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Research Article

Formulation and Evaluation of Herbal Gel Containing Psidium Guajava Linn Leaves Extract

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ABSTRACT

Objective- In the present study an attempt was made to formulate gel containing *Psidium guajava linn* extracts and was evaluated for antimicrobial activity against different microorganism.

Method- The plant were collected and extracted with suitable solvent. Different gels were formulated by using different concentration of extracts. The evaluation was done using Agar well diffusion method. The gel formulation was designed by using Carbopol 940, *Psidium guajava linn* extract, Propylene glycol 400, Methyl paraben, Propyl paraben and required amount of distilled water. Then skin pH (6.8-7) was maintained by drop wise addition of tri-ethanolamine.

Result- The physiochemical parameters of formulations (pH, viscosity, spreadability etc.) were determined. The herbal gel showed that formulation containing *Psidium guajava linn* extract have better Antimicrobial activity.

Conclusion- Our literature survey revealed that the herbal gel of leaves extract from leaves of *Psidium guajava linn* was not investigated in the present study.

Key-words: *Psidium guajava linn,* Carbopol 940, Gel..

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INTRODUCTION

Present time medicinal plants being the effective source of medicines, either it can be modern or traditional medicines, the advantage of medicines are they are useful for health. Guava contains broad spectrum of phytochemicals including polysaccharides, vitamins, essential oils, minerals, enzymes, proteins, sesquiterpenoid alcohols, triterpenoid acids, alkaloids, glycosides, steroids, flavanoids, tannins, saponins. *Psidium guajava linn* or guava is very rich in antioxidants and vitamins and also high in lutein, zeaxanthine and lycopene. Guava is rich in phenols, triterpenes, carotenoids, lectins, fiber, fatty acids, quercetin, anthraquinones, phlobatannins and cardiac glycosides [1]. Much of guava therapeutic activity was attributed to these flavonoids. The flavonoids had demonstrated antibacterial activity. Guava also had antioxidant properties which were attributed to the polyphenols found in the leaves. The present study is carried out by formulation and evaluation of antibacterial and antifungal properties of *Psidium guajava linn* against bacterial and fungal pathogens. The used microorganisms were bacterial and fungal culture *Escherichia coli* and *Candida albicans* respectively and also to check the antibacterial and antifungal activity present in formulated gel whichis responsible for antifungal activity [2-11].

MATERIALS AND METHODS

Plant Materials

The authenticated plant of *Psidium guajava linn* (Lucknow-49) was collected from the Agricultural college of Pune, Maharashtra.

Chemicals

Sodium carboxy methyl cellulose (Merck Ltd),
Carbopol 940 (Merck Ltd),
Methyl Paraben (Suprim chemicals),
Propyl Paraben (Suprim chemicals),
Propylene glycol-400 (SD Fine chemical Ltd),
Triethanolamine (SD Fine chemical Ltd),
Distilled Water

Test organisms

Pure cultures of the bacterial and fungal isolates (*Escherichia coli* and *Candida albicans*) were collected from the Microbiology Laboratory of the Smt. Kashibai Navale college Of Pharmacy, Kondhwa Bk.

Preparation of Leave Extract

50 g of *Psidium guajava linn* leaves are washed with distilled water and keep in dark room for 4 days and grind into powder form. 2 g of powder dissolved in ethanol, methanol, pet ether of 50 ml of solvent respectively in incubator at 40^{o}c for 5 days. The extract was subsequently filtered and concentrated to dryness.

Antibacterial assay

The screening was done by disc diffusion method. The extracts were tested against *Escherichia coli.* A loopful of the pure bacterial culture was suspended in nutrient broth and incubated for 24 hours. Nutrient agar media was sterilized and poured into plates. After solidification, 0.1ml of the inoculum was spread over the agar evenly using L rod. 6mm diameter of Whatman filter paper discs were soaked in plant extracts and dried out. *Chloramphenicol* and *tetracycline* antibiotic discs were used as the control. The discs were carefully placed on the inoculated plates and incubated for 24 hours. Later, the zone of inhibition around the disc was measured and recorded.

Antifungal assay

The assay was performed against *candida albicans*, sabouraud agar was used as the growth media. In each plate 15ml of the sterile media was added allow it to solidify then 0.1ml of the inoculum was spread over media then cavity was made at different position and 0.5 ml plant extract was added and the plate was kept in incubator for 24hrs.

Antibacterial activity

The leaf extracts showed antibacterial activity. They inhibit the growth of *Escherichia coli.* The antibacterial property of the extracts was estimated by measuring the growth of bacteria after 3 days of incubation. In the control plate, the bacteria grew over the entire plate (7cm) in 3 days. Methanol extract showed excellent antibacterial activity against *Chloramphenicol* and *tetracycline,* i.e. it exhibited only 10mm and 18mm of growth in 3 days. Ethanol and petroleum ether extract also showed commendable results. Methanol extract showed good activity against *Chloramphenicol* and *tetracycline,* followed by ethanol and petroleum ether extracts. This study shows that methanol is the best solvent for extraction followed by ethanol and petroleum ether. The data summarized in table 1 showed that guava leaf is an exceptional antibacterial agent.

Antifungal activity

All the three extracts showed excellent antifungal activity against *candida albicans.* The antifungal property of the extracts was estimated by measuring the growth of fungi after 3 days of incubation. In the control plate, the fungi grew over the entire plate (9cm) in 3 days. Methanol extract showed excellent antifungal activity against *nestatin,* i.e. it exhibited only 14mm of growth in 3 days. Ethanol and petroleum ether extract also showed commendable results. Methanol extract showed good activity against *nestatin,* followed by ethanol and petroleum ether extracts. This study shows that methanol is the best solvent for extraction followed by ethanol and petroleum ether. The data summarized in table 2 shows that guava leaf is an exceptional antifungal agent.

Method for Preparation of Gel Containing Extract [20]

1 g of Carbopol 940 was dispersed in 50 ml of distilled water kept the beaker aside to swell the carbopol 940 for half an hour and then stirring should be done to mix the carbopol 940 to form gel. Take 5 ml of distilled water and required quantity of methyl paraben and propyl paraben were dissolved by heating on water bath. Solution was cooled and Propylene glycol-400 was added. Further required quantity of *Psidium guajava linn* leaves extract was mixed to the above mixture and volume made up to 100 ml by adding remaining distilled water. Finally full mixed ingredients were mixed properly to the Carbopol 940 gel with continuous stirring and triethanolamine was added drop wise to the formulation for adjustment of required skin pH (6.8-7) and to obtain the gel at required consistency. The same method was followed for preparation of control sample without adding any *Psidium guajava linn* leaves extract.

Formulation

As per method described above the formulae were tabulated in Table 1. Along with control sample gel were prepared with addition of 1g, 2g and 4g of *Psidium guajava linn* leaves extract to prepared 1%, 2% and 4% *Psidium guajava linn.*gel respectively.

EVALUATION OF TOPICAL GEL FORMULATION

A. Physical Evaluation

Physical parameters such as colour and appearance were checked.

B. Measurement of pH

pH of the gel was measured by using pH meter

C. Spreadability

Spreadability is a term expressed to denote the extent of area to which the gel readily spreads on application to skin or affected part. The spreadability is expressed in terms of time in seconds taken by two slides to slip off from the gel and placed in between the slides, better the spreadability.
Spreadability is calculated by using the formula:

S = ml/t
Where,
m = weight to upper slide
l = length moved on glass slide
t = time taken to separate the slides completely from each other.
In the present experiment, m = 1 gm, l = 5cm, S is recorded in table no. 3

D. Antibacterial activity

The gel showed antibacterial activity. They inhibit the growth of *Escherichia coli.* The antibacterial property of the gel was estimated by measuring the growth of bacteria after 3 days of incubation. In the control plate, the bacteria grew over the entire plate (7cm) in 3 days. Methanol extract gel showed excellent antibacterial activity against *Chloramphenicol* and *tetracycline,* i.e. it exhibited only 10mm and 18mm of growth in 3 days. Methanol extract gel showed good activity against *Chloramphenicol* and *tetracycline,* followed by ethanol and petroleum ether extracts gel. This study shows that methanol is the best solvent for extraction followed by ethanol and petroleum ether. The data summarized in table 5 shows that guava leaf is an exceptional antibacterial agent.

E. Antifungal activity

Methanol extract gel showed excellent antifungal activity against *candida albicans.* The antifungal property of the gel was estimated by measuring the growth of fungi after 3 days of incubation. In the control plate, the fungi grew over the entire plate (9cm) in 3 days. Methanol extract showed excellent antifungal activity against *nestatin,* i.e. it exhibited only 13mm of growth in 3 days. Ethanol and petroleum ether extract also showed commendable results. Methanol extract showed good activity against *nestatin,* followed by ethanol and petroleum ether extracts. This study shows that methanol is the best solvent for extraction followed by ethanol and petroleum ether. The data summarized in table 2 shows that guava leaf is an exceptional antifungal agent.

RESULTS AND DISCUSSIONS

The herbal gel was prepared and subjected to evaluation of the various parameters. The herbal Gel was dark greenish in colour and translucent in appearance and had a cool and smooth feeling on application. pH also maintained constant throughout the study which was found to be 6.90 to 7.0. Spreadability were also measured and found to be good. The herbal gel was showing Antibacterial and Antifungal activity against *Escherichia coli* and *Candida albicans* respectively.

Table 1: Antibacterial activity of leaf extract

Microbial strain	**Mean diameter bacterial growth after 3 days of incubation (mm)**			
Escherichia coli	T – 10 mm	Methanol	Ethanol	Pet ether
	C – 18 mm	16 mm	15 mm	10 mm

T- *Tetracycline*; C - *Chloramphenicol*

Table 2: Antifungal activity of leaf extract

Microbial strain	**Mean diameter fungal growth after 3 days of incubation (mm)**			
Candida albicans	N - 14 mm	Methanol	Ethanol	Pet ether
		16 mm	15 mm	10 mm

N – *Nestatin*

Table 3: Different formulations prepared with this ingredients along with quantity

Formulation	Ingredients	Quantity
Control	Carbopol 940	1 gm
	Methyl Paraben (0.5%)	0.2 ml
	Propyl Paraben (0.2%)	0.1 ml
	Propylene glycol 400 (5%)	5 ml
	Triethanolamine (q.s.)	1.2 ml
	Distilled water	Up to 100 ml

Table 4: Antibacterial activity of the gel formulation

Microbial strain	Blank	STD	1%	2%	4%
Escherichia coli	10 mm	14 mm	22 mm	24 mm	11 mm

Table 5: Antifungal activity of the gel formulation

Microbial strain	Blank	STD	1%	2%	4%
Candida albicans	10 mm	14 mm	12 mm	13 mm	16 mm

CONCLUSION

Herbal formulations have growing demand in the world market. It is an attempt made to establish the herbal gel containing *Psidium guajava linn* leaves extracts at various concentrations (1%, 2%, & 4%).
The study revealed that the developed single herbal formulation consisting 4% *Psidium guajava linn* extract for Antibacterial activity and 2% *Psidium guajava linn* extract for Antifungal activity are comparatively better than later other formulation.

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