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Full Length Research Paper

Anti-dermatophytic activity of eucalyptol rich turmeric somaclone oil against human pathogenic isolates

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Eucalyptol, a compound well known to possess antimicrobial property, reported to be found as a major constituent of essential oil of plants like *Eucalyptus sps*, *Lippia multiflora* and *Myrtus communis L.* but never before in turmeric. De novo occurrence of eucalyptol as major constituent (37.26%) of essential oil in turmeric somaclones in lieu of alpha-phellandrin in its source plant (57.8%), exhibited unanticipated anti-dermatophytic activity against six human skin infecting isolates tested. The diameter of inhibition zones formed by eucalyptol rich oil against *Micrococcus luteus*, *Staphylococcus epidermidis*, *Bacillus substilis*, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aerogenosa,* were found to be 28.66, 33.33, 20.33, 23.66, 24.33 and 7.66 mm, respectively, found totally absent in the mother plants (IZD, 0.0 mm against all isolates). De novo presence of potential anti-microbial eucalyptol, might have been possible due to occurrence of somaclonal variation during tissue culture.

Key words: Eucalyptol, turmeric leaf oil, somaclonal variation, de novo, anti-dermatophytic activity.

INTRODUCTION

Turmeric (*Curcuma longa L.)* leaf oil bestowed with medicinal values, has been used for treatment of various ailments (Usman et al., 2009) and many of its therapeutic properties are experimentally validated (Gur et al. 2006; Naz et al. 2010) including its antimicrobial activity (Aplsarlyakul et al.,1995; Tripathy et al., 2002). Phellandrin (α, β) has been reported to be the major constituent of turmeric leaf oil, the percentage of which has been found to vary with geographic regions like the Himalayan region of northern India (Raina et al., 2005), Uttaranchal (Pande and Chanotiya, 2006), eastern ghat of Orissa (Singh et al., 2010), Kerala (McCarron et al. 1995), Orissa (Behura and Srivastava, 2004) and origin, China (Ma and David, 2006), Nigeria (Oguntimein et al.,1990), Vietnam (Dung et al., 1995) or with variety; Suroma (Singh et al., 2010), Roma and Kasturi (Behura and Srivastava, 2004).

Reports available mainly deal with assay of antimicrobial potential of phellandrin containing oil of conventionally propagated turmeric leaves (Aplsarlyakul et al., 1995; Tripathy et al., 2002) and their constituent analysis including the only article which mentions production of oil from micro-propagated turmeric leaves (Kuanar et al., 2009). Somaclonal variation (Larkins and Scowcroft, 1981) reported in many plant species includes only quantitative and qualitative variation in essential oil constituent (Mathur et al., 1988; Kukreja et al., 1991; Nayak et al., 2003). So far, no information on constituent changes of essential oil from tissue-cultured plants including turmeric, possibly due to somaclonal variation during *in vitro* culture and its consequent variation in bioactivity has been reported.

Eucalyptol, a compound well known to possess

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Abbreviations: BA, 6-benzyladenine; **IAA,** indole acetic acid; **NAA,** naphthalene acetic acid; kin kinetin; **Ads,** Adenine Sulphate; **MS,** Murashige and Skoog medium; **IZD,** Inhibition zone diameter; **MHA,** Muller Hilton agar; **DMSO,** dimethyl solphoxide.

anti-microbial property, found as a major constituent of essential oil of plants like Eucalyptus (Gilles et al., 2010; Karlovi et al., 2000; Akin et al., 2010), *Lippia multiflora* (Owolabi et al., 2009), *Myrtus communis L.* (50.13%), (Akin et al., 2010). In addition, it has other medicinal properties like anti-inflammatory (Juergens et al., 2003), rhinosinusitis (Kehrl et al., 2004), kills leukaemia cells *in vitro* (Moteki et al., 2002) as well as its insecticidal property (Batish et al., 2008). De novo occurrence of eucalyptol, the major changed constituent in micro-propagated turmeric leaf oil which might have resulted due to somaclonal variation and resultant emergence of anti-bacterial activity profile, against human skin infecting isolates such as *Micrococcus luteus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa,* was reported for the first time in the present paper.

MATERIALS AND METHODS

Micropropagation and field establishment of turmeric

Dormant auxillary buds of unsprouted rhizomes, from an elite turmeric cultivar 'Surama' used as explants were thoroughly washed with distilled water. Surface sterilization was done with 0.1% mercuric chloride solution for 8-10 min, followed by a repeated wash with sterile distilled water prior to inoculation. Explants were inoculated to the sterilized basal MS medium (pH 5.7) (Murashige and Skoog, 1962) with varying combinations of 1-5 mg/L of BA, 1-2 mg/L of IAA, 1-2 mg/L of NAA, 1-2 mg/L of Kin and 50-100 mg/L of Ads. Fifteen replications per treatment were used. Each 50 ml culture tube containing 20 ml medium was inoculated with single explant and kept under fluorescent light of 50 $\mu molem^2/s$ light intensity. Micro-propagated plantlets were maintained in culture for two years with regular sub-culturing at 90 days interval. *In vitro* grown plantlets with well developed roots and shoots were transferred to pots containing soil, cow dung and sand mixture in 1:1:1 ratio. These were kept in a green house for acclimatization. After 30 days, pots were transferred to the normal field condition and grown to maturity.

Essential oil extraction and constituent analysis

Essential oil from fresh clean weighed matured turmeric leaves of conventionally grown mother plants and that of field-grown tissue culture derived plants were subjected to hydro-steam distillation for 6 hours, using Clevenger's apparatus (Guenther, 1948). The oil was collected and the volume was recorded and stored in a sterile vial at –20°C. Essential oil collected from leaves of source plant and micro-propagated plants were analyzed by using gas chromatography, to find out the major constituents present in it. The component identification was achieved by the GC-MS analysis using HP 6890 series GC (Hewlett-Packard, USA) coupled with mass selective detector (MSD), HP 5973 series (Hewlett-Packard, USA).

Helium was used as a carrier gas and the sample was injected in split-less mode in a column HP 5 phenylmethyl siloxane [25 µm (film thickness) x 320 µm (internal diameter) x 30 m (length of column)]. Mass spectra were acquired over a 40-400 atomic mass unit range. Compounds were identified by comparing the mass spectral data with those in the NIST Library, provided with software and with commercially available data. Temperature Programming for the analysis was done as given here: Initial temperature, 60°C; ramping rate , 3°/min; final temperature, 243°C; run time, 61 min. For GC-MS evaluation, 10 plants from each group (TP and CP) were randomly selected.

Antimicrobial assay

The test organisms used for screening the anti-microbial activity of the oil were the 22 bacterial isolates collected from skin infected patients of SUM Hospital, Bhubaneswar University. The 22 isolates were identified to be of six bacterial strains; *E. coli* (5 isolates), *B. subtilis* (3 isolates), *S. aureus* (5 isolates), *S. epidermidis* (2 isolates), *M. luteus* (2 isolates), *P. aeruginosa* (5 isolates) etc. The bacterial strains were maintained on Muller Hilton agar medium (Himedia pvt ltd.) at 35°C to 37°C. Active cultures for experiments were prepared by transferring a loop full of cells from stock cultures to test tubes of Muller Hilton Agar (MHA), that were incubated for 24 to 48 hours at 35°C. Cultures were diluted with Muller Hilton broth to attain an inoculum size of 107 to 108 cfu/ml, before it was used for swab.

In vitro anti-microbial activity of essential oil against bacterial strains was screened by agar well diffusion method (Kavanagh, 1972). Wells were prepared in MHA plates by using well borer of 6 mm diameter. The inoculums' suspension was swabbed uniformly and was allowed to dry for 5 min. The oil was diluted in 100% DMSO and evaluated at concentration 50% (V/V) and 20 µl was introduced into the wells. Plates were incubated overnight at 35°C. Each set of experiment was done in three replicates. The anti-microbial spectrum of the essential oil was determined in terms of zone size around each well, using ciprofloxacin (10 µg/ml).

RESULTS AND DISCUSSION

The present study reveals the potential of dormant auxillary buds of an elite cultivar of *C. longa* (Surama) for year round initiation of *in vitro* culture. MS medium containing, BA (3 mg/L) and IAA (1 mg/L) was optimum for shoot multiplication of *C. longa*. Latent buds of rhizome became active and sprouted on this media with subsequent production of 7 to 8 plantlets per culture within a month. The cultures were maintained in the same media with regular sub-culturing at 3 months interval. About 95% of plants transferred to the field during planting season in June, survived successfully showing normal growth. Approximately, forty million plantlets can be produced from single explants in a year whereas multiplication rate through traditional method can be a maximum of 8 plants per annum. The essential oil content, extracted both from leaves of mother plants and tissue culture derived plants of turmeric, were found to be the same that is, 0.5%. The major constituent of essential oil through GC analysis, was found to be alpha-phellandrin (57.8%,) in leaves of conventionally grown source plant but surprisingly Eucalyptol (37.26%,) in case of tissue culture derived field grown plants in addition to other minor constituents as given in the Table 1. Such de novo occurrence of eucalyptol has never been reported earlier as a constituent of essential oil of turmeric.

Unanticipated variation and sudden occurrence of eucalyptol has tempted us to validate the change in

Table 1. Phyto-constituent analysis of conventionally grown and micro-propagated plant.

Sl. no.	Essential oil constituents	Conventionally grown plant in (%)	Micro-propagated plant in (%)
1.	α-Phellandrin	57.80	0.0
2.	Eucalyptol	0.0	37.26
3.	Terpinolene	13.23	27.80
4.	α-pinine	2.79	0.08
5.	β -Farnesene	0.23	0.77
6.	Dehydrocurcumene	0.7	2.37
7.	β- pinene	1.4	1.43
8.	Tumerone	7.18	0.05
9.	3-Carene	3.69	3.33
10.	Caryophyllene	0.56	0.9
11.	α-Farnesene	0.23	0.13
12.	Curlone	2.54	2.58
13.	Phytol	0.11	0.42

Table 2. Comparative anti-dermatophytic activity of essential oil of turmeric cv. Surama mother plant and its somaclone M13 (Inhibition zone diameter in mm) against six human pathogens.

Dermatophytes	Surama mother plant			Surama somaclone M13			Ciprofloxacin (10 µg/ml)		
	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE
E. coli	0	0	0	23.66	0.57	0.33	36	0.57	0.33
S. aureus	0	0	0	24.33	1.15	0.66	0	1.15	0.66
B. substilis	0	0	0	20.33	0.57	0.33	15	2.08	1.20
M. luteus	0	0	0	28.66	0.57	0.33	0	0	0
P. aeruginosa	0	0	0	7.66	0.57	0.33	28	0.57	0.33
S. epidermidis	0	0	0	33.33	2.08	1.20	24	2.08	1.20

SD: Standard deviation, SE: Standard error

in place of phellandrin has tempted us to validate the possible change in antidermatophytic activity through *in vitro* assay of respective essential oil by, finding inhibition zone diameter of respective essential oil, as eucalyptol is known to possess anti-microbial property. From Table 2, it is clear that the turmeric mother plant essential oil showed no activity against all six dermatophytes whereas promising potential activity was exhibited by somaclones, against all the pathogens with respective IZD comparable to the standard anti-bacterial agent ciprofloxacin except *S. aureus* and *M. luteus.* Essential oil from Surama Somaclone showed anti-dermatophytic activity against all the dermatophytic bacteria tested with highest IZD value 33.33 mm against S. epidermidis and lowest against P. aeruginosa with IZD value of 7.66 mm. The oil of the source plant tested, showed no activity at all against any of the test isolates, forming no inhibition zones, as enlisted in Table 2. This is in close agreement with the only available report on anti-dermatophytic activity, of conventionally grown turmeric extract against *E. coli*, *P. aeruginosa*, *B. subtilis* and *S. aureus* showing zero or little activity forming very small IZD of 0.0 mm or 11.0 mm (Gur et al., 2006).

Occurrence of such activity afresh may be attributed to change in the major chemical composition from phellandrin to eucalyptol in micropropagted oil, as there have been reports published which clearly support the above fact, that eucalyptol containing oil bears antimicrobial potential. Variation of eucalyptol (1, 8 cineole) content among essential oils of three Australian Eucalyptus species showed a variable degree of anti-microbial activity with *E. staigeriana* oil showing the highest activity, as it contains the highest percentage of eucalyptol (34.8%)(Gilles et al. 2010). Karlovic et al., 2000, have reported antibacterial effect of eucalyptol (70 to 85%, 1.8 cineole) for *S. aureus* as compared to that of Halothane and orange oil.

High eucalyptol containing *M. communis L.* (50.13%), showed higher anti-bacterial activity against *S. aureus*, *E. coli*, *P. aeruginosa* than oil of *E. camaldulensis,* with 13.73% eucalyptol (Hendry et al., 2009; Akin et al., 2010) concluded that eucalyptol oil or 1.8-cineole may be combined with chlorhexidine digluconate for enhanced synergistic anti-microbial activity against a wide range of

micro-organisms *S. aureus*, methicillin-resistant *S. aureus* (MRSA), *E. coli, C*andida *albicans* and biofilm cultures of MRSA and *P. aeruginosa*.

Conclusion

Thus, the result obtained in the present study demonstrates for the first time, the novel turmeric genotypes rich in eucalyptol with improved anti-dermatophytic activity can be efficiently produced through tissue culture technique.

However, care must be exercised in using these micro-propagated plants and its essential oil for potential medicinal applications because of the pronounced chemical variability in these plants and moreover, retention of the changed property in subsequent generation must be ensured before its actual utilization, the continuous super vision of which has been undertaken in our laboratory.

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