



Chemical Methodologies

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Original Research article

Preparation and Evaluation of Herbal Formulation Using Natural Extract

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ARTICLE INFORMATION

Received: 30 August 2018

Received in revised: 29 December 2018

Accepted: 01 January 2019

Available online: 11 March 2019

DOI: [10.22034/chemm.2018.146968.1079](https://doi.org/10.22034/chemm.2018.146968.1079)

KEYWORDS

Butea monosperma

Psidium guajava

Antibacterial activity

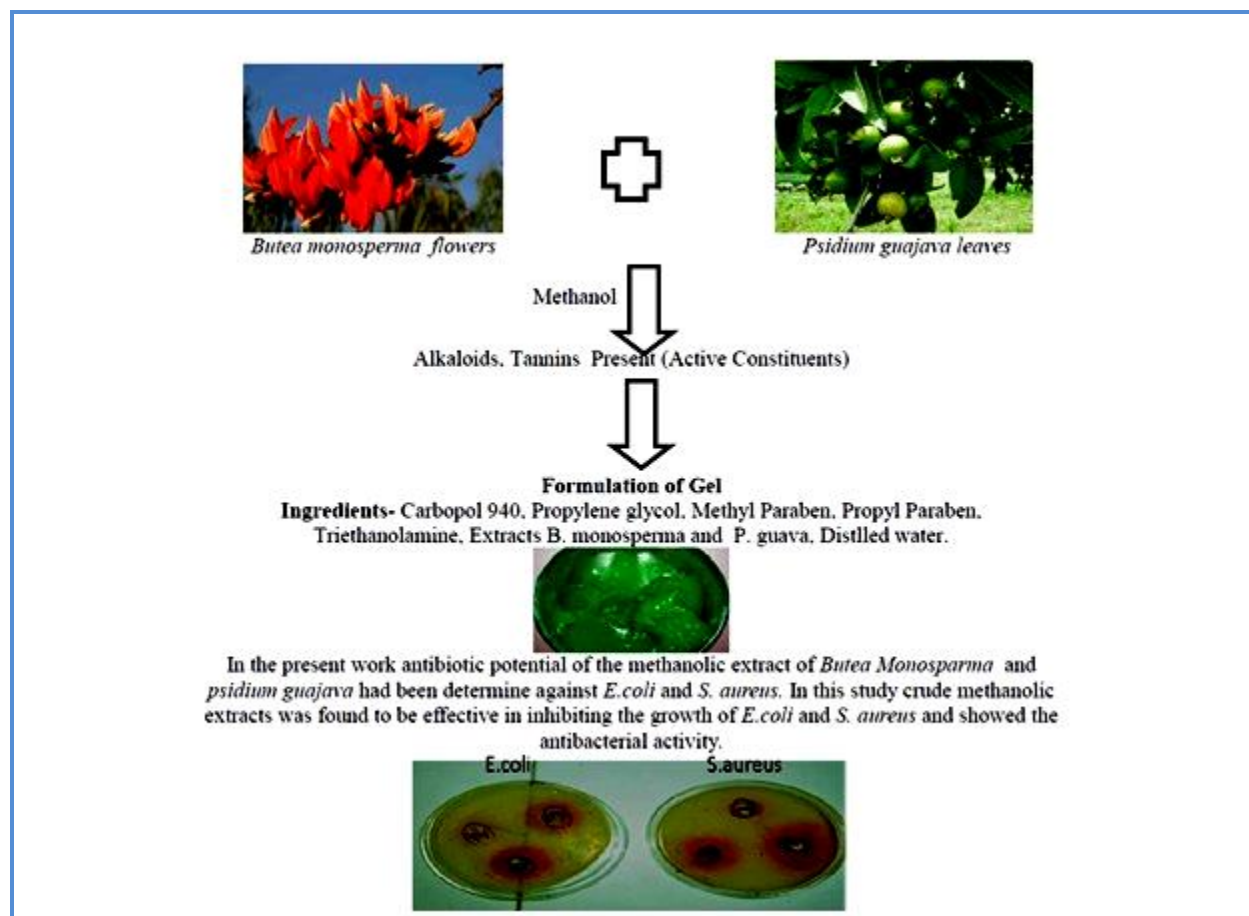
ABSTRACT

Butea monosperma and *Psidium guajava* are traditional important medicinal plants. The plants were used in Ayurvedic unani and Siddha medicine for various ailments. Antibacterial activity was studied using extracts of *Butea monosperma* and *Psidium guajava* by agar well-diffusion method against *E. coli* and *S. aureus*. The ethanolic extracts of *Butea monosperma* flowers showed antibacterial activity against *E. coli* and *S. aureus*. In the same manner *Psidium guajava* leaf, extract showed antibacterial activity against *E. coli* and *S. aureus*. The objective of the present investigation was to formulate and evaluate herbal gel using specified concentration of powdered extracts of *Butea monosperma* flower and *Psidium guajava* leaves.

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Graphical Abstract



Introduction

Butea monosperma (palash) which is a medium-sized deciduous tree belongs to family *Fabaceae* and sub-family papilionaceae. Native to India, also called as "flame of the forest" is a medium sized tree, growing from 20 to 40 feet high, and the trunk is usually crooked and twisted with irregular branches and rough, grey bark. The leaves are pinnate, with an 8-16 cm petiole and three leaflets. It is seen in all its ugliness in December and January when most of the leaves fall: but from January to March it truly becomes a tree of flame, a riot of orange and vermilion flowers covering the entire crown. These flowers, which are scentless, are massed along the ends of the stalks dark velvety green like the cup-shaped calices and the brilliance of the stiff, bright flowers is shown off to perfection by this deep, contrasting color (6, 7). The plant shows various Pharmacological activities like antifungal, antimicrobial, antibacterial (4), anticonvulsive, anti-fertility, anti-inflammatory activity, anti-diabetic activity (1-3) etc.

Guavas are common tropical fruits cultivated in many tropical and subtropical regions. *Psidium* is a small tree in family *Myrtaceae*. It grows 33 ft high, with spreading branches, the *Guava* is easy to recognize because of its smooth, thin, copper-colored bark that flakes off, showing the greenish layer beneath; and also because of the attractive, "bony" aspect of its trunk which may in time attain a diameter of 10 in. The main constituents of *Guava* are vitamins, tanins phenolic compounds, flavonoids, essential oils, sesquiterpene alcohols and triterpenoid acids (8, 9). These and other compounds are related to many health effects of *Guava*.

Experimental

Material and Method

Collection of plant material

The leaves of *psidium guajava* and the flowers of *Butea monosperma* were collected from the Pangali village in Gondia district in the summer season. The plant materials were authenticated by Dr. N.M. Dongarwar of Botany Department; RTM Nagpur University, Nagpur India. A voucher specimen No. 9282, 9283 were deposited at Herbarium, Department of Botany, RTM Nagpur University Nagpur.

Preparation of extracts

The *Guava* leaves were chopped and shade dried at room temperature then grounded to coarse powder. The powdered plant material (350 gm) was packed into soxhlet apparatus and extracted upto (12 hr) with petroleum ether for defatting. It was then extracted with methanol for further 4 hr. The extract was filtered, and the solvent was evaporated under reduced pressure using a rotator, which was kept in desiccators for further use. The palas flowers were also extracted in the same manner by using methanol as solvent (5).

Preparation of media

The media of nutrient agar was prepared in a sterile condition for the antibacterial study.

Table 1. Ingredients and Quantity of medium

Ingredients	Quantity
Peptone	5 gm
Sodium chloride	5 gm
Beef extract	3 gm
Agar	20 gm
Distilled water	1000 mL
pH adjusted to 7.2	

Minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of the extracts of *Butea monosperma* flowers was determined for *Escherichia coli*, *Staphylococcus aureus*. The varied concentration of methanolic extracts of flowers (10 mg/mL, 50 mg/mL, 100 mg/mL, 150 mg/mL, 200 mg/mL and 250 mg/mL) was added to 3 mL of Muelle Hinton broth tubes with 0.1 mL of bacterial suspension. Tubes were incubated at 37 °C for 24 hours. After incubation the tubes were examined for bacterial growth by observing turbidity.

Agar well diffusion method

The antibacterial activity was carried out using extracts from plant parts leaf, bark and flowers of *Butea monosperma* by using agar well diffusion method. Antibacterial activity was determined by measuring the diameter (mm) of zone of inhibition. The concentration of each plant part extract to be used for the method was calculated on the basis of their respective MIC values and twice the MIC values was used to determine the zone of inhibition. The culture was bulk seeded in Mueller Hilton agar and poured into petri plates, four equidistant 8 mm wells were bored into solidified agar plates and then extracts were added to the wells. The diameter of zone of inhibition (ZOI) was recorded in millimeters (mm). Ciprofloxacin (5 µg/mL) was used as positive control and respective solvents were used as negative control. The plates were incubated at 37 °C for 24 hours and zone of inhibition were observed.

Preparation of gel

Table 2. Ingredients and Quantity of gel

Ingredients	Quantity	
Carbopol 940	0.1 gm	
Propylene glycol	0.1 mL	
Methyl Paraben	0.00015 mL	
Propyl Paraben	0.001 mL	
Triethanolamine	0.08 mL	
Extracts	<i>B. monosperma</i>	<i>P. guava</i>
	6.56 mg/mL	1.64 mg/mL
	13.12 mg/mL	3.28 mg/mL
	18.04 mg/mL	6.56 mg/mL
Distilled water	up to 10 mL	

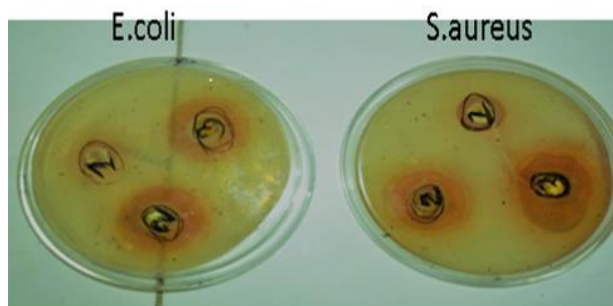
Result and Discussion

Table 3. Antibacterial activity of combined extract of *Butea monosperma* flowers and *Guava* leaf

Sr. no	Organism	Zone of inhibition in mm		
1	Conc. of extract	8.2 mg/mL	16.4 mg/mL	24.6 mg/mL
2	<i>E. coli</i>	0.4 mm	0.8 mm	0.9 mm
3	<i>S. aureus</i>	0.3 mm	1 cm	1.2 cm

Table 4. Antibacterial activity of gel

Sr. no	Organism	Zone of inhibition in mm		
1	Conc. of gel	8.2 mg/mL	16.4 mg/mL	24.6 mg/mL
2	<i>E. coli</i>	-	0.6 mm	0.8 mm
3	<i>S. aureus</i>	-	0.7 mm	1.1 mm



Physical Parameters

In the present work antibiotic potential of the ethanolic extract of *Butea monosparma* and methanolic extract of *Psidium guajava* had been determine against *E.coli* and *S. aureus*. In this study, crude methanolic extracts was found to be effective in inhibiting the growth of *E.coli* and *S. aureus* and showed the antibacterial activity of two extract of *Butea monosparma* and *Psidium guajava* had been determine against *E.coli* and *S. aureus*. The gel formulation taking the combined extract conc. 24.6 mg/mL shows good antibacterial activity. This study indicates that the further attention should be given for research work to identify the active compound for their biological activity.

Table 5. Physical Parameters for gel

Sr. No.		
1	Colour	Yellow
2	Apperance	Transulant
3	Feeling on application	Smooth
4	Homogenicity	No aggregate or clumps
5	pH	6.5-6.8
6	Viscosity	3459 cpm
7	Speradibility	2.34

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How to cite this manuscript: A. Dongarwar, Tulsidas P. Nimbekar*, T. Parshuramkar, N. Indurwade. Preparation and Evaluation of Herbal Formulation Using Natural Extract. *Chemical Methodologies* 3(4), 2019, 451-456. DOI: [10.22034/chemm.2018.146968.1079](https://doi.org/10.22034/chemm.2018.146968.1079).