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Research Article

Evaluation of Fifatrol, Septilin, Giloy (Guduchi, *Tinospora cordifolia*) and Oils of Guggul (*Balsamodendron mukul*) and Holy Basil (Tulsi, *Ocimum sanctum*) for their Antimicrobial Potential

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Abstract

Many herbal antimicrobial drugs, a potential alternative to antibiotics against antibiotic-resistant pathogens, are commercially available in the market in India and abroad. The present study was conducted to evaluate the antimicrobial activity of two most commonly prescribed herbal antimicrobial drugs in India, Fifatrol (Aimil Pharmaceuticals) and Septilin (The Himalaya Drug Company), along with extracts from stems of Giloy (*Tinospora cordifolia*), Holy basil (*Ocimum sanctum*) oil (HBO) and Guggul (*Balsamodendron mukul*) oil (GO) against 80 bacterial strains belonging to 22 species of 18 genera. None of the aqueous extracts (Fifatrol, Septilin and Giloy) had antimicrobial activity even at 12.8 mg mL^{-1} concentration. Ethanolic extracts of Fifatrol, Septilin and Giloy inhibited the growth of 33.75%, 26.25% and 13.75% of microbes, respectively, at 1.6 mg mL^{-1} concentration. However, HBO and GO inhibited 86.25% and 23.75% strains of microbes, respectively at the same concentration. Though Fifatrol ethanolic extract and HBO had better activity (p, 0.01) against vancomycin-resistant bacteria than on vancomycin sensitive strains, no such difference was evident on their activity on extended-spectrum-β-lactamase (ESBL) producers, carbapenem-resistant or methicillin-resistant bacteria and ESBL non-producers, carbapenem and methicillin-sensitive strains. The study indicated that Fifatrol and Septilin were not good antimicrobials on *in-vitro* testing; however, HBO may have potential antimicrobial utility. At 0.64% (6.4 mg mL^{-1}) concentration, HBO inhibited the growth of 79 out of 80 microbial strains tested, indicating its potential in the development of topical antimicrobial ointments or inhalants.

Keywords: Herbal Antimicrobials; ESBL; Carbapenem-resistance; Methicillin-resistance; Vancomycin-resistance; *Staphylococcus; Streptococcus; Pasteurella; Brucella; Klebsiella*

Introduction

In the past few decades, emerging infectious diseases are one of the biggest problems to the public health systems globally [1-5]. There should be a rapid, sensitive, specific, and cost-effective diagnostic and therapeutic management system. Molecular diagnostic assays are better options [6-20]. However, for therapeutic management of infectious diseases options are limited. Medicinal plants are a potential source for isolating the antibacterial, antiviral [21-30], antifungal, and anti-protozoan active compounds [31]. Herbal antimicrobials are purportedly claimed as the best alternative antibiotics, particularly to fight multiple drug-resistant (MDR) pathogens. Many herbal formulations are on the market to en-cash the sentiments of people for herbals. Many of the preparation in the market come without quality evaluation, toxicity information,

authentication of the desired antimicrobial activity and clarity in dose requirements. Fifatrol, a medicine in tablet form from Aimil Pharmaceuticals, India, is claimed to contain Apamarga (*Achyranthes aspera L.*), Chirayata (*Swertia chirata Buch.*), Daruharidra (*Berberis aristata DC.*), Giloy or Guduchi (*Tinospora cordifolia,* Willd Miers), Karanja (*Pongamia pinnata* L. Pierre), Kutaki (*Picrorhiza kurroa Royle ex Benth*), Motha (*Cyperus rotandus*) and Tulsi (*Ocimum sanctum*) extracts, and Godanti Bhasam *(selenite,* $CaSO_4.2H_2O$). Fifatrol is professed for its antimicrobial activity to kill even the resistant strains of bacteria by All India Institute of Medical Research of India (https://www.aimilpharmaceuticals.com/product/fifatrol/). Septilin, another tablet form of medicine with claimed anti-infection potential, is marketed by The Himalaya Drug Company, Bengaluru, India. Septilin contains extracts from Drumstick (*Moringa pterygosperma*), Guduchi, Indian Gooseberry (*Phyllanthus emblica*), Guggul (*Balsamodendron mukul*), Licorice, (Glycyrrhiza glabra), Manjistha (*Rubia cordifolia*), Shankh Bhasma (https://himalayawellness.in/products/septilin). Antimicrobial (antibacterial and antiviral) activity of Fifatrol and Septilin is claimed mainly due to the presence of extracts of Guduchi, Guggul and Tulsi, ostensibly considered as Ayurvedic antibiotics. Fiftrol has been shown to be inhibiting antibiotic-resistant *Staphylococcus* (https://www.outlookindia.com/newsscroll/researchers-developing-ayurvedic-alternatives-for-treating-bacterial-fungal-infections/1645450) while Septilin besides being anti-infective has been claimed an immunomodulator to fight infections in general [32].

Antibacterial activity of Guduchi is reported [33] in its ethanolic extract (20 mg discs) against *Escherichia coli, Proteus vulgaris, Enterobacter faecalis, Salmonella enterica* ssp. *enterica* serovar Typhi, *Staphylococcus aureus* and *Serratia marcesenses* (Gram-positive). However, its aqueous extract inhibited *Escherichia coli, Staphylococcus aureus, Salmonella enterica* ssp. *enterica* serovar Typhi, *Enterococcus faecalis* and *Streptococcus pneumoniae* at 50 mg/mL concentration when tested through agar well diffusion assay [34]. Ethanolic extract of its leaves visibly inhibited the growth of *E. coli* at ≥25 mg/mL [35].

Tulsi (Holy basil) is reported to be antibacterial against a wide range of bacteria and its chloroform extract is shown to be more antibacterial against Gram-ve bacteria than against Gram+ve bacteria while ethanolic extract had the reverse picture [36]. The antibacterial activity of Holy basil oil (HBO) has recently been shown effective against a wide variety of Gram+ve and Gram-ve bacterial isolated from various clinical cases and minimum inhibitory concentration (MIC) ranged from 80 µg to >2.56 mg mL^{-1}. However, in the same study MIC of Guggul oil (GO) was 320 to >2.56 mg mL^{-1} [37,38]. The HBO has been shown to inhibit 83.6% of 72 bacterial isolates (belonging to *Escherichia coli* 27, *E. fergusonii* 3, *E. vulneris* 1, *Enterobacter agglomerans* 11, *Hafnia alvei* 4, *Klebsiella oxytoca* 1, *K. pneumonia* 5, *Pragia fontium* 1, *Proteus mirabilis* 8, *P. penneri* 1, *P, vulgaris* 1, *Salmonella enterica* ssp. *enterica* 8, *Serratia ficaria* 1, *Xenorhabdus boviennii* 1) associated with enteric infections in birds at 1.024 mg mL^{-1}; however, >88% of the isolates were resistant to Guggul oil [39]. The HBO has shown bactericidal activity against *E. coli, S. aureus, Bacillus subtilis, B. cereus* and *Candida albicans* at1.56 µl mL^{-1} [40]. Alcoholic extract of Guggul gum is shown to be antibacterial against several pathogenic bacteria [41]. In a study on 128 strains of potentially pathogenic bacteria tested, Gram+ve bacteria were more sensitive than Gram-ve bacteria and guggul gum extract inhibited *Staphylococcus intermedius* and *Streptococcus pyogenes* strains even at 0.5 mg mL^{-1}; however, many of strains including of *E. coli, K. pneumoniae, Burkholderia* spp., *Pseudomonas aeruginosa, Aeromonas sobria, Bacillus polymyxa, B. Subtilis, Citrobacter freundii* and *Enterobacter agglomerans* were not inhibited at even 10 mg mL^{-1} concentration [42].

Thus, it seems plausible that Fifatrol and Septilin might have wide spectrum antibacterial activity due to different antimicrobial ingredients added therein. The present study was planned to test ethanolic and aqueous extracts of the two drugs against 80 strains of commonly encountered pathogens in clinical infections. The study also compared their activity with the component antimicrobial herbs namely Holy basil, Guggul and Giloy, using their essential oils or extracts.

Materials and Methods

Microbial strains

For the study 78 strains of bacteria (of 22 species of 18 genera) and two of *Candida albicans*) (Tab. 1) were revived from their glycerol stocks available at Clinical Epidemiology, Mycology Laboratory, *Pasteurella* Reference Laboratory, National *Salmonella* Centre and National *Brucella* Centre of Indian Veterinary Research Institute, Izatnagar. All the revived strains were grown on blood agar plates,

tested for purity and identity was confirmed using standard protocols and criteria [43-45]. All strains were maintained on nutrient agar slants throughout the study. All the 78 bacterial strains were tested using E-strips (BioMerieux India Pvt. Ltd) for extended spectrum β-lactamases (ESBL) activity against ceftazidime/ceftazidime clavulanic acid, cefotaxime/cefotaxime clavulanic acid, cefepime/cefepime clavulanic acid and also for imipenem and meropenem resistance as per guidelines of the E-strip manufacturer and results were interpreted according to CLSI guidelines for classifying bacterial strains as resistant or sensitive [46,47]. All Gram-positive bacteria isolates were also tested for oxacillin, methicillin and vancomycin susceptibility on Mueller Hinton agar plates using disc diffusion assay following CLSI guidelines [46,47]. All antibiotic discs were procured from BD (USA). A *Pasteurella multocida* type B2 strain (P52), available at *Pasteurella* Reference Laboratory, which was sensitive to most antibiotics, was used as a drug-sensitive control strain in the study.

Preparation of extracts of fifatrol, septilin and giloy (*Tinospora cordifolia*)

Extracts were prepared using already reported methods [33]. Briefly, aqueous extract was made by soaking finely crushed 10 tablets of Fifatrol/Septilin, purchased from the local drug dealer in Bareilly, in 50 mL of triple glass distilled water and kept for 18 h at 4-8°C on magnetic stirrer (50 rpm) in a screw capped 250 mL sterilized conical flask. Thereafter, the contents of flasks were filtered through sterile Whatman filter paper No. 1 and filtrate was vacuum dried to determine the amount of extract. Ethanolic extract was made in the way similar to aqueous extract using 99.9% pure ethanol (Merc India Ltd.) instead of distilled water. Pure Guggul oil was provided by Dr. MZ Siddiqui (ICAR-IINRG, Namkum, Ranchi, India) and 99% pure Holy Basil oil was purchased from Shubh Flavours and Fragrance Ltd, New Delhi. For extraction of Giloy extract, 50 g of dried and finally powdered Giloy stem harvested from the Institute herbal garden was soaked in 200 ml of ethanol/distilled water. The rest of the process was the same as for the Fifatrol/Septilin extracts. All the extracts were weighed and dissolved in dimethyl sulfoxide (DMSO, Sigma, USA) to the appropriate concentration (128 mg mL^{-1} of Fifatrol and Septilin extracts; 256mg mL^{-1} of Giloy extracts). To determine their antimicrobial activity, holy basil oil and Guggul oil were also solubilised in DMSO @ 128 mg mL^{-1} and 512 mg mL^{-1}, respectively.

Determination of minimum inhibitory concentration (MIC) of extracts and oil preparations

For a total of 80 strains of microbes MIC of aqueous and ethanolic extracts of Fifatrol, Septilin and Giloy and oils of Holy basil (HBO) and Guggul (GO) was determined using agar well diffusion assay [48]. To determine MIC nine wells of 6 mm diameter each were cut in Mueller Hinton Agar (MHA, BBL-BD, USA) plates under sterile environment and bottoms of wells were sealed with molten MHA. Six hour old actively growing culture (trypticase soy broth, BBL-BD) of test microbe (OD_{260} 0.5) was swab inoculated and wells were filled with 50 µL of serially diluted extracts/oils to be tested in sterile in DMSO so that well number one to nine contained 0, 100, 200, 400, 800, 1600, 3200, 6400, and 12800 µg of Giloy extracts; 0, 200, 400, 800, 1600, 3200, 6400, 12800, 25600 µg of Guggul oil; and 0, 50, 100, 200, 400, 800, 1600, 3200 and 6400 µg of Fifatrol, Septilin extracts and Holy basil oil separately. Plates were incubated under appropriate growth conditions for 2 h without inversion to get the well contents adsorbed in the medium and then overnight after inversion in appropriate environment required for the optimum growth of the microbe [44-46]. Measurable zone of growth inhibition around the well containing the highest dilution of the test extract or oil was marked as MIC value of the preparation for each of the strains. Tests were conducted in triplicate for confirmation. An isolate inhibited at ≤1.6 mg mL^{-1} was classified as sensitive to the tested extract or oil and those not inhibited were considered resistant.

Evaluation of the effect of extracts on serum and bacterial growth media (TSB, trypticase soy broth; MHB, Mueller Hinton broth, and LB, Luria Bertani medium)

To assess the effect of ethanolic extracts of Fifatrol and Septilin on serum and different media, their solvent-free extracts were dissolved in DMSO and then mixed with 3 samples each of horse, dog, cattle, buffalo, goat and pig serum collected from the repository of Sero-Epidemiology laboratory of the Institute, two foetal calf serum from BD and Sigma, USA, three tubes each of MHB, TSB and LB in concentration starting from 8, 16, 32, 64, 128, 256, 512 and 1024 µg mL^{-1}. All tubes were incubated at 37°C for two h and then observed for any precipitate, haziness, loss, or transparency. For negative control, DMSO was used in an equal amount as in test preparations with all test serum and media. All tests were repeated for consistency.

Statistical analysis

For finding the difference in sensitivity patterns of microbes of different genera and with different antimicrobial resistance patterns, odds ratio, and Chi-square ($\chi 2$) tests were used.

Results

From 10 tablets of Fifatrol 1.936g and 1.724g of ethanolic and aqueous extracts, respectively, were recovered while from 10 tablets of Septilin ethanolic and aqueous extracts were 2.675g and 2.12g, respectively. The extracts were soluble in DMSO completely. From 50 gm of dried Giloy stems 1.56g and 1.29g of ethanolic and aqueous extracts could be made.

None of the aqueous extracts had any detectible antimicrobial activity on reference sensitive (P52) *Pasteurella multocida* as well as on any of the other bacterial strains tested at a concentration of 12.8 mg mL^{-1}. Because of no antimicrobial activity in aqueous extracts of both of the medicines, further studies for evaluation of their MIC were abandoned. Ethanolic extracts of Septilin caused precipitation in MHB, LB, TSB and all the serum samples at concentration ≤128 µg mL^{-1} while Fifatrol extract induced precipitation similar to Septilin extracts at 512 µg mL^{-1}. Due to turning of the MHB, LB and TSB in to milky opaque or translucent broth, the broth dilution method for determining MIC was not practicable and was not followed in the study; instead, an agar-well diffusion assay was used [17].

The results of MIC of different antimicrobials tested (Table 1) revealed that MIC of ethanolic extract of Fifatrol (Figure 1) and Septilin (Figure 2) for different microbes ranged between 0.05 to >6.4 mg mL^{-1}. However, both the extracts differed in activity against different microbes. The MIC of Fifatrol ethanolic extract was minimum for *Enterococcus faecalis* strains while MIC of Septilin ethanolic extract was minimum for *Streptococcus suis, S. milleri* and *Staphylococcus aureus* strains.

Microbes	Strain Tested	Nos. tested	MIC in mg/mL determined through agar well diffusion assay				
			Fifatrol extract	Septilin extract	Guggul Oil	Holy basil oil	Giloy extract
[1]*Aeromonas trota*	F6T	1	6.4	3.2	3.2	1.6	6.4
[2]*Brucella abortus*	B412462, B418401	2	0.8, 3.2	1.6, 3.2	12.6, 6.4	6.4	3.2
[3]*Candida albicans*	CA5, CA9	2	>6.4	>6.4	>25.6	0.4	0.4
[1]*Edwardsiella hoshniae*	4339HWSW	1	>6.4	>6.4	6.4	1.6	>12.8
[1]*Enterobacter agglomerans*	1712BFSW	1	3.2	>6.4	3.2	1.6	>12.8
[1]*Enterococcus faecalis*	4339HWSβ, CDU 20202	2	0.05, 0.8	>6.4, 6.4	0.05, 1.6	1.6, 0.4	>12.8, 6.4
[1]*Enterococcus faecium*	4241DPS1-2	2	0.4, 0.8	0.8	3.2, 25.6	1.6	6.4
[1]*Escherichia coli*	3 ETEC, 1 Uropathogenic, 8EPEC	14	1.6->6.4	3.2->6.4	0.4->25.6	0.4-1.6	>12.8
[1]*Hafnia alvei*	ADS2020M	1	3.2	>6.4	3.2	0.8	>12.8
[1]*Klebsiella pneumoniae ssp. pneumoniae*	F1M	1	6.4	3.2	25.6	1.6	>12.8
[1]*Kluyvera ascorbata*	DVSU6434α	1	3.2	3.2	>25.6	0.8	>12.8
[1]*Pasteurella canis*	PC1, PC2, PC3, PC4, PC5, PC6	6	0.4-0.8	1.6	0.4	0.2	0.8

[4]*Pasteurella multocida type B2*	P52B2	1	0.4	0.4	1.6	0.4	3.2
[1]*Proteus mirabilis*	ADS2020W	1	1.6	0.8	6.4	3.2	>12.8
[1]*Pseudomonas aeruginosa*	CMT, 4339HWSW, DVS 4634 NHS, DVS 4634 W	4	1.6- 6.4	0.8- >6.4	6.4- 25.6	3.2 - >6.4	>12.8
[1]*Raoultella terrigena*	938PFC2	1	6.4	>6.4	25.6	1.6	>12.8
[5]*Salmonella enterica* ssp. *enterica* 6,7:-:-	6085ST	1	>6.4	6.4	1.6	1.6	>12.8
[5]*Salmonella* Enteritidis	E2478	1	>6.4	>6.4	12.8	0.8	>12.8
[5]*Salmonella* Infantis	19RJ001	1	>6.4	>6.4	12.8	1.6	>12.8
[5]*Salmonella* Kentucky	E408, E5927	2	>6.4	>6.4	25.6	0.8	>12.8
[5]*Salmonella* Miyazaki	6091ST	1	>6.4	6.4	1.6	1.6	>12.8
[5]*Salmonella* Typhimurium	ST1-5, D6ST, M6ST	8	3.2->6.4	1.6->6.4	0.8-25.6	0.8- 1.6	>12.8
[5]*Salmonella Virchow*	SV1-5, UT36, UT72	7	3.2->6.4	6.4->6.4	12.8->25.6	0.8-1.6	>12.8
[1]*Serratia grimasii*	AI342	1	>6.4	3.2	>25.6	1.6	>12.8
[1]*Staphylococcus arlettae*	TeiBNH2, TeiBNHS	2	1.6	>6.4	>6.4	0.8	6.4
[1]*Staphylococcus aureus*	711TVSB, CMβ, 439PFWβ, 431PFWβ, 44PFWβ, ATCC43300	6	0.1->6.4	0.1->6.4	1.6-25.6	0.8-6.4	>12.8
[1]*Staphylococcus cohni ssp. urealyticus*	DV-SU6434NHL	1	0.8	0.8	12.8	0.8	6.4
[1]*Staphylococcus equorum*	AKHUβ	1	>6.4	3.2	25.6	1.6	>12.8
[1]*Staphylococcus haemolyticus*	4241DPSβ	1	1.6	0.4	12.8	0.8	6.4
[1]*Staphylococcus intermedius*	4339HWSNH, 1910DSWβ	2	1.6, 0.8	>6.4	6.4, 1.6	0.8, 1.6	6.4, >12.8
[1]*Streptococcus milleri*	Sangeeta TS, TeiBNH1	2	0.2, 3.2	0.1, >6.4	0.2, >6.4	0.2, 0.8	0.8, 6.4
[1]*Streptococcus suis*	MSS22, 346	2	0.05, 1.6	0.05, 0.1	>6.4	0.2	0.8

Table 1: Minimum inhibitory concentrations (MIC) of ethanolic extracts of Fifatrol, Septilin, Giloy (Guduchi, *Tinospora cordifolia*) and oils of Guggul (*Balsamodendron mukul*) and Holy basil (Tulsi, *Ocimum sanctum*) for different microbes.

Source of the isolates shown as superscript; [1]Clinical Epidemiology Laboratory, [2]National *Brucella* Centre, [3]Mycology Laboratory, [4]*Pasteurella* Reference Laboratory, [5]National Salmonella Centre (Vet) at Indian Veterinary Research Institute, Izatnagar.

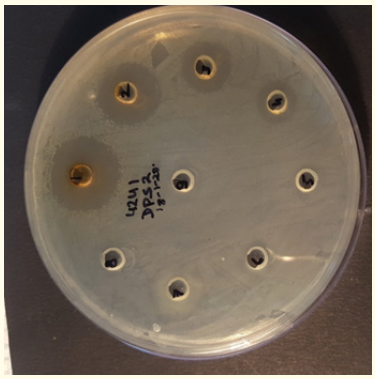

Figure 1: Inhibition of *Enterococcus faecium* by ethanolic extract of Fifatrol up to 4th dilution equivalent to MIC 0.8 mg mL^{-1}.

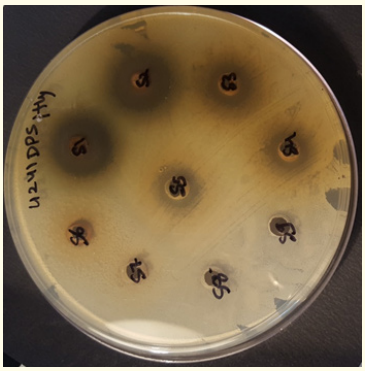

Figure 2: Inhibition of *Staphylococcus haemolyticus* by ethanolic extract of Septilin up to 4th dilution equivalent to MIC 0.8 mg mL^{-1}.

The MIC of Giloy extracts was higher (04 to >12.8 mg mL^{-1}) than both Fifatrol and Septilin extracts for bacteria and, it was minimum for *Candida albicans* (0.4 mg mL^{-1}). The MIC of Guggul oil (Figure 3) varied from 0.05 to >25.6 mg mL^{-1} with different strains of bacteria. An *Enterococcus faecalis* strain was the most sensitive one. Holy basil oil (HBO) inhibited growth of bacteria and yeast both. For *Candida* and bacterial strains MIC of HBO ranged from 0.2 to 6.4 mg mL^{-1}. Strains of *S. suis, S. milleri* and *Pasteurella canis* were the most sensitive strains of bacteria for HBO.

Though both Fifatrol and Septilin ethanolic extracts had a wide spectrum antimicrobial activity (Table 2), only 33.75% and 26.25% strains, respectively were sensitive to their extracts with MIC ≤1.6 mg mL^{-1}. On the other hand, HBO inhibited (Figure 4) growth of 86.25% strains at the same concentration. Guggul oil and Giloy extracts inhibited (Figure 5) only 23.75% and 13.75% strains, respectively at ≤1.6 mg mL^{-1} concentration. Bacteria were more often resistant to both Fifatrol (p, 0.01; OR, 12.3; CI, 4.4-34.7) and Septilin (p, 0.01; OR, 17.6; CI, 6.1-51.1) ethanolic extracts than to HBO. However, test strains of microbes were less often resistant to both Fifatrol (p, 0.01; OR, 0.3; CI, 0.1-0.9) and Septilin (p, 0.1; OR, 0.4; CI, 0.2-0.9) extracts than to Giloy extract. Both, Septilin and Fifatrol extracts not differed in their antibacterial activity significantly than Guggul oil.

Fifatrol extract was significantly more often inhibitory to G+ve bacteria than to G-ve bacteria (p, 0.01; OR 10.5; CI, 1.01-4.2), how-

Type of Microbes	Strain tested	Percent resistant (MIC>1.6 mg mL^{-1}) strains of microbes to				
		Fifatrol extract	Septilin extract	Guggul oil	Holy basil oil	Giloy extract
Gram-positive bacteria	21	23.81	57.14	76.19	19.05	85.71
Gram-negative bacteria	57	80.70	78.95	75.44	12.28	89.47
Oxidase-positive bacteria	14	35.71	28.57	42.86	42.86	57.14
Oxidase-negative bacteria	66	72.73	83.33	84.85	7.58	92.42
Candida albicans	2	100.00	100.00	100.00	0.00	0.00
Escherichia coli	14	92.86	100.00	85.71	0.00	100.00
Pasteurella (*P. canis 6, P. multocida 1*)	7	0.00	0.00	0.00	0.00	14.29
Pseudomonas aeruginosa	4	25.00	50.00	100.00	100.00	100.00
Staphylococcus (*S. arlettae* 2, *S. aureus* 4, *S. Chromohenes* 1, *S. cohnii* 2, *S. equorum* 1, *Haemolyticus* 1, *S. intermedius* 2)	13	30.77	76.92	84.62	30.77	100.00

Salmonella enterica ssp. *enterica*	21	100.00	95.24	76.19	0.00	100.00
Streptococcus (*S. milleri* 2, *S. suis* 2)	4	0.00	25.00	75.00	0.00	25.00
Carbapenem-resistant bacteria	7	57.14	57.14	71.43	14.29	57.14
Carbapenem-sensitive bacteria	71	66.20	74.65	76.06	14.08	90.14
Vancomycin-resistant bacteria	11	9.09	54.55	72.73	9.09	100.00
Vancomycin-sensitive bacteria	10	40.00	60.00	80.00	30.00	70.00
ESBL producing bacteria	22	66.67	80.95	85.71	9.52	100.00
ESBL non- producing bacteria	56	66.07	71.43	73.21	16.07	80.36
All microbes tested	80	66.25	73.75	76.25	13.75	86.25

Table 2: Resistance to ethanolic extracts of Fifatrol, Septilin, Giloy (Guduchi, *Tinospora cordifolia*) and oils of Guggul (*Balsamodendron mukul*) and Holy basil (Tulsi, *Ocimum sanctum*) among different types of microbes.

ESBL, Extended Spectrum β-lactamase.

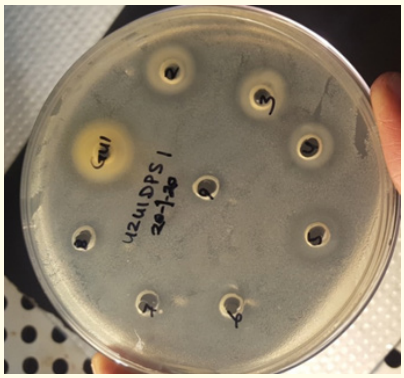

Figure 3: Inhibition of E*nterococcus faecium* by Guggul (*Balsamodendron mukul*) oil up to 5th dilution equivalent to MIC 1.6 mg/mL.

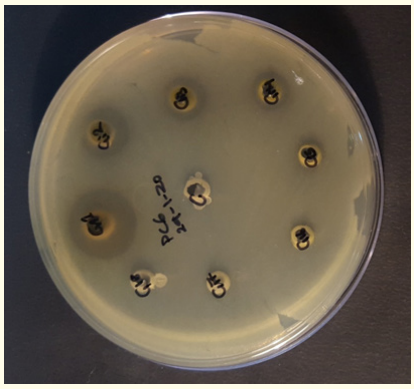

Figure 5: Inhibition of *Salmonella enterica* ssp. enterica serovar Miyazaki by Holy basil (*Ocimum sanctum*) oil of Giloy up to 3rd dilution equivalent to MIC 1.6 mg/mL.

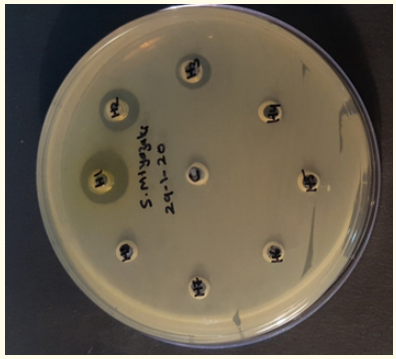

Figure 4: Inhibition of *Pasteurella canis* by ethanolic extract of Giloy (*Tinospora cordifolia*) up to 4th dilution equivalent to MIC 1.6 mg/mL.

ever, for other herbal antimicrobials no such difference was significant among G+ve and G-ve bacteria. Similarly, Fifatrol extract was more often inhibitory to oxidase+ve bacteria than oxidase–ve bacteria (p, 0.1; OR, 4.8; CI 1.7-13.4). Though Septilin extract didn't differed in its antibacterial activity on G+ve and G-ve bacteria but oxidase+ve bacteria were more often sensitive than oxidase–ve bacteria (p, 0.01; OR 12.5; CI, 2.2-71.8) similar to the ethanolic Giloy extract (p, 0.01; OR, 9.2; CI, 1.5-57.6) and Guggul oil (p, 0.01; OR, 7.5; CI, 1.4-38.9). However, oxidase-ve bacteria were less often resistant to HBO than oxidase+ve bacteria (p, 0. 01; OR, 0.1; CI, 0.02-0.69). The ESBL strains were more often resistant to Fifatrol (p, 0.01; OR, 17.5; CI, 1.9-162.6) and Septilin (p, 0.01; OR, 34.0; CI,

3.3-346.1) extracts than to HBO. Vancomycin-resistant (VR) bacteria were more often (p, 0.1; OR, 10.5; CI, 1.04-106.0) sensitive to Fifatrol extract and HBO than vancomycin-sensitive (VS) strains, but for other preparations no such difference was statistically apparent. Further, no difference in sensitivity of methicillin-resistant and methicillin-sensitive bacteria was detectible with any of the herbal preparations. The VR G+ve bacteria were 12 times more sensitive to HBO (p, 0.05; OR, 12; CI, 1.1-128.8) than Septilin extract. Similarly, VR isolates were less often resistant to Fifatrol than to Septilin extract (p, 0.05; OR, 0.08; CI, 0.01-0.89) and Guggul oil (p, 0.01; OR, 0.04; CI, 0.0-0.9). There was no significant difference in sensitivity of carbapenem-resistant (CR) and carbapenem-sensitive (CS) strains with respect to their susceptibility to herbal preparations except for Giloy extract, for which CS strains were 6.9 times more often resistant than CR strains (p, 0.05; OR, 6.9; CI, 1.8-37.1).

The HBO, being the most effective herbal antimicrobial in the study, inhibited >86% of the microbes tested at concentration below 0.2% and at 0.64% concentration of HBO none of the 80 microbes tested but a strain of *P. aeruginosa* was able to grow.

Discussion

Medicinal plants are highly useful and already proven source of antibacterial, antiviral [21-30], antifungal, and anti-protozoan active compounds [31] and several preparations are already in the market. However, *in vitro* studies on two herbal preparations marketed in India and protectant against infections could not be proved as potent antimicrobials in the present study.

In the study neither Fifatrol nor Septilin extracts could inhibit more than one third of the microbes tested, that is majority (>66.6%) were resistant at concentration 1.6 mg mL^{-1}. Similarly, Guggul and Giloy had the similar pattern of antimicrobial efficacy. Ostensibly herbal antimicrobials are often claimed to be an alternative to antibiotics for treatment of antibiotic resistant bacteria [31,49]. Reports of herbal antimicrobial resistance are not scarce probably due to common resistance mechanism for herbal antimicrobials and antibiotics [50].

In the study, Fifatrol extract inhibited 76.2% of G+ve bacteria including staphylococci (69.2%) and streptococci (100%). However, the observations are not in concurrence to reports for AIIMS, Bhopal reporting all staphylococci susceptible to Fifatrol (https://www.outlookindia.com/newsscroll/researchers-developing-ayurvedic-alternatives-for-treating-bacterial-fungal-infections/1645450), it might be due to difference in bacterial strains tested in the two studies. Besides, Fifatrol was also significantly more effective against VR staphylococci than VS staphylococci but was not more active against methicillin-resistant strains. Though HBO (Oil of Tulsi, an active ingredient in Fifatrol) was equally good both on G+ve and G–ve bacteria and had similar pattern of significantly higher activity on oxidase+ve (p, 0.01) and VR strains (p, 0.1) as the Fifatrol extract. Similar antibacterial pattern of HBO has been reported on bacteria associated with different ailments earlier [36-40].

Though Septilin extract had a wide spectrum of antibacterial activity similar to earlier claims [32], it failed to restrict the growth of 73.75% bacteria without any significant difference in activity against G+ve or G–ve bacteria but had 12.5 times more odds of activity against oxidase+ve bacteria than against oxidase–ve bacteria (p, 0.01). Its activity was quite similar to that observed for Guggul oil (an active antibacterial ingredient in Septilin), that inhibited 23.8% of bacteria and was significantly (p, 0.01) more inhibitory to oxidase+ve bacteria. Similar pattern of activity of Guggul oil against bacteria of diverse origin especially against oxidase+ve bacteria has been reported earlier too [35,37-39].

Apparently, both Fifatrol and Septilin possess some antibacterial activity against important potentially pathogenic bacteria but what may be its utility in the systematic infections specially when both Fifatrol and Septilin extracts induced *in-vitro* precipitation of serum samples of different animals at several times low concentration than required to inhibit the most sensitive bacteria. Though doubtful in recommended dosages, utility of both the drugs as antibacterial (Fifatrol and Septilin) may be at the most in gastrointestinal tract and needs careful consideration after *in-vivo* assays to consider these drugs as useful herbal antimicrobials as widely claimed in the literature given by the producers of both of the drugs and also the researchers after testing these drugs at concentrations as they wish to claim their antibacterial potential. There appears to be no reported study on effect of Fifatrol and Septilin extracts or as such on serum or growth medium and needs further investigation to understand the *in-vivo* processing of both the drugs to assess their antimicrobial potential.

The HBO proved to be the best antimicrobial in the study inhibiting >86% of isolates at 0.16% concentration, and at 1% concentration level it inhibited almost all different types of 80 strains of microbes (except a strain of *Pseudomonas aeruginosa*) including

yeasts, and bacteria. The observations are in concurrence to earlier observations on HBO indicating its potential as herbal antimicrobial [36-40].

The ethanolic extracts of stem of *Tinospora cordifolia* failed to inhibit growth of 56 (70%) of the microbes at concentration below 1.28% (12.8 mg mL^{-1}) and was the least effective antimicrobial in the study. In earlier studies too extracts from different parts of *Tinospora cordifolia* are reported to show antimicrobial activity at or above 5% concentrations [34-36].

Conclusion

The present *in-vitro* study concludes that Fifatrol and Septilin were not be good antimicrobials under *in vitro tests*; however, Tulsi (Holy basil) oil had a potential utility for at least topical use antimicrobial. The HBO in concentrations as low as 0.64% (6.4 mg mL^{-1}) inhibited almost all different types of microbes tested. Further investigations may be undertaken to evaluate antibacterial potential of HBO as an ingredient in antimicrobial ointments or inhalants.

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Conflict of Interest

None to declare.

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