

Assessment of Antimicrobial Efficacy of Saffron - Withania Somnifera Herbal gel Against oral ulcers- An Invitro Study

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How to cite this article: Madhumitha M, Devika S Pillai, Rajesh Kumar S (2024) Assessment of Antimicrobial Efficacy of Saffron - Withania Somnifera Herbal gel Against oral ulcers- An Invitro Study. *Library Progress International*, 44(3), 14551-14558.

ABSTRACT

Background: An ulceration is a full-thickness epithelial break. This may cause discomfort and make it harder to speak, eat and drink. It might be difficult to identify oral ulcerative lesions due to the wide range of presenting symptoms and causal variables. There are both systemic and local causes that might lead to ulcer development

Aim and Objective: To evaluate the efficacy of herbal extracts of saffron, withania somnifera, Dry ginger, Tulsi as an antimicrobial agent in oral ulcers

Materials and methodology: Mueller Hinton agar plates were prepared and sterilized using an autoclave at 121°C for 15-20 minutes. After sterilization, the bacterial suspension was made to spread evenly onto the agar plates using sterile cotton swabs. Wells of 9mm diameter were created in the agar plates using a sterile polystyrene tip. The wells were then filled with different concentrations of withania somnifera, dry ginger, tulsi and saffron. The plates were incubated at 37°C for 24 hours and 48 hours for fungal cultures and then antimicrobial properties were evaluated.

Results: Antimicrobial activity of the gel was highest against E.coli when the amount of concentration increased from 25 ug/ml to 100 ug/ml while the lowest antimicrobial activity was against Streptococcus aureus.

Conclusion: This study gives as a clear understanding that the naturally occurring constituents of medicinal herbs will be able to resolve oral ulcers irrespective of their etiology. Hence, herbal medicines can be used as an adjunct to synthetic drugs available commercially.

Key words: Withania somnifera, Tulsi, Dry ginger, Saffron

Introduction

Oral diseases manifest in different degrees as mucosal alterations, ranging from pigmentation to white or hyperkeratotic patches to ulceration, erythema, or atrophy. Mucosal ulceration and anomalies of the lips and tongue are the most frequent appearances. An ulceration is a full-thickness epithelial break. This may cause discomfort and make it harder to speak, eat and drink. A single resolving event, a single permanent ulcer, recurrent but intermittent episodes, and more extensive persistent ulceration are the four primary forms of ulceration. ^[1] It might be difficult to identify oral ulcerative lesions due to the wide range of presenting symptoms and causal variables. There are both systemic and local causes that might lead to ulcer development.^[2] They are rather common, and while most are caused by trauma or recurrent episodes, some may be an indication of an underlying systemic disease or the outcome of a malignant condition, mainly oral cancer.^[3]

Even though many new treatment approaches for the disease have been emerging it seems to be challenging due to its recurrence. Plants are the main source of therapies in contemporary complementary and alternative medicine, and any part of a plant, including the seeds, root, stem, leaves, and fruit, may contain bioactive components. One

of the most revered and holistic herbs used in Indian traditional medicine for a long time is Tulsi, it has been discovered to have healing effects in practically every part of the plant.^[4] There are several different medical benefits associated with tulsi. It has many beneficial qualities, including antibacterial, anti-inflammatory, and antioxidant.^[5]

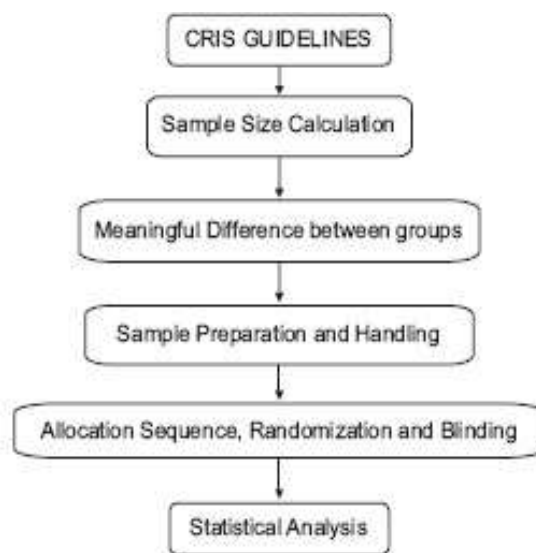
The Zingiberaceous plants are distinguished by their tuberous or non-tuberous rhizomes and contain potent fragrant and therapeutic characteristics. Ginger can be used to treat bacterial infections since it has direct antimicrobial properties.^[6] The plant *Withania somnifera* (L.) Dunal, often known as "Ashwagandha," is a member of the Solanaceae family. It is a component of several prescription formulations for a range of musculoskeletal problems. Recently, several authors examined the antibacterial effectiveness of *W. somnifera* on control isolates.^[7] The costliest spice in the world, saffron (*Crocus sativus* Linn.), which belongs to the Iridaceae family, is frequently used in traditional medicine. Saffron has potent antioxidant, anti-inflammatory, antimicrobial and analgesic properties, due to the presence of Crocin as a unique carotenoid.^[8]

The aim of this study is to evaluate the antimicrobial efficacy in herbal gel containing Saffron, *Withania somnifera*, Tulsi, and dry ginger as an alternative therapeutic modality in the management of oral ulcers.

Materials and methodology

The study was approved by Institutional Human Ethical Committee (Ref No. IHEC/SDC/OMED-2002/21/54). Checklist for Reporting *in-vitro* Studies (CRIS) guidelines was followed for conducting this study.

Flowchart 1 shows the items in CRIS guidelines.



Flowchart 1: Showing items in CRIS (checklist for reporting *in vitro* studies) guideline

The extract is prepared from *Crocus sativus*, *Withania somnifera*, *Ocimum sanctum*, *Gingiber officinale* roscole which have therapeutic properties and potential benefits to health.

Sample preparation and Handling

This herbal formulation consists of a combination of 4 components - Saffron, *Withania somnifera*, Dry ginger, and tulsi.

1g powder of tulsi, withania somnifera, dry ginger were mixed with 100ml of distilled water separately and boiled in heating mantle at 50 to 60 degree for 15 minutes, then filtered and 90ml of extract was obtained. On the other hand 0.5 g saffron was added to 200ml of distilled water, kept in orbital shaker for 24hrs and then boiled at 50 to

60 degree for 15 min and then filtered, extract was obtained. 50 ml of all four components are combined and concentrated for 20ml. Finally anti-inflammatory, antimicrobial, antioxidant, cytotoxic activities were tested by adding 5 ml extract to empty gel containing a combination of carboxylate methyl cellulose 5g, carbapel 5g.

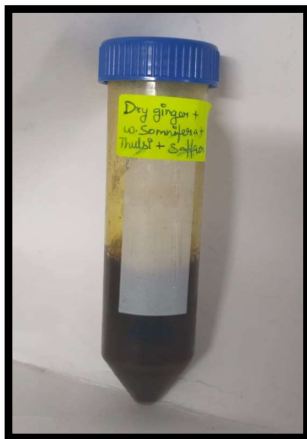


Diagram 1-Extract preparation of Withania somnifera, dry ginger, tulsi, saffron

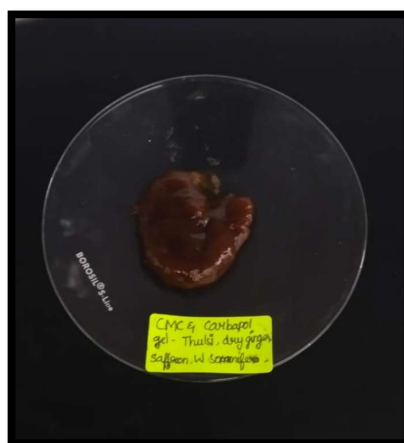


Diagram 2- Extract added to carboxylate methyl cellulose and carbapel gel

Antimicrobial activity

The antimicrobial activity of saffron, withania somnifera, tulsi, dry ginger was evaluated using the agar well diffusion technique. Mueller Hinton agar plates were prepared and sterilized using an autoclave at 121°C for 15-20 minutes. After sterilization, the medium was poured on to the surface of sterile Petri plates and allowed to cool to room temperature. The bacterial suspension (*S.aureus*, *E.coli*, *E.faecalis*, *P.acidophilus*) spreads evenly onto the agar plates using sterile cotton swabs. Numerous studies have sought to link Recurrent Aphthous stomatitis(RAS) to a variety of microbes, such as Epstein-Barr virus, *Streptococcus sanguinis* 2A, *Helicobacter pylori*, *Lactobacillus*, and other bacteria in the genus *Streptococcus*. RAS is the type of ulcerative lesion commonly seen in oral cavity, some of these microbes were used for testing antimicrobial activity of oral ulcers.[9]

Wells of 9mm diameter were created in the agar plates using a sterile polystyrene tip. The wells were then filled with different concentrations (25 µg, 50 µg, 100 µg) of Withania somnifera, saffron, dry ginger, tulsi. An antibiotic (e.g *Bacteria*-Amoxyrite, *Fungi*- Fluconazole) was used as a standard. The plates were incubated at 37°C

for 24 hours and 48 hours for fungal cultures. The antimicrobial activity was evaluated by measuring the diameter of the inhibition zone surrounding the wells. The diameter of the zone of inhibition was measured using a ruler and recorded in millimeters (mm) and the zone of inhibition was calculated.

Statistical analysis

The normality was checked based on the Shapiro–Wilk Test ($\alpha = 0.05$). The zone of inhibitions of Tulsi, Withania, Dry Ginger and Saffron were evaluated by the parametric One-way ANOVA and Turkey Kramer test for multiple independent groups and independent t-test for two independent groups. The significance (P -value) was set at 0.05.

Results

Antimicrobial activity of ingredients like saffron, tulsi, withania somnifera and dry ginger has been tested after preparing a gel against many pathogens like *S. aureus*, *E. coli*, *E. Faecalis*, *P. Acidophilus* against a standard antibiotic. The agar well diffusion technique was used for evaluation. The results indicate that at concentrations of 22 micrograms and 20 micrograms, Withania somnifera, Tulsi, Saffron, Dry ginger gel showed greater Antimicrobial activity against *S. aureus* and *E. Coli* when compared with that of standard antibiotic. The study involves a gel containing Tulsi, dry ginger, saffron, and *Withania somnifera* at different concentrations (25 $\mu\text{g/mL}$, 50 $\mu\text{g/mL}$, 100 $\mu\text{g/mL}$), compared to a standard (Std) and a control. Each bar represents the optical density (OD) values measured at different time points, with the control showing the highest OD, indicating less bacterial inhibition, while the tested concentrations demonstrate varying degrees of bacterial growth suppression (graph 2). Graph 3 presents two bar charts showing the optical density (OD) over time (from 1 to 4 hours) for bacterial growth inhibition of *Staphylococcus aureus* (top chart) and *Enterococcus faecalis* (bottom chart). The effects of a gel containing Tulsi, dry ginger, saffron, and *Withania somnifera* at different concentrations (25 $\mu\text{g/mL}$, 50 $\mu\text{g/mL}$, 100 $\mu\text{g/mL}$) are compared against a standard (Std) and a control. The control group exhibits higher OD values, indicating less inhibition of bacterial growth, while the treated groups show varying levels of growth suppression, with some doses demonstrating more effectiveness over time.

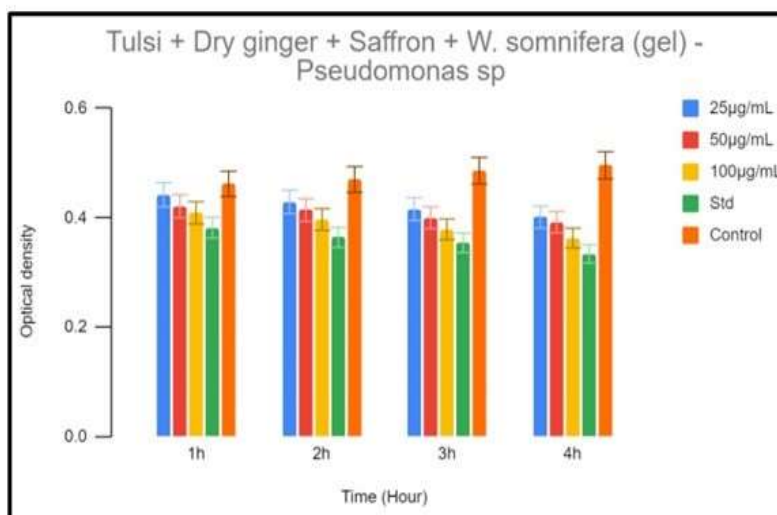
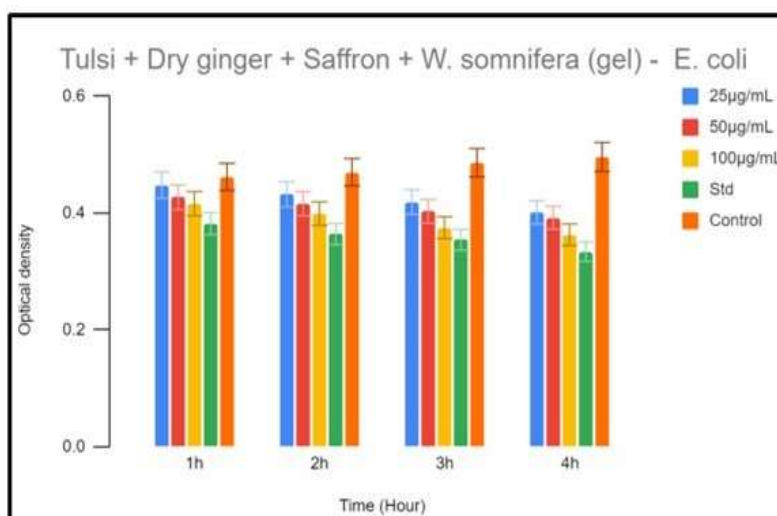
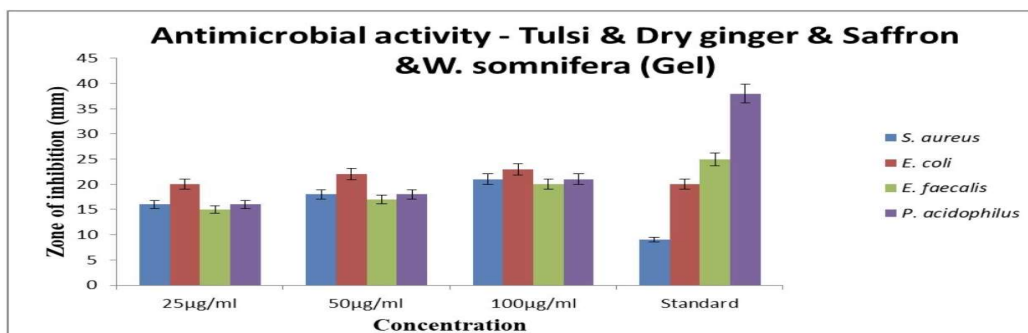
Table 1 shows the activity of the formulation against *S. aureus* with prevalence value of 0.868

	1h	2h	3h	4h	F value	p-value
25 $\mu\text{g/mL}$	0.438	0.422	0.409	0.39	0.238	0.868
50 $\mu\text{g/mL}$	0.418	0.406	0.391	0.379		
100 $\mu\text{g/mL}$	0.403	0.393	0.378	0.364		
Std	0.382	0.364	0.354	0.334		
Control	0.457	0.478	0.491	0.506		

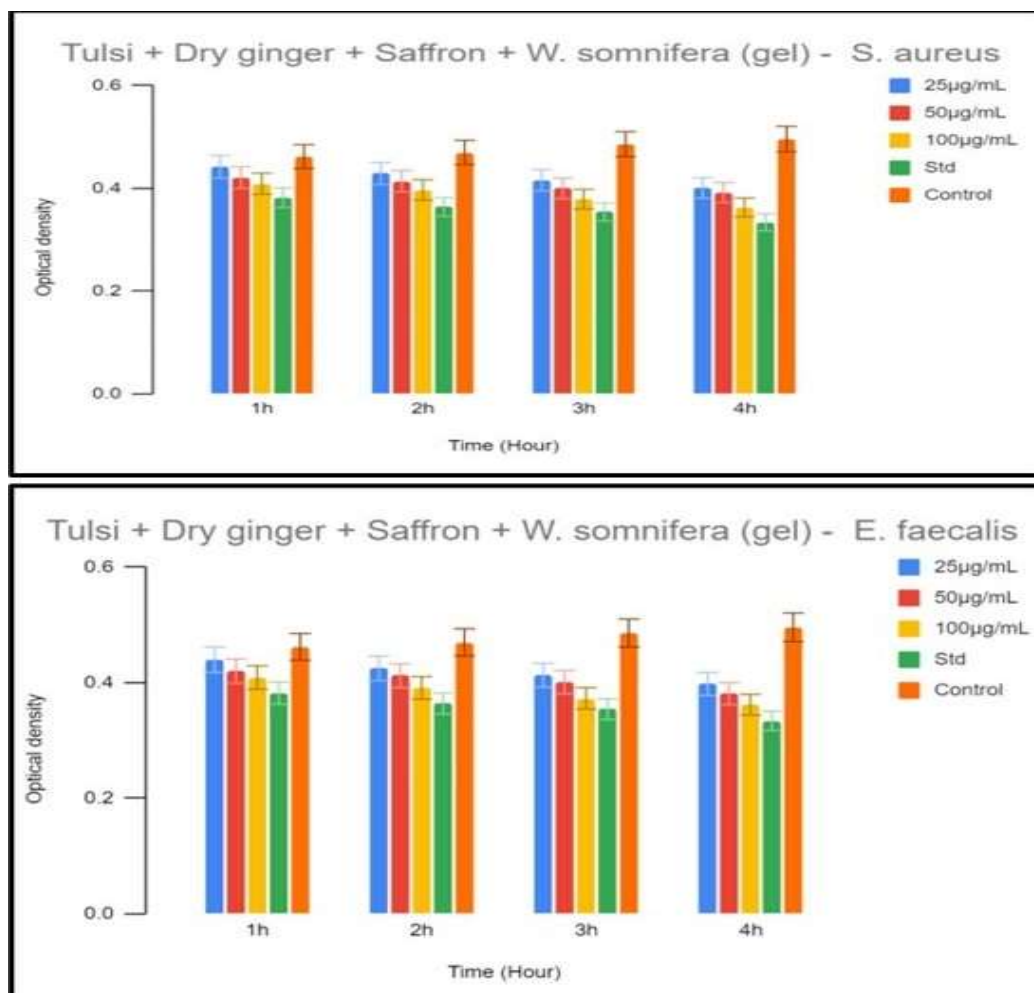
Table 2 shows the activity of the formulation against *E. faecalis* with prevalence value of 0.891

	1h	2h	3h	4h	F value	p-value
25 $\mu\text{g/mL}$	0.438	0.427	0.41	0.396	0.206	0.891
50 $\mu\text{g/mL}$	0.413	0.402	0.389	0.375		
100 $\mu\text{g/mL}$	0.401	0.39	0.373	0.359		
Std	0.382	0.364	0.354	0.334		
Control	0.463	0.486	0.499	0.511		

Graph 1 shows Zone of inhibition , it is higher (39mm) in *p.acidophilus* in standard group , At 25 mug/ml Zone of inhibition for *S.aureus* at is 16mm at 50 microgram/dl it is 18mm, at 100mug/dl it is 20mm, zone of inhibition for *E. Coli* is 20mug/dl ,at 50 microgram/dl it is 27mm, at 100mug/dl it is 24mm, zone of inhibition for *E.coli* is 14mm at 25 microgram/dl, 17mm at 50 microgram/dl, 20mm at 100mug/dl , for *acidophilus* it is 16mm at 25 microgram/dl, 18mm at 50 microgram/dl, 21mm 100mug/dl (graph1), optical dentistry were tested for *S. aureus*, *E. coli*, *E. Faecalis*, *P. Acidophilus*



Graph 2 shows optical density for E. Coli and Pseudomonas Sp which showed that optical density is nearly coinciding (0.5mm) with that of standard group at 1hr and it decreases after 2nd, 3rd and 4th hour.



Graph 3 shows optical density for S.aureus and E faecalis

Discussion

Since oral ulcers are recurring disease occurring due to multifactorial etiology, identification of drug formulation is difficult. Traditional medicine has various advantages like less side effects and cost effectiveness. This study is carried out to identify whether different traditional ingredients are useful in the management of oral ulcers.

Ocimum gratissimum, also known as Nimba tulasi locally, is a common plant in South Africa and India as well as other tropical parts of the world. It is a well-known herb with many different phytochemical components and has been noted for having a variety of physiological qualities.^[10,11] According to Hanaa A Yamini et al Tulsi oil has bacteriostatic activity against *S. aureus*, including MRSA and *E. coli*, but reduced activity against *P. aeruginosa* at concentrations of 2.25-2.5 g/ml.^[12] The present study demonstrated the antimicrobial activity of tulsi against *S. aureus* and *P. aeruginosa* at different concentrations. According to Miri park et al, by using activity-guided assays, it was discovered that [10]-gingerol and [12]-gingerol from the ginger rhizome had powerful antibacterial properties in vitro against anaerobic bacteria linked to periodontitis of the human oral cavity.^[13] According to kochuthressia et al, the ethanolic extract from the leaves of ginger was rated as being only somewhat effective, whereas the ethanolic extract from the rhizome was the most successful at inhibiting bacterial development.^[14] According to Punam Bisht et al, at a dosage of 2 mg/ml, a good inhibitory zone against *Enterococcus* spp. and *S. aureus* was seen.^[15] The antimicrobial activity of ginger was evaluated in the present study and when used with other components, the activity was higher against *Pseudomonas* followed By *E. Coli*. OvaisM et al stated that, Salmonellosis can be treated along with other bacterial infections with the active chemical compounds found in *W. somnifera*.^[16] According to Mir MA et al, an active ingredient which is common in stigma, corolla and stamen which may be responsible for the antimicrobial activity against the selected pathogens.^[17] According to Jadouali SM et al, A source of chemicals with antibacterial action against

the tested bacteria, particularly against *Listeria* spp., is *Crocus sativus* leaf extract. ^[18]

The present research showed increased Antimicrobial activity at 20 and 22 micrograms and optical density of 0.42 in 25 micrograms at 1hr which showed that it has increased Antimicrobial activity at that rate against *E.coli*, *S.Pseudomonas*, *S.aureus*, *E.faecalis*, hence it is proved and combination of *Withania somnifera*, dry ginger, tulsi and saffron has increased Antimicrobial activity when compared the effect individually.

Conclusion

In this formulation, 4 herbal components are included like Saffron- analgesic property, *Withania somnifera*- anti inflammatory and anti-stress rejuvenator, Tulsi & Dry ginger with antimicrobial properties. This study gives a clear understanding that the naturally occurring constituents of medicinal herbs will be able to resolve oral ulcers irrespective of their etiology.

Hence, herbal medicines can be used as an adjunct to synthetic drugs available commercially.

Limitations

This study has performed the antimicrobial efficacy of *withania somnifera*, tulsi, dry ginger and saffron, further studies like Anti-Inflammatory, cytotoxic properties are required to proceed with gel formulation in treatment of recurrent aphthous stomatitis

Acknowledgement

Nil

Funding

Nil

Conflicts of interest

Nil

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