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Evaluation of bioprospective potentiality of *Ocimum gratissimum* Linn. through microbial exploitation — An innovative approach for environmental fragrance development

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'Tulsi' is a familiar name used domestically when we talk about curing common ailments. Use of this plant in domestic medicine is due to its antagonistic effect on disease causing microorganisms. Scientifically this plant is known as *Ocimum* and in India it is represented by six species. Volatile oil obtained mainly from the leaves of this plant has been proved as the bioactive principle. The GC analysis of oil sample reveals that eugenol is the most common constituent among the species except *O.kilimandscharicum*. The quantity of eugenol is highest in *O.gratissimum* (47.45%) which has been proved as one of the inhibitory substance for microbial growth. Eugenol is used industrially as a substrate for bioconversion into vanillin – a flavour enriching compounds used in confectionary. In a bid to search out the potentiality of eugenol present in oil sample of *O. gratissimum* for vanillin production, 10 bacterial and 4 fungal isolates from different putrescent environment were allowed to grow in presence of leaf extract and essential oil respectively. Despite the inhibitory nature of eugenol in such sample 3 bacterial and 1 fungal isolates show comprehensive growth acceleration conferring their resistance to eugenol being pervaded in their metabolic stream. Therefore it is amenable to reason that such microbial inocula could be amended to the soil along with the crude leaves of the plant to abate the stink of fermentative bad odour emanating due to microbial decomposition of waste materials through vanillin production.

Key words: Eugenol, biotransformation, Gas Chromatogram, vanillin

INTRODUCTION

India is considered as a treasure house of medicinal and aromatic Plants (MAPs). The diversity has immense potentiality to improve socioeconomic status of the country. Aromatic compounds obtained from natural plant resources are being exploited greatly in flavour industry because of their easy availability in a cost effective manner and their ineffectiveness to cause health hazards as well. Eugenol is one of such aromatic compounds present in different species of *Ocimum* (Family : Lamiaceae) which has great industrial and pharmaceutical value. In flavour industry it is used in biotransformation to vanillin; a compound which is used to boost up the fragrance of confectionary products making it more acceptable. Besides, the antifungal activity of this aroma compound has been well documented. It has been proved to be responsible for amelioration of plant essential oil against the pathogen *Botrytis cinerea* (Rattanapitigorn, 2006). Eugenol is usually obtained from *Eugenea caryophyllata*. Though its eugenol productivity is reportedly very high never-

theless, to obtain it in a maximum quantity it will require a long span, since being tree like habit the plant has to attain maturity to maximize the yield. Thus an alternative, cheap source of eugenol is to be searched out to expedite the procurement of vanillin. In this regard no attempt has so far been made to test the potentiality of volatile oil of *Ocimum* as a natural additive in the cultural milieu for bioconversion of its eugenol into vanillin. The growing culture of *Pseudomonas aureginosa* isolated from soil has been proved to generate vanillin in presence of isoeugenol after a 72 hrs of incubation at 30°C and 200 rpm.

In our present investigation attempts have been made to quantify the eugenol yield of different species of *Ocimum* reported so far in India. Despite of growth inhibitory property of eugenol present in the essential oil sample, efforts have been made to select certain microbial strains from putrefied environment which could metabolize the compound effectively as vindicated by their growth promotion in the culture medium supplemented with eugenol in the form of natural volatile oil sample extracted from the leaves of the plant concerned.

MATERIALS AND METHODS

Extraction of essential oil

Essential oil sample was extracted from fresh leaves of different species of *Ocimum* following hydro distillation method using Clevenger's apparatus (Clevenger, 1928). Petroleum ether was used as a solvent for extraction (b.p.40°-60°C). The oil sample was anhydrate with Na_2SO_4 (300 g/l).

GC analyses of oil sample and quantification of eugenol content

Gc analysis of oil samples of different species of *Ocimum* were made with the help of CE-8000 top model chromatogram using liquid nitrogen as a carrier gas. The oven temperature of the chromotogram was raised from 60°C to 220°C at the rate of 5°C/min. The holding time of the final temperature in the oven was 10 min. The injector and detector temperature was 220°C for each. The column used for GC analysis was DB-5 MS type of capillary column of 30 mt length. The film thickness and internal diameter of the column was 25 µm. The concentrated essential oil sample was diluted properly up to a particular concentration using n-hexane as a solvent and 1µl of diluted sample was injected into the chromatograms for analysis. The authentic sample was also diluted similarly and the same volume was injected into the column. The peak produced by authentic sample was compared to the peaks obtained from the test samples with respect to their retention time (RT) in order to the identification as well as qualification of the eugenol present in the oil sample (Fig1). To quantify the amount of eugenol in oil sample the following formulation was adopted:

$$\frac{M x A2 x 100}{A1 x N}$$

where, M = Standard stock concentration (ppm); A1= Area of the standard authentic sample (obtained from chromatogram); A2= Area of the test sample (obtained from chromatogram); N= Stock concentration of the test sample (ppm)

Methods for preparation of leaf extract

An amount of 10 g of dry leaves of the plant species were washed 2-3 times with tap water and distilled water and then surface sterilized with 90% alcohol. Subsequently, the plant materials were grinded in a mortar pestle using 10 ml of petroleum ether as solvent. The macerates were kept at room temperature to evaporate the solvent. In the remaining residue, 10 ml of distilled water was added. Macerates were squeezed through double layered muslin cloth and filtered through filter paper. The aliquots were then centrifuged at 10,000 rpm for 20 min. The supernatant were filtered through Whatman No. 1 filter paper and then sterilized by passing through 0.2 µm disposable filters.

Evaluation of growth incentive properties of leaf extract and eugenol on bacterial isolates

For evaluation of growth incentive properties of plant extract and eugenol, microbial bioassay method was followed. Nutrient broth medium was prepared and distributed in several culture tubes, each with 10 ml of medium. The medium was sterilized by autoclaving. To each tube containing 10 ml of medium, 0.2 ml of 24 hrs old culture of test organism (grown in nutrient broth) and 1 ml of filter sterilized extract was added. One blank control was prepared. Another control was prepared by

adding 1 ml of sterilized distilled water with the tube containing 10 ml of medium plus 0.2 ml of inoculum. Three replicas were taken for each organism. To test the growth incentive properties of eugenol, the latter was added to the inoculum in place of leaf extract. These tubes were then incubated in BOD incubator with shaker for 24 hrs at 37°C. The optical density of each set was measured at 540 nm and compared with the control.

Evaluation of growth incentive properties of essential oil on bacterial isolates

It is evaluated by following the same procedure for leaf extract as outlined above. One ml of volatile oil sample was added instead of leaf extract. The oil sample was sterilized by passing through disposable filter before addition.

Evaluation of antifungal properties of essential oil

10 ml of culture medium was taken in a conical flask. The medium was sterilized. To the medium, 0.1 ml of fungal spore suspension and 1 ml of filter sterilized essential oil sample and leaf extract was separately added. One control set was prepared by adding 0.1 ml inoculum and 1 ml of sterilized distilled water. For each treatment three replicas were prepared. The control and treatment sets were incubated in shaking condition for 24 hrs at 32°C in a BOD incubator. The mycelial mass was filtered and then dried in hot air oven. The difference in the dry weight from the control set was considered as growth inhibition.

Statistical analysis

The statistical analysis of the experimental data were made with the help of SPSS software for windows.

RESULTS AND DISCUSSION

Being acquainted with the fact that eugenol is a constituent in the essential oil, quantification of the same was carried out in six available species growing in India (Banerjee, 1996).The GC analysis reveals that eugenol occurs in highest quantity in *Ocimum gratissimumin* in comparison to others(Fig 2).The compound however is entirely absent in *O. kilimandscharicum*. Therefore whenever industrial exploitation of eugenol is concerned, emphasis is to be given on the mass cultivation and conservation of the species following advance biotechnological protocol to ensure enhanced level of productivity of the compound. Whether eugenol from natural plant resources could be exploited as substrate for large scale production of vanillin through the extravagant metabolic amenities in microbial system, microbial isolates from different putrescent environment were allowed to grow in the nutritional milieu supplemented with essential oil and leaf extract of *O.gratissimum*. Nevertheless, being enriched with the eugenol, the essential oil as well as leaf extract demonstrates proficient growth acceleration in three bacterial isolates

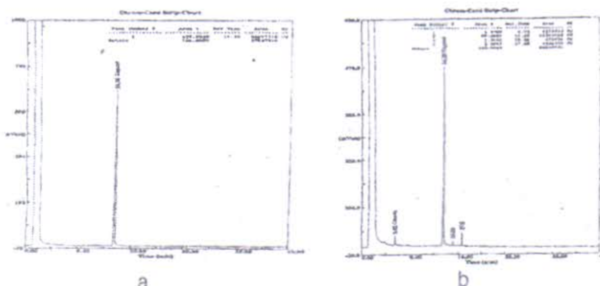

Fig. 1a : Gas Liquid chromatogram of standard authentic sample of eugenol.

Fig. 1b : Gas Liquid Chromatogram of the essential oil sample of *Ocimum gratissimum*.

(SDM101,MID103,KLR103), despite of its antagonistic action on microbial growth (Table 1). One fungal isolate also demonstrated growth proliferation in presence of essential oil and leaf extract in its cultural environment (Fig 3). That the only eu-

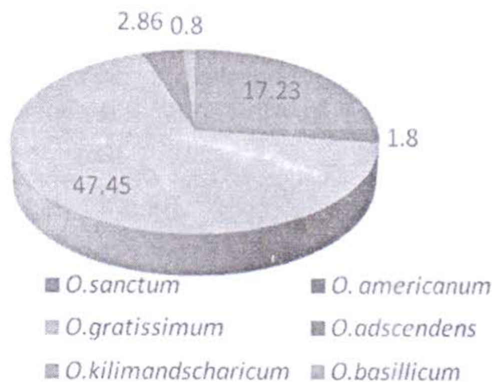

Fig. 2 : Percentage of eugenol content in different species of *Ocimum*.

genol in essential oil is growth promoting in nature could be substantiated by the fact of the resistance and similar kind of proliferation of microbial population even in the presence of authentic eugenol as an additive to the nutritional constituents in lieu

Table 1 : Effect of leaf extract and volatile oil of *Ocimum gratissimum* on the growth of different bacterial isolates from putrescent environment

Isolate Number	Optical density of reaction mixture at 540 nm after incubation			
	Control	Reaction mixture with leaf extract	Reaction mixture with volatile oil	Reaction mixture with eugenol
SDM101	0.59 ± 0.02^{b}	*0.72 ± 0.1^{ab}*	*0.74 ± 0.002^{a}*	*0.72 ± 0.001^{ab}*
KJV102	0.31 ± 0.01^{a}	0.21 ± 0.03^{bcd}	0.24 ± 0.001^{bc}	0.25 ± 0.002^{b}
MID103	0.34 ± 0.05^{b}	*0.78 ± 0.11^{a}*	*0.78 ± 0.005^{ab}*	*0.78 ± 0.004^{abc}*
RMV104	0.36 ± 0.002^{a}	0.28 ± 0.05^{bcd}	0.25 ± 0.01^{b}	0.27 ± 0.02^{bc}
MDR211	0.46 ± 0.011^{a}	0.36 ± 0.06^{b}	0.35 ± 0.04^{bc}	0.36 ± 0.014^{b}
KLR103	0.42 ± 0.02^{b}	*0.75 ± 0.04^{ab}*	*0.76 ± 0.03^{a}*	*0.72 ± 0.005^{abc}*
DID001	0.64 ± 0.04^{a}	0.51 ± 0.02^{b}	0.48 ± 0.002^{bc}	0.49 ± 0.006^{bcd}
PAN002	0.52 ± 0.03^{a}	0.33 ± 0.01^{b}	0.32 ± 0.05^{bc}	0.33 ± 0.004^{b}
SDM102	0.44 ± 0.11^{a}	0.39 ± 0.05^{bc}	0.39 ± 0.004^{bc}	0.38 ± 0.011^{b}
SDM103	0.68 ± 0.06^{a}	0.44 ± 0.012^{bc}	0.43 ± 0.001^{bcd}	0.46 ± 0.012^{b}

Values are mean ± SD; Different superscripts represent mean comparison by Duncan's test at 5% level of significance. Similar alphabet shows homogeneous mean.

of essential oil.

Thus, it could be concluded that natural environment has many potent microorganisms capable of transforming natural substrate into valuable aroma compound. Our observation also corroborate with the findings that natural compounds are the major interest to the flavor industry (Shimoni, 2000). Quantitative analysis of the bio transforming ability is to be worked out with the view to determine the feasibility of the volatile oil of *O. gratissimum* as substrate for industrial production of vanillin. Though extensive research is to be carried out, still it is absolutely true that there is a distinct possibility to exploit the essential oil of this species as a substitute of synthetic eugenol for industrial production of aroma compound i.e. vanillin. It is further to be investigated whether crude leaves as a reservoir of eugenol in the form of essential oil could be amended the soil to rapture the stink of bad odour of putrefied environment through vanillin production.

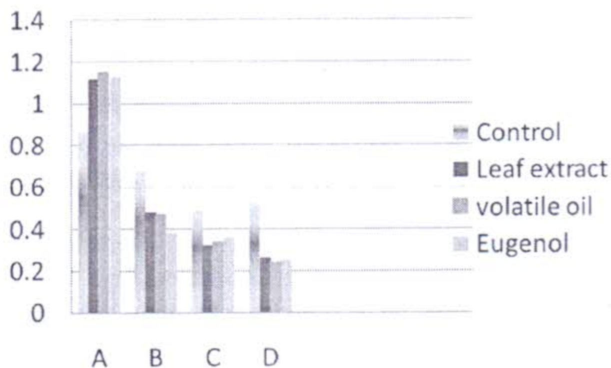

Fig. 3 : Effect of leaf extract and essential of *O. gratissimum* on the growth of four fungal isolates

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