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EVALUATION OF ANTIOXIDANT AND ANTIMICROBIAL PROPERTY OF POLYHERBAL HYDROGEL

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ABSTRACT

The main objective of this study to evaluate the antimicrobial and antioxidant properties of a polyherbal hydrogel formulated using leaf extracts of *Passiflora edulis Sims* and *Psidium guajava* L. The plant extracts were incorporated into a hydrogel base and assessed for physicochemical characteristics, stability, and biological activity. Antimicrobial activity was evaluated against selected bacterial strains using standard in vitro methods, while antioxidant potential was determined through established free radical scavenging assays. The formulated polyherbal hydrogel exhibited significant antimicrobial activity against tested microorganisms and demonstrated notable antioxidant capacity, attributable to the presence of flavonoids, phenolic compounds, and other phytoconstituents in the extracts. The results suggest that the polyherbal hydrogel possesses promising antimicrobial and antioxidant properties and may serve as a potential topical therapeutic formulation for managing microbial infections and oxidative stress–related skin conditions.

KEYWORDS: *Psidium guajava L.*, *Passiflora edulis sims*, polyherbal hydrogel, Antimicrobial and antioxidant activicty.

INTRODUCTION

Medicinal plants have been used in healthcare since time immemorial. Studies have been carried out globally to verify their efficacy and some of the findings have led to the production of plant - based medicines. The use of plant based products for treating diseases and rejuvenating the body can be traced back over five thousand years, with evidence of their application in India.[1] Topical herbal gels are a semisolid preparation containing herbal extracts for skin application. These have emerged as effective alternatives for skincare, combining the therapeutic benefits of natural ingredients with advanced formulation techniques. It was helpful for treating skin rashes, swelling, and skin cracks.[2]
Guava (*Psidium guajava*) is an important economic fruit widely used as food and folk medicine. It contains flavonoids, alkaloids, tannins, triterpenoids, reducing sugars, essential oils, carotenoids, polyphenols, etc. It is used traditionally for medicinal purposes, mainly for antioxidant, antimicrobial, antispasmodic, antidiabetic, anticancer, antiallergy, anti-inflammatory, and hepato-protective properties.[3]

Passion fruit (*Passiflora edulis*), a member of the Passifloraceae family, is a tropical fruit native to South America, with Brazil being considered its primary center of origin. Recent studies have demonstrated that Passiflora edulis is rich in secondary metabolites such as polyphenols, flavonoids, and anthocyanins, which are responsible for its potent antioxidant and antibacterial activities higher levels of phenolic compounds associated with enhanced antioxidant capacity.[4]

Hydrogels loaded with herbal extracts were developed and studied for their possible application as materials for wound dressing and in the management of some skin diseases by many researchers The aim of this work was to formulate and evaluate polyherbal hydrogel with *Psidium guajava L., Passiflora edulis sims.*

2. MATERIALS AND METHODS

2.3 Plant collection and authentication

Fresh leaves of: *Psidium guajava L., Passiflora edulis sims* was collected from Thirumittacode, Pattambi, Kerala. Authentication and certified by Sree Neelakanta Govt Sanskrit College, Pattambi, Palakkad, kerala-679306.

Figure 1: *Psidium guajava L.*

Figure 2: Passiflora edulis sims.

2.3 Drying

The collected fresh leaves of *Psidium guajava L.,Passiflora edulis sims* were washed well using running water and distilled water. This helped to remove all impurities present in the collected leaves. They were then dried under shade for a period of 10 days to remove the excess moisture present in them and this process helps to avoid destruction of active compounds. After drying, they were ground completely and stored.

Figure 3: Drying.

2.3 Preparation of plant extract

2.3.1 Passiflora edulis Sims leaf extraction

The extraction process is conducted using the Soxhlation method. A sufficient amount of crushed dried *Passiflora edulis Sims* is packed into a porous cellulose thimble about 20-30g in experiment. 70-30% ethanol and distilled water is introduced into a round bottom flask, connected to the Soxhlet extractor and condenser. The thimble containing the Passiflora edulis Sims is inserted into the Soxhlet extractor. The solvent is heated with an isomantle at 50-60°C, evaporating and moving through the apparatus to the condenser. The condensed liquid then drips into the reservoir containing the thimble. Once the solvent level reaches the siphon tube, it returns to the flask, restarting the cycle. This process continues for 12 hours. Continue until the solvent in the siphon tube becomes nearly colorless. Upon completion, a small yield of Passiflora edulis Sims is collected in the round bottom flask. Cool the extract to room temperature. Filter and concentrated to obtain a crude ethanolic extract rich in flavonoids and polyphenols.

Figure 4: *Passiflora edulis Sims* leaf extraction.

2.3.2 *Psidium guajava L.* leaf extraction

The extraction process is conducted using the Soxhlation method. A sufficient amount of crushed dried *Psidium guajava L.* Leaf is packed into a porous cellulose thimble about 20g in our experiment. 70:30% ethanol and distilled water is introduced into a round bottom flask, connected to the Soxhlet extractor and condenser. The thimble containing

the *Psidium guajava.L*.Leaf is inserted into the Soxhlet extractor. The solvent is heated with an isomantle at 50-60°C, evaporating and moving through the apparatus to the condenser. The condensed liquid then drips into the reservoir containing the thimble. Once the solvent level reaches the siphon tube, it returns to the flask, restarting the cycle. This process continues for 8 hours. Continue until the solvent in the siphon tube becomes nearly colorless. Upon completion, a small yield of *Psidium guajava.L*.Leaf is collected in the round bottom flask. Cool the extract to room temperature. Filter and concentrated to obtain crude ethanolic extract rich in flavonoids and polyphenols.[5]

Figure 5: *Psidium guajava.L.*leaf extraction.

2.3 phytochemical screening of plant extract

Table 1.

Phytochemical test	**Results**	
	Psidium guajava L.	*Passiflora edulis sims*
ALKALOIDS Mayer's test	+ve	+ve
Wagner's test	+ve	+ve
Dragendroff's test	+ve	+ve
FLAVANOIDS Aqueous sodium Hydroxide test	+ve	+ve
Filter paper test	+ve	+ve
PROTEINS Biuretic test	+ve	+ve
TRITERPINOIDS Horizon test	+ve	+ve

2.3 PREPARATION OF POLYHERBAL HYDROGEL

A measured amount of carbopol -940 was gradually sprinkled into a specified quantity of water with continues stirring to achieve a uniform dispersion. The dispersion was left overnight for hydration. Into the beaker 5ml propylene glycol and 1g methyl paraben were taken and stirred then add glycerin into the mixture An accurately weighed plant extract along with above additives was incorperated into the hydrated carbopol-940 dispersion under constant mechanical stirring. Triethanolamine solution was slowly added with continuous stirring to neutralise the solution and improve the consistency of gel and maintain the pH at 6-7.[6,8,10]

Figure 6: Poly herbal hydrogel.

Table 2.

Ingredients	Formulation 1	Formulation 2	Formulation 3
Passiflora edulis Sims leaf extract	1ml	2 ml	3 ml
Psidiumguajava L Leaf extract	3ml	2 ml	1 ml
Carbopol 940	0.18 gm	0.18 gm	0.18 gm
Propylene glycol	1.5 ml	1.5 ml	1.5 ml
Methyl paraben	0.045 gm	0.045 gm	0.045 gm
Triethenolamine	1-2 drops	1-2 drops	1-2 drops
Glycerine	0.8 ml	0.8 ml	0.8 ml
Distilled water	q.s	q.s	q.s

Figure 7: Formulation of polyherbal formulation (F1, F2, F3).

2.6. EVALUATION TESTS FOR POLY HERBAL HYDROGEL

2.6.1. PHYSICAL APPEARANCE

Visual inspection is done to determine the polyherbal hydrogel physical characteristics, odour, colour, texture.

Colour: Upon inspection against a white background, the formulation exhibited a pale yellow colour.

Odour: The gel's odor was evaluated by mixing it with water and sniffing the mixture, revealing a natural fresh plant scent in the gel.

Consistency: The consistency was assessed by applying the gel to the skin, and it was determined to have the best consistency.

Texture: Take a small amount of hydrogel in your fingers and spread it to, and it was determined to have good texture.

2.6.2. pH TEST

The pH of the hydrogel was determined by a digital pH meter at room temperature. The pH meter was calibrated by using a buffer solution of pH 7.0 & 9.2 then the electrode washed with distilled water. 1gm of hydrogel was dissolved in 100ml of distilled water. This process was repeated three times to obtain the average pH value. The pH of hydrogel was found to be 6.36.[7]

2.6.3. VISCOSITY

Viscosity of hydrogel was done by using rotational viscometer at a temperature of 25 degree celcius using Spindle No: 2 at 6 rpm.

2.6.4. SPREADABILITY

Spreadability was measured by using a definite weight over the hydrogel which isuniformly spreaded between two glass slide. The time required to separate the slides was noted. A weight of 20g is placed over glass slides of equal dimension.[8] Then the upper glass slide is separate from the lower slide and the time required for separation is noted**.**

Spreadability was calculated by using this equation:$S= (m \times l) / t$

Where S is spreadability, m is weight tied on upper slide, l is length of glass slide and t is time taken in seconds.

2.6.5. WASHABILITY

To test the washability of the hydrogel, apply a small amount of hydrogel on to the rough surface of an old glass slide and let it to dry. Once it dry, pour water over the gel to see how easily it washes off.

2.6.6. ANTIMICROBIAL ACTIVITY

Preparation of agar well diffusion method:

Agar well diffusion method was performed to determine the zone of inhibition. In this to the above inoculated agar plates well of 5mm diameter were prepared by using a sterile cork borer. The definite quantity of herbal cream, herbal hydrogel, extract and control was placed into each well. The plates were then incubated for 72 hours at 37°C and observed for zone inhibition. All the experiments were repeated 3 times to get an accurate results.[8,6]

2.6.7. ANTIOXIDANT ACTIVITY

Principle: DPPH (2, 2-diphenyl-1-picryl-hydrate) free radical method is an antioxidant assay based on electron - transfer that produces a violet solution in ethanol. This free radical, stable at room temperature, is reduced in the presence of an antioxidant molecule, giving rise to colorless ethanol solution.

Procedure: 0.1mm DPPH was prepared by dissolving 4mg DPPH in 100ml ethanol. The sample solution 20µl, 40µl, 60µl, 80µl, 100µl concentration was made. The concentration made up to 3 ml using methanol and add 1ml of DPPH solution to it[6][9]. After 30 minutes of incubation the absorbance is measured at 517 nm using spectrophotometer. The procedure was repeated for standard ascorbic acid and the assay is calculated as:

Radical scavenging activity = (A control – A test) / (A Control) 100

The results are given in the table no.8

IC50 (inhibitory concentration 50%) value determined by plotting a graph, various concentrations of standard and extract verse percentage inhibition. The IC 50 value denotes the concentration of sample required to scavenge 50% of the DPPH free radicals.[6]

3. RESULT AND DISCUSSION

3.3 Physical appearance

Table 3.

Evaluation parameter	F1	F2	F3
Colour	Pale yellow colour	Pale yellow colour	Pale yellow colour
Odour	Fresh leafy odour	Fresh leafy odour	Fresh leafy odour
Texture	Smooth	Smooth	Smooth
Homogenity	Homogenous	Homogenous	Homogenous

3.2. Viscosity

Table 4.

Hydrogel	F1	F2	F3
Viscosity (cPs)	1300	1330	1300

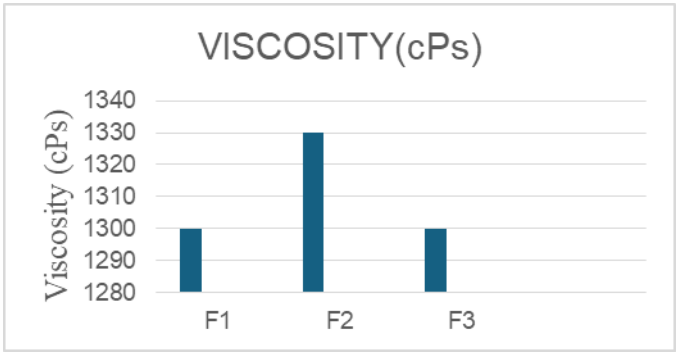

Figure 8: Viscocsity.

3.3 pH

Hydrogel	F1	F2	F3
Trail 1	6.32	6.36	6.34
Trail 2	6.30	6.33	6.36
Trail 3	6.33	6.34	6.32
Average	6.31	6.34	6.34

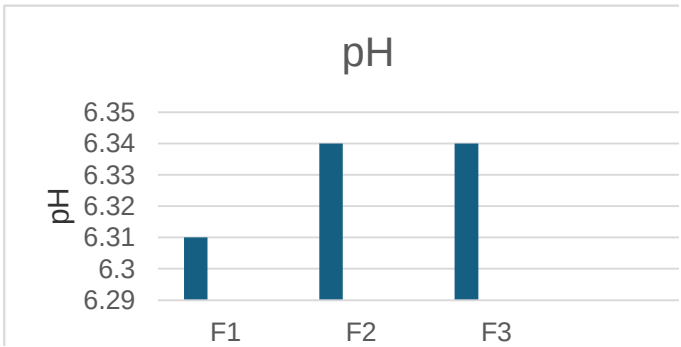

Figure 9: pH.

3.4 Spreadability

Table 5.

Hydrogel	F1	F2	F3
Spreadability (gcm/sec)	22	22	24

Figure 10: Spreadability.

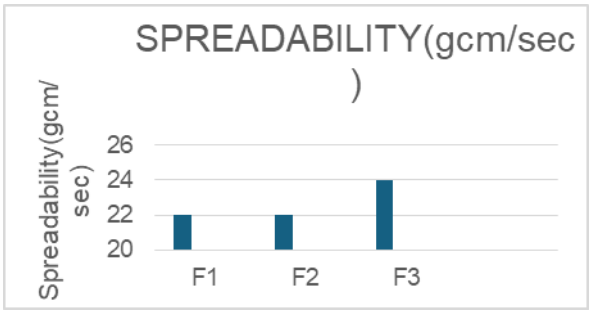

Figure 11: Spreadability.

3.5 Washability

Table 6.

Hydrogel	F1	F2	F3
washability	Easily washable	Easily washable	Easily washable

3.6 Invitro activity of formulation

3.6.1 Result of antimicrobial activity

Table 7.

Sample	Zone of inhibition (mm)Staphylococcus aureus	Zone of inhibition (mm)Escherichia coli	Zone of inhibition (mm)candida albicans
Standard	(Doxycyclin) 20	(Gentamycin)18	(clotrimazole)24
Passiflora edulis.sims extract	12	11	15
Psidium guajava L. extract	15	13	17
Hydrogel	18	15	21

Figure 12: Zone of inhibition of Escherichia coli, Staphylococcus aureus, Candida albicans.

3.6.2. Antioxidant activity-DPPH Assay

Table 8.

Conc.(μg/mL)	Absorbance of hydrogel	% scavenging of hydrogel	Standard(ascorbic acid) absorbance(517)	% scavenging of standard
20	0.282	26.17	0.232	39.26
40	0.235	38.48	0.201	47.38
60	0.206	46.07	0.134	64.92
80	0.156	59.16	0.102	73.29
100	0.132	65.44	0.076	80.10

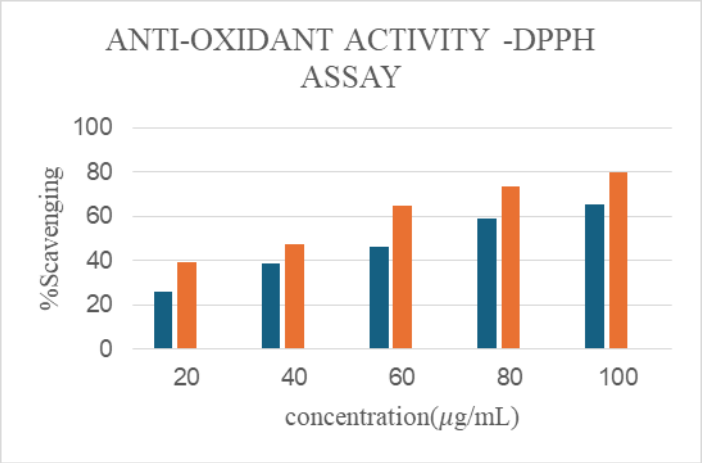

Figure 13: Antioxidant activity.

DISCUSSION

The study formulated and evaluated a polyherbal hydrogel containing Psidium guajava L and passiflora edulis Sims extracts, assessing its phytochemical composition, physical properties, and rheology, anti-oxidant and antimicrobial efficacy.

Phytochemical Analysis

Phytochemical screening confirmed alkaloids, phenols, flavonoids, tannins and terpinoids in *Psidium guajava L*. and *Passiflora edulis Sims*, enhancing the gel's antimicrobial and antioxidant properties.

Physical and Rheological properties

The prepared formulations F1, F2, and F3 where observed to have a pale yellow colour, all formulations had a fresh leafy odour, and a smooth texture.The homogeneous nature of the gel ensures uniform distribution of active ingredients.

Viscosity values ranged from 1300 cPs (F1) to 1330 cPs (F2), indicating good consistency and ease of application.
pH values were in the range of 6.31-6.34, which is suitable for topical application, ensuring no irritation.
Spreadability tests confirmed that the gel was easily spreadable, enhancing ease of use.
Washability was found to be efficient, ensuring easy removal without residue.

Antimicrobial Activity

The antimicrobial study demonstrated the gel's effectiveness against Staphylococcus aureus, Ecoli. and Candida albicans.

The zone of inhibition for Staphylococcus aureus was highest in hydrogel (18mm), followed by extract of *Psidium guajava L*. (15mm), and extract of *Passifora edulis sims*(12mm), showing strong antibacterial activity.

The zone of inhibition for Ecoli. Was highest in hydrogel (15mm), followed by extract of Psidium guajava L. (13mm), and extract of Passiflora edulis sims (11mm), showing antibacterial activity.

The zone of inhibition for Candida albicans was highest in hydrogel (21mm), followed by extract of Psidium guajava L. (17mm), and extract of Passiflora edulis sims (15mm), confirming antifungal potential. The antimicrobial efficacy was comparable to standard drugs (Doxycycline, and Gentamycin for bacteria and Clotrimazole for fungi).

Anti-oxidant Activity

Anti-oxidant activity was performed using DPPH scavenging method for plant extract loaded hydrogel.

The antioxidant activity of hydrogel increased with increasing concentration from 20 to 100μg/mL as indicated by the gradual decrease in absorbance and corresponding increase in percentage inhibition. The standard anti-oxidant showed higher radical scavenging activity than the hydrogel at all concentration. However, the hydrogel exhibited notable, concentration dependent antioxidant activity.

These results confirm that the extract –loaded hydrogel possesses significant free radical scavenging potential.

4. SUMMARY AND CONCLUSION

This study involved the formulation and evaluation of a polyherbal hydrogel containing leaf extracts of *Passiflora edulis Sims* and *Psidium guajava L*. Phytochemical screening confirmed the presence of bioactive compounds with antioxidant and antimicrobial properties. The hydrogel showed acceptable physicochemical characteristics and demonstrated antioxidant as well as antimicrobial activity against Staphylococcus aureus, Escherichia coli, and Candida albicans.The results indicate that the developed polyherbal hydrogel has potential for topical therapeutic use. However, this study serve a foundation in vivo evaluation, stability studies, and clinical trials to standardize the formulation for large scale pharmaceutical production and commercial distribution.

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