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Evaluation of antiviral and cytotoxic activities of methanolic extract of *S. grandiflora* (Fabaceae) flowers

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ABSTRACT

Objective: To investigate the cytotoxicity and antiviral activity of methanolic extract of *S. grandiflora* flowers using different cell lines and viruses. **Methods:** The methanolic flower extracts were prepared and evaluated for their antiviral and cytotoxic activities using viruses like herpes simplex-1 and 2, vaccinia, vesicular stomatitis, cox sackie, respiratory syncytical, feline corona, feline herpes, para influenza, reo-1, sindbis and punta toro viruses in different cell lines, like Hel, HeLa, Crandell Reus feline kidney and Vero cell cultures. **Results:** Among the viruses used the extract possessed strongest antiviral activity against herpes simplex 1 and 2, repiratory syncytical, para influenza, reo, sindbis, cox sackie and punta toro viruses that was (EC_{50} =20 μg/mL and 45 μg/mL) and moderate activity for remaining viruses (EC_{50}= 100 μg/mL). The antiviral activities assessed by calculating the selectivity index may be due to the presence of flavonoids in the extracts there by inhibit the virus cell fusion in the early and replication stages. The cytotoxicity effect was evaluated using MTT assay and the results revealed that the extracts exhibited cytotoxicity from the range of 20 to 100 μg/mL. **Conclusions:** Present results confirmed that the *S. grandiflora* used as a good antimicrobial agent in future.

1. Introduction

S. grandiflora (*S. grandiflora*) belonging to the family of Fabaceae is used traditionally for the treatment of broad spectrum of diseases in India. *S. grandiflora* syn. *Aeschynomene grandiflora* commonly known as sesbania and agathi (Tamil) in ayurvedic and indigenous Indian system of medicine belongs to the family Fabaceae. The bark, leaves, gums, flowers and fruits were used to treat multifactorial diseases like leprosy, gout[1], rheumatism, cancer, liver disorders[2], inflammation, ocular diseases[3], epilepsy and anemia[4]. It possesses anti inflammatory, analgesic, anti pyretic[5], hypolipidemic[6], antibacterial[7], free radical scavenging[8], anti ulcer[9], anti urolithiatic[10], hepatoprotective[11] and chemo preventive[12] activities. The leaves of this plant are the richest source of amino acids, minerals and vitamins like vitamin A, vitamin C, thiamine, riboflavin, and nicotinic acid[6].

It also possesses active biomolecules, and can be used to treat various ailments[13]. The famous ancient text of ayurveda such as Dravyaguna has mentioned the medicinal value of *S. grandiflora*. All parts of this plant including preparations from the plant parts are used as medicine in south eastern Asia and India[7]. Herbal medicines are considered to be one of the most important fields of new antimicrobial drug discovery for pathogenic infectious diseases like common cold, influenza, chickenpox, AIDS, avian, influenza etc. Reactive oxygen species generation is the profound metabolic change in viral infections[14]. The mortality of influenza virus is due to the high singlet oxygen generation, and therefore the use of antioxidant property possessing plant (*S. grandiflora*)[8] will be the ideal choice for anti viral therapies. In recent decades in immunocompromised patients emergent virus and bacterial strains resistant to antibiotics are available clinically thus the prevalence of virally related diseases is of growing concern[14]. Glycoprotein mediated entry of herpes simplex virus in to epithelial cells may rapidly reaches the trigeminal ganglia establishing lifelong latency within the

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sensory neurons[15]. Since medicinal plants are an important social and cultural component to treat health problem and the urgency is to develop new antiviral drugs for complex viral infections in the present scenario.

The aim of this study is to evaluate the antiviral and cytotoxic activities of methanolic extract of *S. grandiflora* flowers growing in southern parts of Tamilnadu, India.

2. Materials and methods

2.1. Plant materials

The plant materials were collected from the tropical areas of Western Ghat regions of Erode and Nagercoil, shade dried at room temperature and a voucher specimen (SC 5/23) was deposited in Herbarium of Laboratory of Botany, Coimbatore, Tamilnadu, India.

2.2. Extraction of plant material

Coarsely powdered flowers were weighed and placed in 8.5 cm diameter glass conical flask, plugged with cotton and extracted by cold maceration process using methanol. The solubility of active principle gets increased by increase in the temperature of solvent, which expects to enhance the concentration gradient and mass transfer of the active principle. After 48 hrs the extracted solution was concentrated by heating at constant temperature (100℃) in heating mantle. The concentrated extract was centrifuged, filtered through methanol resistant filters[16].

2.3. Preliminary phytochemical screening

The various solvent extracts of *S. grandiflora* were screened for the presence of various phytoconstituents such as steroids, alkaloids, terpenoids, glycosides, flavonoids and carbohydrates[17].

2.4. Viruses and cells

The different viruses used in the present study were herpes simplex virus-1, herpes simplex virus-2, vaccinia virus, vesicular stomatitis virus, coxsackie virus, respiratory syncytical virus, feline corona virus, feline herpes virus, para influenza virus, reo virus-1, sindbis virus and punta toro virus. The virus stocks were grown in human embryonic lung (HEL) cells, human epithelial (HeLa) cells, crandell reus feline kidney (CRFK) cells and Vero cells.

2.5. Cell lines and growth conditions

The different cell cultures used for our study were grown in Dulbecco's modified eagle medium with sodium bicarbonate 3.7 g/L, glucose 4.5 g/L, hydroxyethyl piperazine ethane sulphonic acid buffer 15 mM, glutamine 2 mM, gentamicin 16 μg/mL, penicillin 12 μg/mL and foetal calf serum. Cells were grown in humidified atmosphere of 5% CO_2 in air[18].

2.6. Antiviral assays

Confluent cell cultures in microtiter trays were inoculated with virus stock dilution[19]. After 1 hour of virus adsorption to the cells, residual virus were removed and replaced by eagle minimal essential medium containing 3% fetal calf serum and various concentrations of the methanolic extracts ranging from 200 μg/mL to 2 μg/mL[20,21]. Viral cytopathogenicity was recorded as soon as it reached completion in the untreated virus-infected cell cultures. Antiviral activity was expressed as minimal inhibitory concentration (MIC50) required reducing virus induced cytopathogenicity by 50%.

2.7. Cytotoxicity

The confluent cell monolayers in 96-well plates were incubated with 4- fold dilutions of the *S. grandiflora* methanolic extract in growth medium and were observed microscopically for changes in cell morphology and viability at 24, 48 and 72 hrs of incubation[18]. The cytopathic effect was scored under an inverted microscope. The dilution causing microscopically detectable alteration of normal cell morphology of the confluent cell cultures were estimated as 50% cytopathogenic effect (TC50) with respect to cell control[19].

3. Results

3.1. Preliminary phytochemical screening

The preliminary phytochemical screening of *S. grandiflora* was carried out for the detection of various phytoconstituents. The following solvent extracts were used for the study, petroleum ether, chloroform, ethyl acetate, methanol, ethanol and water. The chemical tests used for the study were shinoda test (flavonoid), phlonatanins test (tannins), wagners test (alkaloids), and salkowskii test (glycosides). Among these methanolic extract was found to contain high amount of flavonoids as shown in Table 1. The preliminary phytochemical screening of methanolic extract revealed the presence of alkaloids, flavonoids, tannins, triterpenes, gums and mucilage.

3.2. Antiviral and cytotoxic activity

An antiviral drug should be active against the virus without inducing significant toxicity to the host cell. The 50% cytotoxic concentration (CC_{50}) of the methanolic extract was calculated[22]. As prerequisite for antiviral tests, the cytotoxicity of the methanolic extract of *S. grandiflora* against virus cells was investigated. The methanolic extract

of *S. grandiflora* flowers was found to be not toxic with minimum cytotoxic concentration at 20 μg/mL in Vero cells and 100 μg/mL in remaining cell lines being tested. The results indicated that strong antiviral activities (≥20 μg/mL) against herpes simplex 1 and 2, respiratory synchytical, para influenza, reo, sindbis, cox sackie and punta toro viruses in HEL, HeLa and Vero cell lines were observed when compared to other viruses used in the study. It possessed moderate antiviral activities especially in CRFK cell lines. The results were shown in Table 2 and 3. The reference drugs (brivudin, ganciclovir and ribavirin) possessed antiviral activity in the concentration range between 0.06 to 250 μg/mL, The cytotoxic effects produced by the reference drugs (brivudin, ganciclovir and ribavirin) was >250 μg/mL in Hel, HeLa, CRFK and Vero cell lines. The results were shown in Table 4. The exact mechanism by which the methanolic extract exerts its antiviral activity has not been fully addressed. It is reported that the antiviral activities of the methanolic extract of *S. grandiflora* flowers may occurs due to higher amount of phenolic compounds particularly flavanoids/tannins, which are reported to possess good antiviral activities, immunostimulant radical scavenging activity. It can enhance the innate immunity like superoxide anion and antioxidant property of the extract which protects the cells from the free radicals.

Table 1
Results of preliminary phytochemical screening.

Extract fractions	Flavonoids	Tannins	Alkaloids	Anthroquinone glycosides	Steroids
a	+	+	+	+	−
b	+	+	+	+	−
c	+	+	++	++	−
d	+++	+	+	++	−
e	++	+	+	+	−
f	+	+	+	+	−

a− Petroleum ether, b− Chloroform, c− Ethyl acetate, d− Methanol, e− Ethanol, f− Water.
−: Absent, +: Trace amounts, ++: Presence, +++: High.

Table 2
Antiviral activities1 of methanolic extract of *S. grandiflora* in HEL and CRFK cell lines.

Viruses (Strain)	Cells	SG extract	Brivudin (μg/mL)	Ganciclovir (μg/mL)
Herpes simplex−1	HEL	>20	0.04	0.06
Herpes simplex−2	HEL	>20	50	0.1
Vaccina	HEL	>100	10	>100
Vesicular stomatitis	HEL	>100	250	>100
Feline corona	CRFK	>100	NA	>100
Feline herpes	CRFK	>100	NA	0.1

SG− *S. grandiflora;* 1− Minimum inhibitory concentration (μg/mL) required to reduce virus−induced cytopathogenicity by 50%. NA− Not Applicable.

Table 3
Antiviral activities1 of methanolic extract of *S. grandiflora* in HeLa and Vero cell lines.

Viruses (Strain)	Cells	SG extract (μg/mL)	Ribavirin (μg/mL)
Vesicular stomatitis	HeLa	>100	12
Coxsackie	HeLa	>100	146
Respiratory syncytical	HeLa	>45	10
Parainfluenza−3	Vero	>20	146
Reo−1	Vero	>20	>250
Sindbis	Vero	>20	>250
Cox sackie	Vero	>20	>250
Punta toro	Vero	>20	50

1−Minimum inhibitory concentration (μg/mL) required to reduce virus− induced cytopathogenicity by 50%. Vero− Kidney epithelial cells.

Table 4
Results of cytotoxicity1 of methanolic extract of *S. grandiflora* (μg/mL).

Cell lines	SG extract	Brivudin	Ganciclovir	Ribavirin
HEL	>100	>250	>100	NA
CRFK	>100	NA	>100	NA
HeLa	>100	NA	NA	>250
Vero	>20	NA	NA	112

1− Minimum cytotoxic concentration (μg/ml) required to reduce to cause a microscopically detectable alteration of normal cell morphology.

4. Discussion

The present study clearly shows that the cytotoxicity and antiviral activity of total *S. grandiflora* methanolic extract are not necessary due to same compounds. It may be due to synergistic activity of few compound present on the extract and that the cytotoxicity of some plant compounds may mask the antiviral properties of other plant substances[23]. Our result suggests the scientific data for the usage of this plant. The respective affinity of the constituents present on the extract to specific viral protein partners may be strongly dependent on the amino acid composition and hydrophilicity of the target proteins eliciting the action. The separation of apolar from polar components can increase the chance to find a highly active antiviral compounds with low cytotoxicity. Here the in−vitro results have been demonstrated that it is not known however what these results signify for *in− vivo* effectiveness. The traditional utilization of these plants is thus validated.

The reduction of virus infection (both virus replication and immunomodulatory activity) by the methanolic extract of *S. grandiflora* is very interesting. From the data available it is reasonable to speculate that different antiviral mechanism were involved in the activity of *S. grandiflora* flowers. By the action against both DNA and RNA viruses, it is considered that polar compound (flavonoids) may be responsible for antiviral activity. Moreover cinnamoyl moiety of flavonoids has been reported to possess strong HSV−1 inhibitory activity[24]. The data remains suggestive but not conclusive. Summarizing these data, the *S. grandiflora* methanolic extract is assessed to be an antiviral system, which can be produced. Further preclinical and clinical investigations should clarify the clinical potential of such extracts for therapeutic use.

The methanolic extract used in the present study showed

a great potential of antiviral activity. This activity could be attributed to the presence of major components (flavonoids) and or minor components present in the methanolic flower extracts of *S. grandiflora*. The results of the present study suggest the possibility of using *S. grandiflora* as natural antimicrobials in pharmaceutical research. Usage of plant extracts by consumers and regulatory agencies is expected and reported to be increased in the future due to the risk of "green consumerism"[25]. The results of the present investigation provides an evidence for the potentiality of medicinal plants to represent a reservoir for pharmacologically active substances, but further studies should be adopted to fractionate the methanolic extracts from *S. grandiflora* aiming to identify the active compounds and to determine the exact mechanism of action of the constituents possessing antiviral and cytotoxic activities.

Conflict of interest statement

We declare that we have no conflict of interest.

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