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Full Length Research Paper

In-vitro efficacy of charmil plus gel and av/cps/19 against some pyogenic bacteria and fungi

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The present study was aimed to evaluate topical herbal preparations Charmil plus gel and AV/CPS/19 spray against some pyogenic bacteria and fungi. For antimicrobial *in vitro* activity *Staphylococcus aureus*, *Streptococcus pyogenes* and *Pseudomonas aerogenosa* pyogenic isolates were used. All the three isolates were from clinical cases presented in the Clinical Complex of Veterinary College, Bidar. The isolates were cultured on 5% sheep blood agar after isolating them in pure cultures and further inoculated on selective media. For *in vitro* antibacterial activity, the test products Charmil plus gel and AV/CPS/19 spray were tested at 2%, 5%, 10% and 20% concentrations. Results were recorded by measuring zones of inhibition with the help of Vernier calliper/scale. The results of the present study concluded that Charmil plus gel and AV/CPS/19 are effective against the pyogenic bacterial isolates and fungal isolates.

Keywords: In vitro, herbal, pyogenic, media, skin, zone of inhibition.

INTRODUCTION

The discovery of antibiotics as potentially life saving drugs led to the belief that the scourge of infectious diseases would be gone forever. But, the development of bacterial resistance has meant that antibiotics are now being rendered useless by the very bacteria they were meant to destroy. In addition to this the search for newer drugs has been significantly slowed as pharmaceutical companies are not only finding it increasingly difficult to keep up with the pace at which bacterial resistance renders them useless but are also finding it more difficult to get approval for newer drugs (McKenna, 1997). in the last couple of years, there has been a lot of reports on treatment failures due to emergence and spread of bacterial resistance (Mordi and Erah, 2006). Bacterial resistance to antibiotics poses a great threat to animals health Great efforts are being made to reverse this trend, and one of them is the widespread screening of medicinal plants from the traditional system of medicine hoping to get some newer, safer, and more effective agents that can be used to fight infectious diseases (Natarajan et al., 2003). The traditional medical practitioners use a variety of herbal preparations to treat different kinds of diseases including microbial infections (Mann et al., 2008). The scientific literature is full of reports of studies on roots, stem bark, seeds, flowers and fruits of higher plants having bioactive substances such as peptides, alkaloids, tannins, phenols, sterols, flavonoids, glycosides amongst others which confer healing properties for their use in medicine (Levin et al., 1979: Benli et al., 2008; El-Mahmood et al., 2008). Ayurvedic Medicine is an ancient system based medicine, which evolved among sages of ancient India. The focus of Ayurveda is to integrate and balance the body, mind, and spirit, rather than focusing on individual symptoms. Herbal medicines are widely

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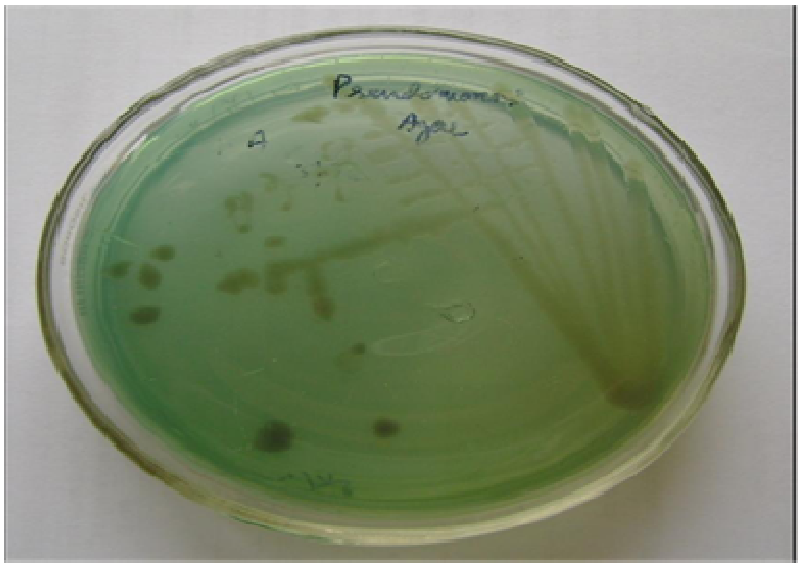

Plate I. *Pseudomonas aerogenosa* isolate used for present study (grown on nutrient agar)

used in veterinary practice as feed additives, immune-modulators, and growth promoters and for dressing of wound and external application. The present study was aimed to evaluate two such ayurvedic medicine, Charmil plus gel and AV/CPS/19 against some pyogenic bacteria and fungi.

MATERIALS AND METHODS

For antimicrobial in vitro activity *Staphylococcus aureus*, *Streptococcus pyogenes* and *Pseudomonas aerogenosa* pyogenic isolates were used in present study. All the three isolates were from clinical cases presented in the Clinical Complex of Veterinary College, Bidar. The isolates were cultured on 5% sheep blood agar after isolating them in pure cultures and further inoculated on selective media. The *Staphylococcus* colony was cultured on Mannitol salt agar (MSA, Hi-media, Mumbai), *Streptococcus* colony was selected and cultured on Edwards medium (Hi-media, Mumbai) and Mac Conkeys medium (Hi-media, Mumbai). For identification of *Pseudomonas* species, it was sub cultured on Nutrient agar (Hi-media, Mumbai) for distinct pigmentation which is the characteristic of this organism (Plate I). On the basis of morphology, staining characters and haemolytic pattern on blood agar and biochemical properties, respective isolates were identified. (Cowan and Steel 1970; Cruickshank *et al.* 1975). Fungal isolates used in the present study were procured from the Department of Veterinary Microbiology, Mumbai Veterinary College, Mumbai, Maharashtra. The isolates were sub cultured on Emmon's Sabouraud's dextrose agar (ESDA, Hi-media, Mumbai) and also Dematophyte test agar (DTMA, Hi-media, Mumbai) for further investigation. The above three pyogenic bacterial sp. and two fugal sp. were selected for further investigation.

In vitro antimicrobial activity

For *in vitro* antibacterial activity, plates of Mueller-Hinton agar (MHA, Himedia, Mumbai) was prepared**.** Overnight nutrient Broth (Himedia, Mumbai) culture of the test organism was smeared onto the agar plates. Two to three wells of 13 mm diameter were punched in each agar plate and the base of the wells was sealed with the agar. The test material Charmil plus gel AV/CPS/19 at 2%, 5%, 10% and 20% concentrations were made in dimethyl sulphoxide (DMSO) and was loaded into the punched wells. The plates were incubated at 370C for 18-24 hours in case of bacterial isolate. However, the ESDA/ DTMA agar were used for fungal isolates which were incubated for 4-7 days duration at room temperature. Wells without the drugs served as controls. Results were recorded and zones of inhibition were measured with the help of Vernier calliper/ scale. Results were analyzed by statistical methods by using Student 't' test.

RESULTS

The present research trial was conducted to evaluate the antimicrobial activity of Charmil plus gel and Charmil spray against pyogenic bacteria and fungi. The efficacy of the drugs under trial as antimicrobial agents was evaluated by measuring the zone of inhibition around the

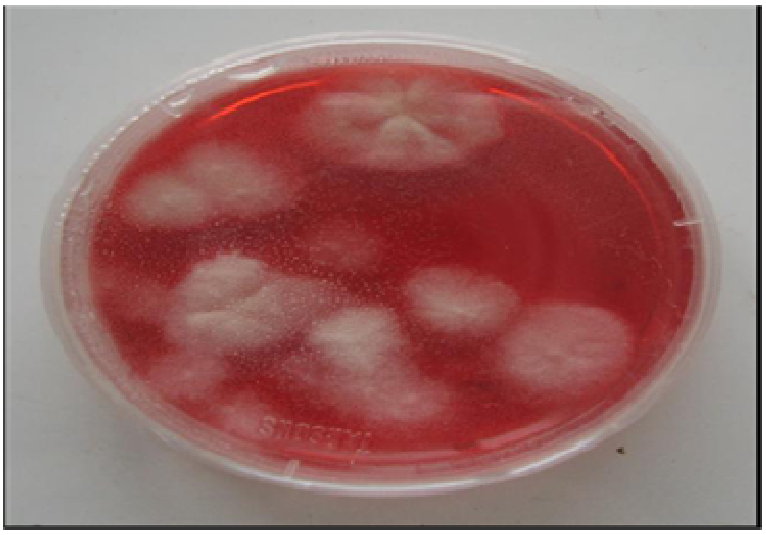

Plate 2. *Microsporum* isolates used for present study (grown on DTMA)

Plate 3. *Trichophyton* isolate used in the study (grown on ESDA

well. The antibacterial activity of Charmil plus gel and AV/CPS/19 against the bacterial isolates was tested in MHA was used (Plate 1). The fungal isolates were cultured on ESDA/ DTMA (Plate 2 and 3). The wells of size of 13 mm were punched in the agar sealed with the same agar and the drug under trial was loaded into wells. The drug Charmil plus gel and AV/CPS/19 was diluted as per the protocol suggested by the manufacturer (Plate 4 and 5). The wells were incubated at 370 C for 18 to 24 hours and the results were recorded by measuring the zone of inhibition around well. Antimicrobial activity Charmil plus gel and AV/CPS/19 was tested against *Staphylococcus aureus*. The results are presented in Table 1 and 2. Six replicates were used for each drug concentration and the experiment was repeated thrice, thus recording a total of 18 observations for each drug concentration and organism. The zones of inhibition shown by Charmil plus gel and AV/CPS/19 against *S. aureus* is presented in Table 1 and 2 (Plate 6, 7 and 8). On perusal of the table, it was observed that Charmil plus gel showed a zone of 15.22 ± 0.78, 21.22 ± 0.69, 26.50 ± 0.45 and30.44± 0.58 with 2, 5, 10, and 20 percent concentrations respectively against *S. aureus*. The zones of inhibition recorded against AV/CPS/19 were 18.66 ± 1.00, 22.94 ± 0.74, 28.38 ± 1.19, and 35.72 ± 0.59 with 2, 5, 10, and 20 percent concentrations respectively against

Table 1. Mean values of zone of inhibition (in mm) in different concentrations of Charmil Plus Gel against test organism

S No.	Name of the organism	No. of observations with each concentration	Zone of inhibition (mm)			
			2%	5%	10%	20%
1.	*S. aureus*	**18**	15.22 ± 0.78	21.22 ± 0.69	26.50 ± 0.45	30.44± 0.58
2.	*S. pyogenes*	**18**	18.83 ± 0.72	22.00 ± 0.65	22.11 ± 0.70	28.33 ± 0.72
3.	*P. aeruginosa*	**18**	22.78 ± 1.19	24.28 ± 0.91	32.00 ± 1.11	38.28 ± 0.91
4.	*Microsporum sp.*	**18**	17.11 ± 0.85	18.56 ± 0.89	21.17 ± 0.67	25.61 ± 0.49
5.	*Trichophyton sp*	**18**	15.78 ± 0.50	18.67 ± 0.61	23.67 ± 0.74	26.22 ± 0.57
6.	*S. aureus*	**18**	15.22 ± 0.78	21.22 ± 0.69	26.50 ± 0.45	30.44± 0.58

Table 2: Mean values of zone of inhibition (in mm) in different concentrations of AV/CPS/19 Spray against test organism

S No.	Name of the organism	No. of observations with each concentration	Zone of inhibition (mm)			
			2%	5%	10%	20%
1.	*S. aureus*	**18**	18.66 ± 1.00	22.94 ± 0.74	28.38 ± 1.19	35.72 ± 0.59
2.	*S. pyogenes*	**18**	16.39 ± 0.79	19.83 ± 0.80	23.67 ± 0.62	29.50 ± 0.54
3.	*P. aeruginosa*	**18**	18.28 ± 1.30	23.17 ± 0.89	26.39 ± 0.82	29.22 ± 0.70
4.	*Microsporum sp.*	**18**	17.11 ± 0.85	18.56 ± 0.89	21.17 ± 0.67	25.61 ± 0.49
5.	*Trichophyton sp*	**18**	14.94 ± 0.40	16.39 ± 0.63	18.61 ± 0.72	24.56 ± 0.72
6.	*S. aureus*	**18**	15.39 ± 0.45	16.94 ± 0.55	18.56 ± 0.53	22.06 ± 0.34

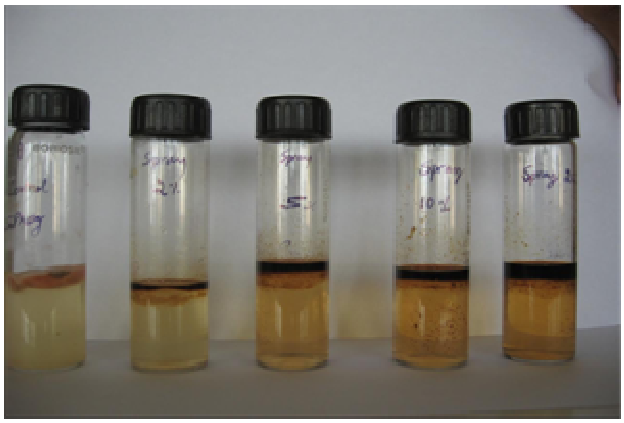

Plate 4. Different dilutions made in SDB of *Microsporum* isolate used in the study

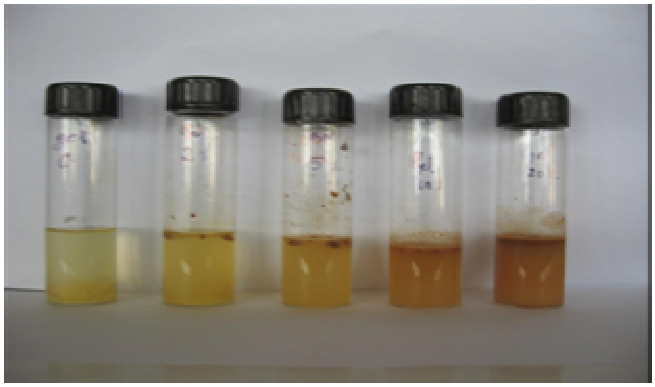

Plate 5. Different dilutions made in DMSO of *Microsporum* isolate used in the study

Plate 6. *Staphylococcus aureus* isolate showing zone of inhibition against different concentration of Charmil gel

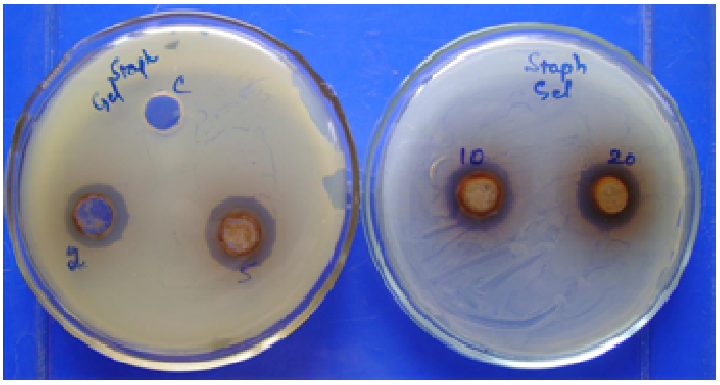

Plate 7. *Staphylococcus aureus* isolate showing zone of inhibition against different Concentration of Charmil

S. aureus. The zones of inhibition shown by Charmil plus gel and AV/CPS/19 against *Str. pyogenes* is presented in Table 1 and 2 (Plate 9 and 10). On perusal of the table, it was observed that Charmil plus gel showed a zone of 18.83 ± 0.72, 22.00 ± 0.65, 22.11 ± 0.70 and 28.33 ± 0.72 with 2, 5, 10, and 20 percent concentration respectively

Plate 8. *Pseudomonas aerogenosa* isolate showing zone of inhibition against different concentration of AV/CPS/19

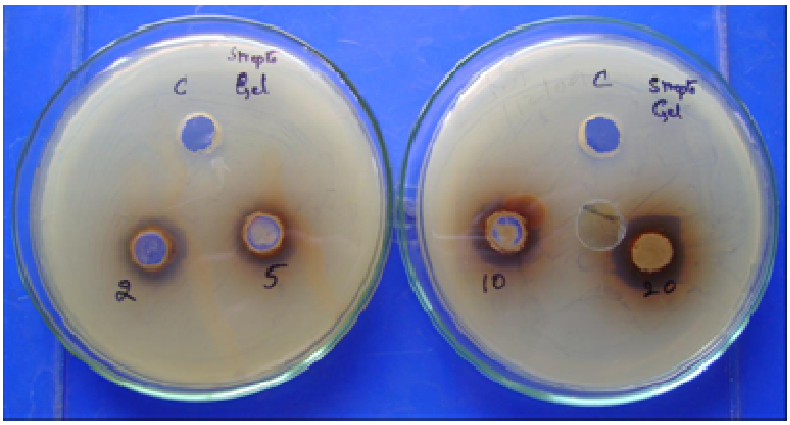

Plate 9 and 10. *Str. pyogenes* isolate showing zone of inhibition against different concentration of AV/CPS/19

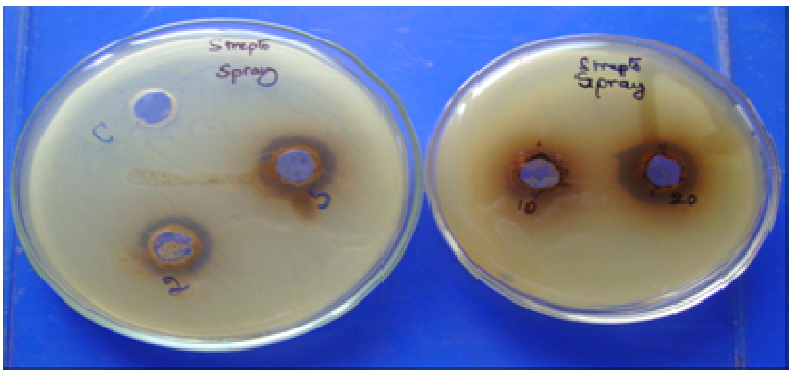

Plate 11. *Microsporum canis* isolate showing zone of inhibition against different concentration of Charmil plus gel

against *Str. pyogenes*. The zones of inhibition recorded against AV/CPS/19 were 16.39 ± 0.79, 19.83 ± 0.80, 23.67 ± 0.62 and 29.50 ± 0.54 with 2, 5, 10, and 20 percent concentrations, respectively, against *Str. pyogenes*. Zone of inhibition exhibited by Charmil plus gel and AV/CPS/19 against *P. aeruginosa* is presented in

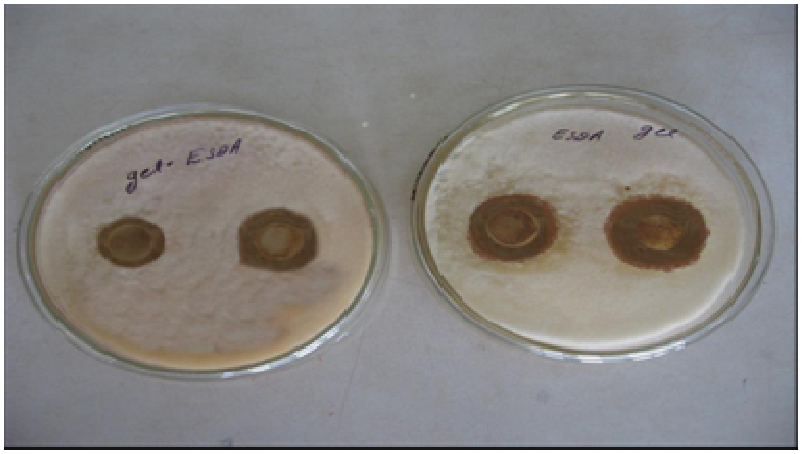

Plate 12. *Microsporum canis* isolate showing no zone of inhibition in control

Plate 13. *Trichophyton* isolate showing zone of inhibition against different concentration of Charmil gel and

Table 1 and 2. Study of the values in the table, it was observed that Charmil plus gel showed a zone of 22.78 ± 1.19, 24.28 ± 0.91, 32.00 ± 1.11 and 38.28 ± 0.91 with 2, 5, 10, and 20 percent concentration respectively against *P. aeruginosa*. The zone of inhibitions recorded against AV/CPS/19 were 18.28 ± 1.30, 23.17 ± 0.89, 26.39 ± 0.82 and 29.22 ± 0.70 with 2, 5, 10, and 20 percent concentration respectively against *P. aeruginosa.* For evaluating antifungal activity of Charmil plus gel and AV/CPS/19 13 mm wells were punched in ESDA. The dilutions of the test drug were made in DMSO as per the protocol suggested by the manufacturer. The wells in the agar were sealed as per the procedure used earlier. The broth culture of fungal isolates was then smeared onto the plate. The plates were incubated for 7 days at room temperature. Wells without the drugs served as controls. ESDA was also used as replica plates to avoid clear expression of the zone of inhibition. The results of the experiment are presented in Table 1 and 2. On perusal of the results it was observed that both the drugs are effective against the fungal isolates tested. Charmil plus gel and AV/CPS/19 both showed distinct and clear zones of inhibition on the agar plates. Six plates were used for each replication and three replications were made thus recording 18 observations in total. On perusal of the table 1 and 2 it was evident that, Charmil plus gel recorded the zones of inhibition against *Microsporum* species and were in the range of 17.11 ± 0.85, 18.56 ± 0.89, 21.17 ± 0.67 and 25.61 ± 0.49 with 2, 5, 10, and 20 percent concentration respectively. AV/CPS/19 showed mean zone of inhibition of 14.94 ± 0.40, 16.39 ± 0.63, 18.61 ± 0.72 and 24.56 ± 0.72 at 2, 5, 10 and 20 percent dilution respectively. On perusal of the table 1 and 2 it was evident that, Charmil plus gel recorded the zones of

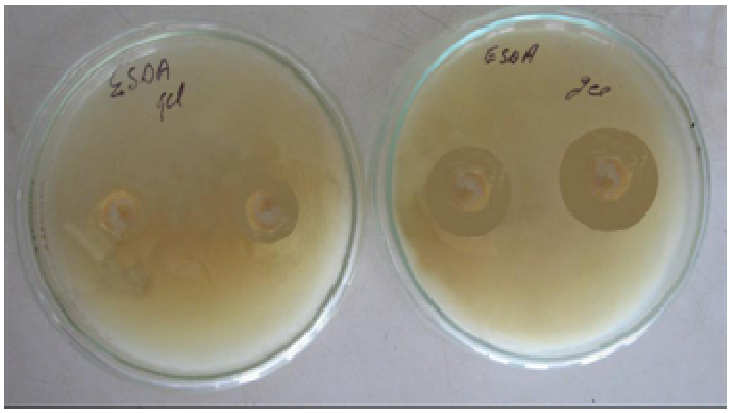

Plate 14. *Trichophyton* isolate showing zone of inhibition against different concentration of Charmil gel and spray

inhibition against *Microsporum* species and were in the range of 17.11 ± 0.85, 18.56 ± 0.89, 21.17 ± 0.67 and 25.61 ± 0.49 with 2, 5, 10, and 20 percent concentration respectively against *Microsporum species.* AV/CPS/19 showed mean zone of inhibition of 14.94 ± 0.40, 16.39 ± 0.63, 18.61 ± 0.72 and 24.56 ± 0.72 at 2, 5, 10 and 20 percent dilution respectively. Antifungal activity *Tricophyton species* was tested against Charmil plus gel and AV/CPS/19. Six ESDA plates were used for one replication. The experiment was repeated and a total of 18 observations were taken. Antifungal zone of inhibition for Charmil plus gel and AV/CPS/19 was shown in Table 1 and 2. For Charmil plus gel at the concentrations of 2, 5, 10 and 20 showed mean zones of inhibition as 15.78 ± 0.50, 18.67 ± 0.61, 23.67 ± 0.74 and 26.22 ± 0.57. For AV/CPS/19, same concentrations showed average zone of inhibition 15.39 ± 0.45, 16.94 ± 0.55, 18.56 ± 0.53 and 22.06 ± 0.34 (Table 1 and 2, Plate 11). The results were subjected for statistical analysis and were statistically significant at 1 and 5 percent level.

DISCUSSION

In vitro efficacy of Charmil plus gel and AV/CPS/19 for having antimicrobial activity against the pyogenic bacteria and fungi was assessed. Both the polyherbal constituents comprises oils of *Cedrus deodara, Azadirachta indica* and *Pongamia Pinnata* in specific proportions. It is evident from present study that Charmil plus gel and AV/CPS/19 possess potent antibacterial and antifungal activity. This may be attributed to the antibacterial and antifungal activity of different constituent herbs/herbal ois of two formulations. The results in the present study are in confirmation with those reported by Chopra et al., (2004), who reported about antibacterial activity of root, stem and leaf extract of *Cedrus deodara* against *E.coli in vitro*. Panday et al., 2009 also reported various activities of Cedrus deodara loud extract including antimicrobial and antifungal properties. In a study on Indian Medicinal Plants as a source of antimycobacterial agents, Azadirachta indica and Cedrus deodara were identified to possess potent antimycobacterial properties (Gautam et al., 2007).

In another study conducted on effect of *Azadirachta indica* on the growth pattern of dermatophytes, it was found to be efficacious to inhibit gorwth of dermatophytes thus suggestive of antifungal activity of *Azadirachta indica.* Similar results on antifungal properties of *Azadirachta indica* were reported against Poria monticolad-a wood destroying fungus (Dhayani *et al*., 2004). Studies on properties of *Pongamia pinnata* also revealed antibacterial and antifungal properties of this herb. Jean, 1999 also reported similar properties of *Pongamia pinnata.* It can be summarized that the indvidual constituents of polyherbal formulations Charmil Plus Gel and AV/CPS/19 possess antimicrobial and antifungal properties and this can be well correlated with the potentially similar properties of the two topical herbal products with non-significant differences in their efficacy.

CONCLUSION

Polyherbal spray formulation AV/CPS/19 combination showed larger zones of inhibition than the gel against the pyogenic bacterial isolates *S. aureus* and *Str. pyogenes*. However, topical herbal gel Charmil Plus Gel showed larger zones of inhibition than the spray against the fungal isolates. It can be concluded from the present study that AV/CPS/19 and Charmil Plus Gel are highly effective against the pyogenic bacterial and fungal isolates.

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