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PHYTOCHEMICAL ANALYSIS AND EVALUATION OF ANTIMICROBIAL ACTIVITY OF PERESKIA BLEO (KUNTH) DC.

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ABSTRACT

Antimicrobial resistance has emerged as a cause of public health threat all over the world at a terrifying rate. The emergence of these global issues compels the continuous exploration of natural products containing antimicrobial activity. *Pereskia bleo*, a medicinal plant belongs to the Cactaceae family. This plant has been used traditionally for various medicinal purposes. However, previous studies regarding antimicrobial activity of *Pereskia bleo* leaves are still lacking. Hence, the present study was carried out to identify the phytochemical constituents and investigate the antimicrobial activity of *Pereskia bleo* leaves extract in different concentrations. Several phytochemical test were conducted to ascertain the presence of various phytoconstituents in the dichloromethane extract of *Pereskia bleo* leaves. The antimicrobial activity of four different concentrations of dichloromethane extract (50, 100, 150 and 200 mg/mL) and its ointment formulations (5, 10, 15 and 20% w/w) were evaluated by using agar well diffusion method against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. The phytochemical screening depicted that the leaves extract of *Pereskia bleo* revealed the presence of fats and oils, phytosterols/sterols, flavonoids, terpenoids, and phenolic derivatives whereas both alkaloids and lactones were absent in it. The extract showed antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus and Pseudomonas aeruginosa* whereas no activity was observed for *Escherichia coli*. However, when the dichloromethane extract incorporated into petroleum jelly, all the extract ointments revealed no inhibitory effect against the selected bacteria. In summary, it is believed that dichloromethane extract of *Pereskia bleo* leaves might be beneficial towards bacterial infection related disease and has much potential to be developed as a phytomedicine. The inhibitory effect of this particular plant might be attributed by the presence of flavonoids and terpenoids. However, further investigation of *Pereskia bleo* are required in terms of *in vitro* and *in vivo* approaches in order to ensure the effectiveness and safety of the plant.

KEYWORDS: *Pereskia bleo*; phytochemical screening; antimicrobial activity; agar well diffusion method.

INTRODUCTION

There is a presence of Microbial infections in human beings since the past decades. While there are diverse synthetic antimicrobial agents accessible in the market for the remedy of microbial infections but pervasive and abusage of these medications may lead to evolution of antimicrobial resistance. As per World Health Organization (WHO), antimicrobial resistance has emerged as a cause of public health threat around the globe at a formidable rate. This is because antimicrobial resistance may lower the effectiveness of treatment resulting in extended duration of illness and infection, which would pre-eminently surge cost for therapy, death risk and disability of patients. The emergence of these global issues compels the nonstop exploration of natural products containing antimicrobial activities. It is reported to be widespread among the rural communities in the Chinese, Indian and South East Sub-continent. As per Mojiol et al., (2010), around 1,200 species in Malaysia have been proven to contain medicinal properties. The Pereskia bleo is one of these plants comprising discrete medicinal properties.

Pereskia bleo (Kunth) DC. belongs to the family of *Cactaceae*. This specific leaf cactus is sourced from Latin America and promptly dispensed to the oriental region (Khan et al., 2013). Pereskia bleo is widely cultivated in various countries for therapeutic and non-therapeutic purpose (Wiart, 2006) due to its heterogeneous composition. Being one of the most recognisable medicinal plants, Pereskia bleo is broadly cultivated and utilised by indigenous groups in different parts of the world. It is prominently believed from ancient times until now that this plant can be used as a

natural remedy to treat a variety of health conditions including diabetes, hypertension and cancer related ailments. Moreover, its effectiveness also has been proven in treating rheumatism, inflammation, gastric pain, ulcers and body revitalization (Khan et al., 2013; Er et al., 2010; Abd Malek et al., 2009, 2008; Tan et al., 2005). Traditionally, Pereskia bleo has been consumed in the form of concoction brewed from dried plant or conventionally eaten raw as vegetables (Sim et al., 2010a; Abd Malek et al., 2008; Er et al., 2007). Pereskia bleo was found to be rich in alkaloids, flavonoids, phytosterol glycosides, lactones, phenolic compounds, sterols, terpenoids and carotenoids (Zareisedehizadeh et al., 2014).

The purpose of the study is to analyse the phytochemical components and examine the antimicrobial activity of Pereskia bleo leaves extract in various concentrations.

The objectives of the study are:

1. To obtain dichloromethane extract from the leaves of Pereskia bleo by using Soxhlet apparatus.
2. To identify the phytoconstituents present in the Pereskia bleo leaves extract using standard phytochemical screening.
3. To formulate Pereskia bleo leaves extract ointment using petrolatum jelly as ointment base.
4. To scrutinize the antimicrobial activity of Pereskia bleo leaves extract and its ointment formulations against selected microbes.

Using this study as a reference, substantial data on antimicrobial activity of Pereskia bleo can be used to facilitate exploration in terms of academic contribution or research development. This study will also impart a better insight on Pereskia bleo and favours individuals interested in this field of study. Despite the fact that there are plenty of synthetic drugs readily available in the market for the treatment of infections, some of these drugs appeared to have severe side effects as compared to herbal medicine. Mentioned observations will therefore be able to analyse the possibility of extracting a new, inexpensive and reliable natural drug from Pereskia bleo. As it is more accessible and beneficial compared to synthetic drugs, the usage of Pereskia bleo is predicted to serve as a better alternative healing agent to be used for infection treatment.

MATERIALS AND METHODS

Plant material and sample preparation

Collection and identification of plant material: Pereskia bleo's leaves were identified and assembled. For identification and authentication, a sample was sent to the CMAP(Center for medicinal and aromatic plants), Hyderabad.

Site of experimental study: The experimental study was conducted in the laboratory of Sultan-Ul-Uloom College of Pharmacy.

Preparation of plant extract

The fresh green leaves of Pereskia bleo were assembled and washed with distilled water and dried for about a week under the shade at ambient temperature. Using electronic blender, the dried leaves were then grounded into powder form. In a Soxhlet extractor, 445 g of dry finely grounded leaves were then extracted using dichloromethane as a solvent. The extraction method was established as per the technique. The extraction procedure was repeated 3 times to achieve sufficient extract quantity for further testing. The extract was subsequently mixed and filtered to remove impurities using filter paper. Following filtration, dicloromethane extract was concentrated under low pressures at 45 ° C using rotary evaporator and left until fully dry (Er et al., 2010) for evaporation. For further testing, the resulting extract was then stored in a plastic container.

Percentage yield of plant extract

The percentage yield of plant extract was determined using the following formula (Dawoud et al., 2015):

Percentage of extraction yield (%)

$$= \frac{\text{weight of sample extract obtained (g)}}{\text{weight of finely grounded plant material used (g)}} \times 100$$

Phytochemical screening

Numerous phytochemical studies were performed to evaluate the concentrations of different phytoconstituents like those of alkaloids, phytosterols / sterols, flavonoids, terpenoids, fats and oils, phenolic derivatives, and lactones in the Pereskia bleo leaves dichloromethane extract.

Determination of alkaloids

The Mayer's and Hager's experiments were conducted to identify the presence of alkaloids in Pereskia bleo's dichloromethane leaves extract. A tiny part of the extract of dichloromethane was transferred to a tidy test tube. Then dilute hydrochloric acid was added to the test tube and the mixture was shaken well to mix the contents thoroughly. The solution was further filtered using filter paper and the following reagents were added to the filtrate:

(i) Mayer's test: A few drops of Mayer's reagent (potassium mercuric iodide solution) was added into 2 mL filtrate and the mixture is stirred well. Formation of precipitate in the test tube indicates the presence of alkaloids.

(ii) Hager's test: 2 mL filtrate was treated with a few drops of Hager's reagent (saturated picric acid solution). The presence of alkaloids is identified by the formation of yellow coloured precipitate.

Determination of phytosterols/ sterols

Detection of phytosterols/ sterols in the dichloromethane extract of Pereskia bleo leaves was carried out via Salkowski test, Liebermann-Burchard reaction and Liebermann's reaction.

(i) Salkowski test: 2 mL of chloroform was added to a small amount of dichloromethane extract. The solution was subsequently added with 2 mL of concentrated sulphuric acid. The mixture was then stirred thoroughly and allowed to stand for few seconds. The presence of phytosterols / sterols is indicated by brown colour.
(ii) Liebermann-Burchard reaction: In a experimental tube, a small portion of dichloromethane extract was added to 2 mL of chloroform. The extracted solution of chloroform was treated with 1 mL of acetic anhydride and some drops of concentrated sulphuric acid from the side of the test tube are added to the solution. The bluish green formation shows the availability of phytosterols / sterols.
(iii) Liebermann's reaction: 2 mL of dichloromethane extract and 2 mL of acetic anhydride were mixed together. The test tube was eventually heated on water bath and allowed to cool for sometime. From the sides of the test tube, a few drops of concentrated sulphuric acid were then added to the solution. Blue colour appearance indicates the occurrence of phytosterols / sterols.

Determination of flavonoids
An alkaline reagent test has been used to identify the presence of flavonoids. With a few drops of sodium hydroxide solution, a relatively small amount of dichloromethane extract was treated. Initially, an intense yellow coloration would form and disappear immediately after the addition of dilute hydrochloric acid, suggesting the presence of flavonoids.

Determination of terpenoids
Terpenoid determination was performed using the Salkowski test. With a few milliliters of dichloromethane extract, 2 mL of chloroform was added. On the side of the test tube, 2 mL of mixed sulphuric acid was further added to the solution. The appearance of reddish brown color at the interface shows terpenoid presence.

Determination of phenolic compounds
Folin-Ciocalteu reagent and Ferric chloride test were performed to detect phenolic compounds in the Pereskia bleo leaves dichloromethane extract.
(i) Ferric chloride test: In the dichloromethane extract, 1 mL of 5% ferric chloride solution was added. The appearance of phenolic compounds is detected by the formation of bluish black or bluish green colour.
(ii) Folin-ciocalteu reagent: Initialy 1 mL of Folin-Ciocalteu reagent was added to 1 mL of extract and 0.8 mL of 7.5% sodium carbonate was added later on. The mixture was stirred well and 30 minutes are taken for it to stand. Deep blue color appears indicating the presence of phenolic compounds.

Determination of lactones
Lactone determination can be performed using the Legal test. A minimal amount of dichloromethane extract was mixed with 2 mL of pyridine. Then the mixture was added with two drops of nitroprusside and 20% of sodium hydroxide solution. Deep red color formation shows the occurrence of lactone.

Determination of fats and oils
In a test tube containing 1mL of chloroform, 1 mL of dichloromethane extract was added drop wise and mixed well till the solution is completely dissolved. Just few drops of Sudan red lll reagent have been consequently added to the above mixture. The transparency on the filter paper shows the occurrence of fatty acids. A red color appearance shows the existence of fats and oils.

Preparation of dichloromethane extract ointments
Petroleum jelly was used as ointment base to prepare the simple ointment from dichloromethane extract. Using palette knife, the formulation was performed by levigation. The experiment was conducted for four varying dichloromethane extract ointment formulations with a concentration of 5, 10, 15 and 20 percent w / w. By introducing 1 g of dichloromethane extract into the ointment base for a total weight of 20 g, 5% w / w of dichloromethane extract ointment was prepared. The same technique was performed using 2, 3 and 4 g dichloromethane extract for the ointment of 10, 15 and 20 percent w / w of dichloromethane extract.

Evaluation of formulated ointment
Previously prepared ointment formulations were assessed through physical appearance and homogeneity.

Physical appearance test: The prepared ointments have been screened for their physical appearance in the color and appearance aspects of the ointments.

Homogeneity test: The homogeneity of the prepared ointments was noticed through visual inspection and the existence of any aggregations was analyzed.

Evaluation of antimicrobial activity of Pereskia bleo
Bacillus subtilis (gram-positive bacteria), Staphylococcus aureus (gram-positive bacteria), Pseudomonas aeruginosa (gram-negative bacteria) and Escherichia coli (gram-negative bacteria) have been screened for its antimicrobial behavior using agar well diffusion technique (Kaur et al., 2011; Cheruiyot et al., 2009).

Plant extract dilution: Reconstitution with 2.5 mL of dimethyl sulphoxide (DMSO)of 2 g of dichloromethane extract was preceded by adding 7.5 mL of sterile distilled water to make 200 mg / mL concentrated solution. The sample was then diluted to the concentration of 150, 100 and 50 mg / mL with sterile distilled water.

Isolation of a pure culture of microorganism by subculture onto an agar plate: Using Bunsen burner, a wire loop was sterilized till the loop turns red. The wire loop was withdrawn off the flame and allowed to cool. The sterilized wire loop was used to inoculate a loopful of microorganism culture onto a new agar plate and part

of the inoculum was further streaked through streak plate techniques over an agar surface. The wire loop was resterilized, cooled and used until the entire surface of the agar plate was used to further dilute the inoculum in about three more streaks. For another culture of microorganisms, the steps were repeated. The agar plates were then covered with parafilm and secured tightly. The agar plates were marked and incubated for 24 hours at 37 ° C.

Preparation of inoculum

To obtain bacterial suspension, a single colony of nutrient agar was taken using a previously sterilized wire loop and inoculated into the nutrient broth. For another culture of bacteria, this step has been repeated. The broth of nutrients was incubated at 37 ° C overnight.

Antimicrobial assay of dichloromethane extract using agar well diffusion method

At the center of each plate containing Mueller-Hinton agar, 100 µL of standardized inoculum was pipette out. The suspension was then distributed uniformly using cotton swab and allowed to stay in contact for 10 minutes. In the agar medium, a conventional well borer was used to bore a hole of 8 mm in diameter. Three wells were developed in each plate and micropipette was used to add 100 µL of sample compounds with varying concentrations as shown in Table 3.1. Then the agar plates were incubated at 37 ° C for 24 hours.

The antimicrobial behavior was assessed by developing an inhibition zone all across the well. Tetracycline was chosen as positive control while for the negative control DMSO has been used. Vernier calipers were used to examine and record the diameter of the inhibition zone (Kaur et al., 2011; Cheruiyot et al., 2009).

Table 1: Test compounds that prepared for antimicrobial testing.

No. of petri plate	Test compounds
1	DMSO - Negative control
2	Tetracycline (0.05 mg/mL) – Positive control
3	Pereskia bleo leaves extract – 50 mg/mL
4	Pereskia bleo leaves extract – 100 mg/mL
5	Pereskia bleo leaves extract – 150 mg/mL
6	Pereskia bleo leaves extract – 200 mg/mL

Antimicrobial assay of dichloromethane extract ointment by agar well diffusion method

100 µL of standardised inoculum was pipetted onto the centre of each plate containing Mueller-Hinton agar. The suspension was then spread evenly using cotton swab and allowed to remain in contact for 10 minutes. A standard well borer was used to bore a 8 mm diameter hole in the agar medium. Three wells were made in each plate and sufficient amount of sample ointment with different concentrations as shown in Table 2 was added into the well. The agar plates were then incubated at 37℃ for 24 hours. The antimicrobial activity was evaluated through the formation of inhibition zone around the well. The diameter of inhibition zone was observed and recorded using vernier calipers.

Table 2: Sample ointments that prepared for antimicrobial testing.

No. of petri plate	Sample ointments
I	Petroleum jelly – Negative control
II	Mupirocin 2% w/w ointment – Positive control
III	5% w/w of Pereskia bleo leaves extract ointment
IV	10% w/w of Pereskia bleo leaves extract ointment
V	15% w/w of Pereskia bleo leaves extract ointment
VI	20% w/w of Pereskia bleo leaves extract ointment

Statistical analysis

All outcomes were statistically analyzed using version 23 of the statistical Package for Social Science (SPSS) program. The acquired information is represented as the standard error mean ± (S.E.M). For statistical research comparison, one-way analysis of variance (ANOVA) and post-hoc testing were used. A difference of less than 0.05 p-value will be regarded as statistically significant.

RESULTS AND DISCUSSION

Soxhlet extraction of Pereskia bleo leaves

Using dichloromethane, a total quantity of 445 g of Pereskia bleo powdered leaves were obtained. The choice of solvent extraction relies primarily on the polarity and solubility of the secondary metabolites of known interest. Dichloromethane was selected for Pereskia bleo leaves extraction in the experiment. Extraction mass and dichloromethane extract percentage yield are shown in Table 3.

Table 3: Percentage yield, colour and consistency of Pereskia bleo leaves extract.

Extract	Mass of extraction (g)	Percentage yield (%)	Colour	Consistency
Dichloromethane	35.81	8.05	Dark green	Semisolid, sticky

The Pereskia bleo leaves study by S.I.A et al. (2009) reveals that the percentage yield of conventional methanol maceration was 9.47 percent. In addition, methanol extract from Pereskia bleo leaves has been recorded to have a 10.60% yield by maceration (Sim et al., 2010a, Abd Malek et al., 2008).

Evaluation of formulated ointments

Four Pereskia bleo dichloromethane extract ointment formulations were prepared using petroleum jelly as the base for ointment. Different dichloromethane extract concentrations were used for varying formulations depending on calculation. Prepared ointments include extract of Pereskia bleo leaves from 5, 10, 15 and 20 percent w / w. Then for their physical appearance and homogeneity, these formulations were assessed.

Table 4: Physical evaluation of formulated ointment.

Sr. No.	Formulations	Colour	Homogeneity
1	5% w/w of Pereskia bleo leaves extract ointment	Dark green	Good
2	10% w/w of Pereskia bleo leaves extract ointment	Dark green	Good
3	15% w/w of Pereskia bleo leaves extract ointment	Dark green	Good
4	20% w/w of Pereskia bleo leaves extract ointment	Dark green	Good

The intensity of the color improved slightly as the extract concentration increased. 20% Pereskia bleo leaves ointment extract has the darkest green colour as it has the greatest concentration while 5% w / w ointment has the lightest green colour because it has the slightest concentration.

Antimicrobial activity of dichloromethane extract

Due to its presence and antimicrobial effect, tetracycline (0.05 mg / mL) was chosen as a reference while DMSO was used as a negative control. The antimicrobial activity intensity relies on the microbes tested and the extract concentration. In the current research, to avoid anomalous outcomes, each experiment was evaluated in triplicate.

In this research, four varying levels of dichloromethane extract, 50, 100, 150 and 200 mg / mL, were used. Table 2.3 summarizes the diameter of the extract inhibition area at varying concentrations and the reference drug against particular bacteria. Experimental findings in this research indicate that Pereskia bleo leaves ' dichloromethane extract was discovered to have notable antimicrobial properties against Bacillus subtilis, Staphylococcus aureus, and Pseudomonas aeruginosa, but showed no antibacterial activity against Escherichia coli. Based on the outcomes, as the concentration of dichloromethane extract of Pereskia bleo leaves increased, the inhibition region slowly raised. On Bacillus subtilis, Staphylococcus aureus and Pseudomonas aeruginosa, the growing trends have been noted.

Table 5: Mean of zone of inhibition (mm) of different concentrations of dichloromethane extract, tetracycline and DMSO on bacteria.

Extract		Zone of inhibition (mm)		
(mg/mL)	B. subtilis	S. aureus	P. aeruginosa	E.coli
Dichloromethane				
50	8.67 ± 0.29	N/A	11.33 ± 0.58	N/A
100	12.00 ± 0.00	9.67 ± 0.58	12.00 ± 0.00	N/A
150	12.67 ± 0.58	11.67 ± 0.58	12.33 ± 0.58	N/A
200	14.33 ± 0.58	14.00 ± 0.00	13.67 ± 0.58	N/A
Positive control				
Tetracycline				
0.05	24.00 ± 0.00	22.33 ± 1.53	23.00 ± 1.00	23.67± 0.58
Negative control				
DMSO	N/A	N/A	N/A	N/A

Note: N/A – Indicate no activity

Each value represent mean ± SD, n=3

Based on the results, 200 mg/mL of dichloromethane extract has exhibited largest zone of inhibition against Bacillus subtilis (14.33 ± 0.58 mm) followed by Staphylococcus aureus (14.00 ± 0.00 mm) and

Pseudomonas aeruginosa (13.67 ± 0.58 mm). However, no zone of inhibition was observed on Escherichia coli at concentration 200 mg/mL.

At concentration 50 mg/L, dichloromethane extract of Pereskia bleo leaves exhibited smallest zone of inhibition against Bacillus subtitis (8.67 ± 0.29 mm) followed by Pseudomonas aeruginosa (11.33 ± 0.58 mm). However, 50 mg/mL of dichloromethane extract showed no zone of inhibition on Staphylococcus aureus and Escherichia coli.

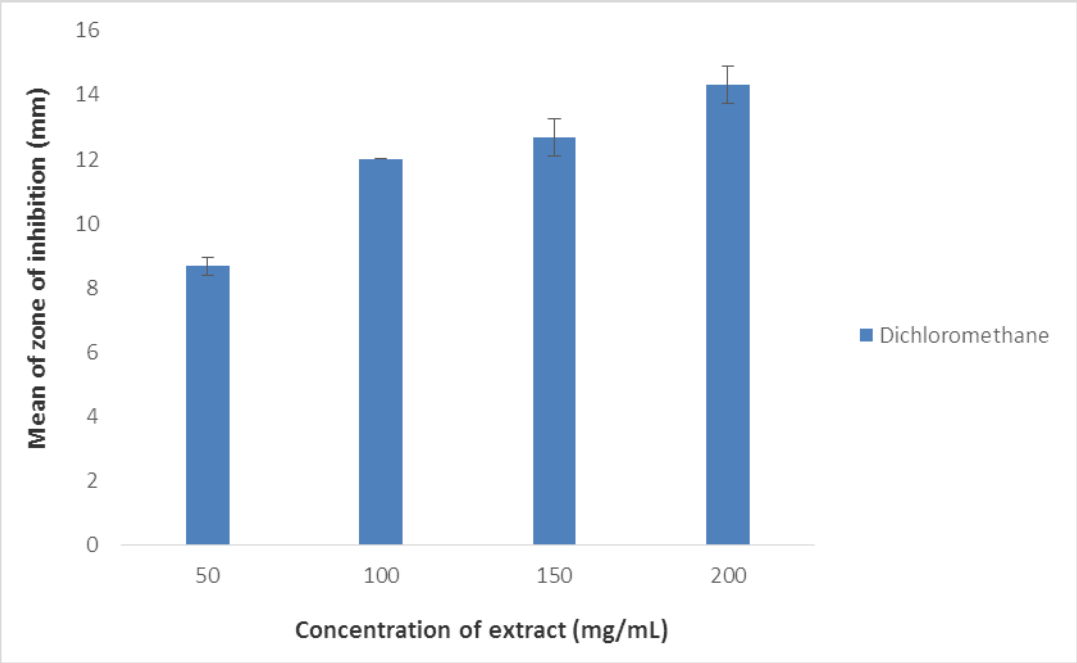

Figure 1: Mean of zone of inhibition (mm) of different concentrations of dichloromethane extracts against Bacillus subtilis.

As shown in Figure 1, the results of this study showed that dichloromethane extract of Pereskia bleo leaves exhibited greatest antimicrobial activity against Bacillus subtilis at concentration of 200 mg/mL with mean inhibition zone of 14.33 ± 0.58 mm whereas the lowest mean inhibition zone was observed at concentration of 50 mg/mL with the value of 8.67 ± 0.29 mm. Additionally, the moderate antibacterial activity against Bacillus subtilis was showed at concentration of 100 mg/mL and 150 mg/mL with the mean inhibition zone of 12 ± 0.00 mm and 12.67 ± 0.58 mm respectively. A study by Philip et al., (2009) on antimicrobial activity of Pereskia bleo also showed results that both hexane and ethyl acetate extracts of Pereskia bleo leaves had inhibitory effect on Bacillus subtilis.

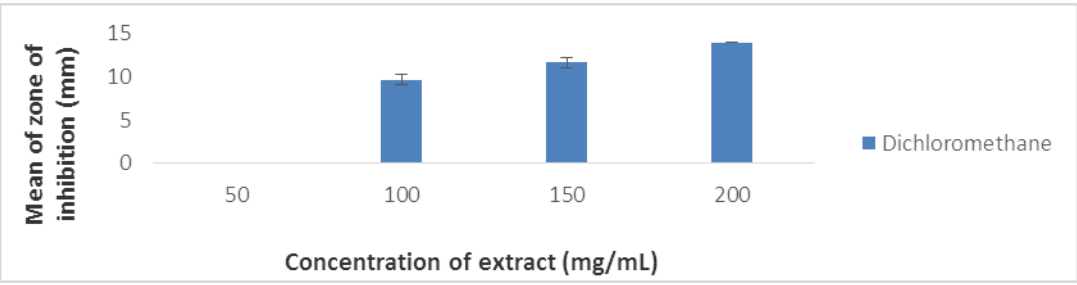

Figure 2: Mean of zone of inhibition (mm) of different concentrations of dichloromethane extract against Staphylococcus aureus.

According to Figure 2, at the concentration of 200 mg/mL, Staphylococcus aureus exhibited highest susceptibility towards the antibacterial activity of dichloromethane extract with mean inhibition zone of 14 ± 0.00 mm. When the concentration of dichloromethane extract lowered to 150 mg/mL and 100 mg/mL, a slightly decline in the antimicrobial activity against Staphylococcus aureus were showed with the mean inhibition zone of 11.67 ± 0.58 mm and 9.67 ± 0.58 mm respectively. However, antimicrobial activity of Pereskia bleo leaves dichloromethane extract at concentration 50 mg/mL showed no inhibition against Staphylococcus aureus.

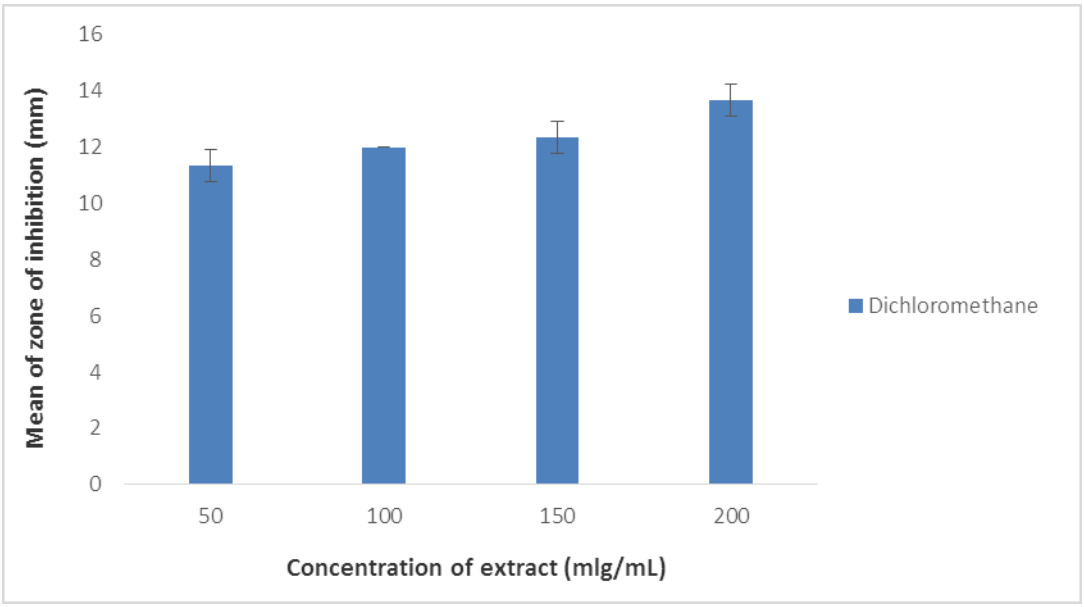

Figure 3: Mean of zone of inhibition (mm) of different concentrations of dichloromethane extract against Pseudomonas aeruginosa.

The antibacterial activities of dichloromethane extract against Pseudomonas aeruginosa showed the same increasing trend as Bacillus subtilis starting from the concentration of 50 mg/mL. As shown in Figure 3, dichloromethane extract revealed a remarked sensitivity towards Pseudomonas aeruginosa at concentration 50 mg/mL with mean inhibition zone of 11.33 ± 0.58 mm. When the concentration of dichloromethane extract increased from 100 mg/mL to 150 mg/mL, a slight increase in mean inhibition zone were observed with the values of 12 ± 0.00 mm and 12.33 ± 0.58 mm respectively. Pseudomonas aeruginosa had the largest susceptibility to the antibacterial activity of dichloromethane extract with a mean inhibition zone of 13.67 ± 0.58 mm at a concentration of 200 mg / mL. The results in this research were endorsed by Philip et al. (2009) and S.I.A et al. (2009), where Pereskia bleo with multiple extracts such as methanol, hexane, ethyl acetate and dichloromethane showed influential inhibitory activity against Pseudomonas aeruginosa.

Apart from that, antimicrobial activity of Pereskia bleo leaves dichloromethane extract showed no inhibitory effect against Escherichia coli at any concentration that is used in this study. In a separate research on the antimicrobial activity of some medicinal plants from Malaysia by Philip et al., (2009), the presence of antimicrobial activity of methanol, hexane, ethyl acetate and water extracts against Escherichia coli was not found in the leaves of Pereskia bleo.

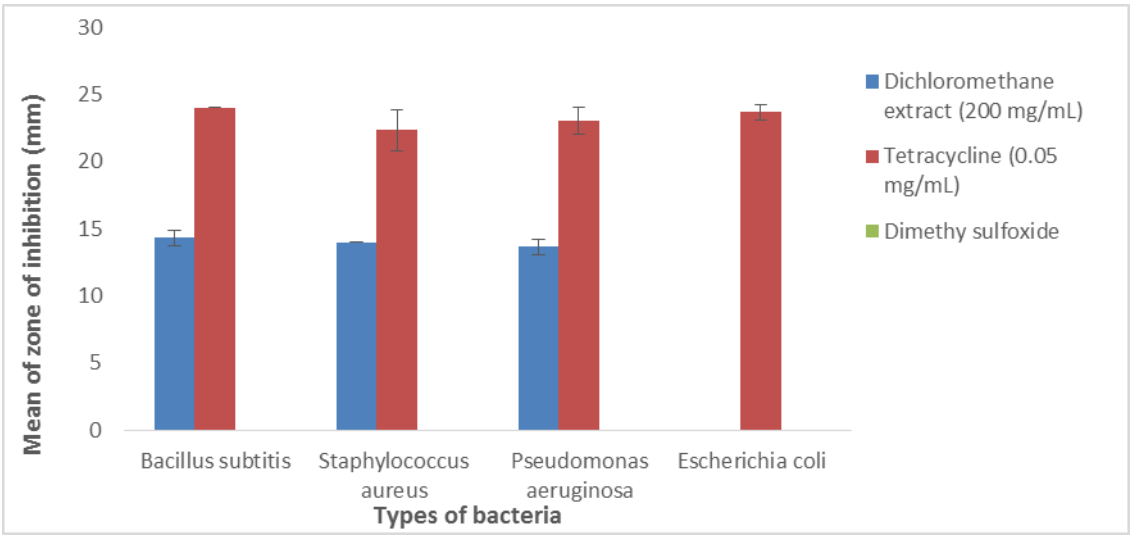

Figure 4: Mean of zone of inhibition of dichloromethane extract (200 mg/mL), tetracycline (0.05 mg/mL) and DMSO against each bacterium.

Based on Figure 4, the concentration of 200 mg/mL of dichloromethane extract against Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli was used to compare with the mean of inhibition zone of tetracycline (positive control) and DMSO (negative control). Dichloromethane extract shows remarkable mean inhibition zone of 14.33 ± 0.00 mm against Bacillus subtilis which are closely related to the mean of inhibition zone of tetracycline (22.33 ± 1.53 mm). Apart from that, both Staphylococcus aureus and Pseudomonas aeruginosa exhibited moderate susceptibility towards the antibacterial activity of dichloromethane extract when compared to tetracycline. However, dichloromethane extract revealed no inhibitory effect against Escherichia coli at concentration 200 mg/mL. Although the concentration of 200 mg/mL of dichloromethane extract has shown the strongest inhibitory effect against Bacillus subtilis, Staphylococcus aureus and Pseudomonas aeruginosa when compared to the concentration of 50, 100 and 150 mg/mL, but the result obtained has showed significant differences when compared to tetracycline. This revealed that the tetracycline has better antimicrobial activity when compared to dichloromethane extract at the concentration of 200 mg/mL.

Antimicrobial activity of dichloromethane extract ointment

Mupirocin 2% w/w ointment was selected to be a reference while petroleum jelly was served as negative control. The intensity of antimicrobial activity depends on the tested microorganisms and concentration of extracts. In the present study, each test was tested in triplicate in order to prevent anomalous results. Four different ointment formulations of Pereksia bleo extract with concentration of 5, 10, 15 and 20% w/w were prepared for this study. The diameter of inhibition zone of Pereskia bleo leaves extract at various concentrations and reference drug against different bacteria are summarised in Table 4. and 5. In this study, experimental results revealed that all Pereskia bleo leaves extract ointments showed no antibacterial activity against Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli. Thus, the results showed that the dichloromethane extract incorporated into the petroleum jelly exhibited poor inhibitory effect compared to dichloromethane extract. This might be attributed to the poor diffusion of phytoconstituents for the formulated ointments compared to the crude extract (Pawar and Nabar, 2010). Due to poor diffusion of phytoconstituents, dichloromethane extract formulated as ointment exhibited less effectively interact with ointment and hence unable to penetrate through the agar medium. However, an extensive study is required to establish the diffusion mechanism of phytoconstituents.

CONCLUSION

This current research provides evidence to inhibit microbial development for the therapeutic advantages of Pereskia bleo. Three types of bacteria: Bacillus subtilis, Staphylococcus aureus and Pseudomonas aeruginosa, except for Escherichia coli, showed sensitivity to dichloromethane extract. Pereskia bleo's antimicrobial activity against these three strains of bacteria was predominantly influenced by extract concentration. Higher dichloromethane extract concentration led in a stronger inhibitory effect compared to the smallest concentration in this research. The research results are consistent with the past research because the maximum extract concentration has peak antibacterial activity. (Philip et al., 2009). Therefore, the extract of Pereskia bleo leaves can be used as a source of antimicrobial product. However, the dichloromethane extract ointment did not reveal any inhibitory impact against the chosen bacteria when the dichloromethane extract was added into the petroleum jelly. It may therefore imply that there may be low diffusion of phytoconstituents relative to the extract for the formulated ointments.

Because of its capacity to isolate non-polar and polar phytoconstituents, dichloromethane is well known to be an efficient extraction agent and thus helps to obtain all the different chemical groups. Pereskia bleo leaves extracts ' qualitative analysis outcomes revealed the presence of fats and oils, phytosterols / sterols, flavonoids, terpenoids, and phenolic derivatives. However, in the Pereskia bleo leaves, the presence of alkaloids and lactones was not discovered. The inhibitory impact of this specific plant could be ascribed to the presence of flavonoids and terpenoids based on the phytochemical consequence acquired. Consequently, it is thought that Pereskia bleo leaves ' dichloromethane extract may be useful in treating bacterial infection-related diseases and has great potential to develop as a phytomedicine. However, in terms of in vitro and in vivo approaches, further inquiry of Pereskia bleo is needed to guarantee the efficacy and safety of the plant. Overall, natural plant phytoconstituents play a significant part in the pharmaceutical industry's drug research and should be regarded as powerful therapeutic agents against multiple diseases.

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