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Effect of different growth conditions on essential oil content from *Cymbopogon martini* (ROXB.) W. Watson and its application

Gauri Sawant*, Jessy Pius
Department of Botany, Ramnarain Ruia Autonomous College, Matunga, Mumbai, Maharashtra, India

Abstract
Cymbopogon martini (Roxb.) W. Watson of Poaceae is an aromatic plant best known as Palmarosa as it smells rose–like. In the present work, essential oil composition and yield of *Cymbopogon martini* (Roxb.) W. Watson (Palmarosa) was evaluated in response to three different growth medium (soil, hydroponics and aggregate hydroponic system). Air dried aerial part of the plant was hydro-distilled and the essential oil was studied by GC-MS. High percentage oil was obtained from Palmarosa grown in hydroponics followed by that grown in aggregate hydroponic system and in soil. According the essential oil profile, the main components were Geraniol, Citral and Geranyl acetate. Total terpenoids determined was found to be significantly high in hydroponically grown Palmarosa (52.31±2.06mg/gm) followed by that in aggregate hydroponic system (47.78±3.33mg/gm) and in soil (42.39±2.45mg/gm). Palmarosa grown in hydroponic cultivation technique had higher percentage of Geraniol (75.64%) than the other two growth media. The oil extracted from Palmarosa was used for making soap. The formulated soap was graded according to BIS (Annex IS 13424:2001). Antimicrobial activity of the formulated soap was also evaluated for its efficacy.

Keywords: *Cymbopogon martini, hydroponics, essential oil, gc-ms, antibacterial*

Introduction
Aromatic plants are in great demand in market due to the presence of pharmacologically important essential oil content. The essential oil holds enormous applications as raw material in the field of pharmaceutical, agronomic, food, sanitary, cosmetic, and perfume industries [1]. Essential oils have such huge applications due to their various biological properties like antimicrobial, antioxidant, anti-inflammatory, etc. [2]. Hence, in the present study efforts were made to develop methods by which quality and quantity of essential oil can be enhanced.
Cymbopogon martini (Roxb.) W. Watson belongs to the family Poaceae. It is popularly recognized as Palmarosa as it smells Rose - like. It is cultivated for its essential oil which is a copious source of Geraniol [3]. The essential oil extracted is pale yellow in colour with viscosity nearly similar to water [4].
Palmarosa oil has been reported to carry antimicrobial [5, 6, 7], antioxidant [8, 9, 4] and antihelmentic properties [10, 11]. Literature survey on properties of *C. martini* essential oil revealed antiparasitic [12], anti-inflammatory [13] and anticonsulvant [14] activities. This paper presents cultivation of *C. martini* in different growth media (soil, hydroponics and aggregate hydroponics), extraction, identification of essential oil components, formulation of a product (Herbal Toilet soap) and testing its antibacterial activity.

Material and Methods
Cultivation
C. martini was grown in automatically controlled Greenhouse. The seeds of *C. martini* were germinated in cocopeat. The seedlings were then transferred to respective growing conditions. Plants were grown in well drained Loamy soil. However, in soilless methods: Nutrient film technique (NFT) and aggregate hydroponic system (Drip irrigation method) were set up. In NFT, Clay balls were used as the support medium and cocopeat for aggregate hydroponics. Nutrients solution of Flora series (General Hydroponics) was used for soilless technology as per the growing stages of the plant. The nutrient medium was maintained at EC 800 to 1000 microsiemen according to the growing stage of the plants.

Determination of Total Terpenoids
Methanolic extracts were made from aerial parts of *C. martini* grown in various growing conditions. Estimation of total terpenoids was carried out according to Ghorai *et al.* [15]. The absorbance of the reddish brown coloured solution was measured at 538 nm. 95% Methanol was considered as blank. Linalool was used as standard. Each extract was prepared in triplicate.

Hydro-Distillation of Palmarosa Essential oil
The collected aerial parts of the plant were shade-dried at room temperature for 24 hours and were subjected to hydro-distillation using Clevenger apparatus for 3 hours [16].

GC-MS of Palmarosa Essential oil
The Essential oils of *C. martini* grown in various growing conditions were analysed by Shimadzu GCMS-QP2010 Ultra system equipped with Rtx-5MS (5% diphenyl/ 95% dimethyl polysiloxane) capillary column of 30m length, 0.25 µm internal diameter and 0.25 µm film thickness. Helium was used as a carrier gas. The ionization energy of 70 eV was used for electron ionization system. The ion source temperature was 220°C, injection temperature was 270°C and interface temperature was 280°C. The oven Temperature was initially set at 80°C with the hold time of 2 mins, then it was raised to 180°C at 4°C/min with the hold

time of 6 mins and finally it was raised to 230°C at 6°C/mins with the hold time of 19 mins.
1.0 µl diluted oil samples of *C. martini* (10:100 in n-Hexane, v/v) were individually injected by automated syringe in the split mode. Individual essential oil compounds were identified by comparison of their mass spectra with those given by Wiley and NIST Libraries.

Formulation of toilet Soap from extracted oil
Sodium hydroxide solution was made by carefully dissolving 100gms of Sodium Hydroxide was in 230gm of distilled water. 700 gm coconut oil was heated with *C. martini* leaves till it reached 100°C. The oil was filtered and the filtrate was used for the further procedure. The sodium hydroxide solution was carefully added to filtered coconut oil. This mixture of coconut oil and sodium hydroxide solution was mixed thoroughly by whisk. 2ml *C. martini* essential oil was added and then it was blended till entire mixture became light trace. It was then allowed to set for 3 days in a mould.

Soap Grading
The formulated soap was tested by Solvent extraction and evaporation as per the standard method in Annex IS 13424:2001.

Determination of Minimum inhibitory concentration (MIC) by Micro dilution broth method
The MIC of formulated *C. martini* soap, Medimix herbal soap and Dettol soap was determined by micro-dilution broth method as given by Valgas *et al.* (modified) [17]**.** Two positive strains of bacteria (*Bacillus subtilis (B. subtilis)* and *Staphylococcus aureus* (*S. aureus*)) and two gram negative strains of bacteria (*Escherichia coli* (*E. coli)*, *Klebsellia pneumonia* (*K. pneumonia*)) were used for the study. Nutrient broth with bacterial culture and 1% TTC was used as positive control and nutrient broth with 1% TTC was used as Negative control**.** All test tubes were then incubated at 37°C for 24 hrs. MIC was determined based on the development of pink colour.

Agar well diffusion Method
It was carried out as per Attaurrahman *et al.* [18]. Antimicrobial activity of prepared soap was compared with that of Medimix soap and Dettol soap. The antimicrobial activity was evaluated by measuring the diameter (mm) of the zone of inhibition (Mean± SD).

Statistical Analysis
Data obtained were subjected to ANOVA.

Results and Discussion
Percentage oil extracted and total terpenoids content
The total amount of essential oil extracted from the *C. martini* cultivated in soil, Hydroponics and aggregated hydroponics system showed a significant difference in their yield. The essential oil (1.32%) extracted from *C. martini and* Total terpenoids content (52.31±2.06mg/gm) were found to be significantly high in hydroponic grown plants followed by that grown in Aggregate hydroponic and soil (Table 1) (Fig. 1).

Table 1: Percentage of essential oil and total terpenoids content in *C. martini* in different cultivation conditions. (Total terpenoids: Mean ± S.D)

Cultivation conditions	Essential oil extracted (%)	Total terpenoids (mg/gm)
Soil	0.85	42.39±2.45
Hydroponics	1.32	52.31±2.06
Aggregate Hydroponics	1.04	47.78±3.33

Fig 1: *C. martini* grown in different cultivation conditions

GC-MS analysis
The essential oil obtained from *C. martini* grown in soil, hydroponics and aggregate hydroponics was pale yellow in colour. The percentage composition of the essential oils, were analyzed by Gas Chromatography-Mass Spectroscopy (GC-MS) technique (Shimadzu GCMS-QP2010 Ultra system). The analysis revealed 60 different components in essential oil extracted from plants grown in soil system, 57 components in hydroponically grown plants and 41 components in plants grown in aggregate hydroponic system. Of all essential oil components, Geraniol was found to be the major essential oil in *C. martini* grown at different conditions. Similar observation was also reported by Rao *et al.* [16] in *C. martini*. Geranyl acetate was the second major component in all cultivation conditions. Hence, geraniol and Geranyl acetate can be considered as the chief constituents of the essential oil. Mallavarapu *et al.* [3] reported Geraniol and Geranyl acetate as dominant constituents of the essential oil under normal cultivation conditions. In addition to these, percentage area of Citral, Epoxylinalol, Caryophyllene oxide, Trans-Farnesol, Geraniol butyrate and linalool were also found to be slightly higher compared to other components. Few unique constituents of essential oil extracted from the plants cultivated under different conditions were found in GC-MS analysis. However most of these unique constituents were in trace amounts (Fig 2, table 2).
Comparative study of essential oils of *C. martini* grown under different cultivation conditions exposed, higher area percentage of geraniol in hydroponic grown plants (75.64 area %) compared to aggregate soil and hydroponic grown plants. The area percentage of geranyl acetate was found to be lesser in soil grown plants (3.9 area %) compared to plants grown in aggregate hydroponic and hydroponics. However, the essential oil of soil grown plants showed greater area percentage of citral (6.4 area %) in comparison to hydroponic and aggregate hydroponics grown plants (Fig 2, Table 2).

Table 2: GCMS analysis of *C.martini* grown in soil (S), hydroponics (H) and aggregate hydroponics (AH)

Name	Retention Time	S	H	AH
		Area%	Area%	Area%
1-Cyclohexylethanamine	2.52	0.38	0.1	-
2-Amino-N-methylpropanamide	2.61	-	0.25	-
DL-2-Amino-1-propanol	2.615	0.26	-	-
Nitrous oxide	2.748	-	0.47	-
3,6-Difluoro-4-[1-hydroxy-2-(Methylamino) ethyl]-1,2-benzenediol	2.92	-	0.03	-
5-Hepten-2-one, 6-methyl-	3.162	0.11	0.05	-
Linalyl isobutyrate	3.213	0.04	-	-
.Beta.-Myrcene	3.221	-	0.27	0.01
2-Methyl-6-hepten-1-ol	3.247	0.05	-	-
3-Undecyne	3.27	0.01	0.02	-
Furan, tetrahydro-2,2-dimethyl-5-(1-methylethyl)-	3.356	0.01	0.01	-
.alpha.-Pinene epoxide	3.5	-	0.01	-
1,5-Hexadiene-3,4-diol, 2,5-dimethyl-	3.673	-	0.01	-
D-Limonene	3.885	0.12	0.11	0.03
.alpha.-Pinene	3.98	-	-	0.02
trans-.beta.-Ocimene	3.988	0.01	0.09	-
2,2,4-Trimethyl-2,5-dihydrofuran	4.119	0.04	-	-
4-Sec-butyl-2,3-dihydrofuran	4.13	-	0.02	-
cis-.beta.-Ocimene	4.183	0.02	-	0.08
.beta.-Ocimene	4.189	-	0.21	-
Citronellol	4.312	-	0.04	0.01
6-Methyl-6-hepten-2-one	4.315	0.1	-	-
.gamma.-Terpinene	4.45	-	0.01	-
6-Methyl-2-(2-oxiranyl)-5-hepten-2-ol	4.73	0.09	0.12	-
.alpha.-Terpineol	5.051	-	-	0.01
Linalool	5.288	1.01	0.85	0.33
Nonanoic aldehyde	5.365	0.02	-	-
3-Octen-2-ol, 2-methyl-, (Z)-	5.833	0.04	-	-
1-Methyl-1-(2-methyl-2-propenyl) cyclopentane	6.17	0.02	-	-
.alpha.-Terpineol	7.659	-	0.02	-
cis-Geraniol	8.726	-	-	0.08
Oxiranecarboxaldehyde, 3-methyl-3-(4-methyl-3-pentenyl)-	8.824	0.44	0.15	0.09
Geraniol	9.834	70.96	75.64	70.85
Citral	10.062	6.4	2.55	1.13
Epoxy-linalooloxide	10.452	0.42	0.05	-
Linalyl formate	10.921	1.76	0.56	0.12
Oxiranemethanol, 3-methyl-3-(4-methyl-3-pentenyl)-	12.006	1.2	0.2	0.14
Geranic acid	12.807	0.09		-
Neric acid	12.866	-	0.09	-
Epoxy-.alpha.-terpenyl acetate	13.3	0.21	0.01	0.01
Geranyl acetate	13.483	3.9	10.2	12.23
Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S (1.alpha.,2.beta.,4.beta.)]-	13.665	-	0.03	0.05
Geranyl vinyl ether	13.751	0.08	-	-
2,6-Dimethyl-1,7-octadiene-3,6-diol	14.184	-	0.01	-
Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-	14.49	0.15	0.77	-
5,8-Decadien-2-one, 5,9-dimethyl-, (E)-	14.822	0.01	-	-
Linalool oxide	14.973	0.16	-	-
2,6-Octadiene-1,8-diol, 2,6-dimethyl-	15.092	0.11	-	-
1,5,9,9-Tetramethyl-1,4,7-cycloundecatriene	15.518	-	-	0.09
Humulene	15.524	0.02	0.07	-
(E)-.beta.-Famesene	15.646	-	0.04	-
2-Norpinene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-	16.83	-	0.02	-
Farnesene	17.221	-	0.03	-
6-Methyl-6-nitroheptan-2-one	17.58	0.1	-	-
2,3,6-Trimethyl-7-octen-3-ol	17.695	1.1	-	-
.alpha.-Limonene diepoxide	18.615	0.26	0.03	-
Linalool oxide	18.698	-	-	0.12
Epoxylinalol	18.72	2.23	0.16	-

Geraniol butyrate	18.79	-	-	0.38
(.+/-.)-trans-Nerolidol	18.886	-	0.06	0.04
Caryophyllene oxide	19.438	1.28	0.54	1.64
3-Methyl-5-propylnonane	19.906	-	-	0.01
3,5-Dimethylcyclohex-1-ene-4-carboxaldehyde	20.191	-	-	0.01
1,3-Hexadiene, 3-ethyl-2,5-dimethyl-	20.204	-	0.02	-
2,6-Dimethyl-3-cyclohexene-1-carbaldehyde	20.207	-	0.06	-
Tetracyclo [6.3.2.0(2,5).0(1,8)] tridecan-9-ol, 4,4-dimethyl-	21.007	-	0.03	0.02
1,4-Methanoazulen-7-ol, decahydro-1,5,5,8a-tetramethyl-, [1s (1.alpha.,3a.beta.,4.alpha.,7.beta.,8a.beta.)]-	21.636	-	0.02	-
Longifolenaldehyde	22.014	0.05	-	-
Viridiflorol	22.024	-	0.04	-
trans-Farnesol	23.477	0.44	2.94	5.94
trans,trans-Farnesal	24.006	-	0.05	0.04
Geraniol butyrate	24.318	0.82	1.91	-
Hexanoic acid, 3,7-dimethyl-2,6-octadienyl ester	24.335	-	-	3.85
Carotol	25.741	0.02	-	-
1(2H)-Naphthalenone, octahydro-4a,8a-dimethyl-7-(1-methylethyl)-, [4aR-(4a.alpha.,7.beta.,8a.alpha.)]-	25.755	-	0.01	-
Phytol, acetate	26.534	-	-	0.04
Farnesyl acetate	26.652	-	-	0.75
lilac alcohol epoxide	27.914	0.2	-	0.04
2-Isopropenyl-5-methylhex-4-enal	28.375	0.08	-	-
Farnesol	28.571	0.07	0.49	0.01
Butanoic acid, 2-methyl-, 3,7-dimethyl-2,6-octadienyl ester, (E)-	29.838	-	0.16	1.27
Hexadecanoic acid	30.422	-	-	0.04
7,11-Dimethyldodeca-2,6,10-trien-1-ol	34.234	-	0.39	-
Nonadecanol	35.1	-	-	0.21
cis,trans-Farnesol	35.917	-	-	0.06
3,7,11-Trimethyl-dodeca-2,6,10-trienoic acid	36.02	0.08	-	-
Phytol	36.045	-	-	0.04
Pulegone	36.09	0.02	-	-
Hexadecamethyl-cyclooctasioxane	36.778	-	-	0.04
Carbonic acid, methyl ester, [(E)-3,7-dimethyl-2,6-octadien-1-yl] ester	37.67	0.47	-	-
2,6-Octadiene, 3,7-dimethyl-1-(2-propenyloxy)-	37.684	-	0.03	-
Tetraprenol	38.169	-	-	0.1
2,2-Dimethyl-3-(3,7,16,20-tetramethyl-heneicosa-3,7,11,15,19-pentaenyl)-	38.182	0.05	-	-
4,8-Dimethylnona-3,7-dien-2-ol	38.414	0.19	-	-
Carbonic acid, methyl ester, [(E,E)-3,7,11-trimethyl-2,6,10-dodecatrien-1-yl] ester	38.587	-	-	0.02
9-(3,3-Dimethyloxiran-2-yl)-2,7-dimethylnona-2,6-dien-1-ol	38.632	0.32	-	-
3,7-Dimethyl-2,6-octadien-1-ol	38.715	0.04	-	-
Cyclopropanemethanol, 2-methyl-2-(4-methyl-3-pentenyl)-	38.848	0.17	-	-
6,10,14-Hexadecatrien-1-ol, 3,7,11,15-tetramethyl-, [R-(E,E)]-	39.009	-	0.02	-
2,6,10-Undecatrien-8-ol, 2,6-dimethyl-	39.098	0.26	-	-
Tetracosa-2,6,10,14,18-pentaen-22-one, 2,6,10,15,19,23-hexamethyl-, all (E)-	39.4	-	0.02	-
Oxirane, 2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15,19-heneicosapentaenyl)-, (all-E)-	39.553	1.11	0.03	-
Octadeamethyl-cyclononasiloxane	40.258	-	-	0.04
4,9,13,17-Tetramethyl-4,8,12,16-octadecatetraenal	40.599	0.03	-	-
2-[(Methylamino)methyl]cyclohexanol	41.47	0.01	-	-
Farnesol isomer a	41.738	0.88	0.05	-
Squalene	41.855	-	0.02	-
Docosa-2,6,10,14,18-pentaen-22-al, 2,6,10,15,18-pentamethyl-, all-trans	41.946	1.22	0.05	-
cis, trans-Farnesal	42.128	0.15	-	-
Cyclodecasiloxane, eicosamethyl-	46.865	-	-	0.05
		100	100	100

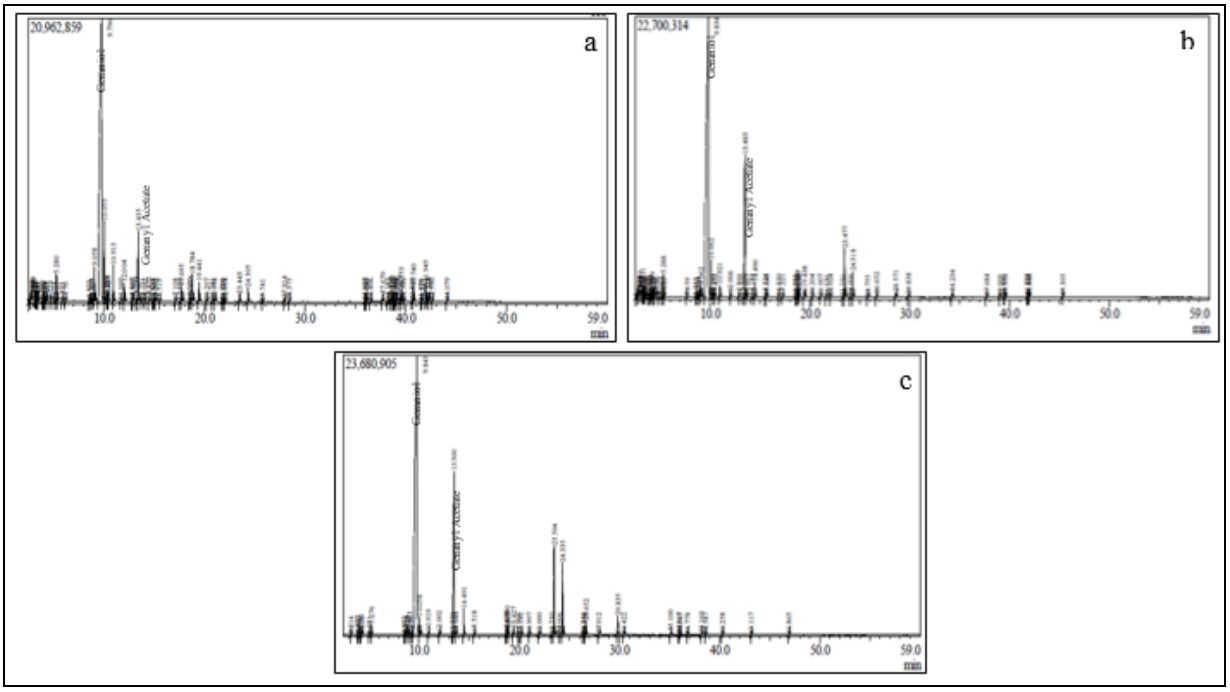

Fig 2: Chromatograms of GCMS analysis of *C. martini* essential oil: a - Soil grown plant essential oil, b - Hydroponically grown plant essential oil and c – aggregate hydroponic grown plant essential oil.

Grading of formulated Soap

Due to high concentration of Geraniol in essential oil of Hydroponic grown *C. martini*, it was used in formulating Soap. Literature survey of geraniol [19, 20], citral [21] and geranyl acetate [20, 22] which are important component of *C. martini* essential oil revealed antibacterial property.

The formulated soap was graded according to BIS (Annex IS 13424:2001). As per the grading, the soap was found conform to the standards of toilet Soap grade 3 prescribed in the method of test, Ref. to IS: 286:1978 and IS2888:2004.

Antibacterial analysis of formulated Soap

The antibacterial activity of the formulated soap was tested against two gram positive bacteria: *B. subtilis*,*S. aureus* and two gram negative strains of bacteria: *E. coli* and *K. pneumonia.* The Minimum inhibitory concentration of formulated soap is shown in Table 3. As per the results, the formulated soap showed more potency against gram positive bacteria than gram negative bacteria. The antimicrobial activity exhibited by the formulated soap was higher than Medimix and Dettol soap available in the Market.

Table 3: Minimum Inhibitory concentration of *C. martini* soap, Medimix Soap and Dettol soap extract against *B. subtilis, S. aureus, E. coli* and *K. pneumoniae.* (mean±S.D.)

	Minimum Inhibitory concentration (mg/ml)			
	B. subtilis	***S. aureus***	***E. coli***	***K. pneumoniae***
Formulated soap	0.5±0.05	1.1± 0.17	7.1±0.2	9.3±0.2
Medimix soap	2±1.93	2.5±0	8±0	11.3±0.57
Dettol Soap	5±0	2.4±0.1	8.5±0.5	12.1±0.28

Antimicrobial activity by Agar well diffusion Method (Table 4) showed higher zone of inhibition for *S. aureus* (1mm±0.2) compared to the other strains of bacteria studied. The zone of inhibition of formulated soap when compared with Commercial available antibacterial soaps (Medimix and Dettol) revealed more antimicrobial activity.

Table 4: Zone of inhibition (mm) exhibited by *C. martini* soap, Medimix Soap and Dettol soap extract against *B. subtilis, S. aureus, K. pneumoniae and E. coli.*

Sample Used	***B. subtilis***	***S. aureus***	***E. coli***	***K. pneumoniae***
Formulated soap	0.78±0.08	1±0.2	0.5±0.1	0.18±0.07
Medimix soap	0.17±0.06	0.4±0.1	0.02±0.0	0.02±0
Dettol Soap	0.06±0.05	0.09±0.0	0.07±0.02	0.14±0.08

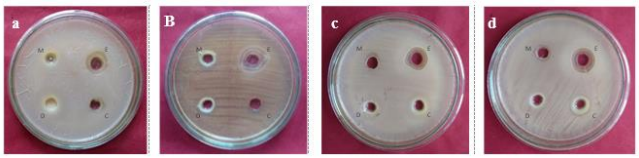

Fig 3: Zone of inhibition exhibited by *C. martini* soap, Medimix Soap and Dettol soap extract against *B. subtilis, S. aureus, K. pneumoniae and E. coli*.a - *Bacillus subtilis,* b - *Staphylococcus aureus,* c - *Klebsiella pneumonia,* d - *Escherichia coli.* M - Medimix soap, E - formulated *C. martini* Soap, D - Dettol, C – Control.

Conclusion

From the current study, it can be concluded that cultivation of *C. martini* by Hydroponic system is a better alternative method to soil system. The essential oil extracted from the plant can be exploited in formulating Soap, which was found to be efficient against gram positive strains of bacteria. Therefore, Hydroponic system can be recommended for farming in urban areas as well as rural areas for growing *C. martini.*

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