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ORIGINAL ARTICLE

Formulation And Evaluation of Antimicrobial Herbal Gel Containing *Piper betel* Leaf Extract with Aloe Vera

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ABSTRACT
Herbal remedies are more tolerable by society in the trust that they are safer with very less adverse effects as compared to synthetic or semi-synthetic drugs. Herbal medicinal formulations have growing demands not only in India but in the world market also. Our present study aims to formulate and evaluate an herbal gel containing the leaf extract of piper betel in combination with Aloe Vera gel for antimicrobial activity against various skin infections. There are number of topical formulations are available in market which includes creams, ointments, pastes, gels etc. Nowadays gels are getting more popular than other topical formulations because of higher stability, controlled release than other semisolid preparations and significant bioavailability due to its enhanced absorption characteristics. Piper betel leaf extract is having antimicrobial activity, Aloe Vera gel has already reported to have an influence on the wound healing. Therefore extract of Piper betel leaf and aloe vera gel were used for the formulation of antimicrobial herbal gel for various skin infections. This herbal gel could be useful for topical application and also safer for use.
Key words: *Piper betel,* Aloe Vera gel, antimicrobial, wound healing.

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INTRODUCTION

Bacterial and fungal infections are very common in India. Even though number of antibiotics are available in market, there is a development of multidrug resistance by many skin infecting pathogens and also severe side effects are associated with these antibiotics have now drown the attention of researchers towards herbal medicine system.[1].India is one of the leading populated countries in the globe, and has around eight different geographical zones [2]. Normally Plant territories represent a massive reservoir of biologically active compounds and near about 60% of the drugs used in medicinal preparations are obtained from plant source. Most of the herbal sources are used as home remedy, it can also be used as a over the counter drug products and also provided as a raw material for the various pharmaceutical industries and cosmetics industry throughout the world and even it signifies a extensive proportion of the world drug market and hence it is significant to set up their quality. Hence it is important to use phytochemical methods for screening and analysis of biologically active components and their therapeutic mechanisms.[2]

Herbal remedies are conventionally used globally for the management of numerous contagious diseases. The use of herbal drug as novel antibiotics have several advantages related to safety, efficacy, accessibility etc even they minimizes the risk of unwanted adverse reactions and also obsession. The World Health Organization (WHO) has adopted a major strategic change in accepting that most of the emerging countries would have to make use of more conventional medical practices for primary health care. In modern years, multi-drug resistance has developed in human beings, animals and also in plant pathogens owing to misuse of various antibiotics which are generally applied in the management of contagious diseases. In modern era the prevalence of resistance to most of the antimicrobials has increased.

Moreover, this frightening situation has led microbiologists to look for the newer antimicrobial drugs from various sources, which includes herbal sources also.[3]

Piper betel Leaves (*P. betel L.*) are dioecious, annual creeper, escalate by many small advantageous rootless, the plant grows very high to a height of about one meter, generally the plant grown in hotter and damper parts of the country and is a widely available plant[4,7,9].Piper betel belongs to piperaceae family. More than 100 varieties of *P. betle L*. has been distributed throughout the world and from that around 40 species have been recorded in India. Various plant parts of *P. betel L*. utilized viz; leaves roots, stems, stalks and fruits. The leaves are deep green in color and heart shaped betel vine are popularly known as Paan, in India. *Piper betle L*. has been use in Chinese, Indian traditional medicine for centuries and it has antimicrobial property [7]. Betel leaf has been described from prehistoric times as an pungent, stimulo-carminative (katu), astringent and aphrodisiac (kamagni sandipanam)[4]. In Ayurveda, the properties of betel leaf described as: Guna (Quality) :Laghu, Ruksha, Tikshan, Rasa (Taste) :Tikta, Vipak (Metabolism) : Katu, Virya (Potency) : Ushan, Prabhav (Impact) : Hridya [4]

P. betel L. is a Vedic medicinal plant and its Vedic name is Saptasira and in sankrit it is known as Tambool, Nagvelleri, Nagani and were used as remedy against various diseases.[4] *P. betle L.* is called by various names in India, betel leaf is called by Pan in Hindi and Bangla, Villayadela in Kannada, Vettilakkoti in Malayalam, Vettilai in Tamil, Tamalapaku in Telugu, Videch-paan in Marathi, Nagarbel in Gujrati.[7] It is also known as Tanbol in Arabic and Burg-e-Tanbol in Persian. [7]

This plant is cost-effective, and has a lot of medicinal value, considered as conventionally important plant in the whole world. [4] *P.betel* L. is also known as betelvine and is a 'green gold of India'. [7] About 20 million people directly or indirectly develop their employment from the manufacture, processing, handling, transportation and marketing of betel leaves in India [7]. Betel leaves contain large number of biomolecules [2]. On the basis of chemical constituents of leaf essential oils, P. betel L are of five prominent groups of landraces, namely Bangla, Kapoori, Meetha, Sanchii and Desawari. [9]

In rural area, the use of betel nut (Areca nut) with betel leaf is regular practice [2]. However, according to Ayurveda, the betel nut with betel leaves is a post mealtime digestive tonic, oral deodorant, natural antiseptic, astringent, diuretic, mood elevator, aphrodisiac and also act as nervine tonic.[7,9]

The betel leaves are primarily used as mouth freshener, to treat bad breath, boils and abscesses, eye problems like conjunctivitis, gastrointestinal infections such as constipation, headache, allergic reactions like itching, mastitis, mastoiditis, leucorrhoea, otorrhoea, swelling of gum, bones and joint problems like rheumatism, and minor cuts and injuries.[4] It is well known for treating many communicable and non-communicable diseases which includes cold, cough, bronchial asthma, rheumatism, stomachalgia [7,9]. The leaves are attributed with wound healing property [4] and the leaf juice is used to disinfect wounds externally.[2] The fresh betel leaves acquire antimicrobial, ringworm, antifungal, antiseptic and antihelminthic effects.[4]

The chief constituents of piper betel leaf are Piperol-A, Piperol-B, methyl piper betlol. Essential oil composing of terpinen-4-ol, safrole, allyl pyrocatechol monoacetate, eugenol, piperbetol, cineole, estragol, etc.[6] Phytochemical analysis on leaves revealed the presence of Alkaloids, Tannins, Carbohydrates, Amino acids and Steroidal components [7].

The aqueous extract of betel leaves showed the presence of preliminary phtytochemicals such as alkaloids, saponins, coumarin and glycosides substance.[2]

The leaf possesses the broad spectrum of antimicrobial activity against various strains of bacteria including bacillus cereus, Pseudomonas Aeruginosa, E.coli, Micrococcus luteus, Stahylococcus aureus, Aeromonas hydrophila etc. [9] Result of different studies indicate the potency of Piper betel leaves in improving wound healing process. [5]

Aloe vera plant has been known as "the healing plant" and Aloe vera gel has reported to have a influence on the wound healing.[19] It is known that Piper betel and Aloe vera are able to accelerate the healing process of burns.[10]

Present work includes formulation of antimicrobial herbal gel with wound healing property as a safe alternative to the existing synthetic antibiotic creams.

MATERIAL AND METHODS

Materials:

The leaves of piper betel were purchased from Globelar Trade hub Pvt. Ltd., Satara, Maharashtra and authentication was carried out from Department of Botany and Plant protection, S. G. M. college Karad. Carbopol 940 is used as gelling agent and is purchased from Shree chemicals, Gujrat. Aloe vera powder from A M Neutratech Pvt Ltd, New Delhi, Triethanolamine was used as pH modifier and methyl paraben and propyl paraben are used as preservatives.

Methods

Phytochemical screening

Phytochemical screening of *Piper betel* leaves extract and Aloe vera extracts was carried out for the detection of various secondary metabolites viz, tannins, alkaloids, flavonoids, terpenoids, steroids, essential oils and Saponin.[11]

Preparation of herbal extract-

For the preparation of herbal extract, betel leaves were selected and chopped into small sizes, chopped leaves undergoes drying at room temperature to preserve the compounds present in betel leaves. After the process of drying the leaves were finely powdered [15] subsequently, 20 grams of dried powdered betel leaves were put into 2 flasks; each flask contained 10 grams of dried powdered betel leaves. Then, dried betel leaves powder were soaked into 95% ethanol where the volume of each ethanol solution was 100 ml, and the extracts were macerated for 48 hours. Finally, the extract in flask undergoes filtration, and the extract is filtered using muslin cloth and then evaporated until dry [5].

Selection of herbal active concentration-

The concentration of herbal extract, 3% w/v was selected on the basis of preformulation studies.

Preparation of trial batches [8,12, 18,22, 23, 24, 25]

Step 1: Preparation of gel base

Gels were formulated using Aloe vera powder and carbopol 940 in different ratios [Table 1] and excipients like trimethanolamine, methyl paraben and propyl paraben. Accurately weighed quantity of Carbopol-940 and Aloe vera powder were dispersed in water with continuous stirring by using mechanical stirrer at 1000rpm (Remi motor RQT-127 HP1/8) at the temperature between 60-80^{0}C for 1hr in the reactor vessel and cooled, then triethanolamine was added drop by drop with constant stirring till pH was neutralized and gel was formed. Methyl paraben and propyl paraben were added as preservatives.

Step 2: Preparation of formulation

The herbal extracts (3% w/v) of betel leaves were added with continuous stirring using mechanical stirrer at 1000 rpm (Remi motor RQT-127 HP1/8) to different gel bases till the uniform dispersion of the ingredients was achieved. All these batches were allowed to equilibrate for 24hrs at room temperature. The prepared gel was filled and stored in a wide mouth container. The pharmaceutical studies were performed

Table 1: Trial batches for gel bases A1-A5

Sr. No.	Ingredients (g)	Formulation code					
		F1	**F2**	**F3**	**F4**	**F5**	**F6**
1.	Dried Aloe Vera gel powder	0.25	0.25	0.25	**0.25**	0.25	0.25
2.	Carbopol 940	0.15	0.25	0.30	**0.35**	0.40	0.45
3.	Triethanolamine	0.025	0.025	0.025	**0.025**	0.025	0.025
4.	Methyl paraben	0.020	0.020	0.020	**0.020**	0.020	0.020
5.	Propyl paraben	0.020	0.020	0.020	**0.020**	0.020	0.020
6.	Distilled water	25	25	25	**25**	25	25

Batch F4 is showing satisfactory consistency. Hence 10 gm of gel base was taken accurately from F4 batch and 0.3 gm of piper betel leaf extract was incorporated to it and mixed well.

Table 2: Formula for Gel

Sr. No.	Ingredients	Quantity Taken
1.	*P. betel L.*extract	3%
2.	Gel base	10%

EVALUATION

1) Organoleptic evaluation:[10]

Organoleptic properties of gel preparations were observed for every change such as color, odor, appearance, consistency and feel were checked.

a. Color- The color of the formulation was checked out against white background.
b. Consistency- The consistency of formulation was checked by its application on skin.
c. Greasiness- The greasiness of gel was assessed by the application on to the skin.
d. Odor- The odor of the gel was checked by mixing the gel in water and taking the smell [10].

2) Washability:
In this study the washability was checked by applying the formulation on the skin. Here the ease and extent of washing with water was checked manually [10].

3) Determination of pH:
The pH of the formulation was determined by using digital pH meter. 1 g of gel was dissolved in 100 ml of demineralised water and stored for about two hours. The measurement of pH of formulation was done in triplicate. Instrument was calibrated before use with standard buffer solutions at pH 4, 7 and 9[10].

4) Stability study:
A stability test was performed by observing any change to consistency, color, and odor in gel preparation placed at 25^0 C for 30 days. [10]

5) Spreadability:
One of the most important criteria for a topical formulation is to meet the ideal characteristics, like it should acquire good spreadability. This term is used to represent the extent of area to which the formulation readily spreads on application to skin or any affected part of skin. The therapeutic efficacy of a formulation also depends upon its spreading value. Moreover, to determine the spreadability of prepared formulation, 0.5 g of gel was placed within a circle of 1 cm diameter pre-marked on a glass plate of 20 × 20 cm, and over which a second glass plate was placed. A specific weight of gel (approx. 500 g) was allowed to rest on the upper glass plate for 5 min. The increase in the diameter due to gel spreading was noted.[8][19]

6) Viscosity measurement:
The viscosity of formulated gel was measured with the help of Brookfield viscometer. Here the formulation was poured into the adaptor of the viscometer at 20 rpm. Temperature was maintained at 20^0C.[10]

7) Determination of Homogeneity:
The prepared gel undergoes Homogeneity test by visual inspection. Here the formulation is set in the container and they were tested for their appearance and presence of aggregate.[13]

Table 3: Homogeneity study

Formulation code	Days	Consistency	pH	Homogenecity
F4	0	6	6.8	Excellent
	1	5.9	6.7	Excellent
	2	5.7	6.7	Good
	3	5.7	6.6	Good

8) Primary Skin Irritation test:
Skin irritation test was performed on human volunteers. Five volunteers were selected for this test. 1.0gm of gel was applied on the marked region on the left hand and dorsal surface. Then the specific area is covered with cotton and secured firmly with adhesives [13], So that the gel was allowed to remain in contact with skin for over 24hrs. Irritancy was checked if any for regular interval up to 24 hrs and reported [10].

9) Antimicrobial Activity [14] [20] [21][25]
The antimicrobial activity of the prepared gel against various strains of aerobic as well as anaerobic microbes was evaluated by using standard cup-plate method. The micro-organisms viz Bacillus subtilis, Staph aureus, E.coli &P. Aerugenosa were used for the screening of antimicrobial activity. Nutrient agar medium was used for bacterial cultures, and the compositions required to prepare nutrient agar medium is mentioned in (Table 4).

For this assay plates were incubated at $37^0\pm 0.2^0$C for 24hrs under strict aerobic conditions. Each plate is filled with 100µl of bacterial culture, and is poured with the help of micro pipette and then spread all over the plate uniformly by means of sterilized spreader. After that a well was made by the sterile cork borer which has a diameter of 8mm. 0.1ml of standard [0.3% Gentamicin] and test samples were poured into the well. At last, the plates were kept in refrigerator for 2 hrs and then incubated at 37^0Cfor 24 h.

Table 4: Composition of nutrient agar medium for antimicrobial study

Sr. No	Ingredient	Quantity
1	Beef extract	0.6
2	Peptone	1
3	Agar-Agar	3
4	NaCl	1
5	Distilled water	Quantity 200ml

The zone of inhibition of bacterial growth around the cup is measured in mm by using scale. The values were taken at four different planes and finally the mean was calculated. The results are shown in Table 6.

RESULT AND DISCUSSION

Herbal formulations are considered to be safe with less or no side effect(s). Herbs or Plants are considered be a vital source of potentially useful constituents for the development of new therapeutic agents. Nowadays, gels have been widely used as a vehicle for topical delivery of drugs. In order to get additional benefits extracts of plants and herbs with specific medicinal properties can be incorporated in this dosage form as active ingredients [26] Topical application of gels offer great advantages in a faster release of a drug directly to site of action as compared to cream and ointment [26]. S. aureus, E. coli, B. subtilis are amongst the commonest pathogens that can cause skin infections.[26] The antimicrobial and wound healing properties of *Piper betel* [2,4,5,9,10,19,21] and aloe vera [10,19,26] have been previously investigated. Direct application of *Piper betel* extract in raw form on the skin surface is difficult therefore gel formulation was developed with incorporation of *Piper betel* extract into aloe vera gel.

Though Herbal formulations are safe, quality control tests are carried out for the prepared herbal gel to control efficacy and safety of the formulation. Stability studies and skin irritation test are well known methods to prove efficacy and efficiency of the herbal formulations [26]

The results of evaluation tests are mentioned in Table 5.

Table 5: Evaluation of Gel

Sr. No	Parameter	Result
1.	Organoleptic evaluation	
a.	Color	Dark green
b.	Odor	Characteristic
c.	Consistency	Semi- Solid
2.	Washability	Good
3.	pH	6.8
4.	Stability	Good
5.	Spreadability(cm)	12.50 cm
6.	Skin irritation test	Non-Irritant

The pH of the prepared formulation determines the irritancy of formulation to the skin and the person should be sure that the formulation can be used without the risk of irritancy to the skin. Hence, pH of all the prepared formulation lies in between 6.6-6.8, which is compatible to normal pH of skin. Spreadability denotes the extent of area to which the gel readily spreads. Even the bioavailability of drug depends on spreadability value. The value of spreadability for optimized gel was found out to be 12.50 cm indicating that the gel is easily spreadable by small amount of shear. The skin irritation studies of formulated gel were carried out on human volunteers. Result of this test verifies the non irritant property of gel and hence it confirms that the gel is purely safe for application on human skin. After this test the formulation undergoes stability and homogeneity study which confirms that the formulation has excellent stability and homogeneity profile by observing Table no 3.

Antimicrobial activity

Literature surveys revealed that Piper betel leaf extract and aloe vera gel has potentially been known for its antimicrobial activity and wound healing activity [2,4,5,9,10,19]. Therefore herbal gel of Piper betel leaf extract with aloe vara gel was formulated as a safe alternative to the existing synthetic antibiotic creams. However, no literature is available related to the formulation and evaluation of antimicrobial activity of herbal gel containing the extracts of Piper betel leaf and alove vera gel.

The antimicrobial activity of formulated gel was compared with the standard antibiotic, Gentamicin and the results were shown in Table 6. According to this, the zone of inhibition was found to be maximum, 24mm at 80 µg/ml for *Staphylococcus aureus* followed by 19mm at 80 µg/ml for *E.coli*, and 20mm, 14mm at 80 µg/ml for *Bacillus subtillis* and *P. aeruginosa* respectively. 80 µg/ml concentration was effective in killing the microorganisms which includes gram positive and gram negative bacteria as per this study.

The relative efficiency of antimicrobial activity by herbal gel of *Piper betel* leaf extract with alove vara gel to that of standard, a broad-spectrum antibiotic on the above mentioned microorganisms suggest the possibility of a more cost effective and potentially harmless antimicrobial agent.

Table 6: Zone of inhibition

Drug Concentration	Zone of Inhibition (mm)			
	Staphylococcus aureus	***E.coli***	***Bacillus subtillis***	***P. aeruginosa***
20 µg/ml	10	8	11	9
40 µg/ml	14	12	12	11
60 µg/ml	20	18	14	13
80 µg/ml	24	19	20	14
Standard	26	21	24	16

CONCLUSION

Skin disease is very common and people need remedy for skin disease without side effects The herbal anti-microbial gel containing piper betel leaf extract with aloe vera was prepared and checked for its efficacy using standard cup plate method. The microorganisms used in this study are the major cause of number of skin infections. As per our study betel leaf extract with aloe vera gel was effective against Staphylococcus aureus, E.coli, Bacillus subtillis and Pseudomonas aeruginosa. The main ideology following the combination of betel leaf extract with aloe vera gel is to study its additive effect and the combination proved to be effective to combat the antibiotic resistance of pathogenic organism and even provide safe and healthy living through germ free skin. Moreover, from this study it can be concluded that the formulated antimicrobial gel containing betel leaf extract was effective in considerable reduction in microbial growth which causes the number of skin infections and almost no side effects like skin irritation and rashes can be expected due to use of herbal drugs.

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