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Role of Essential Oils of Some Higher Plants in Management of Food-borne Pathogenic Bacteria

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Annotation: Five food grade plants were used to extract essential oils for screening against three food-borne human pathogenic bacteria, namely Bacillus cereus, Enterococcus faecalis and Escherichia coli. Preliminary studied were performed by disc diffusion method which showed diverse result. Basil oil was found to be efficient against all tested bacteria exhibiting higher effectiveness against B. cereus (51.10±5.46) and E. faecalis (44.47±4.52) while, canum oil-controlled E. coli (37.63±6.33). During dilution studies, Ocimum basilicum oil was found to be prominent as zone of inhibition was persisted and comparable after dilution. MIC evaluation by broth microdilution technique, basil oil was found to be exceedingly effective against amoxicillin resistant E. faecalis (MIC 2.0 µl/ml), while unproductive against E. coli (MIC 16.0 µl/ml). These values were comparable with that of used reference antibiotics namely; amoxycillin, ceftriaxone and kanamycin.

Keywords: Antibacterial activity, Ocimum basilicum, Volatile oil, Bacillus cereus, Enterococcus faecalis and Escherichia coli, food-borne bacteria.

INTRODUCTION

Medicinal importance of aromatic plant products is known to man since the dawn of civilization. W.H.O. estimated that about three quarters of world population currently use herbs and other forms of traditional medicines (Inamdar *et al.*, 2008). India has a great wealth of traditional knowledge and wisdom. Over 2000 medicinal and essential oil bearing plants have been recorded in India.

Only a small fraction of the existing plant species has been investigated for medicinal and antimicrobial properties till date (Parimelazhagan *et al.*, 2005). Thus, the plant kingdom represents an untapped reservoir of medicinal and variety of natural compounds. Besides, natural plant products impart some additional features over pharmaceutical drugs and antibiotics, as these are affordable, easily available, free from side effects (Kumar *et al.*, 2007) and easy solution to antibiotic resistance (Singh *et al.*, 2002).

The herbs taken in study have rich value and usage in traditional Indian medicinal systems. *Mentha arvensis* is used in rheumatism, and as an antispasmodic, carminative, diuretic, emmenagogue, refrigerant, stimulant and stomachic (Ambasta, 1986; Chopra, Nayar and Chopra, 1956). *Murraya koenigii* is used for diarrhoea, dysentery and for checking vomiting. It is applied externally to cure eruptions and is also used as carminative, stomachic and tonic (Ambasta, 1986; Chopra, Nayar and Chopra, 1956). *Ocimum basilicum* is used for treatment of croup; leaf extract is used as nasal douch and for ringworm. It is reported as alexipharmac, anthelmintic, antipyretic, carminative, diaphoretic, expectorant, stimulant and stomachic (Ambasta, 1986; Chopra, Nayar and Chopra, 1956). *Ocimum canum* is used in parasitical skin diseases, as a diuretic and tonic (Ambasta, 1986; Chopra, Nayar and Chopra, 1956). *Ocimum gratissimum* is used in the treatment of gonorrhoea, paralysis and rheumatism. It is also used as anthelmintic, antiseptic, demulcent, digestive, diuretic, stimulant, stryptic and tonic; employed in cough mixtures (Ambasta, 1986; Chopra, Nayar and Chopra, 1956).

Thus present work aims to screen out 5 essential oils against three food borne pathogenic bacteria and selection of the most potent oil. Further, detailed evaluation of the most potent oil in terms of MIC and MBC values were made. The prevalent antibiotics are used as control.

MATERIALS AND METHODS:

Collection, extraction and storage of essential oils: Leaves of *Mentha arvensis* L., *Murraya koenigii* (L.) Spreng., *Ocimum basilicum* L., *Ocimum canum* Sims and *Ocimum gratissimum* L. were collected from local area of Khagaria district. Plant materials were characterized as per botanical characters. The oil from these plant materials were extracted separately using Clevenger's apparatus by hydro-distillation method. The oils were dried over anhydrous sodium sulphate and stored in cool, dark and dry place in air-tight screw-capped tubes until used.

Test pathogens: Strains of *Bacillus cereus*, *Enterococcus faecalis* and *Escherichia coli* were isolated and purified from the contaminated food as per Indian Standards IS:5887 (Part 6:2012), IS:15186-2002 and IS:5887 (Part 1:2005) respectively). These isolates were maintained on TSA (Trypticase Soy Agar) and modified NA (Nutrient Agar) medium and sub-cultured in two months. For experimental purpose, overnight inoculated Nutrient Broth (adjusted to 0.5 McFarland standard) having 1-2 x 10^8 cfu/ml (approx.) was used.

Determination of antibacterial efficacy by Disc Diffusion method (CLSI, 2012a): The antibacterial activities of the extracted oils were evaluated by disc diffusion method (CLSI, 2012a) with some modification. NA media (10 mL, pH 7.4) was poured in to 80 mm Petri plate and was allowed to solidify (30 min). It was then seeded by freshly prepared 0.1 mL bacterial suspension (0.5 McFarland Standard) within 15 min of preparation. A single drop of each oil was loaded on disc (Whatman No 1, 6 mm dia). Two such pre-soaked discs were placed on the seeded medium at 30 mm apart within 15 min of inoculation. Amoxycillin (10 mcg), Ceftriaxone (30 mcg) and Kanamycin (30 mcg) were used as reference antibiotics. Each of the oils and antibiotics treatments was set up in triplicates. All the plates were incubated at 35±2°C for 18-20 hr and zones of inhibition (ZOI) were recorded in mm.

Apart from the procedure of recording zone of inhibition of various crude oils, some effective oils were diluted up to 50%, 20%, 10% and 4% to study their antibacterial efficacy in terms of zone of inhibition.

Determination of Minimum Inhibitory Concentration (MIC) by Agar Microdilution method

(CLSI, 2012b): For evaluation of MIC of oils, Agar microdilution method as proposed by CLSI, 2012b was adopted with some modification. A range of doubling dilutions of oils (0.06-32.0 µl/ml) was prepared in NA medium (pH 7.4, 10 ml). Tween 80 (0.5% v/v to the final volume) was added to enhance the dispersion of oil in medium. Another set of plates containing recommended range of antibiotic was also prepared. Each plate was spot inoculated by 5 µl overnight broth (a loopful inoculum of 2 mm dia, HiMedia, Mumbai) inside the three premarked circles. Positive (inoculated NA plate) and negative (uninoculated NA plate) were also maintained. The experiment was organized in triplicate. All control and treatment sets were incubated at 35±2°C for 18-20h. The MIC was read as the lowest concentration which inhibited the visible growth of test organisms.

Determination of Minimum Bactericidal Concentration (MBC): MBC of oils was determined following Mishra *et al.* (2008). In this, inoculum was taken from different poisoned plates (prepared during MIC determination) exhibiting no growth. Inoculum was streaked on fresh oil/antibiotic free NA medium. Control set was maintained by streaking test bacteria. All the set was performed in triplicate. All control and treatment sets were incubated at 35±2°C for 18-20h. The MBC was read as the lowest concentration which inhibited the visible growth of test organisms on fresh medium.

RESULTS AND DISCUSSION

General antibacterial screening of different oils and antibiotics as well as effect of dilutions on potency of selected oils was recorded in Table 1. Table 2 depicts the MIC and MBC values of the most potent oil (*Ocimum basilicum*) and antibiotic against all three test pathogens.

The disc diffusion assay indicated mixed result with high degree of variation as basil oil was found to be effective against all tested bacteria exhibiting higher efficacy against *B. cereus* (51.10±5.46) and *E. faecalis* (44.47±4.52) while, canum oil inhibits *E. coli* (37.63±6.33). Oils of *M. koenigii* and *O. gratissimum* exhibits lower degree of inhibition in the terms of zone of inhibition. During MIC evaluation, *O. basilicum* oil was found to be highly effective against *E. faecalis* (MIC 2.0 µl/ml), while ineffective against *E. coli* (MIC 16.0 µl/ml). These values were comparable with that of reference antibiotics namely; amoxycillin, ceftriaxone and kanamycin. Basil oil exhibited the marked activity against amoxicillin resistant *E. faecalis* (MBC being 8.0 µl/ml versus >128 µg/ml).

The findings of present study shows the concurrent similarities of the several studies reviewed by Wirtu *et al.* (2024) in which the basil oil have capability to control the various strains/species of *Bacillus, Eneterococcus, Staphylococcus, Pseudomonas* and *Salmonella*. Some studies in the above mentioned review contradict the findings as *E. coli* exhibited very low degree of inhibition in present findings, while others reported prominent control of *E. coli* by *O. basilicum* oil. This variation may be caused due to the source of strain isolation like clinical or food borne. The discrepancy might be observed as the oil composition may have seasonal variation as suggested by Hussain *et al*. (2008). Linalool is the major compound and supposed to be active constituent for antibacterial activity (Hussain *et al*., 2008).

Table 1. Antibacterial activity of essential oils and antibiotics against human pathogenic bacteria

Plant oil*/antibiotics	Plant part used	% yield of oil	% oil [a]	Zones of inhibition **		
				Bacillus cereus	*Enterococcus faecalis*	*Escherichia coli*
Mentha arvensis L. (Lamiaceae)	Dry leaf	1.60	100	24.10 ± 3.40 [b]	27.30 ± 3.66	17.40 ± 4.77
			50	27.00 ± 5.11	32.66 ± 4.54	19.13 ± 1.33
			20	27.15 ± 4.00	26.57 ± 3.61	15.00 ± 3.11
			10	15.24 ± 2.30	17.43 ± 4.35	13.13 ± 1.82
			4	12.07 ± 2.01	16.77 ± 1.82	13.71 ± 1.55
Murraya koenigii	Dry	1.50	100	18.17 ± 2.11	16.61 ± 2.01	10.84 ± 1.95

(L.) Spreng. (Rutaceae)	leaf					
Ocimum basilicum L. (Lamiaceae)	Dry leaf	5.00	100	51.10 ± 5.46	44.47 ± 4.52	26.37 ± 3.32
			50	# [c]	35.85 ± 5.18	23.63 ± 2.23
			20	34.31 ± 3.50	29.23 ± 3.66	17.92 ± 2.51
			10	28.35 ± 2.60	28.00 ± 4.33	17.80 ± 3.16
			4	17.66 ± 2.44	25.77 ± 1.29	15.65 ± 1.33
Ocimum canum Sims (Lamiaceae)	Dry leaf	1.75	100	14.25 ± 2.50	#	37.63 ± 6.33
			50	33.50 ± 6.65	28.25 ± 2.55	19.40 ± 3.33
			20	28.45 ± 4.37	27.87 ± 3.19	15.30 ± 2.85
			10	24.34 ± 2.42	23.87 ± 1.11	13.56 ± 2.27
			4	17.66 ± 2.92	18.54 ± 4.66	13.30 ± 2.50
Ocimum gratissimum L. (Lamiaceae)	Fresh leaf	0.65	100	22.52 ± 6.75	24.80 ± 3.30	10.50 ± 1.15
Amoxycillin 10 mcg	-	-	-	16.90 ± 0.85	37.55 ± 4.62	22.66 ± 1.25
Ceftriaxone 30 mcg	-	-	-	27.80 ± 1.75	25.05 ± 1.14	34.97 ± 2.50
Kanamycin 30 mcg	-	-	-	25.50 ± 3.66	36.62 ± 1.75	32.75 ± 3.33

[*] 5 µl oil per disc were used.

[**] Zone of inhibition including disc diameter.

[a] Dilution studies of those oils which exhibited higher efficacy.

[b] Values represented as mean of three replicate (n=12) with standard deviation.

[c] No growth was present indicating complete inhibition.

Table 2. MIC and MBC value of *Ocimum basilicum* and antibiotics against human pathogenic bacteria

Oil/Antibiotics / Test organism	*Ocimum basilicum* [*]		Amoxycillin [**]		Ceftriaxone [**]	
	MIC	MBC	MIC	MBC	MIC	MBC
Bacillus cereus	4.0	>16.0	NT [a]	NT	32.0	>128.0
Enterococcus faecalis	2.0	8.0	16.0	>128.0	NT	NT
Escherichia coli	16.0	>16.0	NT	NT	4.0	4.0

[*] Values in µl/ml

[**] Values in µg/ml

[a] Not tested

CONCLUSION:

All the tested oils showed definite antibacterial activity on all the test bacteria used. Oils from *O. basilicum* and *O. canum* were highly effective while *M. koenigii* was least effective in inhibiting the growth of test pathogens. *O. basilicum* oil was found to be the most effective against amoxycillin resistant *E. faecalis* with the MIC value of 2.0 µl/ml, which was significantly lower than that of reference antibiotic. The result may be more prominent as their might be the possibility of drug resistance as per MBC values. These findings are important for future medicinal uses of *Ocimum basilicum* oil in place of prevalent antibiotics.

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