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Studies on Comparative Antimicrobial Potential of Cultivated Patchouli Oil and Marketed Eucalyptus Oil

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Abstract

The present investigation was evaluated the comparative potential antimicrobial activity of patchouli oil (procured from fresh and dried *patchouli* leaf extracts, cultivated in Indian acidic soil zone) with marketed eucalyptus oil. Extraction of patchouli oil was carried out by hydrodistillation method using Clevenger apparatus. The content of patchouli alcohol was estimated by Gas chromatography (GC) method. Microbiocides of patchouli oil was compared with marketed eucalyptus oil (2.6%v/v) against several microorganisms viz. *Bacillus substilis*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Enterobacter aerogenes*, *Pseudomonus aeruginosa* , *Escherichia coli*, *Klebsiella pneumoniae* and *Serratia marcescens* by agar diffusion technique. The Minimum Inhibition Concentration (MIC) of the patchouli oil was appointed by the dilution method in the tube and the results revealed the concentration dependent ($p<0.001$) potential antimicrobial activity of both the oils by determined with zone of inhibition against standard ampicillin. At the dose of 300 mcg/ml patchouli oil gave maximum zone of inhibition against *Staphylococcus* (14.53±0.37**) followed by 12.15 ± 0.35** against *Streptococcus* as compared to eucalyptus oil. The eucalyptus oil gave maximum activity of 12.13± 0.08** ($P<0.001$) against the *Staphylococcus,* followed by 11.93± 0.34** against *E. coli*. Such variation may be due to the effects of rich organic carbon content in acidic soil that increased the quality of oil content in patchouli leaves (collected from second year harvested leaves) rather procured marketed eucalyptus oil. It proved patchouli is a strong potential antimicrobial plant.

Key Words: Antimicrobial study, acidic soil, hydrodistillation, *Pogostemon cablin* (F: Lamiaceae), *patchouli* oil, *patchouli* alcohol, *Eucalyptus globulus* (F:Myrtaceae), *eucalyptus* oil.

INTRODUCTION

India is the country with diverse agro-climatic zones, which makes itself a rich storehouse of different types of Flora and Fauna. Besides, different climatic conditions help for introduction, acclimatization and cultivation of a huge number of aromatic plants with minimum efforts but there are few commercial crops which are cultivated without disturbing the existing Flora and they have a high potential to establish as economic crops. Of late Patchouli has been identified as one such essential oil bearing aromatic plant with immense export potential. *Patchouli* (*Pogostemon cablin*), the native of Philippines, belonging to the lamiaceae family, is the most distinctively fragranced herb in the botanical kingdom. In India it thrives well under humid conditions, coastal areas viz. Maharashtra, Goa, Karnataka , Kerala and West Bengal. *Patchouli* leaves contain *patchouli* oil (essential oil) as the major constituent. It has been reported that the essential oil from *patchouli* consists of *patchouli* alcohol (patchoulol) as a major component and several other minor components such as caryophyllene, alpha, beta -patchoulene, pogostol, seychellene, cycloseychellene, and norpatchoulenol [1, 2]. Literature survey already established the potent antimicrobial activity of eucalyptus oil [3-6] against various microorganisms and also revealed that patchouli oil is used for the reduction of stress without any allergic reaction, stimulation of the nervous system to a normal condition and relief of stress. The fresh leaves are very effective in healing burns, calming nerves, controlling appetite, relieving depression, antimutagenic [7, 8]. The *patchouli* oil is having different pharmacological activities likely antidepressant, anti-inflammatory, antifungal, antiseptic, astringent, diuretic, sedative [7, 9]. Scanty reports available related antibacterial activity of the *patchouli* oil [10, 11, 12] but comparative antimicrobial effect of the same with established marketed oil and the effect harvested leaves in different three years in acidic soil in relation to the microbioside activity has not been established so far and hence the present investigation was carried out with the objective to establish the potential antimicrobial activity of *patchouli* oil procured from different year of harvested leaves.

MATERIALS AND METHODS

Plant Material Collection

Three years of *Patchouli* field experiment was conducted in acidic soil zone in Shimoga, pH 6.10, Karnataka, India, during 2007. The bed dimensions of 5 M x 5.50 M. *Patchouli* leaves were periodically collected in every year and separated half parts as fresh leave biomass and remaining half leaves were oven dried at 60° C for 36 hours. Further the fresh and dried leaves were separately stored at 4°C and were used for further antimicrobial investigation.

Microorganisms Used

The bacterial strains used were obtained from stock culture of the department of Microbiology, Bangalore University, Bangalore, India. Few of the strains viz. *Bacillus substilis* ATCC 6633, *Straphyloccus aureus* ATCC 29737, *Streptococcus pyogenes* ATCC 13813, *Enterobacter aerogenes* ATCC 13048, *Pseudomonus aeruginosa* ATCC 25619, *Escherichia coli* ATCC 8739, *Klebsiella pneumonia* ATCC 10031 and *Serratia marcescens* ATCC 13880 were used for the present study, were grown and maintained on nutrient agar medium at St. John's Pharmacy College, Bangalore.

Extraction Of Crude Patchouli Oil And Antibiotic Solution

Water-steam distillation (hydrodistillation) of fresh and dried *patchouli* leaves was carried out by Clevenger apparatus. Separately patchouli fresh leaves (500 g) were finely ground and then extracted by water-steam distillation. Similarly the dried leaves were prepared (500 g) separately from three years of samples and were dried in the oven at 60°C for 36 h, finely ground and then extracted by water-steam distillation. All the extracted crude oil separately stored in labeled sterile screw capped amber colored bottles at freeze temperature of 5° C. The yield of each extracted crude oil was determined and was tabulated in Table-1.

Stock solution of broad-spectrum antibiotic (Ampicillin as standard) was prepared as 30 mcg/ ml (w/v) concentration in sterile distilled water. The concentration of 0.1 ml ampicillin was used for the antibacterial assay in this experiment.

Determination Of Minimum Inhibitory Concentration (MIC)

Dilution method was used to measure MIC. Colony made from 24 hour culture of bacterium inoculated to Mooler Hinton Berath culture medium. This suspension was inoculated at 37°C for about 4 to 6 hours in order to get the bacteria to the dynamic level and compared to Macfarland 0.5 standard at last. As a result the suspension contains 10 bacteria in each ml. Microbial suspension was diluted to the proportion of 1/100 in order to reach 10^6 bacteria in each ml. To measure the MIC, 1 ml of Mooler Hinton Berath culture was poured in different tubes and mixed right after adding 1 ml of the essential oil to the first tube. One ml of first tube was added to the second and 1 ml of the second to the third tube respectively. Then 1 ml of the microbial suspension was added to each tube to make the final concentrations of 800 mcg/ml, 400 mcg/ml, 200 mcg/ml, 100 mcg/ml, 50 mcg/ml and 25 mcg/ml by two-fold dilution. The tubes were incubated at 37°C and MIC was appointed by the growth or non-growth of the bacterium in the tubes [13, 14].

Table 1. Yield of Patchouli leaves and oil harvested from three years of cultivated acidic soil zone of South India

Patchouli Leaves	1st Year of harvest (Kg)	2nd Year of harvest (Kg)	3rd Year of harvest (Kg)
Total cumulative fresh leaves yield	6.40	7.20	5.30
	Oil content (%)	Oil content (%)	Oil content (%)
Fresh leaves	2.32	2.63	2.36
Dry leaves	1.87	1.90	1.74

Antimicrobial Assay

All the oils were subjected to antimicrobial assay by measuring the diameter of zone of inhibition (IZD) using agar diffusion technique. The Petri dishes were washed and sterilized in hot air oven at 160°C for one and half hour and then 1.0% of the inoculum was added to the sterilized nutrient agar medium at 45°C. Three bores were made on the medium using sterile borer (diameter of borer was 6 mm). 0.2 ml of 10 dilution of 24 hours old bacterial cultures were used so as to ensure the concentration of these organisms to contain approximately 1x 10 CFU/ ml. All the extracted crude oils along with marketed eucalyptus oil were taken at different concentration of 50, 100, 200 and 300 mcg/ml.

Statistical Analysis

The experimental results were triplicate and zone of inhibition were determined in mm. All the results were statistically expressed as the mean ± standard error of mean (SEM). Values of $P < 0.05$ were considered statistically significant. Graph Prism software has used for one way ANOVA study.

RESULTS

Production Of Patchouli Oil

Gas Chromatography analysis was revealed the present of patchouli alcohol in crude oil (3.80%). The percentage yield of oil extracted from both fresh and dried leaves of patchouli leaves were as 2.32%, 2.63%, 2.36% and 1.87%, 1.90% and 1.74% respectively (first, second and third year of harvested sample).

Determination of minimal inhibitory concentration (MIC)

The results demonstrated that the MIC of patchouli oil that could inhibit strains of all microorganisms was 50 mcg/ ml. According to the result of the MIC value, further the antimicrobial activity was performed. The *in vitro* antimicrobial activity of patchouli oil extracted (from fresh and dried leaves separately) from different harvested years were compared with marketed eucalyptus oil and were tabulated separately in graphs 2 a, b, 3 a, b and 4 a, b respectively. All the graphs have represented nearly similar antimicrobial potential of eucalyptus oil but much significant variation in results in case of patchouli oil isolated from different year.

Table 2a. Antimicrobial study of Patchouli oil with respect to standard Eucalyptus oil and Ampicilin (Mean of Three readings ± SEM) (First year of harvested fresh leaves)

Samples	Conc. (mcg/ ml)	1	2	3	4	5	6	7	8
P.O	50	6.23 ± 0.37*	6.32 ± 0.54	5.90± 0.47	8.15± 0.60	5.56 ± 2.24	6.78 ± 2.63	5.98± 0.42	5.70± 0.27
	100	7.21 ± 0.56	7.76 ± 0.44	6.19± 0.30*	9.0 ± 0.27*	7.3 ± 2.8	10.76± 0.12**	5.91± 0.40	5.90± 0.67
	200	9.86 ± 0.59	8.88 ± 1.28*	7.06 ± 0.46	9.11 ±1.11*	10.20 ± 0.36	11.73 ± 0.22**	7.21 ±0.36	6.13± 0.19*
	300	10.18 ± 0.25	9.65 ± 0.57	7.85 ± 0.65	10.96 ±0.33*	11.15± 0.35**	12.33± 0.35**	10.23 ±0.74	6.70± 1.10*
E.O	50	6.74 ± 0.32	7.26 ± 0.72	6.97 ± 0.92*	8.80± 0.42	9.33 ± 0.18**	6.43 ± 0.62*	NA	NA
	100	7.36 ± 1.21*	8.57 ± 0.57**	NA	10.81 ± 0.32	10.56 ± 0.74	6.72 ± 0.97*	10.45 ±0.72	7.19± 1.31*
	200	10.83 ±1.14*	9.80 ± 0.32	8.81 ± 0.27**	11.30 ± 0.32	11.12± 0.47**	9.61 ± 0.34	11.64 ±0.41	7.69± 0.11
	300	11.83± 0.34**	10.66 ± 0.62	9.15 ± 0.26**	11.42 ± 0.95	11.55± 0.74*	12.21± 0.18**	11.50 ±0.50	8.60± 0.44
Std	30 mcg/100 ml	22.7	22.7	22.7	22.7	22.7	22.7	22.7	22.7

- **P.O**= Patchouli oil; **E.O**= Eucalyptus oil; **Std**= Ampicilin; **NA**= Not active
- **1**= *E. coli*; **2**= Enterobacter; **3**= Pseudomonus; **4**= Bacillus; **5** = Streptococcus; **6** = Staphylococcus; **7** = K. *pneumoniae*; **8** = Serratia. P< 0.05 = Significant; ** P< 0.001 = Extremely Significant.

Table 2b. Antimicrobial study of Patchouli oil with respect to standard Eucalyptus oil and Ampicilin (Mean of Three readings ± SEM) (First year of harvested dried leaves)

Samples	Conc. (mcg/ ml)	1	2	3	4	5	6	7	8
P.O	50	6.63 ± 0.47*	6.20 ± 0.55	NA	7.35± 0.70	5.76 ± 2.47	6.18 ± 1.23	NA	NA
	100	7.20 ± 0.76	7.48 ± 0.49	5.71± 0.45	9.12 ± 0.24*	6.21 ± 1.31	8.26± 0.52**	5.11± 0.40	5.72± 0.67
	200	9.86 ± 0.54	8.57 ± 0.21*	7.12 ± 0.44	9.39 ±0.07*	8.31 ± 0.46	9.36 ± 0.24**	6.31 ±0.26	6.23± 0.79*
	300	10.28 ± 0.45	9.35 ± 0.77	7.76 ± 0.36	10.17 ±0.13*	9.75± 0.71**	10.67± 0.75**	8.46 ±0.64	6.58± 1.21*
E.O	50	6.71 ± 0.31	7.23 ± 0.62	6.86 ± 0.85*	8.76± 0.40	9.53 ± 0.08**	6.40 ± 0.67*	NA	NA
	100	7.40 ± 0.96*	8.60 ± 0.59**	7.89 ± 0.85	10.91 ± 0.52	10.76 ± 0.64	6.80 ± 0.80*	9.80 ±0.92	7.10± 0.90*
	200	10.80 ±1.07*	9.80 ± 0.30	8.90 ± 0.24**	11.21 ± 0.32	11.21± 0.47**	9.58 ± 0.43	10.54 ±0.44	7.80± 0.74
	300	11.82± 0.44**	10.70 ± 0.52	9.17 ± 0.26**	11.40 ± 0.78	11.50± 0.74*	12.30± 0.48**	11.46 ±0.51	8.42± 0.40
Std	30 mcg/100 ml	22.7	22.7	22.7	22.7	22.7	22.7	22.7	22.7

- **P.O**= Patchouli oil; **E.O**= Eucalyptus oil; **Std**= Ampicilin; **NA**= Not active
- **1**= *E. coli*; **2**= Enterobacter; **3**= Pseudomonus; **4**= Bacillus; **5** = Streptococcus; **6** = Staphylococcus; **7** = K. *pneumoniae*; **8** = Serratia.
- P< 0.05 = Significant; ** P< 0.001 = Extremely Significant.

The patchouli oil procured from fresh leaves showed high significant activities (p< 0.001) against *Staphylococcus* (12.33± 0.35**) followed by *Streptococcus* (11.15± 0.35**) at 300 mcg/ ml concentration, but there were less significant variation with applied eucalyptus oil and was revealed the highest activity (p<0.001) against *Staphylococcus* of 12.21± 0.18** followed by 11.83± 0.34** against *E. coli* at the dose of 300 mcg/ml. Interestingly, there was no response showed by the eucalyptus oil against K. *pneumonia* and *Serratia* where as minimum responses showed by patchouli oil against these two organisms at dose of 50 mcg/ml (Table-2a). In contrast, the patchouli oil showed comparatively less activity than the fresh one. No much variation in results showed by eucalyptus oil from earlier results. Patchouli oil showed the significant higher activity (p<0.001) up to 10.67 ± 0.75** against *Staphylococcus* followed by 10.28 ± 0.45 against *E.coil* at dose of 300 mcg/ml which was lesser than that of activity showed by the eucalyptus oil at same dose level of 300 mcg/ml (Table -2b).

Table 3a. Antimicrobial study of Patchouli oil with respect to standard Eucalyptus oil and Ampicilin (Mean of Three readings ± SEM) (Second year of harvested fresh leaves)

Samples	Conc. (mcg/ ml)	1	2	3	4	5	6	7	8
P.O	50	6.33 ± 0.67*	7.12 ± 0.64	6.78 ± 0.93	8.35± 0.65	5.86 ± 2.94	6.48 ± 2.73	6.28 ±2.03	6.30 ±0.70
	100	8.29 ± 0.56	7.86 ± 0.44	6.90 ± 1.71	10.26 ±1.27*	7.13 ± 2.81	11.76± 0.32**	6.48 ±1.13	6.60 ±0.70
	200	10.76 ± 0.39	8.98 ± 1.98*	8.06 ± 0.46	10.21 ±1.31*	10.30 ± 0.36	13.73 ± 0.24**	9.40 ±0.46	8.03± 0.99*
	300	11.96 ±0.45*	9.85 ± 0.67	8.31 ± 0.66	11.15 ±1.33*	12.15± 0.35**	14.53± 0.37**	10.53 ±0.94	7.13± 1.10*
E.O	50	6.76 ± 0.34	7.06 ± 0.52	6.93 ± 0.98*	8.50± 0.52	9.43 ± 0.08**	6.53 ± 0.82*	NA	NA
	100	7.06 ± 1.01*	8.67 ± 0.17**	7.19± 0.31*	10.50 ± 0.40	10.66 ± 0.84	6.90 ± 0.97*	10.90 ±0.75	7.13± 1.31*
	200	10.83 ±1.14*	9.70 ± 0.42	8.80 ± 0.21**	11.31 ± 0.36	11.43± 0.27**	9.63 ± 0.38	11.60 ±0.40	7.44± 2.71
	300	11.93± 0.34**	11.26 ± 0.65	9.10 ± 0.26**	11.40 ± 0.95	11.75± 0.74*	12.13± 0.08**	11.80 ±0.90	8.80± 0.49
Std	30 mcg/100 ml	22.7	22.7	22.7	22.7	22.7	22.7	22.7	22.7

- **P.O=** Patchouli oil; **E.O=** Eucalyptus oil; **Std=** Ampicilin; NA= Not active
- **1=** *E. coli*; **2**= Enterobacter; **3**= Pseudomonus; **4**= Bacillus; **5** = Streptococcus; **6** = Staphylococcus; **7** = K. *pneumoniae*; **8** = Serratia.
- P< 0.05 = Significant; ** P< 0.001 = Extremely Significant.

Table 3b. Antimicrobial study of Patchouli oil with respect to standard Eucalyptus oil and Ampicilin (Mean of Three readings ± SEM) (Second year of harvested dried leaves)

Samples	Conc. (mcg/ ml)	1	2	3	4	5	6	7	8
P.O	50	6.62 ± 0.64*	7.12 ± 0.60	NA	8.35± 0.65	5.96 ± 1.24	6.48 ± 2.73	NA	NA
	100	8.63 ± 0.56	7.92 ± 0.41	7.17 ± 0.64	10.26 ±1.27*	7.13 ± 1.40	9.46± 0.32**	6.20 ±1.53	6.50 ±0.73
	200	10.26 ± 0.59	8.90 ± 0.98*	8.06 ± 0.48	10.21 ±1.31*	10.00 ± 0.36	12.53 ± 0.28**	9.31 ±0.26	8.03± 0.69*
	300	10.50 ±0.45*	9.80 ± 0.60	8.40 ± 0.60	11.01 ±1.80*	11.70± 0.30**	13.00± 0.33**	9.90 ±0.70	7.23± 1.00*
E.O	50	6.80 ± 0.34	7.42 ± 0.82	NA	8.58± 0.60	9.60 ± 0.08**	6.47 ± 0.82*	NA	NA
	100	7.16 ± 1.00*	8.47 ± 0.17**	6.80 ± 0.90*	10.59 ± 0.43	10.86 ± 0.94	6.78 ± 0.97*	10.84 ±0.75	6.48 ±0.73
	200	10.70 ±1.14*	9.70 ± 0.42	8.78 ± 0.29**	11.33± 0.76	11.38± 0.27**	9.58 ± 0.30	11.67 ±0.40	7.33± 1.33*
	300	11.50± 0.24**	11.20 ±0.05*	9.32 ± 0.24**	11.60 ± 0.25	11.70± 0.54*	12.40± 0.08**	11.88 ±0.70	8.87± 0.67*
Std	30 mcg/100 ml	22.7	22.7	22.7	22.7	22.7	22.7	22.7	22.7

- **P.O=** Patchouli oil; **E.O=** Eucalyptus oil; **Std=** Ampicilin; **NA**= Not active
- **1=** *E. coli*; **2**= Enterobacter; **3**= Pseudomonus; **4**= Bacillus; **5** = Streptococcus; **6** = Staphylococcus; **7** = K. *pneumoniae*; **8** = Serratia.
- P< 0.05 = Significant; ** P< 0.001 = Extremely Significant.

Table 4a. Antimicrobial study of Patchouli oil with respect to standard Eucalyptus oil and Ampicilin (Mean of Three readings ± SEM) (Third year of harvested fresh leaves)

Samples	Conc. (mcg/ ml)	1	2	3	4	5	6	7	8
P.O	50	6.20 ± 0.27*	6.30 ± 0.54	NA	6.15± 0.60	5.26 ± 2.24	6.28 ± 2.40	NA	NA
	100	7.00 ± 0.50	7.80 ± 0.46	6.09± 0.32*	7.90 ± 0.20*	7.40 ± 1.40	8.26± 0.12*	6.00± 0.39*	5.80± 0.30*
	200	8.80 ± 0.45	8.90 ± 1.20*	7.16 ± 0.40	8.81 ±0.09*	10.00 ± 0.56	10.23 ± 0.20**	7.00 ±0.29	6.00± 0.10*
	300	9.88 ± 0.75*	9.30 ± 0.90	7.40 ± 0.45*	10.00 ±0.30*	10.70± 0.55*	11.20± 0.30**	9.68 ±0.64	6.40± 0.10*
E.O	50	6.72 ± 0.34	7.30 ± 0.70	6.10 ± 0.70*	8.78± 0.42	9.36 ± 0.19**	6.48 ± 0.62*	NA	NA
	100	7.40 ± 1.20*	8.60 ± 0.50**	6.24± 0.20*	10.78 ± 0.22	10.66 ± 0.84	6.79 ± 0.95*	9.75 ±0.32*	7.10± 1.30*
	200	10.78 ±1.10*	9.86 ± 0.34	8.78 ± 0.27**	11.20 ± 0.32	11.17± 0.46*	9.63 ± 0.31	10.94 ±0.50	7.56± 0.11
	300	11.78± 0.34**	10.57 ± 0.60	9.30 ± 0.20**	11.44 ± 0.90	11.65± 0.74*	12.00± 0.20**	11.46 ±0.51	8.50± 0.46
Std	30 mcg/100 ml	22.7	22.7	22.7	22.7	22.7	22.7	22.7	22.7

- **P.O=** Patchouli oil; **E.O=** Eucalyptus oil; **Std=** Ampicilin; **NA=** Not active
- **1=** *E. coli*; **2=** Enterobacter; **3=** Pseudomonus; **4=** Bacillus; **5** = Streptococcus; **6** = Staphylococcus; **7** = K. *pneumoniae*; **8** = Serratia.
- P< 0.05 = Significant; ** P< 0.001 = Extremely Significant.

Table 4b. Antimicrobial study of Patchouli oil with respect to standard Eucalyptus oil and Ampicilin (Mean of Three readings ± SEM) (Third year of harvested dried leaves)

Samples	Conc. (mcg/ ml)	1	2	3	4	5	6	7	8
P.O	50	NA	6.00 ± 0.50*	NA	6.20± 0.65	6.76 ± 1.00	6.10 ± 2.40	NA	NA
	100	7.10 ± 0.53	7.00 ± 0.46	NA	7.50 ± 0.35*	8.80 ± 0.40*	8.00± 0.12*	NA	NA
	200	8.10 ± 0.20	8.00 ± 1.20*	7.16 ± 0.40	8.60 ±0.49*	9.80 ± 0.56	10.00 ± 0.20*	7.00 ±0.09*	6.20± 0.40*
	300	8.40 ± 0.70*	9.00 ± 0.40	7.20 ± 0.25*	9.00 ±0.40*	10.00± 0.55*	10.90± 0.30*	8.90± 0.60	7.80± 0.10*
E.O	50	6.90 ± 0.30*	7.80 ± 0.70	6.00 ± 0.20*	8.75± 0.42	9.30 ± 0.10**	6.50 ± 0.50*	NA	NA
	100	7.45 ± 0.90*	8.58 ± 0.52**	6.20± 0.50*	10.68 ± 0.32	10.56 ± 0.74	6.83 ± 0.70*	9.70 ±0.22*	7.20± 1.00*
	200	10.68 ±1.10*	9.80 ± 0.30*	8.70 ± 0.27**	11.29 ± 0.30	11.20± 0.40*	9.60 ± 0.31	10.90 ±0.50	7.66± 0.11
	300	11.76± 0.30**	10.50 ± 0.70	9.35 ± 0.20**	11.40± 0.87	11.60± 0.70*	11.90± 0.20**	11.40 ±0.50	8.40± 0.30
Std	30 mcg/100 ml	22.7	22.7	22.7	22.7	22.7	22.7	22.7	22.7

- **P.O=** Patchouli oil; **E.O=** Eucalyptus oil; **Std=** Ampicilin; **NA=** Not active
- **1=** *E. coli*; **2=** Enterobacter; **3=** Pseudomonus; **4=** Bacillus; **5** = Streptococcus; **6** = Staphylococcus; **7** = K. *pneumoniae*; **8** = *Serratia*.
- P< 0.05 = Significant; ** P< 0.001 = Extremely Significant.

The interesting results showed by patchouli oil extracted from second year of harvested sample. The oil showed much higher activity than that of earlier one and even the activity was much higher than that of activity showed by the marketed eucalyptus oil. The high significant ($p<0.001$) activity of patchouli oil showed maximum zone of inhibition against *Staphylococcus* of 14.53± 0.37** followed by 12.15± 0.35** against *Streptococcus* at dose of 300 mcg/ml (Table -3a). Against *E.coil*, the oil showed much higher activity of 11.96 ± 0.45* which was significant at $p<0.05$ at the same dose. Whereas eucalyptus oil show nearly same activity as earlier reported in Table -2a & 2b. The maximum activity was show 12.13± 0.08** against *Streptococcus* followed by 11.94±0.34** against *E.coil* at the dose of 300 mcg/ml. Similarly the patchouli oil showed little higher activity than that of first year of collected oil. The maximum activity showed against *Staphylococcus* of 13.00± 0.33** followed by 11.70± 0.30** against *Streptococcus* ($p<0.001$) at 300 mcg/ml dose (Table -3b).

Finally, the most interesting results showed from third year of harvested sample. The result drastically changed and compared to former results it showed much lesser activity against microorganisms (Table-4a and 4b). The result showed maximum activity against *Staphylococcus* of 11.20± 0.30** ($p<0.001$) followed by 10.70± 0.55* ($p<0.05$) against *Streptococcus* at 300 mcg/ml with extracted fresh patchouli oil but no significant differences with eucalyptus oil against all the organisms (Graph-4a) whereas the maximum activity showed against *Staphylococcus* of 10.90± 0.30* ($p<0.05$) followed by 10.00± 0.55* against Streptococcus ($p<0.05$) by patchouli oil (Table -4b).

DISCUSSION

Antimicrobial activity of various plant parts have been reported by the many researchers but it is worthwhile to focus on the area where no literatures investigated comparative antimicrobial activity of patchouli oil collected from different year of harvested patchouli plant from acidic soil zone of South India. Keeping this, oils have extracted from fresh and dried leaves sample separately collected from total three years and were evaluated for antimicrobial study and compared among them with marketed established eucalyptus oil for higher microbial activity. The present investigations endow with the basic information about plant extracted oil especially patchouli oil which was found to be strong and potent substantial antimicrobial activity against pathogens like *Streptococcus, Staphylococcus, Bacillus, E.coil* bacteria but no such activities were found with against K. *pneumonia* and *Serratia* with all the patchouli oils. In general however, both the oils (*Patchouli* and *Eucalyptus*) showed a concentration dependent inhibitory effect on all the bacteria species. This finding also correlated with the literatures earlier reported [15, 16] who independently found that various plant extracts inhibits the growth of some bacteria isolates. The variations in such harvested plant biomass in three years was due to the dilution effect of the soil fertility which reduces the size and other relative physical properties of the leaves of patchouli hence in second year more leaf biomass procured and the results also correlated with the literature reported by earlier [12, 17].

In terms of variation in such antimicrobial activities was due to the soil nature of the climatic zone. Acidic soil enhanced the organic carbon content that helped to improve the leaf biomass which has the correlation with the content of crude patchouli oil. It was reported earlier that climatic condition and other environmental factors are the responsible for the growth of plant health and even their respective chemical components too [16, 18, 19, 20]. The present experiment also correlated with the earlier reports. The antimicrobial effect of patchouli oil is also depends on the amount of patchouli alcohol content in crude oil which was revealed in early literature [12]. Since, we also have correlated our present investigation that results high content and good quality of patchouli oil improved the content of patchouli alcohol (estimated by GC analysis) by cultivated patchouli plant in acidic soil zone in second year of harvested plant biomass. The result was also satisfied with the early reported documentations [21, 22].

CONCLUSION

Although all the individual oils extracted separately from patchouli plant (obtained from three years cultivated in acidic soil zone) show potential antimicrobial activity, compared with marketed eucalyptus oil but the activities for both the oils (patchouli and eucalyptus) were lesser than standard drug Ampicillin. All the three years of harvested oil sample show statistically significant antimicrobial activity in separate experiments but the concentration dependent higher activity shown by the oil extracted from fresh patchouli leaves collected from acidic soil zone due to high range of organic carbon content, improved the quality of oil and content of patchouli alcohol. This further concluded that patchouli plant could be proved as future potential and strong antimicrobial agent as non antibiotics sources.

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