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Efficacy of *Psorospermum febrigerum* Stem Bark Extract in Gel Dosage Formulations against the Bacteria Responsible for Aggravation of Acne Vulgaris Skin Disease

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ABSTRACT

Propionibacterium acnes and Staphylococcus epidermidis are the two major bacteria responsible for the exacerbation of acne vulgaris. All antibiotic being used currently to treat these two types of bacteria have become resistant. For this reason, there is urgent need to develop new drug formulations with ability to fight against the resistant acnes causing bacteria. Of recent the stem bark extract of *P. febrifugum* has been found effective against the Propionibacterium acnes and Staphylococcus epidermidis. No study has been undertaken to develop *P. febrifugum* extracts into a gel or other topical dosage formulations and thereafter test its efficacy against the two major acne causing bacteria. This has therefore, limited the acceptability and wide- usage stem bark of *P. febrifugum* extract as an extract of choice to treat bacterial infections of acne vulgaris. The main aim of this study was to therefore to prepare the stem bark extract of *P. febrifugum* into gel dosage formulations and thereafter evaluate their efficacy against *Propionibacterium acnes* and *Staphylococcus epidermidis* acne causing bacteria. Prior to the study, three increasing dosage concentrations of *P. febrigerum* stem bark extracts were selected basing on the studies previously undertaken. The efficacy of the dosage formulations in an increasing manner against Propionibacterium acne and Staphylococcus epidermidis was established. The dosage concentrations were then used to prepare stem bark extract of *P. febrifugum* gel-based formulations (G0-G3) for the study. The efficacy of gel formulations (G0-G3) against Propionibacterium acnes (P.acnes) and S. epidermidis were determined using Agar Well Diffusion method. Findings from the study indicated that all the three gel-based formulations (G1, G2, G3) significantly inhibited Propionibacterium acnes and Staphylococcus epidermidis with gel G3 Formulation showing highest inhibition growth against the two bacteria.The *P. febrifugum* stem bark extract formulated in increasing gel dosage formulations was effective against Propionibacterium acne and Staphylococcus epidermidis bacteria that cause acnes.

Keywords: Stem bark, Acne vulgaris, Skin disease, Gel dosage formulations

INTRODUCTION

Acne vulgaris is the eighth most common disease world-wide. It affects close to 650 million people, of which 90% are adolescents [1]. Up to date, the prevalence and the risk of acquiring acne vulgaris has remained predominantly high amongst the adolescents [2]. For instance, in China the prevalence is at 38% [3, 4], Kuwait 67.1% [5], Lithuania 82.9% [6] and Brazil 96% [7]. In Nigeria, 64.4% [8]. In East Africa, particularly in Uganda and Kenya, the prevalence of acne vulgaris is at about 15%. Previously acne vulgaris was not a life-threatening disease because it never translated into any mortality. However, because it attacks the most attractive visible areas such as the face and the back and it predominates during the time of adolescents when most prefer to look attract [9-12]. Acne vulgaris is as of now the leading cause of humiliations, embarrassment, depression, anger, social withdrawal, feelings of inferiority, suicidal tendency, low self-esteem, and many other negative impressions in the adolescents. Propionibacterium acnes is the major bacteria responsible for the

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manifestation of acne vulgaris. Studies have demonstrated that P. acnes plays the role of breaking down of sebaceous triglycerides into fatty acids, which chemotactically attracts neutrophils and consequently contributing to the formation of comedones, papules and pustules on skin of patients with acne vulgaris [13]. Currently, antibiotics in gel dosage formulations of tetracyclines, erythromycins and clindamycin are majorly being used to eradicate P. acnes bacteria involved in the manifestation of acne vulgaris. Nevertheless, these antibiotics have acquired resistance and so, they are no longer efficacious enough to eradicate bacterial infections of acne vulgaris. Of recent, the stem bark of *P. febrifugum* extract has however demonstrated efficacy effects against eradication of the bacterial infections including all those involved in the manifestations of acne vulgaris [14]. Nevertheless, no study has however been undertaken to develop the stem bark extract of *P. febrigerum* into a gel dosage formulation and there after tested its efficacy against the major bacteria responsible for aggravation of acne vulgaris disease. This has therefore restricted the acceptance and usage of the stem bark extract of *P. febrifugum* as a drug of choice to treat acne vulgaris. This study therefore developed the stem bark extract of *P. febrigerum* into a gel dosage formulation and thereafter tested its efficacy against the treatment of acne vulgaris using in vitro techniques.

RESEARCH METHODOLOGY

Study design

The study designed as a laboratory based qualitative and quantitative experimental design [15]

Collection of plant material and authentication

The plant stem bark of *Psorospermum febrifugum* was collected from Kakoba, Mbarara district western part of Uganda between March 3 2023 and March 30 2023. After the collection of the stem bark material, a voucher specimen was also carefully collected, packaged in a paper bag and immediately transported to the National herbarium, Makerere University for identification and authentication purposes, for which voucher specimen was identified of MW007 provided.

Storage, drying and pulverization

The stem bark material collected was cut into smaller pieces, spread on a dry cemented tables in an isolated room and changed daily until constant weight was achieved. Dried stem bark material was then coarsely powdered, sieved with a size #40 mesh sieve and then stored in an air tight container by room temperature to prevent contaminations.

Preparation of plant material into extracts

A total of 615g of powdered sample material was put into a jar and weighed amount of ethanol added, then the jar was covered. Stirring for 6hrs then the mixture is put on mechanical shaker while stirring often, followed by shaking at intervals up 12 hours to 3 days (72 hours). The resultant mixture was strained with cotton wool and then filtered by a #1-Whatman filter paper. Thereafter the solvent was removed using a rotary evaporator at 40°C under reduced pressure, the filtrate was then further more concentrated, there after allowed to dry at room temperature

The percentage yield (%) of the extract w
as calculated using the equation below:

$$\text{Percentage yield (\% age yield)} = \frac{\text{Weight of concentrated extract}}{\text{Weight of the plant powder}} \times 100$$

The dried crude extract was stored in a refrigerator at 4°C until it was used for actual experimental studies.

Phytochemical analysis

Test for tannins; 2ml of extract was stirred with 2ml of distilled water then 2-3 drops of ferric chloride (5%) was added. A green precipitate indicated presence of tannin. Test for Alkaloids; 2ml of extract was taken and placed into a test tube. Then 2ml of potassium bismuth iodide solution (Dragendorff's reagent) was added and shaken. An orange red precipitate formed indicated the presence of alkaloids. Test for flavonoids; 2ml of extract was taken and placed into a test tube. Then few drops of lead acetate added and shaken. Formation of yellow precipitate signified the presence of flavonoids [16]**.** Test for steroids; To 5ml of extract, add 2ml of choroform and 3ml of concentrated sulphuric acid. A reddish-brown precipitate formed at the interface indicated the presence of steroids. Test for Anthraquinones; 5g of powdered sample of plant material was added to 10ml benzene, filtered and ammonia solution added. A pink, red or violet colouration in ammonical phase indicated the presence of anthraquinones [16-19]. Test for terpenes; to 5ml of extract, add 2mls of chloroform and 3ml of concentrated sulphuric acid. A reddish brown precipitate is formed.

Obtainment of bacteria that aggravate acne vulgaris skin disease

The bacterium Propionibacterium acnes and Staphylococcus epidermidis which were used for experiment were from the Medical Microbiology laboratory of Mulago hospital in Kampala Uganda.

Formulation of the gel

A weighed amount of Carbopol was taken in a beaker and 50-60ml of water added, the mixture was kept in a hot air oven at 100c for 30 minutes with stirring using glass rod for 10-15 minutes to avoid air bubbles, then kept aside for 30 minutes.The mixture was homogenized for 10 minutes and in warm condition, methyl paraben

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was added. Weighed quantity of drug was dissolved in small amounts of water and the remaining ingredients added to drug solution. Then remaining quantity of water was added with Triethanolamine to neutralize the pH.

Preparations of P. acnes and S. epidermidis

Testing efficacy of gels (G0-G3) against P. acnes and S. epidermidis.

The efficacy of the (G1-G3) dosage formulations was tested against P. acnes and S. epidermidis using the agar well diffusion method. The BHI (Brain Heart Media) Agar shall be inoculated by spreading *P. acnes* over the entire agar surface and left to dry. A hole with a diameter of 6 mm was punched aseptically with a sterile tip. For each gel formulations (G1-G3) about 0.5 g was then be introduced into the wells. After then, agar plates were incubated in an anaerobic environment using the Anaerocult a Mini system (Merck, Darmstadt, Germany) at 37 °C for 48 h. Gel base and Clindalin® gel (clindamycin 1% gel) were also introduced into the wells as the placebo and positive controls respectively. The anti *P. acnes* and S. epidermidis activity was thereafter evaluated by measuring the diameter of zones of inhibition (in mm). The experiments was repeated three times and the mean value of the zones of inhibition was then calculated. Comparative percentage inhibitory activity of the gels (G1-G3) and controls against P. acnes bacteria was determined as per the formula.

$$\%\text{Inhibition} = \frac{\text{DZI (control)} - \text{DZI (treatment)}}{\text{DZI (control)}} \times 100\%$$

Where DZI, diameters of the zones of inhibition

Statistical analysis

The statistical significance of differences between the values each P. febrifugum gel (G1-G3) samples against the controls was evaluated using analysis of variance (ANOVA) and significant differences ($p < 0.05$) among means were determined by R commander software.

Ethical Considerations

The research protocols of this proposed were presented, reviewed and approved by the Research Board School of Pharmacy Kampala International University. Disposal of used materials and others was done in a way that ensured environmental safety and the waste materials were placed in waste bins, then disposed off in the right way so as to prevent unethical trepidations during this study.

RESULTS

Table 1: Phytochemical constituents

Phytochemical components	Interpretation
Tannins	+
Alkaloids	++
Flavonoids	++
Terpenes	+
Steroids	+

(+) present, (-) absent

Table 2: Organoleptic properties of the gel formulation.

Parameter	**Results**
Color	Light brown
Appearance	Translucent
PH	6.8
Homogeneity	Homogenous

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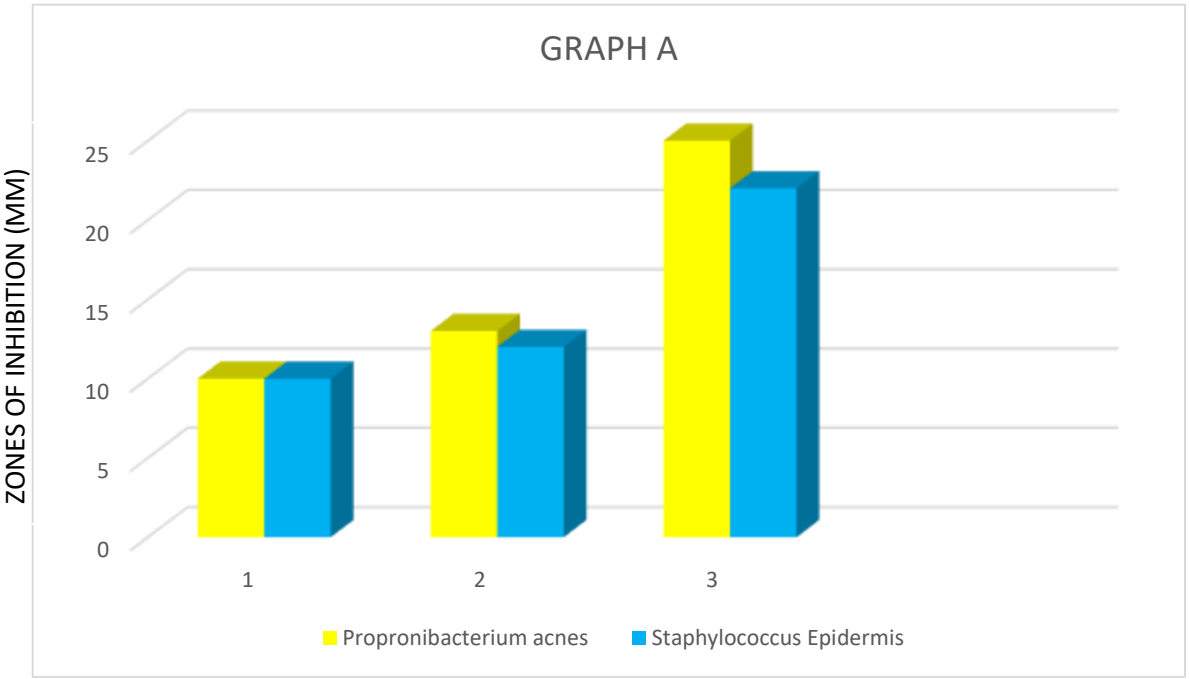
Table 3: Activity of the gel formulations against P. acnes and S.epidermidis

Concentrations of plant extract (grams)	Zones 0f inhibition(a(mm)+-SEM)					
	Extract alone		Plant extract gel formulation		Clindamycin gel (Standard drug)	
	P.acnes	S.epidermis	P.acnes	S.epidermis	P.acnes	S.epidermis
0	0	0	0	0	0	0
1	10	10	6	5	34	33
2	13	12	12	11	34	33
4	25	22	28	26	34	33

P .acnes=Propionibacterium acnes S.epidermis=staphylococcus epidermidis

.

A graph of zones of inhibition of propiobacterium acnes and staphylococcus epidermis against increasing concentrations of gel formulations of plant extract



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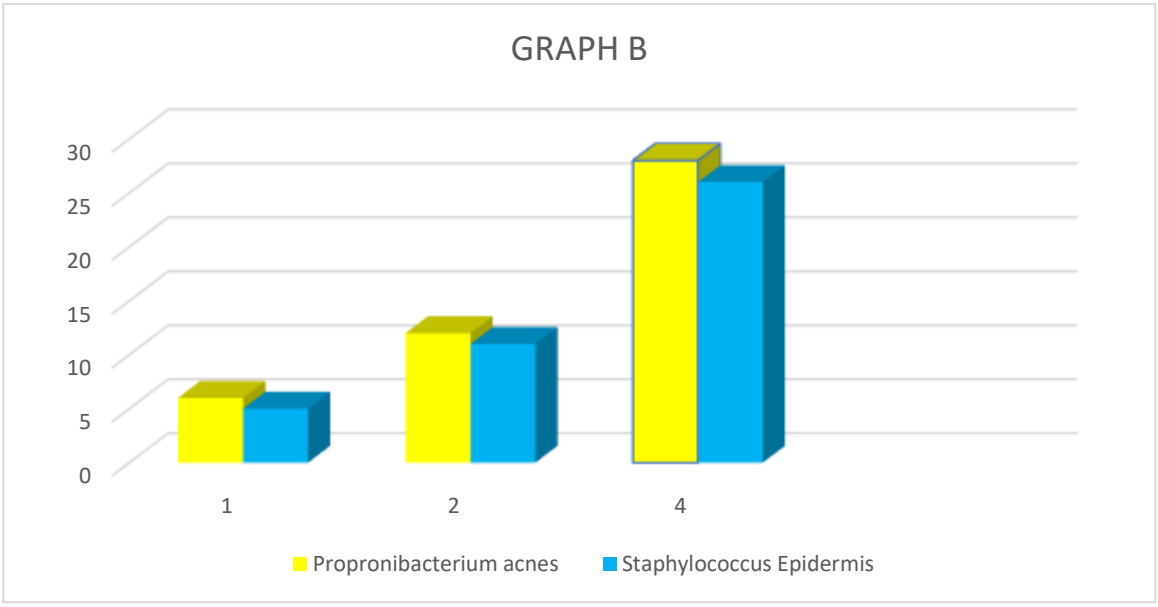

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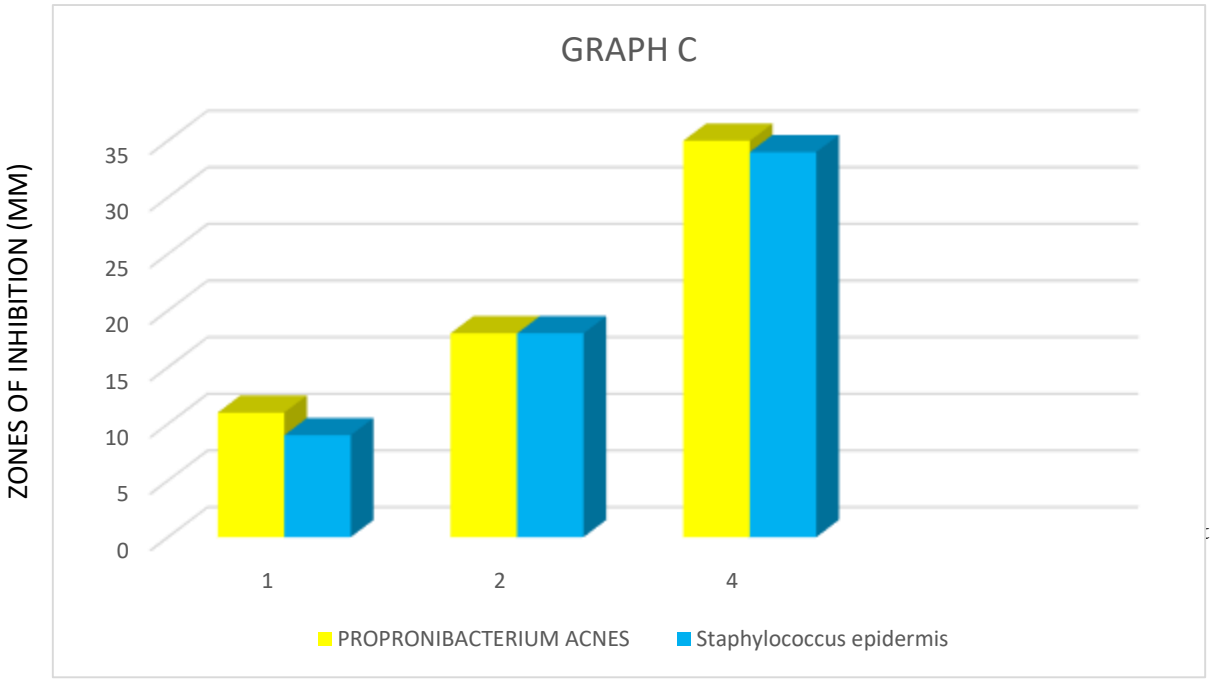
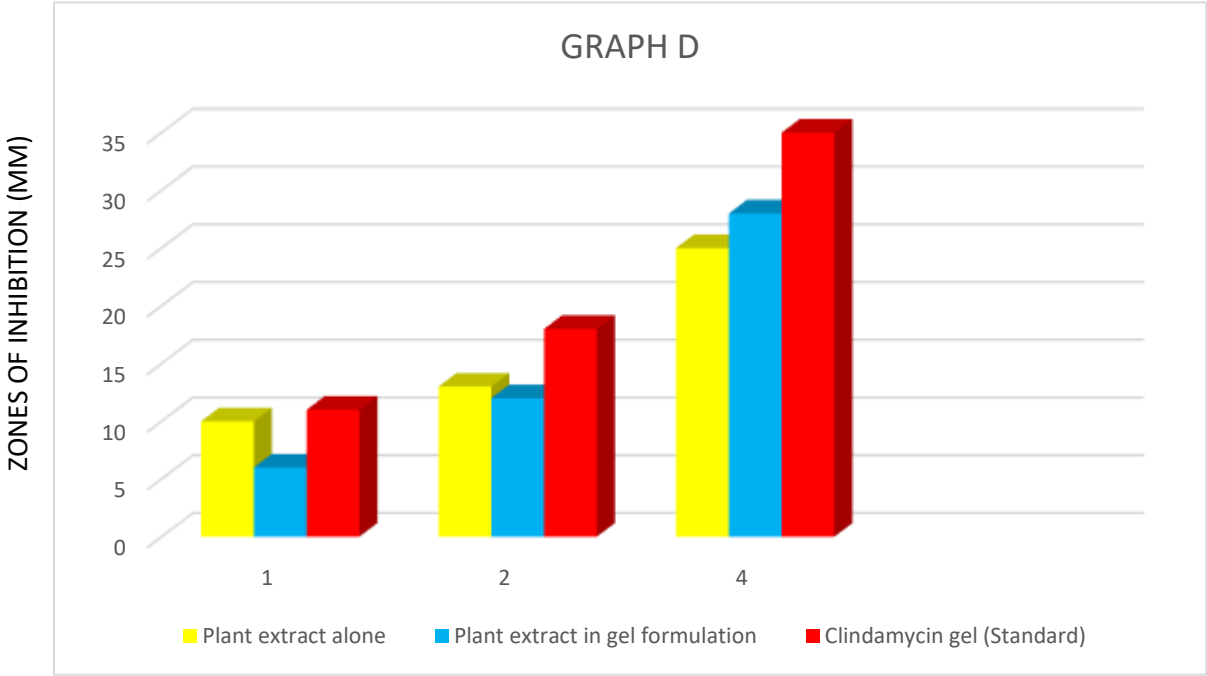


alone, plant extract gel formulation and clindamycin gel(standard)



extract alone, plant extract in gel formulation and clindamycin gel(standard)

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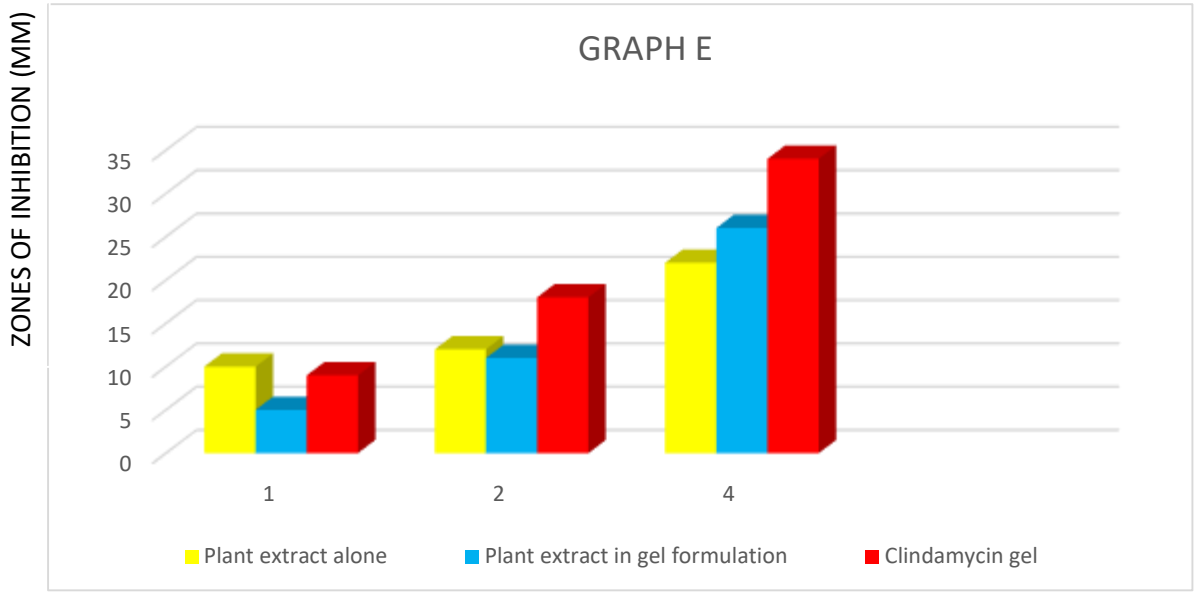

DISCUSSION

In graph A, the antibacterial activity and efficacy of ethanolic stem bark extract of *Psorospermum febrifugum* increased with increasing concentrations of plant extract. The plant extract was more effective against P. acnes than staphylococcus epidermis. In graph B, the antibacterial activity thus efficacy of gel formulation of plant extract increased with increasing concentrations of gel formulation. The plant extract gel formulation was also more effective against P.acnes than S. epidermidis. In graph C, the efficacy of the clindamycin gel (standard) also increased with increasing concentrations. In graph D and E, the zones of inhibition were higher in Clindamycin plant extract alone than gel formulation of plant extract. This is because the concentration (1g) was still very small in the gel components thus reducing the antibacterial activity against P.acnes (graph D) and S.epidermidis (graph E). But with increasing concentrations (4g), the concentration of plant extract in gel become significant and high leading to increased efficacy of plant gel than plant extract alone. Though the efficacy of clindamycin gel is higher than plant extract gel formulation. The difference in zones of inhibition between clindamycin gel (standard) and gel gel formulation of plant extract was small. Therefore the Psorospermum febrifugum stem bark ethanolic extract in increasing gel dosage formulations (G1, G2, G3) was effective against Propionibacterium and staphylococcus epidermis bacteria that cause acnes vulgaris. The antibacterial activity and efficacy of plant extract against P. acnes and S. epidermidis is because of the phytochemical compounds; tannins, alkaloids, flavonoids, terpenes, steroids which were tested and found to be present.

CONCLUSION

Findings from this study demonstrated that P. febrifugum stem bark ethanolic extract in extract and increasing gel dosage formulations (G1, G2, G3) was effective against Propionibacterium and staphylococcus epidermis bacteria that cause acnes vulgaris. All the three gel-based formulations (G1, G2, G3) significantly inhibited Propionibacterium acnes and Staphylococcus epidermidis with gel G3 Formulation showing highest inhibition growth against the two bacteria.

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