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EVALUATION OF ANTI-BACTERIAL ACTIVITY, GC/MS ANALYSIS AND GENOTOXIC POTENTIAL OF *CARUM COPTICUM* ESSENTIAL OIL FRACTIONS AGAINST MULTI-DRUG RESISTANT *STAPHYLOCOCCUS AUREUS*

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ABSTRACT

With the growing problem of emergence of multi-drug resistant bugs the surge for new treatment options has become inevitable. Essential oils contain combination of diverse nature of compounds that possess a variable range of antimicrobial activities. The current study aims to characterize the possible combination of bioactive constituents possessing appreciable activity against multi-drug resistant *staphylococcus aureus*. The essential oil was extracted using steam distillation and fractionated by silica gel gravity column chromatography using various solvent combinations in increasing order of their polarity. In-vitro antibacterial activity of essential oil and its fractions against multidrug resistant *S. aureus* isolates was performed by agar well and micro broth dilution methods. The toxicity of most effective fraction/s was evaluated by performing Comet assay on human lymphocytes. Finally the bioactive molecules present in the essential oil and its fractions were elucidated using GC/MS analysis. Results: *Carum copticum* essential oil and its fractions F1 was found effective against MDR *S. aureus* having zones of inhibition greater than the standards vancomycin and linezolid. Fraction F1 was more effective as the MIC values obtained in case of fraction F1 were lower than pure essential oil. Fraction F1 was found non-DNA damaging using Comet assay. Upon GC/MS analysis of essential oil thirty compounds were eluted with major constituents Carvacrol (24.4%), ϒ -Terpinene (8.8%), Apiol (6.6%), Carvone (3.4%) and p-cymene (3.1%). Fraction F1 contained predominantly following compounds with highest concentration of Carvacrol followed by p-Cymene, ϒ –Terpinene and Apiol as major constituents. It was concluded that *Carum copticum* essential oil fraction F1 with following constituents' carvacrol, p-cymene, ϒ-Terpinene and Apiol was the most effective fraction and have shown superior antibacterial activity as compared to pure essential oil.

Key words: *Carum copticum* essential oil, multi-drug resistant *Staphylococcus aureus*, Minimum inhibitory concentration, Comet assay, GC/MS analysis.

INTRODUCTION

Methicillin resistant *Staphylococcus aureus* has been a leading cause of infections both in the public and health care settings (Weber 2005). This diverse microorganism has been shown to confer resistance against a number of current antibiotics making its treatment more difficult (Gootz 2010). This problem of growing resistance requires to surge for new treatment options and to explore the medicinal plants for their bioactive molecules with antimicrobial properties.

Nature has been the reservoir of medicinal agents for thousands of years and a remarkable number of modern drugs have been isolated from natural sources (Rishton 2008). In last decade screening of active biomolecules from plant extracts have gained tremendous popularity especially from indigenous plants as therapeutic options. Essential oils have gained considerable attention as a potential source possessing promising antimicrobial activity against a number of bacteria and fungi with minimum risk of resistance (Neerrnan, 2003).

Carum copticum seeds are used as a spice in Central Asia and Europe. Plant is annual herb indigenous to India, Iran, Pakistan and Egypt (Agha *et al*., 2010). Traditionally the essential oil obtained from the seeds used as carminative, anti-flatulent and as a flavorant in foods (Boskabady *et al*., 2014). It has strong anti-oxidant properties (Christova-Bagdassarian *et al*., 2013) and potential as anti-cancer drug (Custodia *et al*., 2011). Essential oil from *C. copticum* has been investigated for its antimicrobial potential and found effective against many multi-drug resistant bacteria (Raut and Karuppayil 2014).

Present study was for characterization of bioactive constituents in *C. copticum* essential oil and its different fractions possessing antibacterial activity against selected Multiple drug resistant *S. aureus* and identification of possible constituents in the active fractions using GC/MS.

MATERIALS AND METHODS

C. copticum essential oil was selected for screening of constituents having anti-bacterial activity against selected MDR *S. aureus* isolates. PCR confirmed MRSA isolates resistant to more than two groups of antibiotics were taken from Microbiology Department University of Veterinary and Animal Sciences. Seeds were purchased from the local market and identified by a botanist from Dr Sultan Ahmed Choudhary Herbarium Government College University. The voucher number assigned by the herbarium was GC. Herb. Bot. 2420. Essential oil was recovered by steam distillation (Zarshenas *et al*., 2014).

Fractionation of *Carum copticum* essential oil: Silica gel gravity column chromatography was performed for fractionation of *C. copticum* essential oil in relation to increasing solvent polarity. Silica gel (60mesh) slurry was made in Acetonitrile 40- 60°C and poured into a glass column (50 x 3 cm, TGI Columns with Teflon taps) with glass wool, to make an effective column of 35 x 3 cm size. *C. copticum* essential oil (10 mL) was loaded onto column bed and eluted first with 400 mL Acetonitrile alone followed by 400 ml each of Acetonitrile: Methanol (9:1), Acetonitrile: Methanol (7:3), Acetonitrile: Methanol (3:7), Methanol alone, Methanol: water (9:1), ethanol: water (7:3). Column fractions were collected separately and concentrated by using rotary vacuum evaporator, Buchi type at 50°C and finally weighed.

Antibacterial activity of *Carum copticum* Essential and its fractions: Antibacterial potential of pure essential oil and its seven fractions were determined through agar well diffusion assay against multidrug resistant *S. aureus* at 2, 4, 6, 8, 10, 20, 30, 50, 100µl concentration per well. A lawn of MDR *S. aureus* culture was spread over Muller Hinton agar plates having set for OD values equivalent to 0.5Mcfarland unit. Pure essential oil and all fractions were poured in the wells and plates incubated at 37℃ for 24hrs. Activity was determined by measuring zone of inhibitions and compared with Vancomycin and Linezolid standards. Essential oil and those fractions showing clear zones of inhibition greater than the standards against the selected MDR *S. aureus* isolates were further processed for minimum inhibitory concentration by Micro broth dilution method with few modifications (Calvo *et al*., 2012).

Serial two fold dilutions at concentration 10 – 0.019µl of *Carum copticum* essential oil and its fractions in Muller Hinton broth were prepared. Test organisms inoculated at a concentration of $2x10^6$ CFU/ml in a 96 well microtitration plate. Optical density (OD) values were recorded at zero time and 24hours post incubation. The lowest concentration showing inhibition of growth by decrease in OD value was considered the MIC of essential oil and its fractions against test organisms. Statistical analysis was done using one way ANOVA and post hoc Duncan among the MIC values of *C. copticum* Fraction F1 in all the three sampling groups.

Genotoxicity of *Carum copticum* essential oil fractions: Genotoxic potential of *Carum copticum* essential oil fraction F1 was evaluated on lymphocytes extracted from a healthy human donor's blood. The concentrations tested were 2000, 1000, 800, 600, 400, 200, 100 and 50 µl/mL using 20% DMSO as a positive control where as RPMI (Roswell Park Memorial Institute) medium was taken as negative control. Mean head and tail length of damaged DNA for *C. copticum* essential oil fraction F1 was calculated. Comet scorings for genotoxicity evaluation of *C. copticum* essential oil fraction F1 were based on cells to be categorized in one of the four categories depending upon its tail length. Based on this categorization into different classes the damage index and fragmentation percentage was calculated as described by Valencia-Quintana *et al*. (2012) and genetic damage index by Kousar *et al*. (2014). Statistical analysis using one way ANOVA and post hoc Duncan was applied for comparison of the mean tail lengths at all tested doses from the controls.

GC-MS Analysis of *Carum copticum* essential oil and its fractions: GC-MS Analysis of *Carum copticum* essential oil was performed for the determination of active components showing anti-MRSA activity. The determined constituents were then compared with Wiley (V.7.0) and NIST (National Institute of Standards and Technology V.2.0 GC-MS reference libraries. The essential oil was analyzed by GC-MS on an Agilent Technologies 5975A GC system with Inert XL Mass Selective Detector. An Agilent (DB-1-1022) column (30 m x 320 µm x 0.25 µm) was used with helium as carrier gas at a flow rate of 25cm/s at 40°C. The injector temperature was at 250 °C; split 40:1 and 1µl injection. MS source was 230°C and MS quad 150°C. Mass spectra were recorded at 70 eV. Mass range was from *m/z* 30 to 500. Run time was 62 minutes. The individual constituents of the oil and its fractions were identified on the basis of their retention indices determined with a reference to a homologous series of *n*-alkanes and by comparison of their mass spectral fragmentation patterns (NIST 08.L database/chemstation data system) with data previously reported in literature (McLafferty *et al*., 1991; Joulain and König, 1998).

RESULTS

Pale yellow essential oil obtained from seeds of *Carum copticum* L. having 1.9 percent yield. Silica gel gravity column chromatography performed for fractionation and seven fractions of *C. copticum* essential oil were recovered.

Essential oil and its seven fractions were tested for the antibacterial activity. The fractions F1 and F2 were effective having zones of inhibition greater than pure essential oil and commercial standards of vancomycin and linezolid (Table 1). Fraction F1 of *C. copticum* essential oil showed overall 50 percent activity greater than pure essential oil. Fraction F2 showed 16.6% activity greater than pure essential oil but lesser than as compared to fraction F1 (Table 1).

The MIC of Fraction F1 was found against selected MDR *S. aureus* isolates from patients, health-care workers and community in comparison to pure essential oil. It was found that MIC range of *C. copticum* essential oil against selected MDR *Staphylococcus aureus* isolates from patients, health care worker and community was 2.5-10 µl/mL by micro broth dilution method and for Fraction F1 of essential oil was 1-2µl/mL (Table 2). The mean MIC values for essential oil were 4.38±2.58, 2.03±1.33 and 3.59±3.02 µl/mL respectively and for fraction F1 were 1.25±0.46, 1.37±0.52 and1.37±0.52 µl/mL for isolates from patients, healthcare workers and healthy community. Upon Statistical analysis non-significant difference among the MIC values of *C. copticum* Fraction F1 was observed in all the three sampling groups as p-value > 0.05.

Mean head and tail length of damaged DNA for *C. copticum* essential oil fraction F1 was determined for is given in Table-3 and Figure-1. The mean tail length at highest F1 concentration 2000µl/mL tested was 0.57±0.20µm far lower than produced by positive control (13.3±0.33 µm) and almost half of that obtained for *C. copticum* essential oil at this concentration. Statistical analysis showed highly significant difference between the mean tail lengths at all tested doses.

Comet scorings for genotoxicity evaluation of *C. copticum* essential oil fraction F1 was calculated and genetic damage index recorded at all doses tested was lower than the positive control (Table 4). The GDI recorded at highest concentration of *C. copticum* essential oil tested (2000µl/mL) was 1.00. There was a gradual increase in the GDI values (Figure 2). Statistical analysis using Pearson's Chi-square showed non-significant difference (P>0.05) between the GDI values at all tested doses of *C. copticum* essential oil fraction F1. Most of the cells were in Class I and none were categorized in Class II and III damage at all tested concentrations hence indicating *C. copticum* essential oil fraction F1 non-DNA damaging and safe even at many fold concentration of MIC obtained.

Table 1. Antibacterial activity of *Carum copticum* L. essential oil fractions.

	***Carum copticum* Essential Oil Fractions**			
Column Fractions	**Patients**	**Health care Workers**	**Community**	**Total**
F	N=8	N=8	N=8	N=24
	% Sensitivity in relation to Pure Essential			
F1	4(50%)	5(62.5%)	3(37.5%)	12(50%)
F2	1(12.5%)	2(25%)	1(12.5%)	4(16.6%)
F3	0(0%)	0(0%)	0(0%)	0(0%)
F4	0(0%)	0(0%)	0(0%)	0(0%)
F5	0(0%)	0(0%)	0(0%)	0(0%)
F6	0(0%)	0(0%)	0(0%)	0(0%)
F7	0(0%)	0(0%)	0(0%)	0(0%)
	% Sensitivity in relation to Vancomycin			
F1	8(100%)	8(100%)	8(100%)	24(100%)
F2	8(100%)	8(100%)	8(100%)	24(100%)
F3	1(12.5%)	5(62.5%)	8(100%)	14(58.3%)
F4	0(0%)	1(12.5)	4(50%)	5(20.8%)
F5	0(0%)	1(12.5)	3(37.5%)	4(16.6%)
F6	2(25%)	2(25%)	5(62.5%)	9(37.5%)
F7	0(0%)	1(12.5%)	3(37.5%)	4(16.6%)
	% Sensitivity in relation to Linezolid			
F1	8(100%)	8(100%)	8(100%)	24(100%)
F2	6(75%)	5(62.5%)	8(100%)	18(75%)
F3	0(0%)	1(12.5%)	3(37.5%)	4(16.6%)
F4	0(0%)	1(12.5%)	3(37.5%)	4(16.6%)
F5	0(0%)	1(12.5%)	3(37.5%)	4(16.6%)
F6	0(0%)	1(12.5%)	3(37.5%)	4(16.6%)
F7	0(0%)	1(12.5%)	3(37.5%)	4(16.6%)

Upon GC/MS analysis essential oil contained thirty compounds eluted on a DB-1-1022 column such as 1R-α-Pinene, β-Pinene, D-Limonene, β-Phellandrene, β-Terpinyl acetate, Ţ-Terpinene, p-Cymene, Dehydro-p-cymene, cis-β-Terpineol, Trans-p-Metha-2,8-dienal, 4-Terpineol, Trans-dihydrocarvone, α-Terpineol, D-Carvone, Carvone, Cis-p-Menth-1-en-3-ol, 2,3-Epoxycarene, L-cis-Sabinol, p-Cymen-8-ol, cis-Carveol, Estragole, 2-Pinen-4-one, 2-Allyl-4-methyl phenol, O-tert-butyl Phenol, Thymol, Carvacrol, Elemicin, Myristicin, Apiol, Methoxyeugenol and Limonene-6-ol, pivalate (Table 5). The major constituents were' carvacrol (24.4%), Ţ-Terpinene (8.8%), Apiol (6.5%), Carvone (3.4%) and p-Cymene (3.1%) followed by β-Terpinyl acetate (1.7%), β-Pinene (1.4%), D-Limonene (1.2%) and Thymol (1.1%). Fraction F1 of *C. copticum* essential oil contained main constituents carvacrol followed by p-Cymene, Ţ-Terpinene and Apiol. This fraction showed highest anti-MDR *S. aureus* activity as compared to pure essential oil. Fraction F2 contained thymol as major constituent and was less effective than fraction F1. Rest of the fractions contained solvents with only trace amounts of p-Cymene, Ţ-Terpinene and carvacrol and showed no activity against MDR *S. aureus*.

Table 2. Minimum inhibitory concentration of *Carum copticum* essential oil against selected multi-drug resistant *Staphylococcus aureus* isolates.

Isolates	MIC of *Carum copticum* Essential Oil (µl/ml)					
	Patient (n=8)		Health-Care Workers (n=8)		Community (n=8)	
	E.O	F1	E.O	F1	E.O	F1
1.	10	1	2.5	1	1.25	1
2.	5	1	1.25	1	1.25	1
3.	2.5	1	1.25	1	5	2
4.	5	1	1.25	2	1.25	1
5.	5	1	2.5	1	10	1
6.	2.5	2	2.5	1	0.625	2
7.	2.5	2	1.25	2	5	1
8.	0.625	1	1.25	2	0.625	2
MIC Range	0.625-10	1-2	1.25-2.5	1-2	0.625-10	1-2
Mean±S.D	4.14±2.85	1.25-0.46	1.71±0.65	1.37±0.52	3.12-3.32	1.37±0.52

Table 3. Mean head and tail length of damaged DNA for *Carum copticum* Essential Oil Fraction F1.

Sr. no.	Concentrations (µl/ml)	Mean DNA Head Length (µm) n=25	Mean Tail length (µm) n=25
1	2000	2.06±0.50	0.57±0.20
2	1000	2.23±0.48	0.37±0.38
3	800	2.26±0.65	0.22±0.19
4	600	2.61±0.99	0.17±0.21
5	400	2.69±0.63	0.16±0.20
6	200	2.71±0.61	0.15±0.31
7	100	2.87±0.42	0.05±0.14
8	50	3.98±0.85	0.04±0.11
Positive control	20% DMSO	0.78±0.01	13.3±0.50
Negative control	RPMI medium	5.78±0.16	0.13±0.01

Table 4. Genetic damage index of various concentrations of *Carum copticum* essential oil Fraction F1 in COMET assay using extracted lymphocytes from the blood of healthy donor after incubation of 3hours

Sr No	Concentration µl/ml	Class 0	Class 1	Class 2	Class 3	Damage index	Genetic Damage Index GDI
1	2000	-	25	`	-	25	1.00
2	1000	6	19	-	-	19	0.76
3	800	11	14	-	-	14	0.56
4	600	13	12	-	-	12	0.48
5	400	15	10	-	-	10	0.40
6	200	18	7	-	-	7	0.28
7	100	21	4	-	-	4	0.16
8	50	22	3	-	-	3	0.12
Positive control	20% DMSO	0	10	2	13	53	2.12
Negative control	RPMI medium	23	2	0	0	2	0.08

Max. GDI value is 3 (if all the lymphocytes DNA damage categorized in Class 3)

Table 5. GC/MS analysis of *Carum copticum* L. essential oil and its fractions

Sr.#	Components	RT (mins)	Estimated Kovat RI	Relative Peak Area %							
				E.O	F1	F2	F3	F4	F5	F6	F7
1.	1R-α-Pinene	2.693	948	0.113	-	-	-	-	-	-	-
2.	β-Pinene	3.900	943	1.398	+	-	-	-	-	-	-
3.	D-Limonene	5.771	1018	1.201	+	-	-	-	-	-	-
4.	β-Phellandrene	5.965	964	-	+	-	-	-	-	-	-
5.	β-Terpinyl acetate	5.972	1348	1.745	-	+	-	-	-	-	-
6.	Ƭ-Terpinene	7.408	998	8.801	++*	++*	+	-	+	-	-
7.	p-Cymene	8.094	1042	3.060	+++	+++	++	-	+	-	-
8.	Dehydro-p-cymene	12.878	1073	0.052	+	-	-	-	-	-	-
9.	cis-β-Terpineol	14.246	1158	0.013	+	-	-	-	-	-	-
10.	Trans-p-Metha-2,8-dienal	17.129		0.177	-	-	-	-	-	-	-
11.	4-Terpineol	18.714	1137	0.135	-	+	-	-	-	-	-
12.	Trans-dihydrocarvone	20.471	1179	0.327	-	+	-	-	-	-	-
13.	α-Terpineol	22.554	1143	0.222	+	+	-	-	-	-	-
14.	D-Carvone	22.743	1270	0.040	-	-	-	-	-	-	-
15.	Carvone	23.830	1190	3.369	+	++	-	-	-	-	-
16.	Cis-p-Menth-1-en-3-ol,	23.852	1175	-	+	-	-	-	-	-	-
17.	2,3-Epoxycarene	25.878	961	0.097	-	-	-	-	-	-	-
18.	L-cis-Sabinol	26.456	1085	0.058	-	-	-	-	-	-	-
19.	p-Cymen-8-ol	26.885	1197	0.100	+	-	-	-	-	-	-
20.	cis-Carveol	27.618	1206	0.035	-	-	-	-	-	-	-
21.	Estragole	27.730	1172	0.076	+	-	-	-	-	-	-
22.	2-Pinen-4-one	30.336	1119	0.121	-	-	-	-	-	-	-
23.	2-Allyl-4-methyl phenol	31.028	1316	0.026	-	-	-	-	-	-	-
23.	O-tert-butyl Phenol	36.103	1228	0.513	+	-	-	-	-	-	-
24.	Thymol	36.544	1262	-	-	+++*	-	-	-	-	-
25.	Carvacrol	37.173	1262	24.442	+++*	+	-	+	-	-	-
26.	Elemicin	37.648	1550	0.047	+	-	-	-	-	-	-
27.	Myristicin	38.324	1516	0.209	+	-	-	-	-	-	-
28.	Apiol	40.858	1705	6.479	++	-	-	-	-	-	-
29.	Methoxyeugenol	41.763	1581	0.025	-	-	-	-	-	-	-
30.	Limonene-6-ol, pivalate	44.578	1560	0.027	-	-	-	-	-	-	-

Kovats retention index on DB-1-1022 column, +++ = abundantly present, ++ = Moderately present, + = fairly present, - = absent
*indicate relative abundance

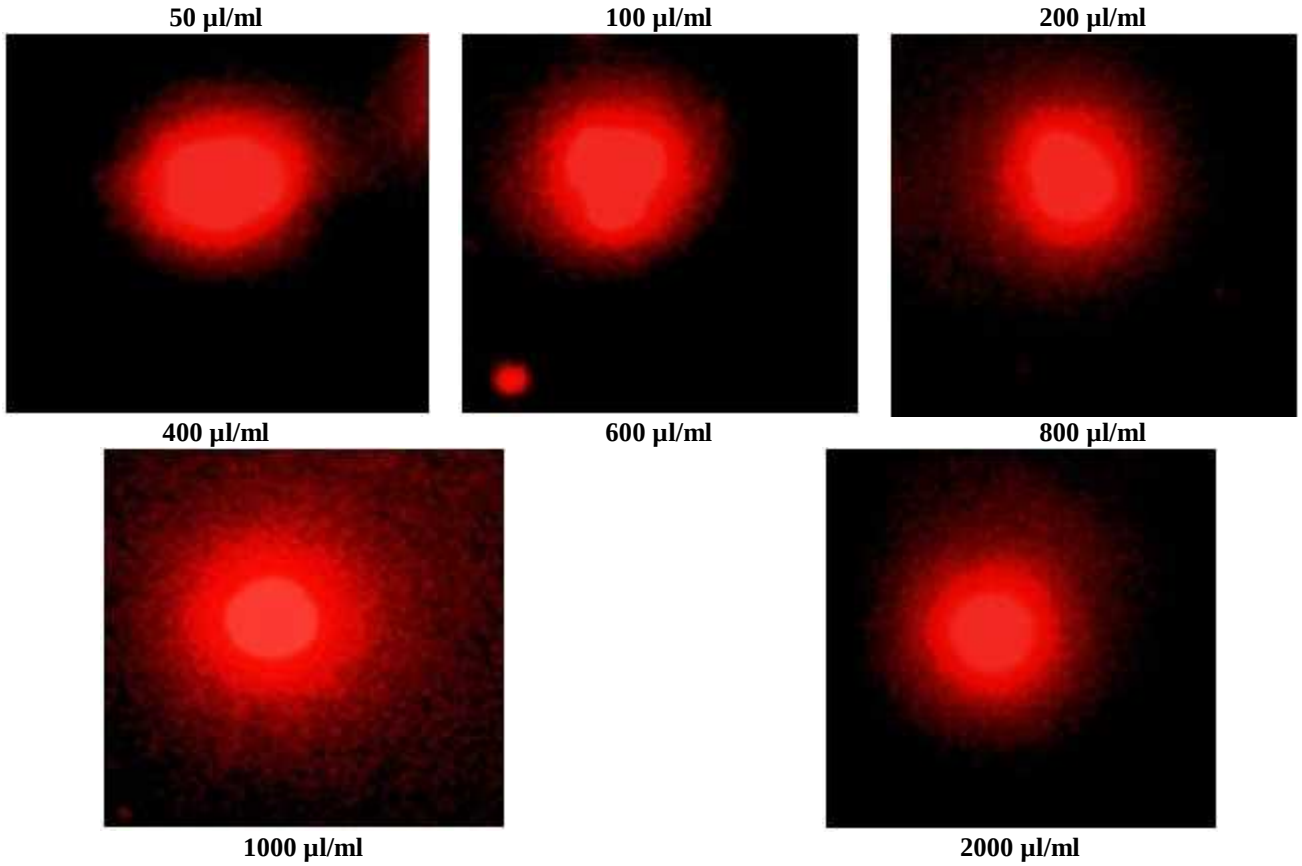


Figure 1. Images of *Carum copticum* essential oil fraction F1 Comets taken from a Fluorescent microscope representing a class 1 DNA damage since the head size is greater the length of tail (Comet formation)

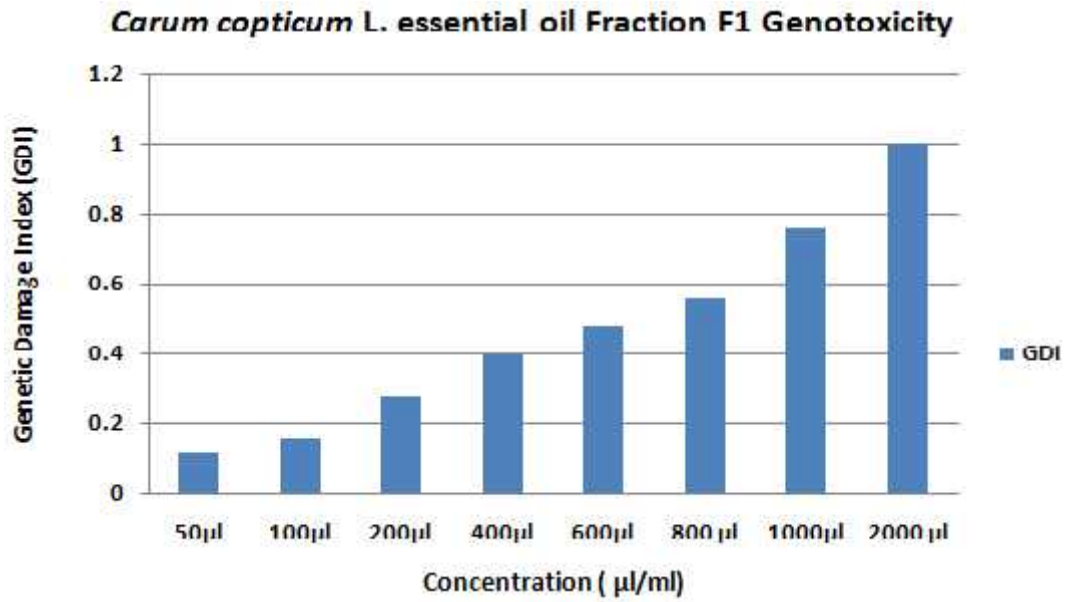
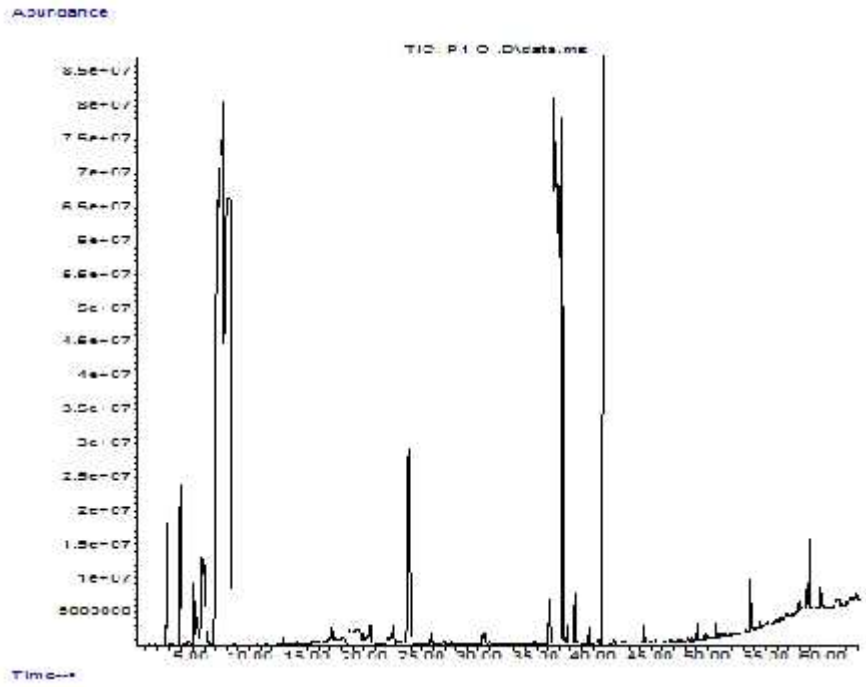


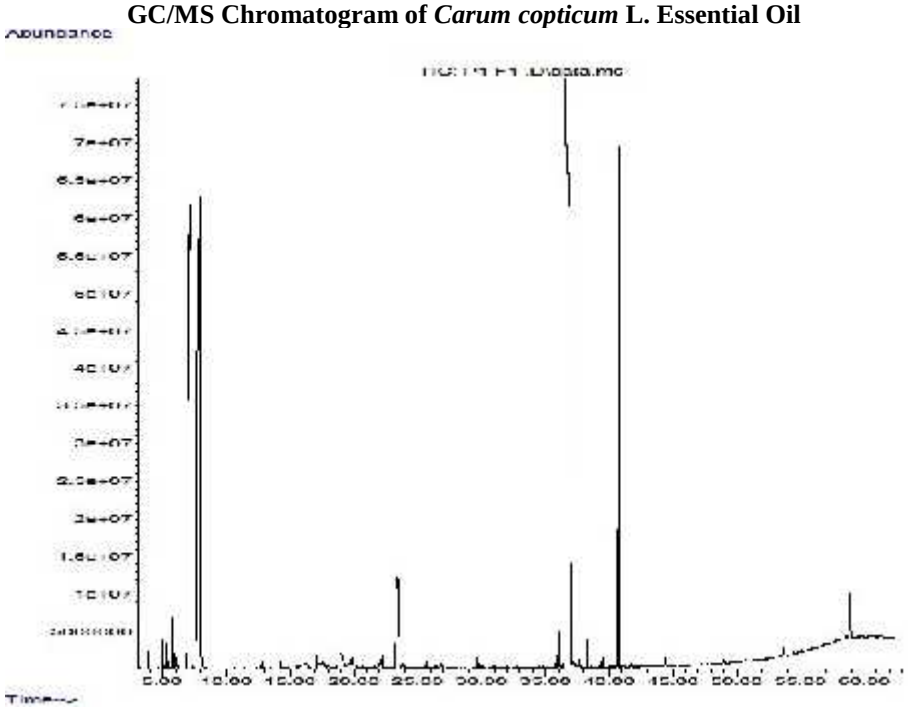
Figure 2. Genetic damage index against various concentrations of *Carum copticum* essential oil fraction F1by COMET assay on human lymphocytes

X axis = Concentration of *Carum copticum* essential oil fraction F1 in µl/ml

Y axis = Genetic damage index against various concentration of *Carum copticum* essential oil fraction F1



GC/MS Chromatogram of *Carum copticum* L. Essential Oil



GC/MS Chromatogram of *Carum copticum* L. Essential Oil Fraction F1

Figure 3. GC/MS Chromatogram of *Carum copticum* L. Essential Oil and its Fraction F1

DISCUSSION

The antibacterial components from indigenous plant essential oil *C. copticum* were evaluated against multidrug resistant *Staphylococcus aureus*. The screening for new antibacterials will guide in the improved management of infectious diseases with adequate treatment options. Many economic benefits can be achieved by exploring the indigenous plants for treatment alternatives as being in reach and cost effective.

Fractionation of *C. copticum* essential oil was performed using silica gel gravity column chromatography and separated into seven different fractions based on increasing solvent polarity using

various solvent combinations. None of the previous studies are available specifically regarding such fractionation pattern of *C. copticum* essential oil. Studies are available on other essential oils fractionated on the same pattern (Rana *et al*., 2011).

The GC/MS of *C. copticum* essential oil eluted almost thirty compounds on a DB-1-1022 column with major constituents as carvacrol (24.4%), Ɣ-Terpinene (8.8%), Apiol (6.5%), Carvone (3.4%) and p-Cymene (3.1%) followed by β-Terpinyl acetate (1.7%), β-Pinene (1.4%) and D-Limonene (1.2%). In the present study the dominant constituent was carvacrol. Oroojalian *et al*. (2010) also reported carvacrol as the major constituent which is consistent with present findings. Goudarzi, *et al*., 2010 reported lesser constituents with the most dominant constituent thymol instead of carvacrol. Zomorodian *et al*., 2011 reported terpinene and cymene as major constituents followed by thymo1 whereas in current study terpinene is the second most dominant constituent from *C. copticum* essential oil. A review article on chemical constituents of *C. copticum* reported comparable findings with the existing study (Boskabady *et al*. 2014). These differences in composition depend upon the chemotype prevalent in a particular geographic region (Zarshenas *et al*., 2014); also factors such as availability of nutrients, climatic conditions, drought and salinity are all factors contributing to this variation in chemical composition of *C. copticum* essential oils (Subramanian *et al*., 2012).

The antibacterial potential of all the column fractions were determined through agar well diffusion assay against selected MDR *S. aureus* strains from all three sampling groups. Out of seven fractions only first two fractions were found effective having zones of inhibition greater than the pure essential oil and commercial standards of Vancomycin and Linezolid. The Fraction F1 of *C. copticum* essential oil containing main constituents carvacrol followed by p-cymene, Ɣ-terpinene and apiol has shown overall 50% sensitivity greater than pure essential oil. The reason being it allowed the purification of oil and elution of those constituents in combination which exhibited maximum activity (Alexopoulos *et al*. 2011). High antimicrobial activity was reported by phenolic constituents like carvacrol and thymol affecting the cell membrane of bacteria (Bassole and Juliani 2012). The combination of these terpenoid components increased the antimicrobial activity (Lv, Liang *et al*., 2011) as evident in case of Fraction F1 and F2 as compared to pure essential oil. Fraction F1 contained mainly phenolic monoterpene carvacrol with monoterpene hydrocarbons p-Cymene and Ɣ-Terpinene which together represented a synergistic combination and enhanced antibacterial activity as also reported by Fournomiti *et al*., (2015). Custodio *et al*. (2011) also reported p-cymene synergistic combination with thymol and carvacrol. Fortunately all these constituents were eluted together in fraction F1 of *C. copticum* essential oil exhibiting synergistic response. The possible reason for inferior activity of fraction F2 as compared to F1 was that it contained lesser content of phenolic monoterpenes as contained only thymol and no carvacrol. Also lesser quantities of p-cymene and Ɣ-Terpinene were present. As other fractions contained only trace amount of these constituents therefore possessed no antibacterial activity.

The MIC of Fraction F1 in case of *C. copticum* essential oil was determined against selected MDR *S. aureus* isolates and compared with pure essential oil. It was found that maximum MIC range of F1 of *C. copticum* essential oil against selected MDR *S. aureus* isolates from patients, health care worker and community was 1-2µl/mL by micro broth dilution method which was lower than10 µl/mL obtained in case of pure essential oil . The reason being the compounds eluted in fraction F1 were in such a combination where acting synergistically as reflected in the decrease in values obtained for MIC of fraction F1 as compared to pure essential oil. The removal of extraneous substances from the essential oil enhanced the anti-bacterial activity (Alexopoulos *et al*. 2011; Bassole and Juliani 2012).

In the present study Fraction F1 of *C. copticum* essential oil exhibited better antibacterial properties when compared to pure essential oil therefore the safety profile of this fraction was evaluated using Comet assay. Overall exhibited better safety profile with mean tail length at highest concentration tested was 0.57±0.20µm which was lower as compared to positive control (13.3±0.33 µm) and comparable with negative control at this concentration.

The GDI values at all tested doses of *C. copticum* essential oil fraction F1 indicated that there was no significant difference in genetic damage caused at both the lowest and highest doses tested declaring the Fraction F1 of essential oil safe even at higher doses. No such studies are available regarding genotoxic analysis of *C. copticum* essential oil and its fractions by Comet assay. Few studies are available regarding other essential oils such as Copaiba oil and its fractions (Almeida *et al*., 2012) and Essential oil (EO) of Alpinia zerumbet leaves showed on DNA damage (Cavalcanti *et al*., 2012) but essential oils from Artemisia lavandulaefolia (Zhang *et al*., 2013) and Rosmarinus officinalis (Maistro *et al*., 2010) showed genotoxic effects. Therefore it is recommended to confirm the results using in-vivo models for dose-response relationships and their possible effective and toxic doses.

Conclusion: It was concluded that *C. copticum* essential oil fraction F1 was the most effective fraction with dominant constituents carvacrol, p-cymene, Ɣ-Terpinene and Apiol and showed highest antibacterial activity as compared to pure essential oil.

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