



Formulation, Evaluation, and Antibacterial Activity of Herbal Gel Containing *Jasminum sambac* Leaves Extract for Ulcer Treatment

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Abstract:

Medicinal From ancient times, plants have been used in traditional medicines for the treatment of different ailments. Plants are one of the richest bioresources for traditional and folk medicines to date. Jasmine is botanically known as *Jasminum sambac* or Jasminie and belongs to the olive family of Oleaceae. Literature reports suggest that Jasmine is analgesic, antidepressant, antiseptic, expectorant, aphrodisiac, sedative, stomachic, diuretic, depurative, astringent, stimulating, anti-oxidizing, anthelmintic, and anti-inflammatory. The objective was to study the antibacterial activity of *Jasminum sambac* extracts against mouth ulcer-causing organisms. The antibacterial activity has been studied against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* & *Enterococcus faecalis* by agar well diffusion method. Leaves extract of *J. sambac* gives effective results against oral pathogens causing mouth ulcers. Acetone and Ethanol extracts displayed good antibacterial activity. The phytochemical studies revealed the presence of Carbohydrates, Proteins, Steroids, Alkaloids, Flavonoids, Phenols, Saponins, Glycosides, and Tannins. *J. sambac* may prove to be an effective medicine for the treatment of ulcers.

Keywords: *Jasminum sambac*, Antibacterial activity, Phytochemical analysis, Oral Pathogens.

Introduction:

From ancient times, plants have been used in traditional medicines for the treatment of different ailments. Medicinal plants are one of the richest bioresources for traditional and folk medicines to date. Around 20,000 medicinal plants have been recorded in India. Only 7,000 - 7,500 plants are used for curing different diseases. The antimicrobial potential and antioxidant activity of plants have attracted the attention of the scientific community since ancient times. This has led to an increase in interest in natural substances exhibiting antimicrobial and antioxidant properties. (1)

The treatment of disease began long ago with the use of herbs. Herbs became the sources of many important drugs due to their wide range of therapeutic and pharmacological effects. (2)

Indian system of medicines comprises Ayurveda, Unani, Siddha, Homeopathy, Naturopathy and Yoga. Each of which uses herbal constituents in some or the other form, crude drug is not so effective because they have not been tested for efficacy according to rigid pharmacological standards. As the constituents derived from the medicinal plants proved to cure human disorders they isolated and used for their pharmacological action.

Jasmine is botanically known as *Jasminum sambac* or Jasminie and belongs to the olive family of Oleaceae. Jasmine is analgesic, antidepressant, antiseptic, expectorant, aphrodisiac, sedative, stomachic, diuretic, depurative, astringent, stimulating, anti-oxidizing, anthelmintic and anti-inflammatory in nature. Furthermore, there are other numerous advantages this amazing plant offers to humanity. These benefits have been attributed to its phytochemical, medicinal, and pharmacological properties. (3)

Jasminum sambac indicated the presence of alkaloids, flavonoids, tannins, terpenoids, glycosides, steroids, essential oils, and saponins. Studies revealed that the plant exerted antimicrobial, insecticidal, antioxidant, antifertility, and dermatological effects. (2)



Fig 1. *Jasminum sambac* (3) **Classification of *Jasminum sambac* (2) Kingdom:** Plantae

Subkingdom:	Viridiplantae
Division:	Tracheophyta
Class:	Magnoliopsida
Order:	Lamiales
Family:	Oleaceae
Genus:	<i>Jasminum</i>
Species:	<i>Jasminum sambac</i>

Health Benefits of *Jasminum sambac*

The aroma of jasmine is calming and soothing without being soporific and is indicated for depression and stress. It is indicated for sensitive skin conditions too. Jasmine also has a reputation as an aphrodisiac and is used for all kinds of sexual problems. *Jasminum* is used to treat skin problems, the leaf juices can be applied to clear up corns and treat mouth ulcerations, and the anti-secretory and anti-oxidant components of *Jasminum* may also treat peptic ulcers. *Jasminum* also produces an antibiotic effect upon typhoid fever and staph infections, They stressed that jasmine oil may serve as a mainstream antibiotic treatment, the juice of the leaf is applied to corns and ear discharges, the leaves and the barks contain salicylic acid and are used as analgesic, febrifuge, etc. The roots are used in the treatment of ringworm, while the flowers are aphrodisiac, antiseptic, antispasmodic, and tonic. One of the uses of *J. sambac* in urinary infections and diuretics the leaves of the stem, bark, and root of *Jasminum* have demonstrated detectable antibacterial activity against many microorganisms.(4)

1) Mouth ulcers

Mouth ulcers are painful sores on the inside lining of the mouth. They usually develop on the inside of the lips and cheeks and on the underneath and edge of the tongue. Medicines from a pharmacist can reduce the pain and help mouth ulcers to heal.

Mouth ulcers include sores, lesions, abrasions, lacerations, or any open break in the mucosa of the lips, mouth, or tongue. Mouth ulcers are also called stomatitis and are a symptom of a variety of mild to serious diseases, disorders, and conditions. Mouth ulcers can result from infection, vitamin deficiencies, trauma, inflammation, malignancy, and other diseases and abnormal processes. (5)

2) Causes

The exact reason of mouth ulcers develop is not yet clearly defined. Approximately 40% of people who get mouth ulcers have a family history of the same. In some cases, the ulcers are related to diseases. These include Injury from badly fitting dentures, harsh brushing of teeth, etc. Changes in hormone levels. Some women find that mouth ulcers occur just before their periods. A lack of iron or a lack of certain vitamins (such as vitamin B12 and folic acid) may be a factor in some cases. Rarely, a food allergy may be the cause. Stress is said to trigger mouth ulcers in some people. Some medicines can cause mouth ulcers. Examples of medicines that can cause mouth ulcers are nicorandil, ibuprofen, etc. Mouth ulcers are more common in people with Crohn's disease, coeliac disease, HIV infection, etc. (6)

3) Bacteriology

In the mouth, there are many good and bad micro-organisms and bacteria, which now have access to the wound surface and produce toxins which in turn allow further cell death causing the ulcer to get larger. Also, at this stage the bacteria lining the ulcer. This situation continues till the causative agent is gone the body's immune system comes up with the solution and the bad bacteria are compressed. How long this takes depends on many factors. *Staphylococcus*, *Pseudomonas*, *Bacillus*, *E.coli*, *Enterococcus*, and *Candida* species are important components of the normal flora of the Oropharynx. (7)

Bacteria:

1. *Escherichia coli*.

2. *Pseudomonas aeruginosa*.

3. *Staphylococcus aureus*.

4. *Bacillus subtilis*.

5. *Enterococcus faecalis*

Fungi

1. *Candida albicans*.

II. Aims & Objective

To prevent side effects from antibiotics on the human body and see the importance of natural antibiotics (Plant extracts) has been undertaken with the following aims & objectives.

- Selection and collection of *Jasminum sambac* leaves from different areas in the Shirur region
- Extraction of plant leaves material.
- Phytochemical analysis of *Jasminum sambac* leaves extract.
- Collection of ulcer samples from the patients having mouth ulcers.
- Isolation and identification of organisms.
- To check the antibacterial activity of *Jasminum sambac* extract against isolated organisms.

III. Materials and Methods

A) Preparation of plant leaf extract

i) Collection of Plant material

Fresh leaves of *Jasminum sambac* were collected from Shirur city. The collected plant leaves were washed with water to remove other undesirable materials. The leaves were spread on the tray and allowed to dry at room temperature under shade for several days. The air-dried leaves of *Jasminum sambac* were crushed. The dried leaves were ground into powder using an electrical blender. The fine powder was stored in an airtight container.

ii) Preparation of extract using soxhlet apparatus (9)

Plant extracts were prepared by using the Soxhlet apparatus. For this 30gm of plant leaves powder was wrapped in muslin cloth and loaded in the thimble of the Soxhlet apparatus. The solvent was added to the round bottom flask. The solvents used were ethanol and aqueous. The boiling point of ethanol is 78°C. The temperature was maintained by using a heating mantle for the recycling of solvent. It was continuously extracted till 3 cycles were complete. The extract was filtered through the Whatman No. 1 filter paper. The Filtrate was collected in a sterilized iodine flask and stored at 5°C in the refrigerator until further use.

Similarly, the same extraction process is carried out using aqueous and acetone solvents.



Fig 2. Powder of *Jasminum sambac* leaves



Fig 3. Extraction of leaves by Soxhlet apparatus

B) Sampling and isolation of oral pathogens

i) Sampling

The samples were collected from different patients suffering from mouth ulcers by using a swab.

ii) Isolation and identification of the organism

The collected samples were inoculated on selective media. HiCrome *UTI Agar*, Eosin Methylene Blue Agar (EMB), MacConkey Agar, *Baird Parker Agar* (B.P), and Nutrient Agar plates were inoculated by swabbing and incubated at 37°C for 24 hrs. The next day colonies which were appeared on Nutrient Agar were further identified by using selective media. Cetrimide Agar for *Pseudomonas aeruginosa* and Luria-Bertani Agar (LB) for *Bacillus subtilis*. The colonies of *Escherichia coli* and *Staphylococcus aureus* appeared on their selective media such as EMB agar, and B.P. Agar respectively. On *UTI Agar* green colour colonies appeared. These isolates were also identified by Gram staining and biochemical tests such as sugar fermentation tests, IMViC, and another test.

After the identification of bacteria, these isolates were inoculated on Nutrient Agar as a pure culture. Then the antibacterial activity of *Jasminum sambac* was tested against these isolated organisms.

C) Phytochemical analysis of *Jasminum sambac* leaves extract (9)

The Phytochemical analysis of plant extracts was carried out by using standard qualitative methods for the identification of active chemical constituents such as sterol, alkaloids, flavonoids, terpenoids, etc., which were identified by characteristics color change development by standard procedures. The ethanolic, aqueous, and acetic extracts of the leaves of *Jasminum sambac* were used for phytochemical analysis.

i) Test for carbohydrates

Benedict's test

To 0.5ml of the leaves extract, 5ml of Benedict's reagent was added and boiled for 5 minutes. The appearance of red precipitate showed the presence of reducing sugar.

ii) Test for Proteins

Biurette's test

To 1ml of the leaves extract, 1ml of 10% sodium hydroxide solution was added and the resulting mixture was heated. To this, a drop of 0.7% copper sulfate solution was added. The formation of a purplish violet color indicated the presence of proteins.

iii) Test for Steroids

Salkowki's test

To 2ml of the leaf extract add 1ml of concentrated sulphuric acid was added carefully along the sides of the test tube. The red color produced in the chloroform layer indicated the presence of steroids.

iv) Test for alkaloids*Wagner's test*

1ml of the leaf extract was acidified by adding 1.5% v/v of HCl and a few drops of Wagner's reagent. The formation of a brown precipitate indicated the presence of alkaloids.

v) Test for Flavonoids

The zinc hydrochloride test to the leaves extract a pinch of zinc dust and concentrated HCl was added. The appearance of a magenta color after a few minutes indicated the presence of flavonoids.

vi) Test for Phenols*Lead acetate test*

1 ml of the leaf extract was diluted with 3 ml of distilled water and to this few drops of 1% aqueous solution of Lead acetate was added. A yellow precipitate was formed which indicated the presence of phenol.

vii) Test for Saponins*Foam test*

To the leaves extract a few drops of sodium bicarbonate solution were added. Shaken vigorously and kept for 3 minutes. A honeycomb-like froth was formed indicating the presence of saponins.

viii) Test for Glycosides*Legal's test*

To the leaves extract, 1ml of sodium nitroprusside solution was added and then it was made alkaline with sodium hydroxide solution. The appearance of pink to red color confirmed the presence of glycosides.

ix) Test for Tannins*Lead acetate test*

To 5ml of the extract add few drops of 1% solution of lead acetate are added. A yellow precipitate was formed indicating the presence of tannins.

D) Formulation of Herbal Gel:-**I) Chemicals:-**

Tincture Benzoin and Glycerine

II) Procedure:-

- Take the drug (extract of leaves)
- Freshly prepare the tincture of benzoin (Benzoin + Ethanol) and mix in the extract.
- Add the glycerine.
- Mix all the ingredients and prepare the gel.

E) Antibacterial Assay (10)

The antibacterial activity was determined by the agar well diffusion method. Muller Hinton Agar and Petri plates were sterilized and cooled. Muller Hinton agar (25 ml) was then poured into the sterilized petri plates and allowed to solidify. Then 8 hours inoculated young cultures of the test organism were spread uniformly with the help of a sterile cotton swab on Muller Hinton Agar plates. After that well was made in the plate with the help of a sterile cork borer (8 mm) and filled with the leaf extract. For comparative study, the well loaded with ethanol serves as a control. The plate was then incubated at 37°C for 24 hrs. and was observed for the zone of inhibition.

Results and Discussion:

From the collected oral samples following bacteria were isolated and identified by gram staining and biochemical tests.

Bacteria found were *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* and *Enterococcus faecalis*

Table 1: Morphological characteristics of organisms

Organisms	Agar	Colony Character	Gram Character	Motility
<i>E. coli</i>	EMB agar	Green metallic sheen	Gram -ve	Motile
<i>P. aeruginosa</i>	Cetrimide agar	yellow-green color	Gram -ve	Motile
<i>S. aureus</i>	BP agar	Black colour	Gram +ve	Non-Motile
<i>B. subtilis</i>	Luria-Bertani agar (LB)	White colour	Gram +ve	Motile
<i>E. faecalis</i>	UTI agar	Green colour	Gram +ve	Motile



Fig 4. *E. coli* on EMB agar



Fig 5. *P. aeruginosa* on Cetrimide agar



Fig 6. *S. aureus* on BP agar



Fig 7. *B. subtilis* on LB agar

Table 2: Biochemical test- Sugar fermentation test

Sugar Organisms	Glucose		Lactose		Mannitol	
	Acid	Gas	Acid	Gas	Acid	Gas
<i>E. coli</i>	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
<i>P. aeruginosa</i>	+ ve	- ve	- ve	- ve	- ve	- ve
<i>S. aureus</i>	+ ve	- ve	+ ve	- ve	+ ve	- ve
<i>B. subtilis</i>	+ ve	- ve	- ve	- ve	- ve	- ve
<i>E. faecalis</i>	+ ve	- ve	+ ve	- ve	+ ve	- ve

Table 3: Biochemical test- IMViC test

Test / Organisms	Indole	Methyl Red	Voges Proskauer	Citrate	Coagulase
<i>E. coli</i>	+ ve	+ ve	- ve	- ve	--
<i>P. aeruginosa</i>	- ve	- ve	- ve	+ ve	--
<i>S. aureus</i>	- ve	+ ve	+ ve	- ve	+ve
<i>B. subtilis</i>	- ve	- ve	+ ve	+ ve	--
<i>E. faecalis</i>	- ve	- ve	+ ve	- ve	--



Fig 8. Biochemical test for *E. coli*



Fig 9. Biochemical test for *P. aeruginosa*



Fig 10. Biochemical test for *S. aureus*

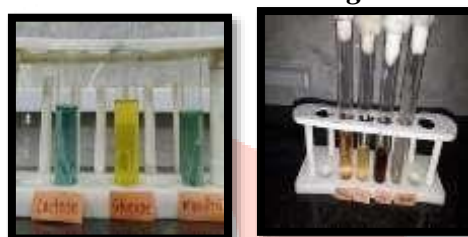


Fig 11. Biochemical test for *B. subtilis*



Fig 12. Biochemical test for *E. faecalis*

Table 4: Effect of extracts on isolated oral pathogens

Sr. No.	Organisms	Zone of inhibition of <i>J. sambac</i> leaves (aqueous extract in mm)	Zone of inhibition of <i>J. sambac</i> leaves (ethanolic extract in mm)	Zone of inhibition of <i>J. sambac</i> leaves (acetic extract in mm)	Control (in mm)
1	<i>E. coli</i>	18	23	13	21
2	<i>P. aeruginosa</i>	12	19	24	20
3	<i>S. aureus</i>	9	17	16	20
4	<i>B. subtilis</i>	10	21	20	15
5	<i>E. faecalis</i>	14	19	25	12

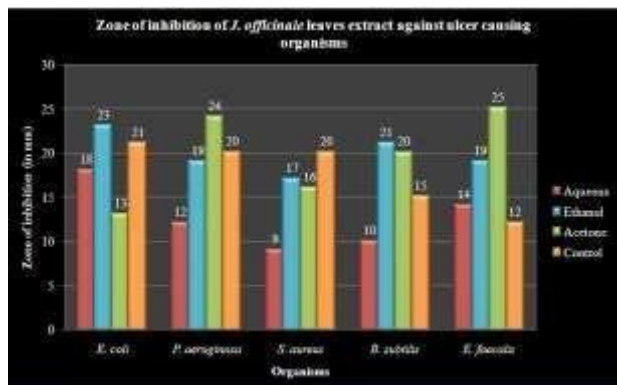


Fig 13. Graphical representation of the effect of *J. sambac* extracts against ulcer-causing oral pathogens. From the above results, it was observed that *J. sambac* give effective result against oral pathogens.

In the antibacterial activity of leaves of *J. sambac* extract, the zone of inhibition shown by *E. coli* was 13mm in Acetone extract, 18mm in Aqueous extract, and 23mm in ethanolic extract. The zone of inhibition shown by *P. aeruginosa* was 12mm in aqueous extract, 24mm in acetone extract, and 19mm in ethanolic extract. The zone of inhibition shown by *S. aureus* was 16mm in Acetone extract, 17mm in Ethanolic extract, and 9mm in Aqueous extract. The zone of inhibition shown by *B. subtilis* was 21mm in Ethanolic extract, 20mm in Acetone extract, and 10mm in Aqueous extract. The zone of inhibition shown by *E. faecalis* was 25mm in Acetone extract, 14mm in Aqueous extract, and 19mm in Ethanolic extract.

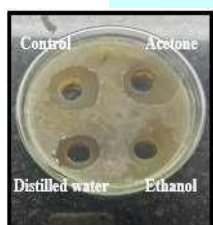


Fig 14. Antibacterial activity shown by *E. coli* **Fig 15. Antibacterial activity shown by *P. aeruginosa***

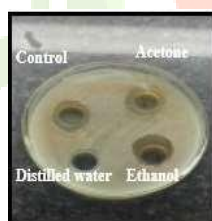
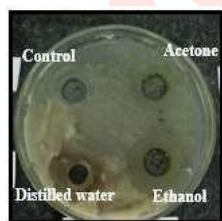


Fig 16. Antibacterial activity shown by *S. aureus* **Fig 17. Antibacterial activity shown by *B. subtilis*** **Fig 18. Antibacterial activity shown by *E. faecalis***

Table 5: Phytochemical analysis

Sr. No.	Bioactive components	Aqueous extract of leaves	Ethanolic extract of leaves	Acetonic extract from leaves
1	Carbohydrates	-	+	-
2	Proteins	+	-	+
3	Steroids	+	-	-

4	Alkaloids	+	+	+
5	Flavonoids	-	+	-
6	Phenols	+	+	+
7	Saponins	+	-	-
8	Glycosides	+	+	+
9	Tannins	+	+	+

The crude extracts of leaves of *J. sambac* indicate the accumulation of alkaloids, saponins, tannins, flavonoids, glycosides, and terpenoids. Thus results in the present study can be attributed to the presence of these chemical constituents.

DISCUSSION

Aqueous extract showed maximum activity against *B. subtilis* (19 mm), *E. coli* (17 mm), *S. aureus* (16 mm), *P. aeruginosa* (16 mm) and *E. faecalis* (15 mm). (8)

Ethanol extract showed maximum activity against *S. aureus* (28.2mm), *E. faecalis* (15.7mm), *E. coli* (22.3mm) and *P. aeruginosa* (23.8mm). (4)

The preliminary phytochemical analysis of the aqueous extract of *Jasminum sambac* leaves indicated the presence of alkaloids, coumarins, flavonoids, tannins, terpenoids, glycosides, emodine, leucoanthocyanins, steroids, anthocyanins, phlobatannins, essential oil and saponins. (2)

In the present study, the bacteria found were *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* and *Enterococcus faecalis* were isolated and identified from collected oral flora of patients. Then the antibacterial activity of *J. sambac* leaf extract in Aqueous, Ethanol, and Acetone was analyzed against these isolated oral pathogens.

On the basis of the results, Acetone extract shows maximum inhibition to *E. faecalis* (25mm), *P. aeruginosa* (24mm) followed by *B. subtilis* (20mm), *S. aureus* (16mm), and *E. coli* (13mm)

Ethanol extract shows maximum inhibition to *E. coli* (23mm) followed by *B. subtilis* (21mm), *P. aeruginosa* (19mm), *S. aureus* (17mm), and *E. faecalis* (19mm)

Aqueous extract shows maximum inhibition to *E. coli* (18mm) followed by *E. faecalis* (14mm), *P. aeruginosa* (12mm), *B. subtilis* (10mm) and *S. aureus* (9mm).

Conclusion:

Antibacterial activity of *J. sambac* leaf extract in Aqueous, Ethanol, and Acetone was analyzed against some ulcer-causing organisms such as *E. coli*, *P. aeruginosa*, *S. aureus*, *B. subtilis*, and *E. faecalis* by agar well diffusion assay on Muller Hinton agar media plate.

On the basis of the results, Acetone extract shows maximum inhibition to *E. faecalis*, and *P. aeruginosa* followed by *B. subtilis*, *S. aureus* and *E. coli*.

Ethanol extract shows maximum inhibition to *E. coli* followed by *B. subtilis*, *P. aeruginosa*, *S. aureus*, and *E. faecalis*.

The aqueous extract shows maximum inhibition to *E. coli* followed by *E. faecalis*, *P. aeruginosa*, *B. subtilis*, and *S. aureus*.

From the above results, we can conclude that *J. sambac* has remarkable antibacterial activity.

The medicinal values of the plant leaves may be due to these specific groups of phytochemicals present in them. Phenolic compounds are well-known as antioxidant and scavenging agents for free radicals associated with oxidative damage. Tannins are known to be useful in the treatment of inflamed or ulcerated tissues and they have remarkable anticancer properties. It has been confirmed that the pharmacological effect of flavonoids is also correlating with their antioxidant activities. (11)

Due to these properties of *J. sambac*, this can be useful in the development of new drugs. We can use natural medicines in place of antibiotics in the future.

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