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Preparation of *Spilanthes acmella* based emulgel: Antimicrobial study and evaluation

Aqsa Afzal[1], Nisar Hussain Shah[1], Irshad Hussain[2], Shaukat Hussain Munawar[3], Asma Mumtaz[1] and Nwal Qureshi[4]

[1]Department of Pharmaceutics, Faculty of Pharmacy, Bahauddin Zakariya University, Multan, Pakistan
[2]Institute of Pharmacy, Shaheed Mohtarma Benazir Bhutto (SMBB) Medical University, Larkana, Pakistan
[3]Faculty of Medicine and Allied Medical Sciences, Isra University, Islamabad, Pakistan
[4]Institute of Food Science and Nutrition, Bahauddin Zakariya University, Multan, Pakistan

Abstract: SSTIs (Skin and soft tissue infections) are the most commonly occurring infections among all age groups. This study aimed to create an herbal emulgel for the treatment of bacterial skin infections as many bacteria have developed strong resistance against antibiotics. *Spilanthe acmella* plant extract contains spilanthol which has strong anti-bacterial properties. Methanolic *S. acmella* extract-based emulgels being promising drug delivery systems have been evaluated for various parameters like physical characteristics, viscosity, pH, spreading coefficient, Bioadhesive strength determination, Extrudability, antioxidant and antibacterial activity. 200µg/100µl exhibited the highest antioxidative activity 60.01±0.28% radical scavenging activity. MIC values of pure extract found in the range of 0.83±0.21 to 1.66±0.41µg/100µl, MBC values found in the range of 1.66±0.41 to 3.33±0.83µg/100µl for all strains of bacteria. Statistically significant antibacterial activity of all extract containing emulgels was observed against *S. aureus, P. aeruginosa, E. Coli* p-value = 0.00, while maximum antibacterial effect all formulations have produaced zone of inhibitions against *E. Coli* p-value = 0.00. The current study thus suggests the use of *S. acmella* extract-based emulgel for the treatment of bacterial skin infections caused by *S. aureus, P. aeruginosa* and *E. coli*.

Keywords: *Spilanthes acmella, Staphylococcus aureus, Pseudomonas aeruginosa, E. coli,* emulgel, plant extracts, antibacterial activity, MIC, MBC, Carbapol 934.

INTRODUCTION

Most of the *Escherichia coli* strains being segregated from infections of SSTIs (skin and soft tissue infections). Strains of *E. coli* being isolated mainly from foot ulcers, surgical and traumatic wounds (Petkovšek *et al*. 2009). SSTIs are the most commonly occurring infections among patients of each age group. Most of the infections of the skin resolve without any treatment and some require antibacterial treatment. However, some moderate to severe cases may need to be admitted to the hospital (Moet *et al*. 2007). Several reports have revealed that omphalitis has been caused by *E. coli* (Fraser *et al*. 2006). *E. coli* has been involved in various skin, bacteremia and soft tissue infections (Russo and Johnson 2000). *Pseudomonas aeruginosa* is an example of Gram-negative bacteria that is most often linked with opportunistic infections. The prevalence of *P. aeruginosa* infections oscillates from local skin infections to serious systemic life-threatening infections. Most *P. aeruginosa* infections are characterized by typical cutaneous manifestations (Wu *et al*. 2011). The cutaneous infections of *P. aeruginosa* infection fluctuate from superficial to deep skin tissue and can occur in both healthy as well as immune-compromised individuals. Notable morbidity and mortality are reported among immunocompromised hosts (Chernosky and Dukes 1963). Various cutaneous manifestations of *P. aeruginosa* have been revealed in literature. It has also been involved in the development of a skin disease called cutaneous botryomycosis. Bacterial botryomycosis is an infrequent, chronic granulomatous skin manifestation often created by *S. aureus, Escherichia coli*, or *P. Aeruginosa* (Meissner *et al*. 2007). Antibiotics usually provide the main source for the therapy of bacterial infections. Since the inventions of many antibiotics and their utilization as chemotherapeutic agents being a belief in the medical profession that these antibiotics eradicate various infectious diseases. Overuse of antibiotics, however, has been the major cause for dissemination and emergence of multidrug resistant strains of various groups of microbes (Harbottle *et al*. 2006). In recent years all over the world drug resistance to various human pathogenic bacteria, is commonly being reported (Piddock and Wise 1989). Indiscriminate use of antibiotics is an alarming situation in both developed as well as developing countries (Ahmad and Beg 2001). In the last 30 years, pharmaceutical industries even though have produced a huge number of new antibiotics and microbial resistance to these antibiotics has been increased. Generally in bacteria, there is genetic ability to acquire and develop resistance to those antibiotics, which have been used as therapeutic agents (Cohen 1992). Many medicinal plants being traditionally used contain a variety of components of known therapeutic properties. The

*Corresponding author: e-mail: irshadpharmacist@yahoo.com

plants have antibacterial, antidiabetic, anti-inflammatory, antioxidant, antipyretic properties. It is reported that extracts of plants act on different target sites those utilized by antibiotics. This provides a great benefit against drug resistant microbial pathogens. There are WHO statements that medicinal plants are the ideal source for acquiring variety of drugs (Nascimento *et al*. 2000) (Chopra *et al*. 1956). *Spilanthes acmella* extract is composed of diverse group of active constituents. Spilanthol is found to be a major active ingredient of *Spilanthes acmella* extract having strong antimicrobial, anti-fungal and antioxidant properties (Prachayasittikul *et al*. 2009) (Ramsewak *et al*. 1999).

MATERIALS AND METHODS

Collection of plant

Spilanthes acmella plant has collected across the tropical areas of Pakistan. It was botanically identified by an expert taxonomist. Parts used were made free from adulterants, dust particles and vegetative debris. The shade-dried plant material has grinded to a coarse powder by the use of a herbal grinder.

Preparation of extract

Powdered plant material (almost 200g) got macerated in methanol- aqueous solution in the ratio of 70: 30 respectively in an amber-colored bottle for 14 days at room temperature with vigorous shaking occasionally at room temperature. After 14 days obtained filtrate by filtering the mixture via muslin cloth. Then the filtrate was again passed through Whatman filter paper qualitative grade 1. Evaporation of filtrate was carried out by using a rotary evaporator at 37ºC and thick paste-like extract was stored at 4°C in an airtight glass jar.

Materials

The pure extract of *Spilanthes acmella*, methylparaben (Merk German), propylparaben (Merk German). Distilled water (BZU Pharmacy distillation apparatus), Span 20 (Merk German), Carbopol 934 (Merk German), Tween 20 (Merk German), Liquid paraffin (Merk German), Ethanol (Merk German), Triethanolamine (Merk German) propylene glycol (Merk German), Muller-Hinton agar (OXOID CM0337), Nutrient broth (OXOID CM0001, NaOH (Merk German), K2H2PO4 (Merk German). All other reagents were of analytical grade.

Equipment

Beakers (50ml, 100ml, 500ml) Pyrex Germany, Conical Flask (50ml, 100 ml, 250ml, 500ml) Pyrex Germany, volumetric flask (50ml, 100ml, 500ml, 1000ml) Pyrex Germany, separating funnel (100ml) Pyrex Germany, Gilson Pipette and tips (Fischer Life Sciences), Pipette sucker Disposable Syringe (1ml, 3ml, 5ml) Terumo & BD, Glass sample vessel 10ml (Kirtikar and Basu), Spatula (Fischer Life Sciences), pH meter (Bechman), UV (Spectrophotometer Perkin Elmer Lambda 25), weighing balance (Analytical grade), Lamy Rheology B-One Plus viscometer (SN.18.12.PB049 France), Magnetic stirrer (Clifton-Germany Origin), Petri dish Pyrex Germany. Falcon tubes, Autoclaved Nutrient Broth, Autoclaved Mueller Hinton Agar, Micropipette ranges from 100 to 1000 µl with autoclaved tips, BSL1 desk, Ethanol, Cotton, Cotton swabs, Transferring loops.

Preparation of emulgel

The preparation of emulgel is comprised of three steps 1st is to make the gel, 2nd is the preparation of emulsion then in the third step we combine emulsion and gel.

Preparation of carbapol gel

About 50 grams quantity of carbapol gel got prepared by dispersing specified amount powder of carbapol 934 in some amount of distilled water (q.s to make it 50g total) then soak it for 24 hrs. After 24 hrs homogenize it with the help of magnetic stirrer about 50 rpm for 15 minutes and then adjust the pH (6-6.5) by using triethanolamine (Mohamed 2004).

Preparation of emulsion

The general method was employed as per Ansel *et al*. (Masar 2004) for preparation of emulsion in which oil phase of emulsion was prepared by dissolving specified quantity of span 20 into liquid paraffin, while the aqueous phase of emulsion was prepared by dissolving the specified quantity of tween 20 into purified water. 2-gram extract of *Spilanthes acmella* was dissolved into 2.5-gram ethanol, 0.03 gram of methylparaben, and 0.01 gram of propylparaben was dissolved into 5 grams of propylene glycol and both these solutions then added to above aqueous phase solution of tween 20 in water. A specified quantity of oil was added to the oily phase. Then both oily and aqueous solutions were separately heated at a temperature of 70-80°C. Finally, the oily phase is added to the aqueous phase with continuous stirring at the rate of 50 rpm for 15 minutes at room temperature (Khullar *et al*. 2012).

The above-obtained emulsion was then mixed with carbapol gel in a ratio of 1:1 (emulsion: gel) gentle stirring was done to obtain a homogenized form of emulgel (Jain *et al*. 2011). Four formulation batches of emulgel were prepared with Clove oil and peppermint oil with varying concentrations of carbapol 934 to develop the optimized formulation. Detail constituent composition of all four batches of emulgel has been being mentioned in table 1 containing 2% *Spilanthes acmella* extract W/W. (table 1).

HLB value of clove oil =9 (Shahavi *et al*. 2019), peppermint oil = 12.3 (Orafidiya and Oladimeji 2002) and of liquid paraffin = 10 (Orafidiya and Oladimeji 2002). Values of span and tween have been calculated by the Griffin method (Pasquali *et al*., 2008). Emulgels are being

characterized for parameters such as Physical Examination, Rheological Studies, pH determination, Spreading Coefficient, Bioadhesive strength measurement, Extrudability Studies, FTIR, antioxidant activity, and antibacterial activity MIC and MBC values of gellified emulsion.

Physical examination

The prepared emulgel batches were inspected visually for phase separation, their color, homogeneity, consistency, and grittiness (fig. 1, table 2).

Viscosity

The viscosity of all formulations was determined by using a viscometer with spindle 7 at 150 rpm for 10 minutes by B-One Plus viscometer. The formulation whose viscosity was to be determined was added to a beaker covered with a thermostatic jacket. Spindle moved freely into the emulgel and the reading was noted (Bonacucina *et al*., 2009, fig. 2).

pH Determination

A digital pH meter is used to measure the pH. It is very important to determine the pH of the topical formulation. The pH of every formulation is checked thrice and an average value of each was calculated (fig. 3) (Ashara *et al*. 2014), (Kasliwal *et al*. 2008; Jain 2016; Singh *et al*. 2009; Rathore and Nema 2008).

Spreading coefficient

The formulation was placed on a glass plate of about 20cm x 5cm. Another plate of glass having the same dimension was placed over the top of the emulgel in such a way that the formulations were sandwiched among the two slides. The weight of 100 grams has been placed uniformly on top of the slides. The weight was removed and excess gel was cleared out. Two slides had been in a position to a stand at an angle of 45º without negligible disturbance and the lower slide had been firmly clamped, permitting the upper slide to freely slip off with the aid of 20-gram weight that was attached to the upper slide. Then time taken by the upper slide to detach from the lower slide was measured in seconds as per guidelines of ICH (Guideline 2003). The experiment was performed in triplicate and the spreadability of all emulgels was calculated as follows:

S = M x L/ T, Where, S = Spreadability, L = Length of the glass plate.

W = Weight tied to the upper plate, T = Time in seconds (Das *et al*. 2009) (table 3, fig. 4).

Bio adhesive strength measurement

The modified method has been used for the quantification of bio-adhesive strength. The fresh hairless skin of the rat was cut into pieces and rinsed with 0.1 N NaOH. Two pans of physical balance were removed. The right side of the pan has been replaced with a beaker of 100 ml and left side of the physical balance a glass slide has been hanged. On the left side, for assembly balancing, 20 g weight has been hanged. Below the hanged slide on the left side another glass slide has been put down. Fragments of fresh hairless rat skin were attached with both glass slides. About one gram of emulgel was settled between both rat skin faces. Little pressure has been applied to create bioadhesion bond, slowly adding water on right side beaker, till the emulgel got separated from both faces of rat skin. The volume of water that was added was converted to grams. In this way, the bioadhesive strength of emulgels had been determined. (Jones, Woolfson *et al*. 1997). The bioadhesive strength has been calculated by the following formula:

Bio-adhesive Strength =Weight required (in gms) / Area (cm^2). (Tasdighi *et al*. 2012), (Manne *et al*. 2014) (fig. 5)

Extrudability determination

Standard collapsible capped aluminum tubes were used to fill emulates, were sealed by pinching to the end. After filling emulates into each tube their weight was measured. The tubes had been placed between two slides made of glass and were braced. A weight of 500 gm was placed above the slides, uncapped. The amount of gel that was extruded got collected and weighed. The percentage of gel extruded was calculated. The percent of the extruded gel was calculated (>90% extrudability: excellent ++++, >80% extrudability: good +++, >70% extrudability: Fair ++) (Wood *et al*. 1963) (table 4, fig. 6).

Antioxidant activity

The antioxidant activity of *Spilanthes acmella* extract was evaluated by the radical scavenging activity of DPPH. DPPH solution of 0.1mM concentration was prepared by dissolving DPPH in methanol and after half an hour its absorbance was measured at 515 nm. Serial dilutions 200µg/100µl, 100µg/100µl, 50µg/100µl, 25µg/100µl, 12.5µg/100µl were prepared. Then 3.9 ml of DPPH solution was added to each tube and placed in orbit shaker for 300 motivation /minute for 15 minutes and after that absorbance of each solution being measured at 515 nm wavelength, the radical scavenging activity percentage was calculated from the following equation:

% Radical Scavanging = (1- Absorbance of sample/ Absorbance of Control) × 100

The absorbance of control is the absorbance of pure DPPH solution (Tanwer *et al*. 2010) (table 5, fig. 7).

Antibacterial Activity

1. Media Preparation

Muller Hinton Agar broth media was used in the study prepared by suspending 38 g in 1 liter of distilled water bring it to boil to dissolve the media completely. Sterilize it by autoclaving at 121°C for 15 minutes. This media will be used for the agar well diffusion method. For bacterial

Table 1: Composition of all batches of emulgels

Ingredients	F1	F2	F3	F4
Extract	2	2	2	2
Carbapol 934	1	2	1	2
Clove oil	8	8	-	-
Pippermint oil	-	-	4	4
Span 20	2.7	2.7	1.7	1.7
Tween 20	0.3	0.3	0.6	0.6
Liquid paraffin	7.5	7.5	7.5	7.5
Ethanol	2.5	2.5	2.5	2.5
Methyl paraben	0.03	0.03	0.03	0.03
Ethyl paraben	0.01	0.01	0.01	0.01
Propylene glycol	5	5	5	5
Triethanolamine	q.s	q.s	q.s	q.s
Water	q.s	q.s	q.s	q.s

Table 2: Physical characteristics of emulgel

S No.	Formulation	Colour	Phase separation	Grittiness	Homogeneity
1.	F1	Dark skin	No	No	Good
2.	F2	Skin	No	No	Good
3.	F3	Light brown	No	No	Excellent
4.	F4	Light skin	No	No	Good

solution preparation Nutrient, Broth media has been prepared by adding 13g powder to 1000 ml of distilled water. Mix it well and sterilize it by autoclaving at 121°C for 15 minutes.

2. Tested Microbes

Staphylococcus aureus (*S. aureus*) (ATCC- 2529), *Pseudomonas aeruginosa* (*P. aeruginosa*) (ATCC 27853), *Escherichia coli* (*E. coli*) (ATCC-27853).

3. 1OD Bacterial solution preparation

The Fresh cultures of *Staphylococcus aureus was taken*, *Pseudomonasaeruginosa*, and *E. Coli.* Add bacterial colonies from their culture. Add maximum colonies to turbid the nutrient broth so that we always have an optical density greater than 1 each for *staphylococcus aureus, Pseudomonasaeruginosa*, and *E. Coli.*

4. Plating

Liquefied Mueller Hinton Agar (11-14 ml) was poured in Petri plates for solidification. Petri Plates regarding their specimen and samples were labeled.

5. Serial Dilution

The main purpose for this step was to obtain the specific numbers of bacteria per 1 ml so here we required 1000 bacteria per 100 µl. Taking 15 Falcon tubes 5 each for each strain of bacterial dilution adding 9ml Nutrient broth to each falcon tube and Label them 10^8, 10^7, 10^6, 10^5, 10^4 for each strain of bacteria. Now took pure 1 ml from 1OD solution to falcon tube labeled 8 and mix it then take 1ml from 10^8 and added to 10^7 falcon tube, then repeated the same procedure until to reach the last one, the falcon tube labeled 4 (as falcon tube labeled 8 contained 10^8 per ml and same for other). Taking 100µl from 10^4 dilution via micro pipette and pouring it on plates containing Muller Hinton Agar as this 100µl from 10^4 containing 1000 bacteria.

6. Spreading and Well Formation

In this step, first, we poured 100 µl from falcon tube labeled 4 in Petri plate according to the specific bacteria. Made a lawn with the swab and now made wells with the help of a loop. Made plates separately for each bacterium. Spreading the bacterial solution on agar by cotton swab in multi directions to form an equally distributes loon of bacteria. After spreading wells made in the agar with the help of 1ml autoclaved micropipette tips according to the arrangement of samples per plate.

7. Sample Loading into well and Incubation

In this step poured 100µg of each sample into well with the help of micropipette ranges 10 to 200µl and incubated them for 24 hours at 37°C in Incubator. 100µg of emulgel formulation which was loaded into wells contained 2 µg of extract. Meropenem 10ug has been used as standard.

8. Obtaining results

After 24 hours removed the plates from the incubator and measured the diameter of zones of inhibition (table 15, fig. 10).

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal

Concentration (MBC)

The lowest concentration that produced no visible turbidity after a total incubation period of 48 hours was regarded as the final MIC. The lowest concentration dilution that yielded no single bacterial colony on the nutrient agar plates was taken as MBC. For MIC and MBC testing of the extract, we had prepared serial

dilutions of extract. The assay has been performed as maintained by Soberon *et al.* (2007) but with little modifications (Soberon *et al.,* 2007).

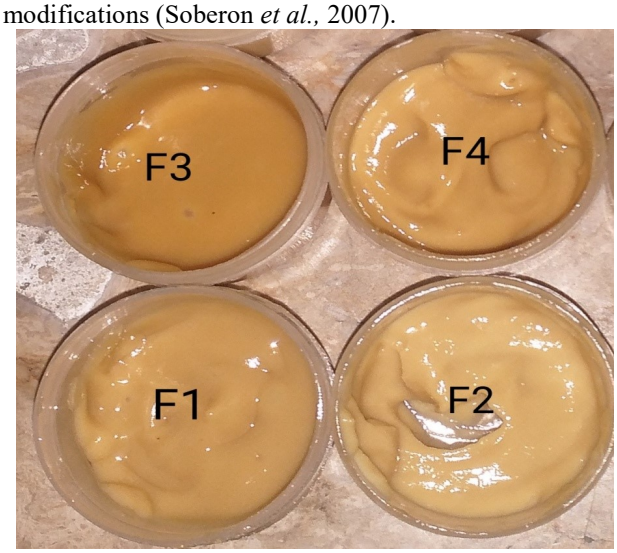

Fig 1: Images showing all emulgel formulations F1, F2, F3, F4

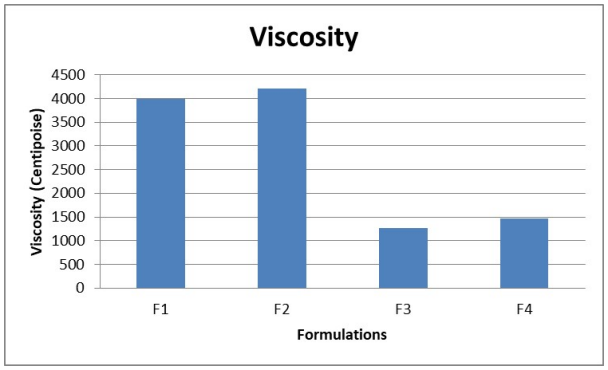

Fig. 2: Viscosities of all formulations checked with spindle 7 and 150 rpm

The microtiter broth dilution method has been used to determine MIC and MBC values each for *Staphylococcus aureus, Pseudomonas aeruginosa* and *E. coli* (fig. 9).

Methanolic extract solution was made 10µg / 100µl. 1st of all filled 100µl nutrient broth to 12 wells of a microtiter plate. Serial dilutions were made within wells. The methanolic extract is made 10µg / 100µl, then 100 µl is added to the 1st well then taking 100µl from 1st well and added to the 2nd well then 100 µl from 2nd and added to 3rd well and so on made dilutions till 10th well. Final concentration of extract in wells was 10, 5, 2.5, 1.5, 1.25, 0.625, 0.312, 0.156, 0.078, 0.039µg. Then 100µl bacteria sample from 10^8 falcon tube dilution for *staphylococcus* strain of bacteria and added to each well no. 1 to 10. After that last 2 wells were taken as the negative and positive control. The negative control contained only 100µl methanolic extract solution while the positive had 100µl bacterial solution. It got incubated at 37°C for 24 hours. MIC and MBC values have been identified. After that, we took 100 µl from wells those were identified as MIC and MBC and poured them on 2 separate nutrient agar plates for about 18-42h at 37°C to confirm the absence of bacteria (Hammer *et al*., 2003), (Song *et al*., 2006). Do the same procedure for *Pseudomonas* and *E. coli* strains of bacteria. The test is performed in triplicate (table 14, fig. 11).

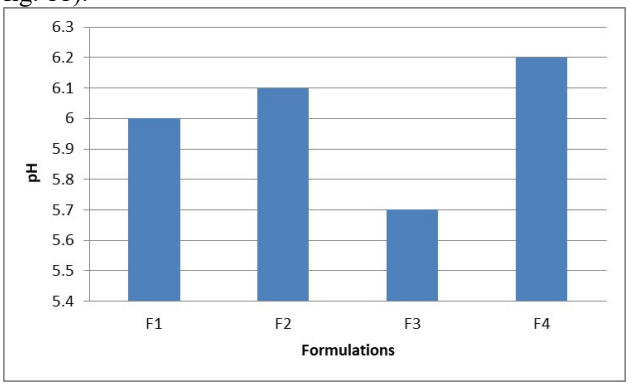

Fig. 3: Graph describing pH of all batches of emulgels

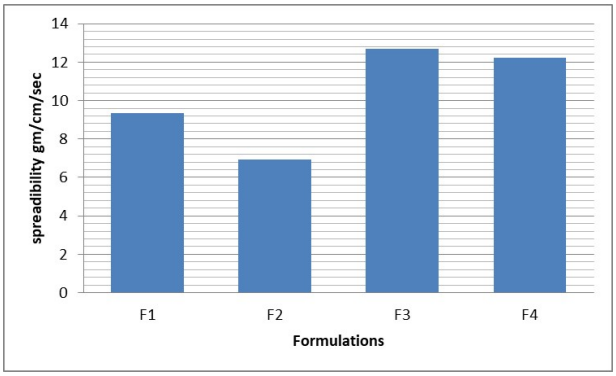

Fig 4: Graph showing Spreadability of all emulgels

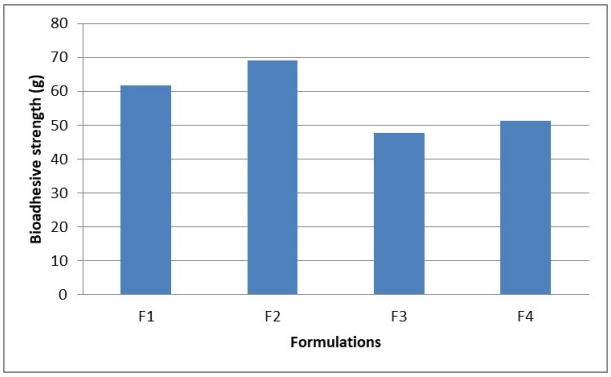

Fig. 5: Bio adhesive strength measurement of all formulations.

Table 3: Spreadability of all emulgel formulations

	Meantime ± SEM	Spreadibility gm/cm/sec
F1	4.33 ± 0.333	9.33 ± 0.666
F2	5.83 ± 0.44	6.94 ± 0.55
F3	3.16 ± 0.166	12.69 ± 0.634
F4	3.33 ± 0.333	12.22 ± 1.111

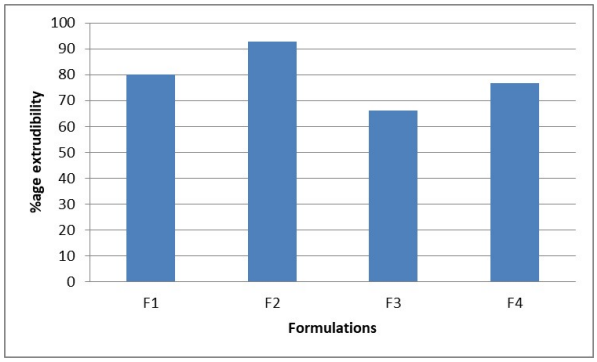

Fig. 6: Graphic representation of extrudability amount in percentage of all batches

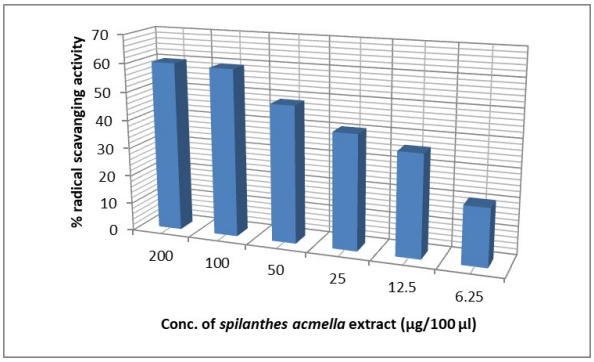

Fig. 7: Graphical representation of antioxidant activity.

RESULTS

The herbal emulgel of *Spilanthes acmella* had been prepared and evaluated for the various parameters. Emulgel color tone range from light skin color to dark brown color and was having a cool feeling while application to the skin and had smooth texture on application (table 2).

The viscosities of all emulgel formulations Mean ± SEM were in the range of 1263.333±6.76 to 4207.667±3.84 were measured in centipoises and were found to get increased with increasing concentration of polymer carbapol 934 fig. 2.

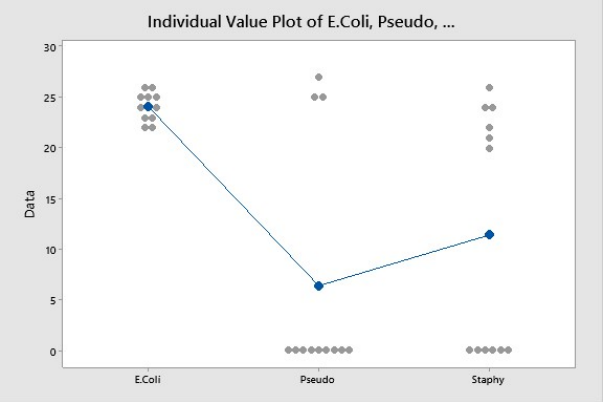

Fig. 8: Individual value plot showing zones of inhibitions produced by all formulations against each bacterium.

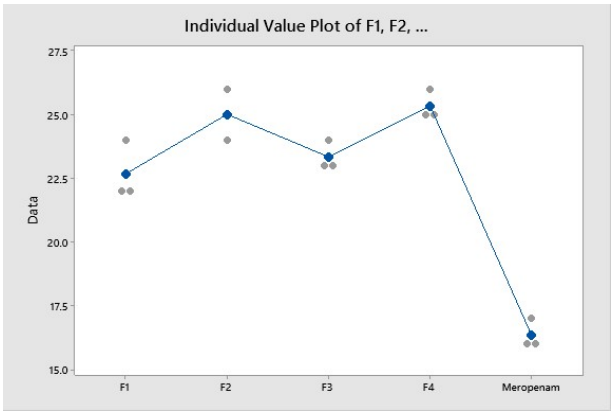

Fig. 9: Individual value plot showing zones of inhibitions produced by all formulations vs standard.

Fig. 10: Image showing mean zone of inhibitions on plates.

pH also got maintained throughout the study. pH range was in the range of 5.7 to 6.2. The emulgel was found non-irritant on application to the surface of the skin. The pH of all batches of emulgel discussed in fig. 3.

Table 5: Percentage radical scavenging effect of dilutions of extract

Concentration of pure extract solution µg/100µl	% Radical scavenging activity ±SEM
200	60.01 ± 0.28
100	59.36 ±1.62
50	48.47 ±1.17
25	40.67 ± 0.43
12.5	36.15 ±0.58
6.25	20.36 ± 1.64

Table 6: Analysis of Variance of all formulations against each bacterium

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	1990	995.11	10.61	0.000
Error	33	3095	93.78		
Total	35	5085			

Table 7: Means of zone of inhibitions of extract against each bacterium (n=3)

Factor	Mean (mm)	St Dev	95% CI
E. Coli	41.667	1.528	(39.669, 43.665)
Pseudo	27.333	1.155	(25.335, 29.331)
Staphy	25.333	1.528	(23.335, 27.331)

Table 8: Means of zones of inhibitions produced by all formulations against *E. Coli, Pseudo* and *Staph* (n=12)

Factor	Mean (mm)	St Dev	95% CI
E. Coli	24.083	1.379	(18.396, 29.771)
Pseudo	6.42	11.62	(0.73, 12.10)
Staphy	11.42	12.02	(5.73, 17.10)

Spreadability of emulgels was found in the range of Mean ± SEM 6.94 ± 0.55 to 12.69 ± 0.634 gm/cm/sec. Those formulations that had the highest viscosity had the minimum level of spreadability (table 3, fig. 4).

The bio-adhesive strength of all emulgels formulations had been determined. It was observed that the concentration of carbapol 934 had a greater influence on the emulgel bio-adhesive strength. More the concentration of carbapol greater will be the bioadhesive strength fig. 5.

Those formulations having a low level of spread ability had a higher level of extrudability. Extrudability of emulgel was ranged from fair to excellent Mean ± SEM (66.01 ± 0.272 to 92.64 ± 0.539) (table 4, fig. 6).

The antioxidant potential of methanolic extract of *Spilanthes acmella* has been measured by using DPPH. The results showed that the highest concentration of parts extracts 200 µg/100µl exhibited the highest antioxidative activity 60.01 ± 0.28 %, while at the lowest concentration 60 µg/100µl have shown 20.36±1.64% radical scavenging activity (table 5, fig. 7).

STATISTICAL ANALYSIS

Statistical analysis had been performed by using MINITAB 17 statistical package software version by using one way ANOVA ($p<0.05$). The pure extract has shown a significant antibacterial effect for each tested bacteria (table 7) while maximum zone of inhibition was observed against *E. Coli* 41.66 mm. All formulations collectively had shown statistically significant antibacterial effect, all tested bacterium p-value=0.00 (table 6, fig. 8). Against *E. coli* 100% formulations had produced zone of inhibitions while maximum zone of inhibition got produced by F4 (25.33mm) which was much greater than meropenem standard 16.33 mm (table 10, fig. 9). In the case of *Pseudomonas*, only F1 formulation had shown zone of inhibition 25 mm (table 10). F3, F4 have shown zone of inhibition against *Staphylococcus aureus* 21, 25.5 mm respectively, while meropenem got failed to show antibacterial effect against staph (table 10).

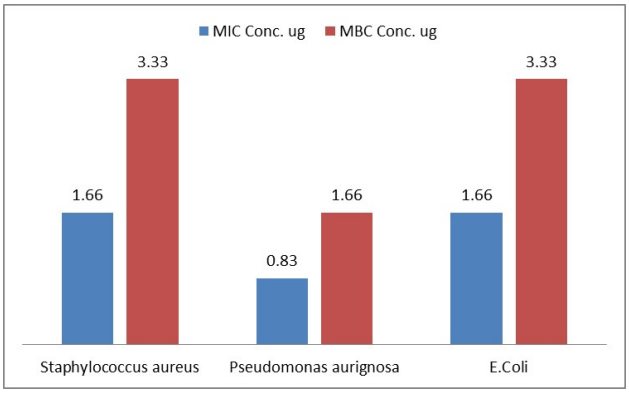

Fig. 11: Graphical representation of MIC and MBC in µg/µl of pure extract against different bacterial strains

MIC values found in the range of 0.83±0.21 to 1.66± 0.41µg/100 µl, MBC values found in the range of 1.66± 0.41 to 3.33±0.83µg/100 µl for all strain of bacteria. The minimum value of MIC and MBC has been reported for *Pseudomonas aeruginosa* 0.83±0.21, 1.66±0.41µg/100 µl respectively. MIC and MBC values found to be equal for both *Staphylococcus aureus* and *E. coli* 1.66±0.41, 3.33±0.83µg/100µl respectively. MBC was found to be 2 fold greater than the MIC value. MIC and MBC values were found to be the lowest for *Pseudomonas aeruginosa* (table 9, fig 11).

DISCUSSION

According to CLSI guidelines, ≥16 mm zone of inhibition shows sensitivity according to CLSI 2009-11 and EUCAST 2011 guidelines (Hombach *et al*., 2012, Wayne 2014). While *Staphylococcus aureus* was found to be completely resistant against meropenem standard as this also has been reported by Kayser in 1989 that meropenem is ineffective against methicillin-resistant *Staphylococcus aureus* (Kayser *et al*., 1989). The pure extract had shown a much higher antibacterial effect as compared to the standard drug meropenem zone of inhibition range of extract found between 25.33±0.88 mm to 41.66±0.88mm (table 10). F1 was found to be active against pseudomonas and *E. coli,* while F2 was found to be active only against *E. coli.* F3 and F4 were found to be sensitive for *Staphylococcus aureus* and *E. coli*. 2(50%) formulations were found to be active against *Staphylococcus aureus* while 1(25%) formulations were found to be active against *Pseudomonas aeruginosa* 25±0.57mm. 4(100%) formulations were found to be active against *E. coli.* Mean±SEM zone of inhibition of all formulations was in the range of 23.33± 0.33 to 25.33±0.33mm in diameter. While in the case of *Staphylococcus aureus* 2(50%) of formulations F3, F4 has shown antibacterial effects 21±0.57, 25.5±0.66 mm respectively. Several reports describing the *Escherichia coli* causing most of the SSTI have been produced (Paolucci *et al*., 2009). Isolates from SSTIs a study has

Table 9: Mean of MIC and MBC values against different bacterial strain

MIC Conc. µg/100 µl Mean ± SEM	MBC Conc. µg/100 µl Mean ± SEM	Bacterial Strain tested
1.66 ± 0.41	3.33 ± 0.83	*Staphylococcus aureus*
0.83 ± 0.21	1.66 ± 0.41	*Pseudomonas aeruginosa*
1.66 ± 0.41	3.33 ± 0.83	*E.Coli*

Table 10: Zone of inhibition diameter Mean ± SEM (mm)

Preparations	*Staphylococcus aureus*	Pseudomonas *aeruginosa*	*E.Coli*
F1	0 ± 0	25 ± 0.57	23.5 ± 0.66
F2	0 ± 0	0 ± 0	25 ± 0.57
F3	21 ± 0.57	0 ± 0	23.33 ± 0.33
F4	25.5 ± 0.66	0 ± 0	25.33 ± 0.33
Extract	25.33 ± 0.88	27.33 ± 0.66	41.66 ± 0.88
Meropenem	0 ± 0	17 ± 0.577	16.33 ± 0.33

revealed that *E. coli* had exhibited a remarkable potential of virulence as compared to *E. coli* isolates from UTI (urinary tract infections). (Petkovšek *et al*., 2009).

CONCLUSION

Emulgel containing herbal plant extract of *Spilanthes acmella* was found to be most active against *E. coli* bacteria. This herbal emulgel could serve as a better treatment option for *E. coli* causing deep skin tissue infections which cannot be treated by antibiotics due to drug resistance.

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