Indian Journal of Fibre & Textile Research
Vol. 44, September 2019, pp. 344-351

Synergic antibacterial effect of *Curcuma aromatica* Salisb and *Ocimum tenuiflorum* Linn herbal extract combinations on treated cotton knitted fabrics against selective bacterial strains

K Chandrasekaran[1,a] & M Senthilkumar[2]

[1]Department of Fashion Technology, [2]Department of Textile Technology,
PSG College of Technology, Coimbatore 641 004, India

Received 29 March 2018; revised received and accepted 20 June 2018

Synergic antibacterial properties of wild turmeric (*Curcuma aromatica* Salisb, WT) and holy basil (*Ocimum Tenuiflorum* Linn, HB) and their combination (100:0, 75:25, 50:50, 25:75 and 0:100) on cotton knitted fabric against *Staphylococcus aureus* (*S.aureus*) and *Escherichia coli* (*E.coli*) bacterial strains have been studied. The effect of aqueous and methanol extract of WT, HB and their combination has also been studied separately. It is observed that the methanol extract of 50:50 proportion yields minimum inhibitory concentration value against *S.aureus* and *E.coli* bacterial strains. The gas chromatography and mass spectrometry (GC-MS) test results reveal that the active components which contribute to the antibacterial performance are present both in individual and 50:50 proportion of herbal extracts. In 50:50 (WT:HB) proportion, besides the active components of wild turmeric and holy basil, new compounds are also identified. Pre-treated single jersey cotton knitted fabrics have been finished with the herbal extracts combination by maintaining the common process parameters. The AATCC 100 test results reveal that the 50:50 (WT: HB) combination exhibits good antibacterial activity against *S.aureus* and *E.coli* bacterial strains and the results correlate with the MIC test results and GC-MS analysis. The test results explore a new combination of herbal based antibacterial finishing agent for the development of antibacterial textile products.

Keywords: Antibacterial textiles, Cotton fabric, Gas chromatography, Holy basil, Mass Spectrometry analysis, Wild turmeric

1 Introduction

On combining several herbs in a specific proportion, the resultant formulation will give a better therapeutic effect and reduce the toxicity[1]. Synergistic interactions between the compounds of individual or mixtures of herbs are a great part of their therapeutic efficacy[2]. *Curcuma aromatic* Salisb (wild turmeric) can be used in treating inflammation, wound and microbial infections[3]. Wild turmeric and its extracts possess anti-inflammatory, wound healing, anti-melanogenic, antioxidant, free radical scavenging, anti-tumor, anti-cancer, anti-repellent, antitussive, anti-platelet and anti-nephrotoxic properties[4]. Phytochemical analysis of *C. aromatica* and *C. xanthorrhiza* revealed the presence of major phytochemicals like flavonoids, tannin, saponin, and terpenoids in both the species[5]. Besides curcuminoids, wild turmeric extracts contains other phytochemicals which are to be explored[6]. It was reported that ethanolic extracts of *C. aromatica* is more potent than *C. fenestratum* [7].

Ayurvedic texts identified *Ocimum sanctum* L. as stimulant, aromatic, antipyretic, antibacterial, antiviral, antifungal, etc[8]. The active ingredients, such as eugenol, ß-caryophyllene, ß-elemene, germacrene D, flavonoids and glycosides were found in *Ocimum tenuiflorum* L. The extracts of the *Ocimum sanctum* L. individually and its combination with other plant extract have been found to have therapeutic activity[9]. The methanol extract of *Ocimum sanctum* L. leaves was reported with higher activity than other organic and aqueous extracts[10]. Among the different leaf extracts of *Ocimum tenuiflorum* L., methanol extract is reported to have more antibacterial activity against *S. aureus* than *E. coli* and *C. albicans*[11]. The presence of steroids, alkaloids, and tannins in phytochemical study, and significant antimicrobial activity have been reported with both aqueous and methanol extracts of root and leaves of *Ocimum sanctum* L. against *E. coli*, *P.mirabilis* and *S. aureus* [12]. The methanol and aqueous extracts of *Ocimum sanctum* L. crude leaf

[a]Corresponding author.
E-mail: kcs.fashion@psgtech.ac.in

powder were reported to be most effective against the pathogenic bacterial strains[13]. Scientists have understood the immense potential in natural products from medicinal plants to fight against infections in human beings[14]. Antimicrobial activity in the cotton fabric, treated with methanolic extracts of neem leaves, prickly chaff flower, tulsi leaves and pomegranate rind, against the bacterial strains *S.aureus* and *E.coli* has been reported; with neem ranked as first, followed by pomegranate and prickly chaff flower[15]. The demand for hygienic textiles, and the consumption of antimicrobial products are increasing day by day. Many research activities are being carried out to achieve more effective and safe solutions. There is an increased interest in natural probable sources for antimicrobial agents, including those from animal (chitosan) and metal (copper and silver) sources[16]. Antimicrobial activity of cotton fabric treated with *Quercus infectoria* extract showed that the treated cotton textiles can successfully be used as bio active textiles from natural eco-friendly materials[17]. Natural antimicrobial agents, due to their eco-friendly nature and non-toxic properties, are still the promising candidates for applications, like medical and healthcare textiles[18]. The application of *Ocimum sanctum* L. (tulsi leaf) and rind of *Punica granatum* L. (pomegranate) herbal extracts on cotton fabric by direct method, micro-encapsulation, resin cross-linking and their combinations shows good antibacterial properties[19]. Recently, the focus on the coating of plant based natural dyes and other bioactive natural extract on fabric as antimicrobial textile finish has gained significant momentum. The major synthetic antimicrobial agents for textile coating are of chemical in nature with toxic and environmental issues. Several plants based natural dyes show strong antimicrobial properties. Therefore, coating of natural dyes and bioactive plant extract on cotton fabrics for antimicrobial effect has been reported as an emerging technology for the production of medical textiles [20].

In view of above, an attempt has been made to explore the synergic antibacterial performance of the herbs, wild turmeric (*Curcuma aromatica* Salisb) and holy basil (*Ocimum Tenuiflorum* L.*)*. These herbs possess many bioactive agents individually and offer medicinal properties such as antimicrobial, antioxidant, wound healing, anti-inflammatory, anti-cancer etc. In order to study their synergic antibacterial properties, the combination of two herbal extracts is used for treatment on single jersey knitted cotton fabrics and then tested for their quantitative antibacterial performance.

2 Materials and Methods

2.1 Materials

Wild turmeric (WT, *Curcuma aromatica* Salisb) and holy basil (HB, *Ocimum Tenuiflorum* L.*)* were sourced from the organic farms in Tamilnadu state, India. The leaves of the holy basil were shadow dried and then powdered using pulverizer. The rhizomes of wild turmeric were shadow dried and the dried tubers were crushed into small pieces. The debris was removed and the remaining part was powdered using a pulverizer.

Pre-treated cotton single jersey fabrics knitted with 30s Ne yarn count, having 180 g/m^2 (GSM) fabric weight, 20 courses/cm, 16 wales/cm , 0.263 cm loop length and 16.87 fabric tightness factor, were used for the finishing treatment.

2.2 Methods

2.2.1 Solvent Extraction and Yield Percentage Evaluation

Fifty gram of finely powdered sample was exhaustively extracted with methanol using soxhlet apparatus. The solvent extract was concentrated to dryness under reduced pressure using rotary vacuum evaporator, and weighed. For aqueous extraction, 50 g of powdered sample was macerated using hot water with occasional stirring for 16 h. The water extract was filtered, concentrated and dried in a desiccator to constant weight. The percentage yield (recovery) of evaporated extract was calculated as follows:

$$\text{Yield (\%)} = \{[\text{Extract + container (g)}] - [\text{Empty container (g)}]\} / \text{Sample weight (g)} \times 100$$

The percentage yield was expressed in terms of the air dried drug.

2.2.2 Minimum Inhibitory Concentration Test

The microorganisms tested for the study were *Staphylococcus aureus* (MTCC 3381) and *Escherichia coli* (MTCC 739). The minimum inhibitory concentration (MIC), which is considered as the least concentration of the sample which inhibits the visible growth of a microbe was determined by the broth dilution method. Organisms were sub cultured on nutrient agar, followed by incubation for 24 h at 37°C. Inocula were prepared by transferring several colonies of microorganisms to sterile nutrient broth. The suspensions were mixed for 15 s and incubated

for 24 h at 37 °C. Required volume of suspension culture was diluted to match the turbidity of 0.5 Mc Farland standard (1.5×108 CFU/mL). Samples were prepared in dimethylsulphoxide (DMSO) at the concentration of 2 mg/mL. A series of 15 tubes were filled with 0.5 mL sterilized nutrient broth. Sequentially, test tubes 2–14 received an additional 0.5 mL of the sample serially diluted to create a concentration sequence from 500 – 0.06µg. The first tube served as the control. All the tubes received 0.5mL of inoculum. The tubes were vortexed well and incubated for 24 h at 37°C. The resulting turbidity was observed and after 24 h, MIC was determined where growth was no longer visible by assessment of turbidity by optical density readings at 600 nm.

2.2.3 Synergy Calculation

Based on the minimum inhibitory concentration (MIC) values of the individual and their combination herbal extracts of wild turmeric and holy basil, the synergy between the two herbs was calculated by using the fractional inhibitory concentration (FIC) index method[21], as shown below:

FIC index= FIC (WT) + FIC (HB)

FIC (WT) = MIC of WT+HB/ MIC of WT

FIC of HB = MIC of HB+WT/ MIC of HB

Combinations were classified as synergistic if the FIC indices were ≤1, additive if the FIC indices were = 1; indifferent if the FIC indices were between 1 and 2, and antagonistic if the FIC indices were ≥ 2.

2.2.4 Gas Chromatography–Mass Spectrometry (GC-MS) Analysis

The GC-MS analysis technique was used to identify the compounds present in the methanol extracts of wild turmeric, holy basil and their combination. The instrument details were: Thermo GC (Trace ultra version 5.0); Thermo MS DSQ II instrument with ZB 5 - MS capillary standard non – polar column; dimension 30 m, ID 0.25 mm; film 0.25 µm, carrier gas He; flow rate 1.0 mL/min; temperature progress from oven temperature 70 °C to 260 °C at 6°C/ min; and injection volume 1 micro litre. Identification of the peaks was carried out based on the computer matching of the mass spectra with that of the National Institute of Standards and Technology (NIST 08 and NIST 08_S) library and also by directly comparing with the published data[22].

2.2.5 Finishing Treatment on Cotton Knitted Fabrics

The combination herbal extracts of wild turmeric (WT) and holy basil (HB) prepared with methanol as solvent in the proportion of 100:0; 75:25; 50:50; 25:75 and 0:100 respectively were applied on to pre-treated single jersey cotton knitted fabrics using pad-dry-cure method. The process parameters used for the study were: methanol extract of combination herbs; wild turmeric + holy basil concentration 2.5% (owf); cross linking agent citric acid (5% owf); immersion time 30 min; wet pick-up 80%; drying conditions 10 min at 60°C; and curing conditions 2 min at 100°C.

2.2.6 Assessment of Quantitative Antibacterial Activity

The assessment of quantitative antibacterial activity of the combination herbal extracts treated cotton single jersey knitted fabrics was carried out by using AATCC 100 test method**.** The microorganisms used for the study are *Staphylococcus aureus* (MTCC 96) and *Escherichia coli (*MTCC 724).

3 Results and Discussion

3.1 Solvent Extraction Yield Percentage

The active components present in wild turmeric and holy basil were extracted with aqueous media using maceration method and solvent media (methanol) in soxhlet apparatus. The results show that the aqueous extraction process yields 11.75% and 15% and methanol extraction process yields 13.5% and 23.75% for wild turmeric and holy basil respectively. Among the two extraction methods, the solvent extraction process yield is found to be higher for both the herbs. Thus, the methanol extract of both the herbs is used to prepare the combination herbal extract formulation containing wild turmeric (WT) and holy basil (HB) in the proportion of 100:0; 75:25; 50:50; 25:75 and 0:100 respectively.

3.2 Minimum Inhibitory Concentration

Table 1 shows the minimum inhibition concentration (MIC) results of aqueous and methanol extract of wild turmeric and holy basil combination herbal extracts. The minimum inhibition concentration results reveal that the combination herbal extracts using methanol as solvent are having lowest MIC values as compared to the aqueous

Table 1 — Minimum inhibitory concentration (MIC) test results

WT:HB extract	MIC, µg/mL			
	Aqueous		Methanol	
	S. aureus	*E.coli*	*S. aureus*	*E.coli*
100:0	62.50	125	15.63	15.63
75:25	125.00	250	62.50	31.25
50:50	62.50	125	3.90	7.80
25:75	125.00	125	62.50	31.25
0:100	15.63	125	7.80	62.50

combination herbal extracts. Among the methanol extract samples, 50:50 (WT: HB) combination yields minimum MIC value as compared to other combinations. The results show that the balanced synergic actions of bio active components in both the herbs are found in this formulation. These results also match with the concept of mixing equal proportion of herbs in a medicinal formulation practiced in the traditional medicine systems, like Siddha and Ayurveda.

3.3 Synergy between Herbs in Combined Extracts

The synergy between wild turmeric and holy basil is calculated based on their MIC values alone and in combination and the resultant fractional inhibition concentration index (FIC) values are given in Table 2 for both aqueous and methanol combination herbal extracts. The FIC index values obtained for methanol extract of 50:50 (WT: HB) combination for *Staphylococcus aureus and Escherichia coli* are 0.7495 and 0.6238 respectively. Since the FIC index values are < 1 for both bacterial strains, this indicates that the constituent herbs in this combination herbal extract proportion are found to be synergic in action against bacterial strains.

Table 2 — Synergy calculation of combination herbal extracts

WT:HB	Fractional inhibitory concentration index (FIC)			
	Aqueous		Methanol	
	S. aureus	*E.coli*	*S. aureus*	*E.coli*
100:0	1.000	1	1.000	1.000
75:25	9.997	4	24.038	4.506
50:50	4.999	2	0.750	0.624
25:75	9.997	2	12.012	2.499
0:100	1.000	1	1.000	1.000

3.4 GC-MS Analysis

3.4.1 Methanol Extract of Wild Turmeric

Figure 1 shows the GC-MS spectra of methanol extract of wild turmeric. Table 3 shows the GC-MS analysis results of the methanol extract of *Curcuma aromatica* Salisb identified with 30 compounds. In the GC-MS analysis, many active components are detected. The identification of phytochemical compounds is based on peak area, molecular weight and molecular formula, match factor (SI), reverse match factor (RSI) and probability values. Among the

Table 3 — GC-MS results of methanol extract of wild turmeric

Retention time, min	Methanol extract constituent	Molecular formula	Molecular weight	Peak area, %
7.09	2'-Hydroxy-6'-methoxychalcone	$C_{18}H_{18}O_5$	314	0.49
9.77	ë-Elemene	$C_{15}H_{24}$	204	0.14
10.81	Zingiberene	$C_{15}H_{24}$	204	1.04
11.36	Cyclohexane	$C_{15}H_{24}$	204	2.85
11.74	trans-á-Farnesene	$C_{15}H_{24}$	204	1.52
12.40	(2S,3S,4aS,8aR)-2-[2-(tert-Butyldiphenylsiloxy)ethyl] oct ahydropyrano[3,2-b]pyran-3-yl 4-Nitrobenzoate	$C_{33}H_{39}NO_7Si$	589	17.04
13.00	1-(4-Trifluoromethylphenyl) nonanone	$C_{16}H_{21}F_3O$	286	24.92
13.90	sesquisabinene hydrate	$C_{15}H_{26}O$	222	1.81
14.92	á-Elemenone	$C_{15}H_{22}O$	218	11.60
15.54	(-)-Spathulenol	$C_{15}H_{24}O$	220	0.29
16.39	á-Bisabolol	$C_{15}H_{26}O$	222	1.42
16.94	Germacrone	$C_{15}H_{22}O$	218	2.02
17.27	(S)-(+)-Curcuphenol	$C_{15}H_{22}O$	218	1.54
18.08	2-Methylbenzimidazole-1-acetic acid hydrazide	$C_{10}H_{12}N_4O$	204	29.18
18.58	Sesquiterpene diol	$C_{15}H_{26}O_2$	238	0.47
18.93	6,8-Dimethoxy-2-methyl-1,4-naphthoquinone	$C_{13}H_{12}O_4$	232	0.44
19.29	Colchiethanamine	$C_{19}H_{23}NO_3$	313	0.11
20.17	2-Fluoro-5-phenoxybenzoic acid	$C_{13}H_9FO_3$	232	0.48
21.03	11,13-Dihydro-7,11-dehydro-13-hydroxy-3-desoxyzaluzanin	$C_{15}H_{18}O_3$	246	0.30
22.55	o-Curcuhydroquinone	$C_{15}H_{22}O_2$	234	0.52
22.89	Xanthinin	$C_{17}H_{22}O_5$	306	0.24
24.22	Hexadecane-1,2-diol	$C_{16}H_{34}O_2$	258	0.18
25.53	4-Amino-2,3-dibromobenzaldehyde	$C_7H_5Br_2N_O$	277	0.20
30.93	Hexacosane	$C_{26}H_{54}$	366	0.10
33.74	Heptacosane	$C_{27}H_{56}$	380	0.14
35.21	Cis-1,2,7,9,10-pentamethoxyaporphine	$C_{22}H_{27}NO_5$	385	0.19
35.64	Octacosane	$C_{28}H_{58}$	394	0.13
36.66	Stigmasterol	$C_{29}H_{48}O$	412	0.23
37.98	Nonacosane	$C_{29}H_{60}$	408	0.13
40.08	STIGMAST-5-EN-3-OL, (3á,24S)-	$C_{29}H_{50}O$	414	0.29

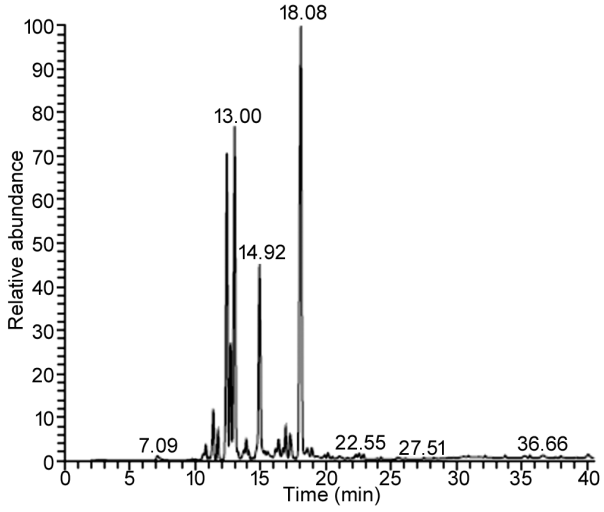

Fig. 1 — GC-MS spectra of methanol extract of wild turmeric

identified compounds, (2S,3S,4aS,8aR)-2-[2-(tert-butyldiphenylsiloxy)ethyl]oct ahydropyrano [3,2-b]pyran-3-yl 4-nitrobenzoate, 1-(4-trifluoromethylphenyl) nonanone, á-elemenone and 2-methylbenzimidazole-1-acetic acid hydrazide are found with prominent peaks.

The rhizome extract of the plant is reported as highly effective against many human pathogens as well as microorganisms causing food spoilage and food borne diseases. Extensive literature survey reveals that the plant has anticancerous, anti-obesity, anti-acne, antitussive, antioxidant, anti-inflammatory, antidiabetic and wound healing properties. The antimicrobial activity against several human pathogens shows its ability to fight against several diseases and skin infections. The use of *Curcuma*

Table 4 — GC-MS results of methanol extract of holy basil

Retention time, min	Methanol extract constituent	Molecular formula	Molecular weight	Peak area, %
9.99	Eugenol	$C_{10}H_{12}O_2$	164	26.93
10.67	3-(Phenylseleno)bicyclo[1.1.1] pentane-1-carboxylic methyl ester	$C_{13}H_{14}O_2Se$	282	7.64
11.25	Caryophyllene	$C_{15}H_{24}$	204	10.12
11.96	à-Humulene	$C_{15}H_{24}$	204	0.86
12.36	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	$C_{15}H_{22}$	202	1.81
12.75	exo-6-Chloro-3-(5-acetyl-2-phenyloxazol-3-yl)benzopyran-4-one	$C20H_{16}ClNO_4$	369	4.09
14.59	(-)-Caryophyllene oxide	$C_{15}H_{24}O$	220	3.23
15.72	Tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol, 4,4-dimethyl-	$C_{15}H_{24}O$	220	0.46
16.14	2-Azido-N-[2-azidobenzyl] benzamide	$C_{14}H_{11}N_7O$	293	2.24
17.18	5à-acetoxymethyl-4a,5,8,8aà-tetrahydro-2,4 aá-dimethyl-1,4-naphthalindione	$C_{15}H_{18}O_4$	262	0.44
17.56	Longipinocarveol, trans-	$C_{15}H_{24}O$	220	1.28
17.94	Phenol, 2-methyl-5-(1,2,2-trimethylcyclopentyl)-, (S)-	$C_{15}H_{22}O$	218	0.83
19.42	Dihydro-á-ionone	$C_{13}H_{22}O$	194	0.87
19.77	Neophytadiene	$C_{20}H_{38}$	278	0.56
21.54	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	0.41
22.28	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	3.28
25.01	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	$C_{20}H_{40}O$	296	1.12
25.56	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278	5.47
30.88	Benzene, 1-methoxy-4-(1-propenyl)-	$C_{10}H_{12}O$	148	13.13
31.72	Tetratetracontane	$C_{44}H_{90}$	618	0.57
32.21	Hexacosane	$C_{26}H_{54}$	366	1.46
33.35	(S)-(2u,6l,3"x)-(1'E)-2-[3'-(3"-Oxocyclohexyl)-1'-propenyl] -6-methyl-3-(1,1-dimethylethyl)-1,3,2-oxazaphosphorinan e 2-Oxide	$C_{18}H_{32}NO_3P$	341	1.26
33.74	Heptacosane	$C_{27}H_{56}$	380	1.61
34.06	11à-hydrozyresibufogenin	$C_{24}H_{32}O_5$	400	0.62
35.22	(E/Z)-2,2-Dimethyl-8-(methoxymethoxy)-4-octen-3-ol	$C_{12}H_{24}O_3$	216	0.56
35.84	Squalene	$C_{30}H_{50}$	410	3.96
36.71	1H-Cyclopropa[3,4]benz[1,2-e]azulene-2,5-dione, 9,9a-bis(acetyloxy)-3-[(acetyloxy)methyl]-1a,1b,4a,7a,7b, 8,9,9a-octahydro-4a,7b-dihydroxy-1,1,6,8-tetramethyl-, [1aR-(1aà,1bá,4aá,7aà,7bà,8à,9á,9aà)]-	$C_{26}H_{32}O_{10}$	504	1.95
37.97	Nonacosane	$C_{29}H_{60}$	408	1.09
38.34	á-Amyrin methyl ether	$C_{31}H_{52}O$	440	0.56
40.09	Stigmast-5-en-3-ol, (3á,24S)-	$C_{29}H_{50}O$	414	1.57

aromatica Salisb in traditional systems of medicines can be supported by the various properties reported in modern scientific literatures published in the past decade[23].

Among the listed compounds, Germacrone is identified as anti-inflammatory and antimicrobial apart from the other activities identified. Zingiberene is identified with anti-inflammatory and immunomodulatory activities[24].

3.4.2 Methanol Extract of Holy Basil

Figure 2 shows the GC-MS spectra of methanol extract of holy basil (*Ocimum Tenuiflorum* L.*).* Table 4 shows the GC-MS results of methanol extract of holy basil (*Ocimum Tenuiflorum* L.*)* identified with 30 compounds. Among the identified compounds, eugenol, 3-(phenylseleno) bicyclo [1.1.1] pentane-1-carboxylic, trans-caryophyllene, (-)-caryophyllene oxide, dihydro-á-ionone, hexadecanoic acid, 9, 12, 15-octadecatrienoic acid, (Z, Z, Z)-, benzene, 1-methoxy-4-(1-propenyl)-, squalene and nonacosane are found with prominent peaks.

From the listed compounds, eugenol, caryophyllene, hexadecanoic acid, methyl ester are reported for reducing the growth of human and fish pathogens[25]. Eugenol is identified with acaricide, antibacterial, anti-inflammatory, antioxidant, cancer-preventive, antispasmodic, antiviral, and insecticide activities. Caryophyllene is identified with anti-tumor, analgesic, antibacterial, anti-inflammatory, and fungicide activities[26].

3.4.3 WT: HB (50:50) Combination

Figure 3 shows the GC-MS spectra of combination methanol extract of 50:50 (WT: HB) proportion.

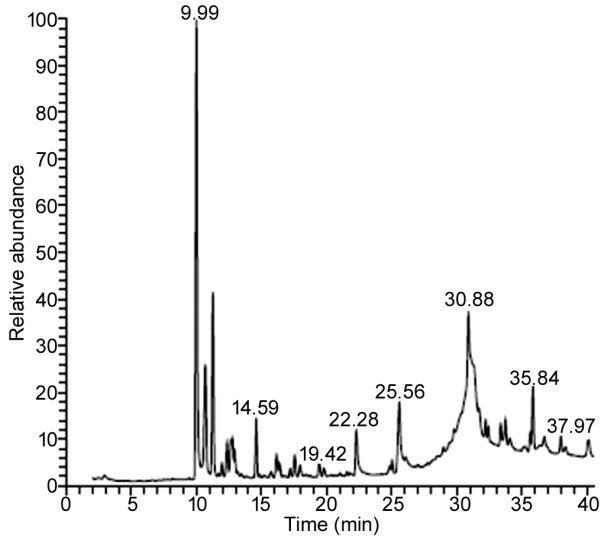

Fig.2 — GC-MS spectra of methanol extract of holy basil

Table 5 shows the GC-MS results of the methanol extract of 50: 50 (WT: HB) combination identified with 30 compounds. Apart from the active components of wild turmeric and holy basil, the new compounds identified are 4-chloro-2,2-dimethyl-7-propoxy-2H-chromene, 4-(cyanomethyl}azulene, ethyl (3R,4R,5S)-4-N-Acetylamino-5-N-allylamino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate, 4H-Furo[3,2-c][1]benzopyran-4-one, 2,3-dihydro-2,2,3-trimethyl-, (.+-.)-,Benzo[e]isobenzofuran-1,4-dione,1,3,4,5,5a,6,7,8,9,9a-decahydro-6,6,9a-trimethyl. The new compounds formation may be due to the mixture of wild turmeric and holy basil extracts.

Among the listed compounds, isocurcumenol is identified with antiviral, antiandrogenic, and platelet derived growth factor inhibitor activities[24]. The good antimicrobial performance of 50:50 (WT: HB) combination may be due to the presence of active components of wild turmeric and holy basil along with the new compounds originated from their mixture.

3.5 Quantitative Antibacterial Test

Table 6 shows the quantitative antibacterial test (AATCC 100) results of wild turmeric and holy basil combination herbal extracts treated cotton single jersey knitted fabrics against *S. aureus and E.coli* bacterial strains for 12 h and 24 h contact time. The test results show that among all the combination herbal extract proportions, 50:50 (WT:HB) proportion has good

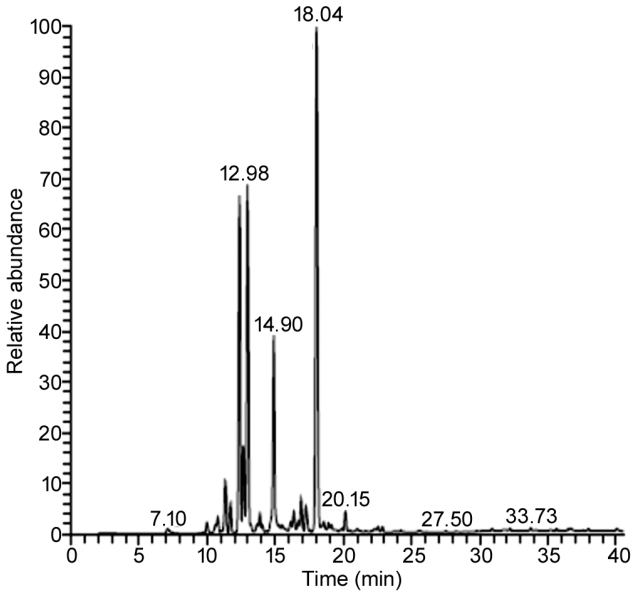

Fig. 3 — GC-MS spectra of combination methanol extract of 50:50 (WT:HB)

Table 5 — GC-MS results of 50:50 (WT:HB) methanol extract

Retention time, min	Methanol extract constituent	Molecular formula	Molecular weight	Peak area, %
7.10	4-Chloro-2,2-dimethyl-7-propoxy-2H-chromene	$C_{14}H_{17}ClO_2$	252	0.46
10.0	Eugenol	$C_{10}H_{12}O_2$	164	0.65
10.79	Zingiberene	$C_{15}H_{24}$	204	1.14
11.34	ç-Elemene	$C_{15}H_{24}$	204	3.01
11.72	trans-á-Farnesene	$C_{15}H_{24}$	204	1.48
12.38	4-(Cyanomethyl}azulene	$C_{12}H_9N$	167	17.20
12.98	Ethyl (3R,4R,5S)-4-N-Acetylamino-5-N-allylamino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate	$C_{19}H_{32}N_2O_4$	352	22.47
13.88	Sesquisabinene hydrate	$C_{15}H_{26}O$	222	1.70
14.90	Germacrone	$C_{15}H_{22}O$	218	11.14
15.52	Isospathulenol	$C_{15}H_{24}O$	220	0.29
16.36	á-Bisabolol	$C_{15}H_{26}O$	222	1.36
16.90	3,7-Cyclodecadien-1-one, 3,7-dimethyl-10-(1-methylethylidene)-, (E,E)-	$C_{15}H_{22}O$	218	1.95
17.26	(S)-(+)-Curcuphenol	$C_{15}H_{22}O$	218	1.51
18.04	4H-Furo[3,2-c][1]benzopyran-4-one, 2,3-dihydro-2,2,3-trimethyl-, (.+-.)-	$C_14H_{14}O_3$	230	30.72
18.56	(-)-Spathulenol	$C_{15}H_{24}O$	220	0.41
18.90	1-Formyl-[2.2]paracyclophane-1,9-diene	$C_{17}H_{12}O$	232	0.72
20.15	Benzo[e]isobenzofuran-1,4-dione,1,3,4,5,5a,6,7,8,9,9a-decahydro-6,6,9a-trimethyl	$C_{15}H_{20}O_3$	248	1.30
21.00	11,13-Dihydro-7,11-dehydro-13-hydroxy-3-desoxyzaluzanin	$C_{15}H_{18}O_3$	246	0.27
22.53	(S)-(+)-Curcuhydroquinone	$C_{15}H_{22}O_2$	234	0.42
22.86	Xanthanin	$C_{18}H_{20}O_5$	316	0.24
23.70	Isocurcumenol	$C_{15}H_{22}O_2$	234	0.11
24.22	Hexadecane-1,2-diol	$C_{16}H_{34}O_2$	258	0.14
25.56	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278	0.17
30.90	7-(2"-Hydroxyisopropyl)-4,5-epoxy-18,19-dihydro-3-hydroxy-6-methoxy-17-allyl-6,14-ethenomorphinane	$C_{25}H_{35}NO_4$	413	0.13
33.73	Octacosane	$C_{28}H_{58}$	394	0.21
35.16	5-Amino-7-bromo-8-cyano-4-methyl-3,4-dihydro-1,6-naphthyridin-2(1H)-one	$C_{10}H_9BrN_4O$	280	0.11
35.61	Nonacosane	$C_{29}H_6O$	408	0.17
36.67	13,17-Diethyl-12,18-dimethyl-21,22-dioxaoxophlorin	$C_{26}H_{24}N_2O_3$	412	0.22
37.97	celidoniol, deoxy-	$C_{29}H_{60}$	408	0.12
40.07	stigmast-5-en-3-ol, (3á,24s)-	$C_{29}H_{50}O$	414	0.17

Table 6 — Quantitative antibacterial test results of combination herbal extracts treated single jersey fabrics

WT:HB	Bacterial reduction ,%			
	12 h contact time		24 h contact time	
	S.aureus	*E.coli*	*S.aureus*	*E.coli*
100:0	50.93	92.55	69.54	96.65
75:25	52.17	92.8	71.25	96.77
50:50	75.91	92.97	82.03	96.59
25:75	47.08	92.37	68.28	92.09
0:100	56.23	92.60	82.56	96.91

bacterial reduction percentage against *Staphylococcus aureus* in both 12 h and 24 h contact time (75.91% and 82.03% respectively). WT: HB (0:100) proportion shows good bacterial reduction percentage (82.56) against *Staphylococcus aureus* in 24 h contact time. All the samples exhibit good bacterial reduction performance against *E.coli* strains both in 12 h and 24 h contact time. The quantitative antibacterial test results obtained for 50: 50 (WT: HB) combination treated cotton single jersey fabrics correlates with the minimum inhibition concentration (MIC), synergy calculation and GC-MS analysis results. However, further trials are required to optimise the finishing process parameters against the tested bacterial strains and other bacterial strains using 50:50 (WT: HB) combination for the development of antibacterial textiles for specific applications.

4 Conclusion

4.1 The minimum inhibitory concentration results of the combination herbal extracts using methanol as solvent are found to be better than that of aqueous media.

4.2 Among the methanol extracts, 50:50 (WT: HB) combination yields minimum MIC value.

4.3 The synergy calculation based on fractional

inhibitory concentration (FIC) index reveals that the wild turmeric and holy basil combination (50:50) proportion is synergic in action against the bacterial strains *S.aureus* and *E.coli* ensuring the balanced performance of active constituents in this combination.

4.4 The GC-MS analysis of the methanol extract of wild turmeric, holy basil and 50:50 (WT: HB) combination show 30 compounds.

4.5 In 50:50 combination, apart from the active components of wild turmeric and holy basil, five more new compounds are identified, which also constituted the good antibacterial performance against the tested bacterial strains.

4.6 The quantitative antibacterial test (AATCC 100) results of the cotton single jersey fabrics show that among the combination herbal extract proportions, 50: 50 (WT:HB) proportion shows good bacterial reduction performance against both *S.aureus* and *E.coli* bacterial strains.

4.7 Further studies are required to optimise the finishing process parameters using 50: 50 proportion against tested and other bacterial strains for developing specific application based antibacterial textile products.

References

1 Parasuraman S, Thing G S & Dhanaraj S A, *Phcog Rev,* 8 (2014)73.
2 Mukherjee P K, Ponnusankar S & Venkatesh P, *Indian J Pharm Edu Res*, 45 (2011) 210.
3 Kumar A, Chomwal R, Kumar P & Sawal R, *J Chem Pharm Res,* 1 (2009) 304.
4 Sikha A, Harini A & Hegde Prakash L, *J Pharmacogn Phytochem,* 3 (2015) 01.
5 Anjusha S & Gangaprasad A**,** *J Pharmacogn Phytochem,* 3 (2014) 50.
6 Rachana S & Venugopalan P, *J Pharmacogn Phytochem* , 2 (2014) 89.
7 Sudharshan S J, Prashith Kekuda T R & Sujatha M L, *J Pharm Res,* 3 (2010) 24.
8 Pandey G & Madhuri S, *Int J Pharm Sci Rev Res,* 5 (2010) 61.
9 Pingalea S S, Firke N & Markandeya A G, *J Pharm Res***,** 5 (2012) 2215.
10 Goyal P & Kaushik P, *Brit Microbiol Res J*, 1 (2011) 70.
11 Gomathinayagam S, Tewari B B & Rekha G, *Am Int J Contemp Res,* 4 (2014) 149.
12 Singh A R, Bajaj V K, Sekhawat P S & Singh K, *J Nat Prod Plant Resour*, 3 (2013) 51.
13 Ali R, Chauhan V, Farooq S, Khan A & Farooq U, *Int J Pharm Sci Rev Res,* 25 (2014)13.
14 Alam Sher**,** *Gomal J Med Sci***,** 7 (2009) 72.
15 Thilagavathi G, Rajendrakumar K & Rajendran R , *Indian J Fibre Text Research,* 30 (2005) 431.
16 Deepti Gupta, *Indian J Fibre Text Res,* 32 (2007)254.
17 Deepti Gupta, *Indian J Fibre Text Res,* 32 (2007)88.
18 Joshi M, Wazed Ali S & Purwar R, *Indian J Fibre Text Res,* 34 (2009)295.
19 Sathianarayanan M P, Bhat N V, Kokate S S & Walunj VE, *Indian J Fibre Text Res,* 35 *(2010)50.*
20 Mahesh S, Manjunatha Reddy A H & Vijaya Kumar G, *Proceedings, International Conference on Advances in Biotechnology and Pharmaceutical Sciences,* Bangkok, 2011, 253.
21 Hanan H A & Eman A M, *Int J Pharmaceut Applic***,** 3 (2012) 387.
22 Rathinamoorthy R & Thilagavathi G, *Fiber Polym*, 17 (2016) 917.
23 Anoop K, *Int J Phytopharm Res*, 6 (2015) 10.
24 Aqeela Afzal , Ghalib Oriqat , Akram Khan M , Jacquilion Jose & Mohammad Afzal, *J Biol Act Prod Fr Nat*, 3 (2013) 1.
25 Arulraj J, Shanmugaiah V & Lakshmanan N, *Int J Ad Lif Sci,* 7 (2014) 27.
26 Balasubramanian S, Ganesh D, Shridhar Reddy P & Surya Narayana V V S , *Asian J Pharm Anal Med Chem*, 2 (2014) 71.