* Leaf extracts [70% ethanol: methanol: chloroform (4:3:3)] of KFA propagated *M. arvensis* showed maximum zone of inhibition against*C. albicans.* Minimum zone of inhibition was shown by MS propagated *M.citrata* and KFA propagated *M. arvensis.*

Keywords: KFA, MS, antimicrobial, *Mentha* species, *Escherichia coli, Candida albicans.*

INTRODUCTION:

About 25% of total medicines used are derived directly or indirectly from plants. Herbs with medicinal properties are in high demand as a source for alternate medicine among people in both developed and developing countries. This ever increasing demand for herbal medicines, poses further increase in demand for higher production of these plants. Medicinal plants represent a rich source of antimicrobial agents. Antimicrobial activity of plants is mostly due to the presence of phytochemicals like phenols, flavones, flavonoids, tannins, coumarins, essential oils, alkaloids etc. which can be extracted from various plant parts using different solvents. However, the

quality of an extract depends on the parts of plant material used, type of solvent used and type of extraction procedure used[1]. On a global basis, around 130 drugs, all single chemical entities extracted from plants or modified synthetically, are currently in use[2]. It has been also documented that various minerals in the plant extract contribute to the antimicrobial properties. Germicidal properties of copper and zinc have been demonstrated against *S. typhi* and *P. aeruginosa*[3]. Mentha is a genus of flowering plants belonging to the family Lamiaceae. It is sub-cosmopolitan in distribution across Europe, Asia, Africa and North America. Mints are aromatic, almost exclusively perennial herbs and are exclusively cultivated for their oils and terpenoid contents. *M. spicata* (Spearmint) oil benefits all respiratory problems, are refreshing to muscles, nervous and glandular systems. *M. citrata* has diaphoretic and vasodilator properties, also recommended for fevers and headaches. The juice of leaves of *M. arvensis* is an effective gargle in ailments of oral cavity. It is also used as an expectorant, uterine tonic, in the diseases of liver and spleen, asthma and for joint pains. In the present study, three species of *M. arvensis, M. spicata* and *M. citrate* were propagated on Murashige and Skoog(MS) medium[4] and on KFA medium(aims at reducing the cost of Plant Tissue Culture media). Various plants have been successfully established on low cost KFA medium by Biswas and Biswas[5].

MATERIALS AND METHODS:

Plant Material: *M. arvensis, M. spicata* and *M. citrata* were cultured on MS medium and on novel KFA medium[6] (10% fly ash+ nitrogenous sources+ plant growth regulators) with the hormone concentration of 0.5mg/L of IAA and 1.0mg/L of BAP.MS medium propagated plants were taken as control.

Preparation of crude extracts: Crude extracts were prepared in two different solvent systems. For first solvent system- 1g chopped leaves of tissue cultured plants were taken in mortar and were crushed in 4ml of 70% ethanol, 3ml of methanol and 3ml of chloroform. The extract was incubated at room temperature for 24 hours and then filtered and stored at 4°C.For second solvent system-100mg chopped leaves of tissue cultured plants were taken in mortar and were crushed in 1ml of 70% ethanol. The extract was incubated at room temperature for 24 hours and then filtered and stored at 4°C.

Preparation of inoculum: Bacterial inoculum was prepared by suspending 6-8 colonies of *E.coli* in 2ml autoclaved distilled water and fungal inoculum was prepared by suspending 3-4 colonies of *C. albicans* in 2ml autoclaved distilled water.

Antibacterial assay: Antibacterial assay was performed by using disc diffusion method. LB agar plates were used for comparing antibacterial activity of various crude extracts of three *Mentha* sp. grown in both KFA and MS media [KFA medium-70% ethanolic; KFA, 70% ethanol + methanol + chloroform (4:3:3); MS, 70%

ethanolic; MS medium- 70% ethanol + methanol + chloroform(4:3:3)]. 200µl of inoculum was spread on LB agar plates. Inoculum was allowed to dry for 5 minutes. Sterile individual discs (Whatman no.4) were placed in the center of the plate and was loaded with 5µl of extract. Ampicillin (10mg/ml) was used as a control. The plates were incubated at 37°C overnight. The plates were observed for the appearance of zone of inhibition and the antibacterial activity was evaluated by calculating diameter of zones.

Antifungal assay: Antifungal assay was performed by using disc diffusion method. Yeast Extract Peptone Dextrose (YEPD) agar plates were used for comparing antifungal activity of various crude extracts of three *Mentha* sp. (KFA medium and MS medium).The solvent system and rest of the method was as described in antibacterial assay. Flucanazole (2mg/ml) was used as a control. The plates were incubated at 27°C for 3 days. The plates were observed for the appearance of zone of inhibition and the antifungal activity was evaluated by calculating diameter of zones.

Statistical analysis: For determining whether there is a significant difference between the media used (MS and KFA) and between solvent systems used (70% ethanol and 70% ethanol: methanol: chloroform), ANOVA (Analysis Of Variance) was carried out. F value was calculated at 5% level of significance.

RESULTS:

Antibacterial activity: Extraction of crude extract was carried out from the leaves of the plants growing on MS media and KFA media using 2 solvent systems and the zone of inhibition against *E. coli* was compared (Table 1). Ampicillin (10mg/ml) was used as a control. The data was analyzed using ANOVA at 5% level of significance. No significant level of difference in the antimicrobial activity was found between different solvent systems used and different media used (Table 3).

Antifungal activity: Extraction of crude extract was carried out from the leaves of the plants growing on MS media and KFA media using 2 solvent systems and the zone of inhibition against *C. albicans* was compared (Table 2). The comparison was made between the solvent systems, species and media used. Flucanazole (2mg/ml) was used as a control. At 5% level of significance, the difference was insignificant for antifungal activity between different solvent systems used and different media used for three *Mentha* species using ANOVA (Table 3).

Table 1. Mean Zone of Inhibition of plant crude extracts (in mm± SD) against *E.coli*

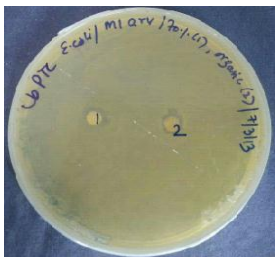
Solvent system	*Mentha arvensis*		*Mentha spicata*		*Mentha citrata*	
	MS	KFA	MS	KFA	MS	KFA
70% ethanol	4.5 ± 0.70	5.0 ± 0	3.0 ± 0	1.95 ± 0.07	6.9 ± 0.14	8.05 ± 0.07
70% ethanol: methanol: chloroform (4:3:3)	3.4 ± 0.56	4.9 ± 0.14	2.1 ± 0.14	2.25 ± 0.35	3.8 ± 0.28	3.95 ± 0.07

Table 2. Mean Zone of Inhibition of plant crude extracts (in mm± SD) against *Candida albicans*

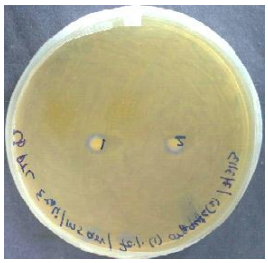
Solvent system	*Mentha arvensis*		*Mentha spicata*		*Mentha citrata*	
	MS	KFA	MS	KFA	MS	KFA
70% ethanol	3.1 ± 0.14	0 ± 0	1.4 ± 0.56	1.95 ± 0.07	1.1 ± 0.14	2.05 ± 0.07
70% ethanol: methanol: chloroform (4:3:3)	4.85 ± 0.56	1.1±0.14	1.95 ± 0.07	2.25 ± 0.35	2.0 ± 0	3.0 ± 0

Table 3. Table showing main ANOVA output

Output obtained for *M. arvensis*					Output obtained for *M. spicata*				Output obtained for *M. citrata*			
Source of variation	SS	df	MSS	R	SS	df	MSS	R	SS	df	MSS	R
Between solvents	0.36	1	0.36	1.44	0.09	1	0.09	0.12	12.96	1	12.96	51.84
Between media	1	1	1	4	0.2	1	0.2	0.27	0.42	1	0.42	1.68
Residual or error	0.25	1	0.25		0.74	1	0.74		0.25	1	0.25	
Total	**1.61**	**3**			1.03	3			**13.63**	**3**		
SS= Sum of squares; df= Degrees of freedom; MSS= Mean sum of squares, R= Ratio n of F												
At 5% level of significance, F_T =161.45; $F_{(Solvent)} < F_T$ and $F_{(Solvent)} < F_T$												



MS *Mentha arvensis*



KFA *Mentha arvensis*

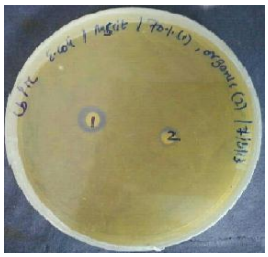
Fig 1. Showing zone of inhibition of MS and KFA *Mentha arvensis* crude extract extracted by solvents 1 and 2 on *Escherichia coli.*

MS *Mentha spicata*

KFA *Mentha spicata*

Fig 2. Showing zone of inhibition of MS and KFA *Mentha spicata* crude extract extracted by solvents 1 and 2 on *Escherichia coli.*

MS *Mentha citrata*



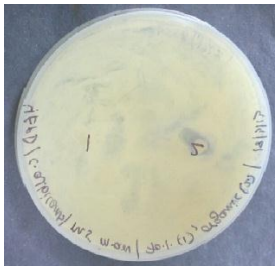
KFA *Mentha citrata*

Fig 3. Showing zone of inhibition of MS and KFA *Mentha citrata* crude extract extracted by solvents 1 and 2 on *Escherichia coli.*

Control Ampicillin (10mg/ml)

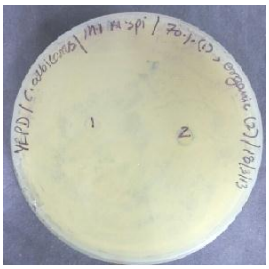
Fig 4. Showing zone of inhibition of control Ampicillin (10mg/ml) on *Escherichia coli.*

MS *M. arvensis*

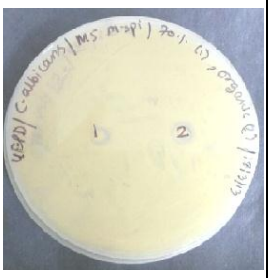


KFA *M.arvensis*

Fig 5. Showing zone of inhibition of MS and KFA *Mentha arvensis* crude extract extracted by solvents 1 and 2 on *Candida albicans.*



MS *M. spicata*



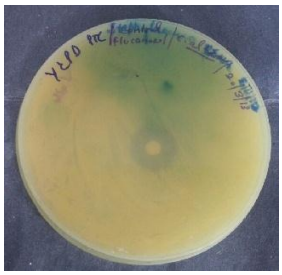
KFA *M.spicata*

Fig 6. Showing zone of inhibition of MS and KFA *Mentha spicata* crude extract extracted by solvents 1 and 2 on *Candida albicans.*

MS *M. citrata*

KFA *M. citrata*

Fig 7. Showing zone of inhibition of MS and KFA *Mentha citrata* crude extract extracted by solvents 1 and 2 on *Candida albicans.*



C. albicans.

Fig 8. Showing zone of inhibition of Control Flucanazole (2mg/ml) on *Candida albicans.*

DISCUSSION:

This study was aimed at comparing the antimicrobial and antifungal properties of organic solvent extracts from three species of Mentha grown in Murashige and Skoog[4] the most widely used plant tissue culture medium and a low cost, high efficiency novel medium KFA[6]containing Fly Ash developed in our laboratory. For **antibacterial studies**, zone of inhibition ranging from 1.95 mm to 8.05 mm were observed, 8.05 mm being the maximum zone of inhibition for *Mentha citrata* followed by *M. arvensis* and 1.95 mm being the lowest zone of inhibition for *M. spicata* grown in KFA medium. In all the three species an increase in the zone of inhibition in KFA grown plant extract as compared to MS grown plant extract was observed. Since this is the first antimicrobial study on *M. citrata* and no previous studies on this species have been reported till date a comparison could not be made. In the previous studies of other *Mentha* species, antimicrobial activity against human pathogens have been reported[7,8]. Various constituents of plant extract like phenols, flavones, flavanoids, tannins, coumarins, essential oils, alkaloids contribute to the antimicrobial property[1]. Changes in microenvironment of both biotic and abiotic factors like pH, temperature, growth hormones, light intensity, media components might lead to changes in these secondary metabolite productions[9]. In the present study increase in the zone of inhibition of KFA grown plant extract, can be attributed to the fact that Fly Ash in the KFA has contributed to the increase in secondary metabolite production which further contribute to the antibacterial property of the plant extracts. The addition of methanol and chloroform in the solvent system did not contribute to any increase in antimicrobial property but instead exhibited reduced zone of inhibition which was not statistically significant as shown by ANOVA. For **antifungal studies**, the microbe chosen was *Candida albicans* because *Candida* is known to cause opportunistic infections when the person is immune compromised. Because of its

eukaryotic origin and tough cell wall, these infections are very difficult to treat. Treatment in the form of synthetic drugs possesses harmful side effects which may affect natural flora and fauna of the human body. So there is a need for an alternative therapy. It has been previously suggested that phytotherapy can replace chemotherapy to a great extent as herbal medicines do not possess any harmful side effects[10]. Zone of inhibitions for *M. spicata* and *M. citrata* ranged from 0 mm to 4.85 mm, 0 mm being the lowest for *M. arvensis* in KFA whereas 4.85mm being the highest for *M. arvensis* in MS against the fungus. Similar results were obtained for other *Mentha* species in previous studies[11,12]. *M. arvensis* grown in KFA did not show any antimicrobial activity against *C. albicans* which can be due to the decrease in the particular secondary metabolite production which inhibited the growth of the fungus. Among the biotic factors genotype of a species plays an important role in its response and this difference in the response of *M. arvensis* could be due to its genetic makeup where forth the genes for the production of the inhibitory secondary metabolite may switch off in the KFA medium. It can be inferred that KFA medium have induced more production of certain secondary metabolites and increased antimicrobial activity.

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