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An efficient edible anti-bacterial aqueous solution to purify food juices

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ABSTRACT

This study was conducted to formulate an economically viable aqueous antibacterial formulation using edible essential oils to purify bacterially contaminated fresh juices sold at roadside outlets in tropical countries, which maintain very low/nil hygiene conditions. Such a formulation was felt necessary to prevent epidemic outbreaks of infective diseases which occur due to consumption of fresh juices (like sugarcane juice) sold at such outlets. As part of the study, the level of bacterial contamination in sugarcane juice sample collected from a roadside outlet in Belgaum, Karnataka was accessed and predicted to have 4000micro-organisms/100ml of juice. Gram positive bacteria Bacillus subtilis & Staphylococcus aureus and Gram negative bacteria E. Coli & Klebsiella contaminants were present in the samples. To reduce this high degree of contamination, four essential oils i.e. Clove, Cinnamon, Thyme and Lemon grass were tested individually and in combination for their antibacterial activity against the isolated contaminants. The best effective formulation was then retested and found to effectively purify the juice sample (1ml/1000ml) within fifteen minutes of its addition.This formulation of essential oils can be added to fresh juices sold at roadside outlets to reduce their degree of bacterial contamination and hence help in preventing outbreaks of infective diseases as well as improve the general health conditions of people.

Key words: *Bacillus subtilis, Staphylococcus aureus, E. Coli, Klebsiella*, Clove, Cinnamon, Thyme and Lemon grass.

INTRODUCTION

During summers in a tropical country like India, in order to gain respite from the rising mercury people tend to consume fresh juices. As a result, several roadside stalls spring up to cater to the growing demand. In view of the unsatisfactory sanitary conditions that prevail in the vicinity of

these stalls there is an increase in outbreaks of infective diseases [1-6]. There is always a need to prevent such outbreaks which can affect hundreds of people in a largely populated country like India. Several studies have shown the sugarcane juice sold at roadside outlets to be contaminated with pathogenic organisms [4]. Innovative and economically viable methods are necessary to prevent such outbreaks. This need forms the basis of this project where in edible essential oils are used to formulate an effective, economically viable aqueous antibacterial solution for fresh juices.

The study was carried out in two phases. The first phase was aimed at isolation and identification of bacterial contaminants from sugarcane juice sample obtained from a roadside outlet. The second phase was aimed at formulating an aqueous solution comprising essential oils including Clove, Cinnamon, Lemongrass and Thyme. These oils were selected on the basis of previous research in plant essential oils [7]

EXPERIMENTAL SECTION

2.1 Sample Collection and MPN (Most Probable Number)

Sugarcane juice sample was collected in 250ml conical sterile flask from a roadside vendor and stored in an ice box. The approximate number of microorganisms was predicted by the Most Probable Number (MPN) method [8]. The samples were diluted upto 10^{-4} dilution in distilled water.

2.2 Isolation and identification of the bacterial contaminants

Bacterial contaminants were isolated from the sample by streak plate method on several differential and selective growth media including Nutrient agar (NA), Mac Conkay agar (MAC), Eosin Methylene Blue agar (EMB), Blood agar (BA) and Xylose Lysine Deoxycholate agar (XLD) incubated at 37˚ C for 24 hours. This was followed by biochemical tests for identification using Himedia IMViC biochemical test kit. Each HiMViC kit is a standardized colorimetric identification system utilizing four conventional biochemical tests and eight carbohydrate utilization tests. The kit divided into twelve slots each for individual tests 1) Indole test 2) Methyl red test 3) Voges proskower 4) Citrate utilization test 5) Glucose test 6) Adonitol Test 7) Arabinose test 8) Lactose test 9) Sorbitol test 10) Mannitol test 11) Rhamnase test 12) Sucrose test

2.3 Antibacterial activity of essential oils

Four essential oils i.e. Lemongrass, Clove, Cinnamon and Thyme commercially purchased from Falcon, Bangalore (Exporters of Essential Oils) were individually and in combination tested for their antibacterial activity using "well-method" on NA plates cultured with isolated contaminants. The diameters of the wells were 5mm and 40 µl of test sample was used for each well. Incubation time 24 hrs at 37 ° C

RESULTS

4000 micro-organisms were predicted to be present per 100ml of the juice sample by the Most Probable Number (MPN) test using faecal coliforms as the indicator organisms. Streaking the sample on NA, MAC, EMB, BA and XLD media and biochemical tests led to the identification

of gram positive bacterial contaminants *Bacillus subtilis* & *Staphylococcus* aureus and gram negative contaminants *E. Coli* & *Klebsiella* were isolated on differential and selective growth media followed by biochemical tests.

Table 1: Zone of inhibition by individual essential oils clove, cinnamon, thyme and lemongrass

Zone of inhibition (in mm)				
	S.aureus	*Bacillus subtilis*	*E.coli*	*Klebsiella*
Thyme oil	29	23	15	13
Clove oil	20.5	16	14	14
Cinnamom oil	29.5	19	23	15.5
Lemongrass oil	14	38	13	24

Table 2: Four essential oils mixed in twenty five different ratios with ethanol as a solvent and water the carrier

Combination number	Thyme%	Lemongrass%	Cinnamon%	Clove%	Enthanol%	Water%
1	1	0.5	0.75	1.3	2	94.45
2	0.5	0.5	0.75	1.3	2	94.95
3	0.75	0.5	0.75	1.3	2	94.75
4	0.25	0.5	0.75	0.67	2	95.88
5	0.5	0.5	0.75	0.67	2	95.58
6	0.5	0.5	0.75	1	2	95.25
7	0.75	0.5	0.75	1	2	95
8	1.25	0.5	0.75	1.5	2	94
9	0.75	0.75	1.1	1	2	94.4
10	2.5	0.5	0.75	2.5	2	91.75
11	2.5	0.5	2.5	0.75	2	91.75
12	2.5	2.5	0.75	0.5	2	91.75
13	0.5	2.25	2.5	0.5	2.25	91.75
14	0.5	0.5	2.5	2.5	2.25	91.75
15	2.5	0.5	2.5	2.5	2.25	89.75
16	2.5	2.5	2.5	0.5	2.25	89.55
17	0.5	2.5	0.5	2.5	2.25	91.75
18	2.5	-	-	-	2.25	95.25
19	0.5	-	-	-	2.25	97.25
20	-	-	2.5	-	2.25	95.25
21	-	-	0.5	-	2.25	97.25
22	-	2.5	-	-	2.25	95.25
23	-	0.5	-	-	2.25	97.25
24	-	-	-	2.5	2.25	95.25
25	-	-	-	0.5	2.25	97.25

Each of the four selected essential oils clove, cinnamon, thyme and lemongrass produced zone of inhibitions against the isolated contaminants (**Table 1).** However, twenty five formulations comprising the four essential oils, ethanol as solvent and water as a carrier were formulated in different ratios to gain maximum efficiency (**Table 2**). These formulations tested for their antibacterial effect against the bacterial contaminants isolated showed significant zone of inhibitions (**Table3**).

Of the twenty five formulations, one formulation no 17 comprising 0.5% Thyme oil, 2.5% lemongrass oil, 0.5% cinnamon oil and 2.5% of Clove oil was found to be the most efficacious formulation producing zone of inhibitions of 14mm (*S aureus*); 11mm (*B subtilis*); 17mm (*E. coli*) and 16mm (*Klebshiella*).

Then, 1 ml of the formulation number 17 was added to 1000ml of sugarcane juice sample and MPN test was setup 15 minutes after the addition. 0 micro-organisms were predicted by MPN test.

Table 3: Zone of inhibition produced by the 25 ratios of essential oils against the isolated bacterial contaminants gram positive bacteria *Bacillus subtilis* & *Staphylococcus aureus* and gram negative bacteria *E. Coli* & *Klebsiella* are shown in millimeters

Combination no	*S. aureus*	*Bacillus*	*E.coli*	*Klebsiella*
1	12	8.5	8.5	12
2	18	10	9	9.5
3	16	8.5	13.5	6
4	16	10.5	8.5	11
5	15	10	17	8
6	12.5	10	19	12
7	16	9	15	17
8	18	7	12.5	18
9	12	11	22	13
10	8	12	17	7
11	6	13	17	13
12	8	11	11	12
13	6	16	17	9
14	12.5	12	8	12
15	7	13	13	12
16	17	12	10	13
17	14	11	17	16
18	9	6	10	12.5
19	10	12	12	12.5
20	8	10	16	17
21	12	23	15	8.5
22	13.5	10	12	12.5
23	12	12	20	18
24	6	6	21	7
25	6	13	12	6

CONCLUSION

The outcomes of this study supports previous reports that sugarcane juice sold at roadside outlets is contaminated with pathogenic organisms. We also show that edible essential oils in combinations rather than individually can efficiently reduce bacterial contamination in food juices. Such a formulation can be used on a large scale as an economically viable potential bacterial purifier of food juices to prevent outbreak of infective diseases from roadside outlets

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