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Original Research Article

Formulation and Evaluation of Antimicrobial Properties of *Psidium guajava* Ethanol Leaf Extract Creams

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ABSTRACT

Psidium guajava is a medicinal plant that belongs to the family Mytaceace. This study was carried out to evaluate the antimicrobial property of creams formulated using the ethanol extract of *Psidium guajava* leaf.

The powdered dried *Psidium guajava* leaf was extracted using cold maceration. The extract was concentrated and evaluated based on antimicrobial activity using test organisms; *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Apergillus niger* and *Candida albicans*. Herbal creams were formulated using the extract and they were evaluated based on antimicrobial and physicochemical properties.

Percentage yield for the extract was 53.4%w/w. The pH of the creams ranged from 5.1 to 6.4 while the viscosity ranged from 40000 to 77500 mPas. The spreadability was from 2.5±0.14 cm to 4.75±0.35 cm. The creams had good homogeneity and they were stable after three months accelerated stability study. The extract inhibited *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus* at 3.125 mg/ml with inhibition zone diameter (IZD) of 6 to 19 mm and *Candida albicans* at 25 mg/ml with IZD of 6 to 9 mm. The extract had no activity against *Aspergillus niger* at the tested concentrations (3.125 to 100 mg/ml). The IZD for the creams against the test bacteria ranged from 3.0±0.00 to 16±1.41 mm while IZD against *Candida albicans* was between 4.5±0.71 and 7.0±1.41 mm. The creams had no antifungal activity against *Aspergillus niger*.

This study showed that the extract and the formulated creams exhibited good antimicrobial activity against the test organisms with the exception of *Aspergillus niger*.

Keywords: *Psidium guajava*, Creams, Ethanol extract, Antimicrobials.

Introduction

Man has utilized medicinal plants as remedies for several human diseases for centuries.[1] Herbal medicines are used as antibacterial,[2] anticonvulsant,[3] antifungal,[4] antioxidant,[5-7] antidiabetic,[8] antiulcer,[9] antiinflammatory[10] and antimicrobials.[1]

In traditional medicine, one plant can be used to treat several diseases. *Psidium guajava* has been used in the treatment of various ailments[11] including treatment of diabetes,[12, 13] treatment of plague,[14] treatment of acne,[15] oral care.[16] Its anti-cancer activity,[17] anti-microbial activity,[18] anti-amoebic and anti malarial effects, as well as antioxidant activities have been documented.[11]

Psidium guajava L is a medicinal plant that belongs to the family Mytaceace. It is also known as guava.[19, 20] It is an ever-green shrub-like tree which reaches to the height of 6-25 feet. It is widely distributed in tropical and warm temperature regions of the world.[19] The plant parts include leaves, flowers, fruit, seeds, bark.[21] The antimicrobial uses of *Psidium guajava* have been studied by many researchers.[19-24] Creams are semi-solid dosage forms. They are viscous semi-solid emulsions for external use, usually the skin and also mucous membrane such as the rectum or vagina. There consistency varies between liquid and solid. They are oil in water (o/w) or water in oil (w/o) creams.[25]

The aim of this research is to formulate herbal creams using *Psidium guajava* ethanol extract and to evaluate their antimicrobial and physicochemical properties.

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Materials and Methods

Materials

Emulsifying wax (Lodha Chemicals India), white soft paraffin (HP Chemical Industries Uttar Pradesh India), Liquid paraffin (Niram Chemicals Mumbai India), Chlorocresol (Lodha Chemicals India), Ethanol (JHD Gunsgdong Guandgus chemical factory Co.ltd, Shautou, Guandghuo, China), Nutrient agar (Titan Biotech, India), Mueller Hinton agar (Titan Biotech India), Sabouraud dextrose agar (Life Save biotech USA), Gentamycin and Ketoconazole.

Organisms used:

Pseudomonas aeruginosa, *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Aspergillus niger and Candida albicans* obtained from the stock preparation of The Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Delta State University, Abraka.

Collection and identification of plant material

Fresh *Psidium guajava* leaves were collected in January 2021 from Asaba, Nigeria, located on latitude 6.1982° N, longitude 6.7319° E. It was identified by Dr Emmanuel Ikpefan of the Department of Pharmacognosy, Faculty of Pharmacy, Delta State University, Abraka

and authenticated by Dr Akinnibosun Henry of Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Nigeria. It was issued voucher number UBH-P378. The fresh leaves were air dried under shade at room temperature. The dried leaves were blended to powder using a grinding machine (Vitamix Blender, Ohio, USA.).

Extraction of the plant material
The method of Okokon *et al*[26] was used with slight modification. A 500 g of the plant material was added to 2000 mL of 70%v/v ethanol and macerated for 72 hours with agitation. The decoction was filtered using muslin cloth and the extracts obtained were concentrated at 70±0.5^{o}C in a water bath (Clifton water bath Serial no. 80105, Nickel electro LTD, Weston-5-Mare Somerset. England). The concentrated extracts were stored in porcelain dish wrapped in an aluminum foil.

Preparation of fungal inoculums
A 0.75 g/5 mL of freshly prepared Sabouraud dextrose agar was sterilized at 121 ± 0.5^{o}C for 15 minutes at 1 atmospheric pressure using bucket autoclave (Apothecaries Sundries Manufacturing Company, New Delhi India). After sterilization, it was left to cool. It was later transferred into sterile bijou bottles and left to stand in a slanted position to solidify. After solidification, *Candida albicans* and *Aspergillus niger* were inoculated into it and incubated for 24 hours at 37^{o}C.[27]

Preparation of bacterial inoculums
A 0.65 g/25 mL of freshly prepared nutrient agar was sterilized at 121^{o}C for 15 minutes at one atmospheric pressure. After sterilization, it was left to cool. It was transferred into sterile bijou bottles and left to stand in a slanted position to solidify. After solidification, strain of *Staphlococcus aureus, Escherichia coli, Bacillus subtilis and Pseudomonas aeroginosa* were inoculated and incubated for 24 hours at 37^{o}C respectively.[27]

Preparation of overnight broth culture
A 0.75 g/50 mL of freshly prepared Sabouraud dextrose broth and 0.65 g/100 mL of freshly prepared nutrient broth were sterilized for 15 minutes at 121^{o}C and 1 atmospheric pressure. After sterilization, the Sabouraud dextrose broth and nutrient broth, were left to cool and transferred into sterile bijou bottles. Strains of *Candida albicans* and *Aspergillus niger* were inoculated into Sabouraud dextrose broth and strains of *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa* were inoculated into nutrient broth and each was incubated for 24 hours at 37^{o}C.[27]

Antimicrobial Susceptibility testing
The method of Emencheta *et al*[1] was used with slight modification. The prepared agars (Mueller Hinton and Sabouraud Dextrose agars) were aseptically poured into six Petri dishes to solidify. Upon solidification, the agars were inoculated with the different organisms. Thereafter, eight holes were bored into each of the agar plates with a 6 mm cork borer and the holes were labeled respectively. Two holes in the middle were for the negative control (ethanol) and the positive control (gentamicin (80 mg/2 mL) for bacteria and ketoconazole (200 mg/10mL) for the fungi) and the other six holes were for the two fold serial dilutions (100, 50, 25, 12.50, 6.25 and 3.125 mg/mL) prepared from ethanol extract. The bacteria inoculated samples were incubated for 24 hours and the zones of inhibition were measured. The fungal inoculated samples were wrapped with aluminum foil and kept at room temperature for 48 hours. After 48 hours, zones of inhibition were then measured to the nearest millimeter along two axes 90^{o} to each other and the mean of the two readings calculated.

Determination of minimum inhibitory concentration (MIC)
Freshly prepared Sabouraud dextrose agar (for fungi) and Muller Hinton agar (bacteria) were sterilized and left to cool. Two fold dilutions of the ethanol extracts were made and the concentrations were 100, 50, 25, 12.50, 6.25 and 3.125 mg/mL. Thereafter, 1 mL each of the various dilutions of ethanol extracts was mixed with 19 mL of the sterilized agar then poured into the different Petri dishes according to their labeled concentrations and rocked to mix well. After that the microorganisms were inoculated into the agar plate by streaking the agar. The culture plate were inverted and incubated at 37^{o}C for 24 hours and the minimum inhibitory concentrations were determined.[28]

Preparation of emulsifying ointment B.P as the cream base
Emulsifying ointment B.P was prepared using the formula in Table 1.[29] A 30 g quantity of emulsifying wax and 50 g of white soft paraffin were weighed and transferred into a porcelain dish. A 23.53 mL (20 g) of liquid paraffin was measured and transferred into the same porcelain dish. The ingredients in the porcelain dish were melted at 70 ^{0}C, stirred to give a homogenous mixture and then allowed to cool.

Preparation of Psidium guajava creams
This was prepared using the formula on Table 2.[29] A 0.0625 g of *Psidium guajava* ethanol extract was weighed and transferred into a beaker. A 34.94 mL of purified water, freshly boiled and cooled was transferred into the beaker and mixed properly with the extract. A 15 g of emulsifying ointment BP was weighed in a beaker and melted in a water-bath at 70^{o}C. The dissolved *Psidium guajava* ethanol extract solution was heated to 70^{o}C and added to the melted emulsifying ointment at the same temperature (70^{0}C). The contents were stirred gently and allowed to cool. It was then transferred into a cream jar and labeled appropriately.

Evaluation parameters for Psidium guajava creams
pH determination
A pH meter (HI 2211 Ph/ORP meter – Hanna instruments) was inserted into the cream in the beaker and the reading was recorded.[27]

Homogeneity
The formulated herbal creams were evaluated for homogeneity by visual inspection and were ranked as follows: +++ = Excellent, ++ = Very good, + =Good, - = Poor.[30, 31]

Ease of removal
The cream was applied on the skin and later washed off by water flowing directly from a tap. The creams were ranked as follows: +++ = Excellent, ++ = Very good, + = Good, - = Poor.[31]

Organoleptic test
The appearance, texture and odour of the creams were determined by physical inspection.[31]

Table 1: Composition of emulsifying ointment B.P[29]

Ingredients	Quantity given	Quantity required
Emulsifying wax (g)	300	30
White soft paraffin (g)	500	50
Liquid paraffin (g)	200	20
Total (g)	1000	100

Table 2: Composition of *Psidium guajava* creams[29]

Ingredient	PG1	PG2	PG3	PG4	PG5
Psidium guajava extract (g)	0.0625	0.0625	1.250	1.250	-
Emulsifying cream base (g)	15	15	15	15	15
Chlorocresol (g)	-	0.05	0.05	-	0.05
Purified water (g)	34.9375	34.8875	33.7	33.75	34.95
Total (g)	50	50	50	50	50

Viscosity
This was evaluated using a Brookfield viscometer (Brookfield RVDV-E Viscometer, Brookfield, MA 02346, USA) that was set at a speed of 6 revolutions per minute (rpm) and with spindle number 4.[10]

Antimicrobial susceptibility
Antimicrobial activities of the formulated creams were determined by their zones of inhibition using the agar well diffusion method. Mueller Hinton agar or Sabouraud dextrose agar was prepared and poured into respective Petri dishes and allowed to solidify. The overnight broth of the test organisms were uniformly spread over the surface of the sterile agar plate with a sterile swab stick. Wells (6 mm) were bored in the solidified agar using a sterile cork borer and the different formulations of the herbal creams were poured into the respective wells. Either gentamicin or ketoconazole served as control. The plates were incubated at 37^{O}C for 24 and 48 hours respectively. The diameter of the zone of inhibition was measured. Clear zones of inhibition show susceptibility of organisms, while absence of such zone indicated resistance.[31]

Stability
Accelerated stability study was done using the method of Gyawali *et al* with slight modification.[32] A 15 g sample of each of the formulations were transferred into a beaker and stored at a temperature of 40^{o}C for 3 months. The physical appearances were evaluated every 30 days for 3 months.

Statistical analysis:
The experiments were done in triplicates for validity of data analysis and they were expressed as mean ± SD. Differences between means were determined with one way analysis of variance (ANOVA) at a level of significance of $P < 0.05$.

Results and Discussion

The percentage yield of the *Psidium guajava* ethanol extract was 53.4%w/w.

Antimicrobial activities of ethanol extracts of Psidium guajava
As shown in Table 3, all the test bacteria were susceptible to the effects of the extract even at the lowest concentration (3.125 mg/mL) used. *S. aureus* has inhibition zone diameter (IZD) of 6-18 mm, *B. subtilis* has IZD of 7-10 mm, *E. coli* has IZD of 6-19 mm and *P. aeruginosa* has IZD of 7-14 mm. This result agrees with the report of Ahmed and Yagoub,[19] where all the test bacteria, *S.aureus* (14-21 mm), *B. subtilis* (14-25 mm), *E. coli* (10-25 mm) and *S. typhi* (15-28 mm) were susceptible to the effect of both the petroleum ether and methanol extracts of *P. guajava*. The work of Biswas *et al*[22] supports the finding that *S. aureus* is susceptible to the effects of ethanol leaves extract of *P. guagava* but disagree with the sensitivity of *E. coli*. Goncalves *et al,*[21] reported of susceptibility of both S. aureus (IZD, 7 – 9.25 mm) and E. coli (IZD, 7 – 9.0 mm) to effect of hexane, ethyl acetate and methanol extracts of P. guajava leaves.
The MIC for *C. albicans* was 25 mg/ml and the IZD was 6-9 mm, while *A. niger* was resistant even at the highest concentration (100 mg/mL) used. This agrees with the work of Ahmed and Yagoub[19] which recorded resistance for *A. niger* at all concentration of *Psidium guajava* methanol and petroleum ether extracts. They recorded that *C. albicans* was sensitive to petroleum ether extract but not susceptible to methanol extract. The higher concentrations of the extract (25 – 100 mg/mL) gave higher IZD (10 -18 mm) against test bacteria with the exception of *B. subtilis* when compared to the lower concentrations (3.125 – 12.5 mg/mL) that gave IZD of 6 – 12 mm against same organisms. However, the IZD produced by the extract concentrations were lower than that produced by the control, 26 -34 mm for gentamicin and 19 – 23 mm for ketoconazole.
The physical properties of aqueous herbal cream formulated from ethanol *Psidium guajava* extract are represented in Table 4. The colours of the formulated creams ranged from light brown to dark brown depending on the concentration of the extract used in the formulation. Formulation PG5 that did not contain any extract was white in colour. The formulations PG2, PG3 and PG5 have slight phenolic odour which may be due to presence of chlorocresol in their formulation.
The pH of the different formulations ranged from 5.1 to 6.4 which is within the normal pH range of the skin (4-6). This shows that the cream will not cause irritation when applied to the skin. This pH range will be effective in preventing the growth of bacteria on the cream.
All the formulated creams were ranked excellent in terms of homogeneity. This showed that the creams were properly mixed and there was presence of lumps. The spreadability ranged from 2.5±0.14 to 4.75±0.35 cm.
The viscosity of the different formulations ranges from 40000 to 77500 mPas.
All the cream formulations showed activity against all the test bacteria (Table 5) *P. aeruginosa* (3.0±0.00 - 13±1.41 mm), *B. subtilis* (5.5±0.71 – 16.0±1.41 mm), *E. coli* (3.0±1.41 - 13±1.41 mm) and *S. aureus* (2.5±0.71 – 11.0±0.00 mm). Formulation PG5 that did not contain extract showed some activity, probably due to the presence of chlorocresol, a preservative in its formulation. The formulated creams retained the antimicrobial property of the extract. Formulations PG1 and PG2 that were formulated with 0.0625 g/mL of the extract had IZD of 2.5±0.71 – 7±1.41 mm for *S. aureus*, 11±1.41 – 13±1.41 mm for *B. subtilis*, 8.0±1.41 – 9.0±1.41 mm for *E. coli* and 10.0±1.41 – 10.5±0.71 mm for *P. aeruginosa*. These values were close to those obtained for the 0.0625 mg/ml concentration of the extract against these bacteria (6, 7, 6 and 7 mm respectively) Formulations PG3 and PG4 that were prepared with 1.25 g/mL of the extract produced IZD of 9±1.41 – 11±0.00 mm for *S. aureus*, 15 – 16±1.41 mm for *B. subtilis*, 11.5±2.12 – 13±1.41 mm for *E. coli* and 11±4.24 – 13±1.41 mm for *P. aureginosa.* The IZD values were close that produced by 1.25 g/mL concentration of the extract (14, 10, 12 and 11 mm respectively).
The different cream formulations were stable after 3 months. There was no separation of the constituents.

Table 3: Microbial sensitivity pattern of ethanol extracts of *Psidium guajava*

S/N	Organism	Zone of inhibition (mm) of different concentrations of *Psidium guajava* extract						
		100 mg/mL	50 mg/mL	25 mg/mL	12.5 mg/mL	6.25 mg/mL	3.125 mg/Ml	Control
1	*S. aureus*	18	15	14	12	9	6	32
2	*B. subtilis*	9	8	10	10	9	7	28
3	*E. coli*	19	16	12	9	9	6	34
4	*P. aeruginosa*	14	12	11	8	7	7	26
5	*C. albicans*	9	7	6	-	-	-	23
6	*A. niger*	-	-	-	-	-	-	19

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Table 4: Physical properties of herbal cream formulated from ethanol extract of *Psidium guajava*.

Physicochemical property	PG1	PG2	PG3	PG4	PG5
Colour	Light brown	Brown	Brown	Dark Brown	White
Odour	Blank	Sightly Phenolic	Sightly phenolic	Blank	Slightly Phenolic
Homogeneity	++++	++++	++++	++++	++++
Spreadability[a] (cm)	2.8 ± 0.14	2.8 ± 0.00	2.5 ± 0.14	2.9 ± 0.14	4.75 ± 0.35
Ph	6.4	6.0	5.5	5.1	6.2
Viscosity (mPas)	43,500	77,500	48,000	62,500	40,000

[a]Data was expressed as mean ± standard deviation (n = 3); Key: ++++ = Excellent; +++ = Good; ++ = Fair; + = Poor

Table 5: Inhibition zone diameters of the formulated creams against test organisms

S/N	Organism	PG1 (mm)	PG2 (mm)	PG3 (mm)	PG4 (mm)	PG5 (mm)	Control (mm)
1	S.aureus	2.5 ± 0.71	7 ± 1.41	9 ± 1.41	11 ± 0.00	4.0 ± 0.00	39 ± 0.00
2	*B. subtilis*	13 ± 1.41	11 ± 1.41	15 ± 0.00	16 ± 1.41	5.5 ± 0.71	35 ± 1.41
3	*E. coli*	8.0 ± 1.41	9.0 ± 1.41	11.5 ± 2.12	13 ± 1.41	3.0 ± 1.41	29 ± 0.00
4	*P. aureginosa*	10.5 ± 0.71	10 ± 1.41	11 ± 4.24	13 ± 1.41	3.0 ± 0.00	40.5 ± 2.12
5	*C. albicans*		7.0 ± 0.00	7.0 ± 1.41	-	4.5 ± 0.71	-
6	*A. niger*	-	-	-	-		-

Data was expressed as mean ± standard deviation (n = 3)

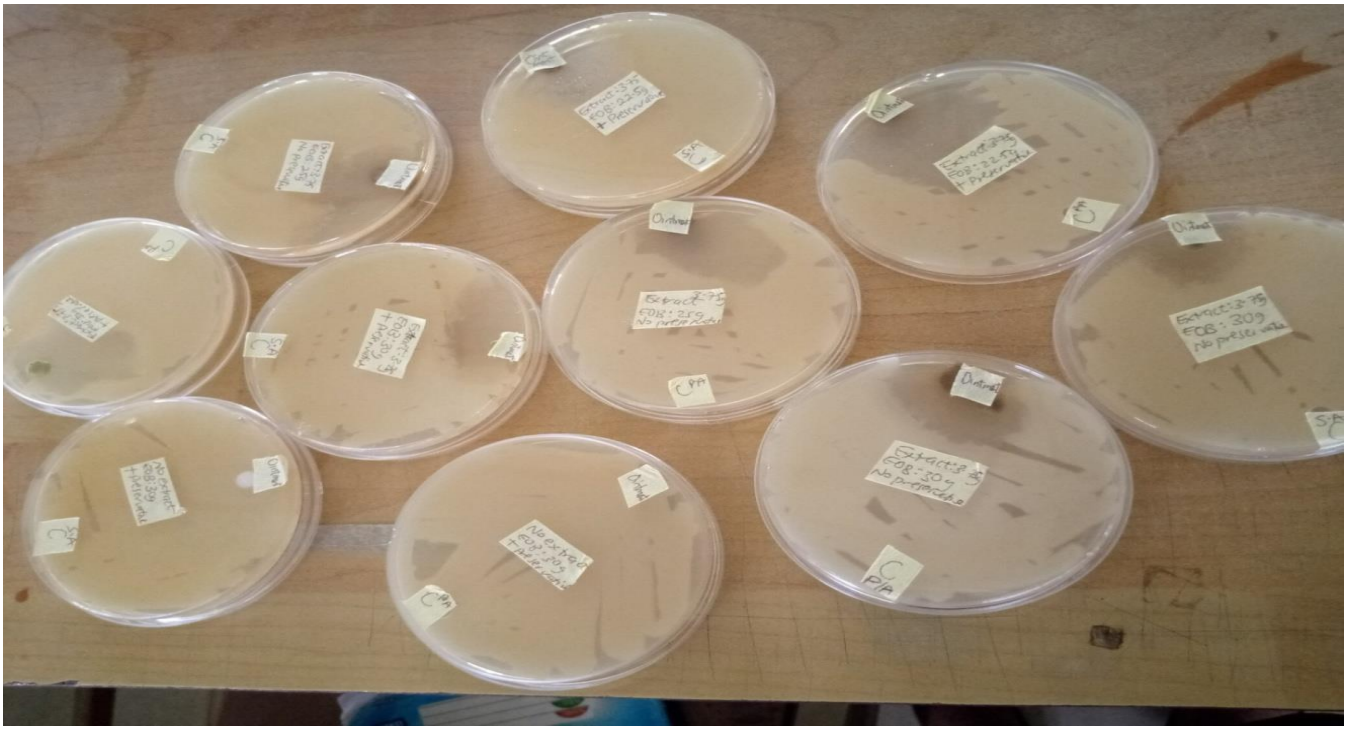

Figure 1: Antimicrobial sensitivity of test organisms to *Psidum guajava* cream

Conclusion

The study showed that *Psidium guajava* ethanol leaf extract exhibited good anti-bacterial activity against the tested bacteria. It has activity against C. albicans at high concentrations but no activity against A. niger. The creams prepared with the *Psidium guajava* ethanol leaf extract retained the antimicrobial activity of the extract against the tested bacteria and C. albicans but had no activity against A. niger.
The formulated creams were stable and have good physicochemical properties.

Conflict of Interest

The authors declare no conflict of interest**.**

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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