J. Pharm. Res. 2022;21(4):123–127

Print ISSN: 0973-7200
Online ISSN: 2454-8405

Journal of Pharmaceutical Research

Research Article

Studies of Antibacterial Activity of the Seed Extract of *Solanum surattense* Burm. F .

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ARTICLE INFO

Article history:
Received 05.12.2022
Revised 23.12.2022
Accepted 27.12.2022
Published 30.12.2022

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https://doi.org/10.18579/jopcr/v21i4.22.24

ABSTRACT

Crude Flavonoids extracted from crushed seeds of dried ripe fruits of *Solanum surattense* Burm F. were screened for antimicrobial activity against two bacteria – *Streptococcus mutans* and *Aggregatibacter actinomycetemcomitans* – which were found to be major contributers for occurrence of Dental caries. Minimum inhibitory concentration, Minimum bactericidal concentration, and Time Kill curve of the extract against each sensitive test pathogen, were evaluated. The flavonoid extract showed good antimicrobial activity against *Streptococcus mutans* than towards *Aggregatibacter actinomycetemcomitans*. MIC & MBC of *Streptococcus mutans* is 25μg/mL & 25μg/mL respectively. MIC & MBC of *Aggregatibacter actinomycetemcomitans* is 50μg/mL & 50μg/mL respectively. Time Kill Curve of *Streptococcus mutans* and *Aggregatibacter actinomycetemcomitans* was 2hr and 2hr respectively.

Keywords: Solanum surattense; Streptococcus mutans; Aggregatibacter actinomycetemcomitans; Minimum inhibitory concentration; Minimum bactericidal concentration; Time kill curve

INTRODUCTION

Microbial infections are one of the important health problems faced by the humanity despite availability of many antimicrobial agents. Hence, there is a continuous effort by scientists in order to identify new antimicrobial agents that help to treat the infectious disease. The need for newer antibiotic sources has increased as the occurrence of multidrug resistant microorganisms has increased. From time immemorial, plants continue to be the most important source of antimicrobial agents as ethnopharmacologically a range of plants are used against different infections. Plants comprise a variety of secondary metabolites, the most important of which are tannins, alkaloids, flavonoids, steroids, and other compounds. As a result, science has stretched its wings to investigate traditional and folk medical agents in order to extract therapeutic benefits from these approaches and discover unique and potent antimicrobial agents for the benefit of humanity. [1–3]

Dental caries, often known as tooth decay, is a prevalent chronic disease that affects people across the world. Dental caries is the decay of teeth caused by generation of acid in the oral cavity as a result of bacterial colonies in the mouth that ferment and digest carbohydrates. Tooth decay is a serious condition that causes excruciating pain, damages the tooth and its surrounding areas. It also has a negative impact on one's quality of life. Streptococcal species like Streptococcus mutans, as well as some acid-forming facultative anaerobes such as *Aggregatibacter actinomycetemcomitans*, cause dental caries. [4–7] *Streptococcus mutans* is a bacterial species that can be found in the mouth and has been linked to disorders such as periodontitis and gingivitis. It is a gram-positive, facultatively anaerobic cocci that is widely discovered in human oral cavity. It uses the enzyme glucansucrase to convert sucrose to lactic acid, which contributes to tooth damage. It's also thought to be a major contributor to the production of dental plaque. *S. mutans* converts sucrose, as well as glucose, fructose, and lactose, to lactic acid, greatly increasing the risk of tooth decay. [8–10] *Aggregatibacter actinomycetemcomitans* is another bacterial species that plays a function in aggressive periodontitis that

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is localised. It's a non-motile, gram-negative, facultatively anaerobic bacteria. Localized aggressive periodontitis is a serious periodontal disease. It is thought that the pathogenic component Leukotoxin A is responsible for its pore-forming action (LtxA). According to Topley & Wilson's classification, it's also known as *Actinobaccillus actinomycetemcomitans*.[5,11,12]

Solanum surattense Burm. F. (Solanaceae) is a medicinal plant native to areas of India's Himalayas. The seeds of *S. surattense* dried ripe fruit, often known as yellow-fruit nightshade is mostly used in traditional medicine, particularly in Ayurveda, to treat finger abscesses, cough, asthma, and chest pain.[13,14] *S. surattense* is also used in topical skin diseases like eczema and scabies, as an anti-diabetic, and in cardiac diseases due to its anti-inflammatory characteristics. The seeds are used to alleviate toothache as a form of fumigation.[15–17]

In view of the above facts, we intended to test the antibacterial potency of the fruit extract of *S. surattense* against bacteria that cause dental diseases.

MATERIALS AND METHODS

Plant Collection

The plant specimen was collected from the ripe berries of *Solanum surattense* Burm F. in Chowdavaram, Guntur Dist., Andhra Pradesh, India. It was identified as the berries belonging to *Solanum surattense* Burm. F. of Solanaceae family by the Department of Botany, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, Guntur Dist. Andhra Pradesh, India.

Phytochemistry

Chemicals and Reagents

The chemicals and reagents used for extraction and detection of constituents of the dried ripe fruit seeds of *Solanum surattense* were laboratory grade Fehling's reagent – A & B, Wagner's reagent; 1% lead acetate solution, Ethanol, Distilled water obtained from Merck Industries Pvt. Ltd.

Preparation of Crude plant extract

The seeds of dried ripe fruits of *S. Surattense* were taken for extraction. A finely powdered sample (100 g) of the seed was extracted with water in ethanol (50 mL each) for 24 h. The extracts were concentrated by drying at 60°C under reduced pressure. The extracts thus obtained were stored in airtight glass vials for further use.[18]

Identification of Phytoconstituents

10 g of the crude seed extract was dissolved in 10 ml of water : ethanol (50:50) mixture and used for the identification of the phytoconstituents. This solution was labelled as test solution.

Identification of Carbohydrates

To 1ml of the test solution, a few drops of Fehling's A & B reagent was added and boiled for a few minutes. Occurrence of red colour indicated the presence of carbohydrates.[19]

Identification of Alkaloids

To 3ml of the test sample, a few drops of Wagner's reagent was added and kept aside for few minutes. Formation of brownish precipitate indicated the presence of alkaloids.[20]

Identification of Flavonoids

1ml of test sample was combined with pieces of magnesium ribbon and concentrated HCl. After a few minutes, appearance of pink tint indicated the presence of flavonoids.[21]

Identification of Tannins and Phenols

To 1 ml of the solution, 0.5 ml of 1% lead acetate solution was added. The mixture is shaken and kept aside for a few minutes. Formation of dark bluish black colour indicated the presence of Tannins and Phenols.[22]

Extraction of Flavonoids

To extract the flavonoids, 50 gm of powdered plant material was placed in 1 litre of ethanol and refluxed for 24 hours. The crude extract was filtered and concentrated by evaporating the solvent in a rotary evaporator.[23]

Study of Antimicrobial activity of Solanum surattense seed extract

Selected test microorganisms

Pathogenic microorganism selected for the study included *Streptococcus mutans* (ATCC 25175), *Aggregatibacter actinomycetemcomitans* [*Actinobacillus actinomycetemcomitans*] (ATCC 33384). The selected microorganisms were procured from Maratha Medical College, Belgaum, India. The bacterial strains were grown and maintained on 'Brain heart infusion broth medium' (HIMEDIA M210 (500g) - calf brain infusion (200g/L) beef heart infusion (250g/L) protease peptone (10g/L) dextrose (2g/L) sodium chloride (5g/L) disodium phosphate (5g/L) Final pH 7.5±0.2)

Minimum Inhibitory Concentration

Using the disc diffusion experiment, the minimum inhibitory concentration (MIC) of the seed extract that showed antibacterial activity against test pathogens was determined. The MIC was studied using the HIMEDIA M210 medium. The extract was diluted nine times in Thioglycollate broth to determine the MIC. 20 microliters of extract was added to 380 microliters of Thioglycollate broth in the first tube. Dilutions were made by adding 200 microliters of Thioglycollate broth to each of the next 9 tubes sequentially. Then 200 microliter was transferred from the first tube to the first tube containing 200 microliter of

Thioglycollate broth. This was regarded as a dilution of 10-1. To create a 10-2 dilution, 200 microliter was transferred from the 10-1 diluted tube to the second tube. The serial dilutions were performed up to a 10-9 dilution. 5 microliter was collected from the needed organisms' stock cultures and added to 2 ml of Thioglycollate broth. 200 microliters of the aforesaid culture suspension were added to each serially diluted tube. The tubes were cultured in an anaerobic jar at 37°C for 48-72 hours and then checked for turbidity. [24]

Minimum Bactericidal Concentration

The first 3 or 5 tubes from the MIC dilutions tubes were plated (which was sensitive in MIC) and incubated for 24 hours, after which the colony count was obtained the next day. MBC is used to determine whether the extract (Drug) has a bactericostatic or bactericidal impact on the organism. If there is no growth, the impact is bactericidal. If there is growth, the bacteriostatic effect is at work. Tubes were incubated in a CO_2 Jar at 37°C for 48-72 hours for facultative anaerobes. Tubes were cultured in anaerobic jars for 48-72 hours for strict anaerobes. [24]

Time Kill Curve

After mixing an equal amount of broth with the organism and chemical, it was immediately plated, and the time was recorded as 0 hours. Tubes were stored in the CO_2 jar until a later time slot was available. It was cultured or plated every 5 minutes, 10 minutes, 30 minutes, and 2 hours, and incubated according to the growth requirements, i.e., in CO_2 and anaerobic jars. The plates were removed after 48-72 hours of incubation and the colony count was recorded. [24]

RESULTS

Phytochemistry

The crude extract from the powdered seed of dried ripe berries of *S. surattense* was subjected to different tests for identification of various phytoconstituents. The results obtained are given in Table 1.

Table 1: Presence of different phytoconstituents in the crude extract of seeds ofSolanum surattense

Sl. No.	Test	Reagent	Presence/ Absence
1.	Carbohydrates	Fehling's A & B	++
2.	Alkaloids	Wagner's reagent	++
3.	Flavonoids	Mg ribbons + Conc. HCl	+++
4.	Tannins and Phenols	1% lead acetate solution	+

(+++) Good amount, (++) Appreciable amount, (+) Small amount

After identification of phytoconstituents, the dried berries were subjected to extraction of flavonoids for the study of antimicrobial activity. The method employed in the extraction of flavonoids was percolation method using ethanol. An opaque translucent powder of group of flavonoids weighing about 63 g was obtained.

Antimicrobial activity

The antimicrobial activity of the seed extract from *S. surattense* was studied against these microorganisms, using 'Disc Diffusion Assay'. The results indicated that the extract was more potential against *A. actinomycetemcomitans* than *S. mutans* among the tested pathogens. In the investigation, the lowest MIC value, 25μg/mL, was recorded against *S. mutans*. The MIC, MBC and Time Kill Curve values are presented in Tables 2–4 respectively.

MIC activity against *A. Actinomycetemcomitans* was 50μg/mL indicating significant antimicrobial potential in the tested extracts. The results of the MIC are indicated in the Table 2.

The Minimum Bacterial Concentration (MBC) was studied against *Aggregatibacter actinomycetemcomitans* and *Streptococcus mutans* using the extract obtained from *Solanum surattense*. The values of MBC are listed in Table 3.

The range of MIC and MBC of the extract was recorded as 25-100μg/mL for *S. mutans* and 50-100μg/mL for *A. actinomycetemcomitans*, respectively.

The Time Kill data of *Solanum surattense* was determined and the results are presented in Table 4.

Date of Tables 2, 3 and 4 demonstrate that the extract of *Solanum surattense* seed has a greater effect on *Aggregatibacter actinomycetemcomitans* as compared to *Streptococcus mutans*.

DISCUSSION

The crude extract derived from the powder of the seeds of dried ripe berries of *Solanum surattense* was subjected to various tests for the identification of different phytoconstituents. The obtained results presented in Table 1 identified good amount of flavonoids. Using percolation method and ethanol as solvent, flavonoids were extracted. Approximately 63 grams of a translucent, opaque flavonoids powder was obtained.

Using the 'Disc Diffusion Assay,' the antimicrobial activity of the *S. surattense* seed extract against selected microorganisms was investigated. Among the tested pathogens, the results indicated that the extract was more effective against *A. actinomycetemcomitans* than *S. mutans*. *S. mutans* recorded the lowest MIC value of 25 μg/mL during the investigation. Hence, it is more effective in inhibiting *A. actinomycetemcomitans* than *S. mutans*.

The MIC activity against *A. actinomycetemcomitans* was 50μg/mL, indicating that the test extracts possess significant antimicrobial potential. The outcomes of the MIC are shown in Table 2. Using the extract the Minimum Bacterial Concen-

Table 2: Antibacterial activity of the seed extract against the tested strains (MIC in μg/mL)

Sl. No.	Samples	100	50	25	12.5	6.25	3.12	1.6	0.8	0.4	0.2
1.	***S. mutans***	S	S	S	R	R	R	R	R	R	R
2.	**A a**	S	S	R	R	R	R	R	R	R	R

*S = Sensitive; R = Resistant, *S. mutans = Streptococcus mutans, A a = Aggregatibacter actinomycetemcomitans*

Table 3: Antibacterial activity of the seed extract against the tested strains (MBC in μg/mL)

Sl. No.	Samples	100	50	25	12.5	6.25	3.12	1.6	0.8	0.4	0.2
1.	*S. mutans*	NG	NG	NG	46	52	60	86	92	116	120
2.	A a	NG	NG	12	18	20	21	30	36	42	50

*NG = No Growth, *S. mutans = Streptococcus mutans, A a = Aggregatibacter actinomycetemcomitans*

Table 4: Time Kill data of the plant extract against the tested strains

Sl. No.	Samples	00hr	5 min	10 min	30 min	02 hr
1.	*S. mutans*	110	98	80	34	NG
2.	A a	81	70	41	12	NG

*NG = No Growth, *S. mutans = Streptococcus mutans, A a = Aggregatibacter actinomycetemcomitans*

tration (MBC) against *Aggregatibacter actinomycetemcomitans* and *Streptococcus mutans* was investigated. The MBC values are shown in Table 3.

The MIC and MBC of the extract were found to be between 25 μg/mL and 100 μg/mL both for *S. mutans* and *A. actinomycetemcomitans*. The Time Kill data of *Solanum surattense* was analyzed as presented in Table 4. The results showed greater activity against *A. actinomycetemcomitans* as compared to *S. mutans*.

Table 2 through Table 4 demonstrate that the seed extract of *Solanum surattense* is more effective against *Aggregatibacter actinomycetemcomitans* than *Streptococcus mutans*.

CONCLUSION

In the present study we extracted flavonoids from the seeds of dried ripe fruits of *Solanum surattense* Burm. F. and evaluated their antibacterial activity against two bacterial species like *Streptococcus mutans* and *Aggregatibacter actinomycetemcomitans* that commonly cause dental infections. The extract was found to be active against both the species. Hence, the extract is useful for further isolation of specific flavonoids responsible for the antibacterial activity.

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