Original Article

Phenolic composition, antioxidant and anti-fibrotic effects of *Sesbania grandiflora* L. (*Agastya*) – An edible medicinal plant

Saravanakumar Arthanari, Parthiban Periyasamy
Department of Pharmaceutical Biotechnology, Vellalar College of Pharmacy, Erode, Tamil Nadu, India

Abstract

Background: *Sesbania grandiflora* (*S. grandiflora*/*Agastya*) is an edible Indian traditional medicinal plant widely used as dietary supplements and possesses various pharmacological activities. **Aims:** The aim is to evaluate aqueous ethanol extract of *S. grandiflora* leaves and flowers for its anti-oxidant and anti-proliferative and anti-fibrotic effects using activated rat hepatic stellate cell (HSC)-T6. **Material and methods:** The antioxidant activities of these plant extracts were assessed as per the standard methods and the total phenolic and flavonoid contents were examined by folin ciocalteu reagent and colorimetric methods respectively. The anti-proliferation assay was conducted by using a cyto X cell viability assay kit. The anti-fibrotic effect was investigated by measuring the hydroxyproline content and gene expression analysis of the two main fibrogenic cytokines in activated HSC-T6 cells: Transforming growth factor-beta (TGF-β) and alpha-smooth muscle actin (α-SMA). **Results:** The aqueous ethanol extract of *S. grandiflora* leaves showed the highest antioxidant activity in a concentration-dependent manner. These findings were well correlated with the total phenols and flavonoids contents. The aqueous ethanol extract of *S. grandiflora* leaf and flower significantly reduced the proliferation of activated HSC-T6 cells. Regarding the anti-fibrotic effect, the hydroxyproline content was significantly reduced in a concentration-dependent manner during the extract treatment. In gene expression analysis, the treatment without extracts drastically up-regulated the fibrogenic cytokines (TGF-β and α-SMA), whereas the treatment with extracts significantly reduced these alterations. **Conclusion:** Results of present study revealed the significant antioxidant potential of the aqueous ethanol extract of *S. grandiflora* leaves and flowers. Among two extracts, *S. grandiflora* leaves demonstrated greater antioxidant, anti-fibrotic capacity with lower inhibiting concentrations corresponding to 50% values than *S. grandiflora* flowers.

Keywords: 2,2-diphenyl-1-picrylhydrazyl agastya, anti-fibrotic, antioxidant, assay, *Sesbania grandiflora* L

Introduction

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) including free radicals play a vital role in oxidation, which may oxidize proteins, lipids, or DNA in tissues and leading to chronic degenerative diseases such as cancer, diabetes, and cardiovascular diseases.[1,2] The main role of antioxidants is the protection of biological systems against oxidation and inhibition or delay of the oxidation process.[3] Liver fibrosis is a common disease that is associated with chronic liver injuries, alcoholism, persistent viral and helminthic infections, toxins, and hereditary metal overload.[4,5] Activation of hepatic stellate cells (HSCs) plays a crucial role in the development of liver fibrosis. Formation of myofibroblast-like phenotypes, extracellular matrix (ECM) proteins, and increased cell proliferation are the main events involved in the pathogenesis of liver fibrosis.[6]

Access this article online

Quick Response Code:

Website: www.ayujournal.org

DOI: 10.4103/ayu.AYU_198_19

Sesbania grandiflora (*S. grandiflora*) L. pers (Fabaceae) is commonly known as "*Agathi"* and "sesbania" (*Agastya* in Sanskrit)[7] and all parts of this plant are widely used in *Ayurveda* for the treatment of leprosy, gout, rheumatism, tumor, and liver disorders.[8,9] Various parts of this plant like leaves and flowers are widely used[10] for the treatment of anti-inflammatory, analgesic, antipyretic, anti-epileptic effects,[11,12] antibiotic, anthelmintic, antitumor, and contraceptive properties.[13,14] *S. grandiflora* leaves are the richest source of amino acids, minerals, and vitamins.[15] Although *S. grandiflora* is used

Address for correspondence: Dr. Saravanakumar Arthanari, Department of Pharmaceutical Biotechnology, Vellalar College of Pharmacy, Maruthi Nagar, Thindal, Erode - 638 012, Tamil Nadu, India.
E-mail: drsaravanakumara@gmail.com

How to cite this article: Arthanari S, Periyasamy P. Phenolic composition, antioxidant and anti-fibrotic effects of *Sesbania grandiflora* L. (*Agastya*)– An edible medicinal plant. Ayu 2020;41:242-9.

Submitted: 06-Aug-2019 **Revised:** 30-Aug-2019
Accepted: 06-Jan-2021 **Published:** 03-Jun-2022

to treat liver disorders, no comparative study has yet been reported to examine the *in vitro* anti-fibrotic activity between flowers and leaves of this plant against rat HSC. The results of this study may provide additional understanding for the usage of *Agastya* in ayurvedic medicine.

Aims and objective

To evaluate *in vitro* antioxidant and anti-fibrotic activity of *S. grandiflora* leaves and *S. grandiflora* flowers extracts.

Experimental Methods

Plant materials and extraction

The plant materials were collected between November and January from the tropical areas of the Western Ghat regions of Erode, Tamil Nadu, India, shade dried at room temperature, authenticated by Tamilnadu Agricultural University, Coimbatore. Dried leaves and flowers of *S. grandiflora* (each 100 g) were mixed in 1 lt of aqueous ethanol (ethanol/water 70/30, v/v) for 1 day in the automatic shaker. The suspension was filtered using a 300-mesh filter paper (50 mm) (Advantec, Toyo RoshiKaisah, Tokyo, Japan) and the filtrate was concentrated in rotary evaporator (Buchi Laboratoriums, Switzerland) and freeze-dried (Clean Vac Freeze dryer, Hanil biomedical laboratory, South Korea).

Total phenolic, flavonoid content and antioxidant assays

The total phenolic (TPC) and flavonoid content of aqueous extracts (water extracts) was determined by taking the average of three using the folin ciocalteu and colorimetric assay method respectively. previous method.[16,17] Various antioxidant assays such as 2,2-diphenyl-1-picrylhydrazyl (DPPH),[18] Superoxide scavenging,[19] Hydroxyl radical scavenging,[20] Nitric oxide scavenging,[21] Hydrogen peroxide scavenging,[22] Iron chelating,[23] Ferric reducing antioxidant power (FRAP)[24] and total antioxidant capacity (TAC)[25] were done by using the standard protocol.

Anti-fibrotic activity

Culture of rat hepatic stellate cells

HSC-T6 cells, an immortalized rat HSCs was kindly provided by Prof. SL Friedman, Mount Sinai School of Medicine, New York. HSC T6 cells were cultured in DMEM supplemented with 10% heat-inactivated fetal bovine serum, 100 IU/ml penicillin, and 100 mg/ml streptomycin at 37°C in a humidified atmosphere of 95% air– 5% CO_2.

Anti-proliferation assay

Anti-proliferation assay was done as per Guo *et al*. with some minor modifications.[26] After HSC-T6 cells (3000 cells/well) (180 µl) were cultured in 96-well plates for 24 h, 20 µL of different extract concentrations (10, 20, 50, 100, 200 and 500 µg/ml) were added to the cells and further incubated for 24 hr. Finally, 20 µL of CCK-8 kit (LPS solutions) solution was added to measure the cell proliferation after 1–3 hr incubation at 37°C in 5% CO_2. The absorbance was measured by an enzyme-linked immune sorbent assay reader (Versamax tunable microplate reader, Sunnyvale, California, USA) at a test wavelength of 450 nm and a reference wavelength of 600 nm.

Hydroxyproline content

Collagen was determined by estimating the hydroxyproline content, which is an amino acid found in collagen. HSC–T6 cells were lysed after treatment with different concentrations of extracts (5, 10, 50, 100 and 200 µg/ml) or standard dimethyl dimethoxy biphenyl dicarboxylate (DDB) (10 µg/ml) ($n = 3$) for 48 hr.[27] The lysates were hydrolysed in 2 mL of 6N HCl for 24 hr at 110°C and evaporated to dryness to remove the acid. Then, 100 µL of 0.05 M $CuSO_4$, 250 µl of 2.5 N NaOH, and 250 µl of 7% chloramine T were mixed with the residue. The solution was kept at room temperature for 5 min. It was heated at 70°C for 10 min in water bath followed by cooling in ice for 5–10 min. Then, 200 µl of 8 N H_2SO_4 and 1.25 ml of 5% p-dimethyl amino benzaldehyde in isopropanol (Ehrlich's reagent) were mixed and again it was heated at 70°C for 40 ± 5 min in the water bath. p-dimethyl amino benzaldehyde in isopropanol was prepared by heating the solution in the water bath at 65°C–70°C for 10 min. Finally, the absorbance was taken at 558 nm in ultraviolet spectrophotometer. DDB (10 µg/ml) was used as standard. Hydroxyproline was used to prepare the standard curve. The values obtained were expressed as µg of hydroxyproline.

Gene expression analysis by quantitative real-time polymerase chain reaction

HSC-T6 cells were maintained in Dulbecco's modified Eagle's medium with 10% fetal bovine serum and incubated at 37°C in a 5% CO2 humidified atmosphere. The cells were treated with different concentrations of (10, 20, 50, 100, 200, and 500 µg/ml) SG extracts or standard DDB (10 µg/ml) ($n = 2$). Total RNA was extracted after 24 h treatment with plant extracts using TRIzol reagent (Molecular Research Center, Cincinnati, OH, USA). After incubation for 6 h, the harvested cells were used to isolate the mRNA. Total RNA was measured by NanoDrop 2000 (Thermo Scientific). Extracted RNA concentration was adjusted to be 2 µg per reverse transcription reaction using high-capacity cDNA reverse transcription kit (Ambion). The primers sequence used were (forward and reverse, respectively) β-actin (CTAAGGCCAACCGTGAAAAGAT and GACCAGAGGCATACAGGGACAA), alpha-smooth muscle actin (α-SMA) (AACACGGCATCATCACCAACT and TTTCTCCCGGTTGGCCTTA), and transforming growth factor beta (TGF-β) (AGGAGACGGAATACAGGGCTTT and AGCAGGAAGGGTCGGTTCAT), respectively. Reactions were performed with 8 µl of iQ SYBR Green supermix, 1 µl of 10 pmol/l primer pairs, 8 µl of distilled water, and 3 µl of cDNA. Each PCR reaction was performed under the following conditions: 95°C for 5 min followed by 40 cycles of 95°C for 1 min, 58°C for 40s and 72°C for 40s, followed by single fluorescence measurement.

Statistical analysis

All of the experiments were done in triplicate. The results were expressed as mean ± standard deviation (SD). Analysis of variance and significant differences were conducted by one-way ANOVA followed by Bonferroni *post hoc* test using SPSS software (version 13.0 for Windows, SPSS Inc., Chicago, IL, USA). Pearson correlation coefficients were calculated between TPCs, flavonoids and antioxidant methods. In all methods, $P < 0.05$ was regarded as significant.

Results

Total phenolics and flavonoids content

Aqueous ethanol extract of *S. grandiflora* leaves and flowers was investigated for the contents of total phenols and flavonoids. The results revealed that both leaves and flowers have a high content of flavonoids, but much lower TPC content. The highest concentration of TPCs was measured in *S. grandiflora* leaves (5.98 ± 2.17 mg), followed by SG flowers (5.23 ± 1.21 mg) of GAE/g DW. The maximum concentration of total flavonoids was measured in *S. grandiflora* leaves (9.56 ± 3.29 mg), followed by SG flowers (8.72 ± 2.32 mg) of QE/g DW. The results are shown in Table 1.

Antioxidant assays

Free radical scavenging activity

The free radical scavenging effect of aqueous ethanol extract of both *S. grandiflora* leaves and flowers was analyzed in terms of DPPH$_{\cdot}$, $O_2^{\cdot-}$, NO$_{\cdot}$, H_2O_2 and OH$_{\cdot}$ scavenging activities. In the DPPH assay, both leaves and flowers of SG had a significant radical scavenging effect with increasing concentration. The studied plant extracts showed scavenging activity in a concentration-dependent manner. The investigated plant extracts demonstrated the lowest inhibiting concentrations corresponding to 50% (IC_{50}) values of 136.5 ± 13.4 µg/mL and 232.7 ± 5.9 µg/mL for the leaves and flowers, respectively. Different concentrations of *S. grandiflora* leaves and *S. grandiflora* flowers were used for measuring the scavenging activity of superoxide radicals. *S. grandiflora* exhibited dose-dependent inhibitory effect on superoxide radicals. The IC_{50} values were 41.8 ± 1.7 and 46.8 ± 0.4 µg/mL for *S. grandiflora* leaves and flowers, respectively. In the tested extracts, the scavenging effect on hydroxyl radicals exhibited concentration-dependent manner. The highest scavenging capacity occurred with the lowest IC_{50} values of 30.5 ± 0.7 and 34.0 ± 0.4 µg/mL for *S. grandiflora* leaves and flowers, respectively. In hydrogen peroxide radicals, the lowest IC_{50} value was in *S. grandiflora* leaves (57.4 ± 1.9 µg/ml), followed by *S. grandiflora* flowers (82.6 ± 6.8 µg/ml). In nitric oxide$_{\cdot}$ scavenging assay, the leaves and flowers exhibited scavenging activity with IC_{50} values of 63.0 ± 1.9, 65.7 ± 0.6 µg/ml, respectively. Vitamin C and gallic acid were used as standard and the results are presented in Table 2. Overall, both *S. grandiflora* leaves and flowers had significant antiradical activity in all the tested methods. In hydroxyl radicals, our extract exhibited a lower IC_{50} value than that of standard gallic acid. The average, free radical scavenging activity was in the order of *S. grandiflora* leaves is more than *S. grandiflora* flowers.

Iron chelating assay

In addition to free radical scavenging activity, the iron-chelating effect was measured and the highest chelation with lowest IC_{50} was observed in *S. grandiflora* leaves (179.7 ± 4.9 µg/ml) followed by *S. grandiflora* flowers (447.4 ± 17.5 µg/ml) [Table 2]. Ethylenediaminetetraacetic acid was used as standard with an IC_{50} of 60 ± 0.0 µg/ml.

Ferric reducing antioxidant power assay

To determine the antioxidant capacity of the samples, the absorbance was measured at 593 nm and the result compared

Table 1: Total phenol, flavonoid and antioxidant abilities of extracts

Extracts or standard	FRAP mmol Fe (II)/g extract	TAC µg GAE/g extract	Total phenols mg GAE/g extract	Total flavonoids mg QE/g extract
S. grandiflora flowers	1418.2±7.5	43.4±0.3	5.23±1.21	8.72±2.32
S. grandiflora leaves	1501.5±8.7	44.3±0.6	5.98±2.17	9.56±3.29

S. grandiflora: Sesbania grandiflora, FRAP: Ferric reducing antioxidant power, TAC: Total antioxidant capacity, GAE: Gallic acid equivalent, QE: Quercetin equivalent

Table 2: Inhibitory concentration 50 values of radical scavenging activity and iron chelation of extracts

Extracts or standard	IC_{50} values of free radical scavenging activity (µg/ml)					Iron chelation (IC_{50} µg/ml)
	DPPH	H_2O_2	Superoxide	Nitric oxide	Hydroxyl	
S. grandiflora flowers	232.7±5.9	82.6±6.8	46.8±0.4	65.7±0.6	34±0.4	447.4±17.5
S. grandiflora leaves	136.5±13.4	57.4±1.9	41.8±1.7	63±1.9	30.5±0.7	179.7±4.9
Vitamin C	1.1±0.4	ND	7.8±2.5	ND	ND	ND
Gallic acid	5.5±1.3	1.9±0.8	18.2±1.7	50.6±3.4	59.2±0.8	ND
EDTA	NA	NA	NA	NA	NA	59.6±1.8

S. grandiflora: Sesbania grandiflora, DPPH: Diphenyl picrylhydrazyl, H_2O_2: Hydrogen peroxide, IC_{50}: Inhibitory concentration 50, NA: Not applicable, ND: Not detected

with those obtained from the linear standard curves of $FeSO_4$ (r^2 = 0.9971). The antioxidant capacity values were expressed as mmol Fe (II) equivalent in g extract (mmol Fe (II) eq/g extract). Within these extracts, the *S. grandiflora* leaves displayed the highest FRA*P* value (1501.5 ± 18.7) followed by *S. grandiflora* flowers (1418.2 ± 17.5) mmol Fe (II) eq/g) [Table 1].

Total antioxidant capacity

Followed by free radical scavenging activity, the antioxidant capacity was measured and the calibration curve was plotted by using gallic acid (r^2 = 0.998) and the results were expressed as µg of GA eq/g extract. The highest antioxidant capacity was found in *S. grandiflora* leaves (44.3 ± 0.6), followed by *S. grandiflora* flowers (43.4 ± 0.3 µg of GA eq/g) [Table 1].

Anti-fibrotic activity

Anti-proliferation assay

The anti-proliferation activity of the extracts was investigated by assessing the cell viability in fully activated rat HSCs. In both leaf and flower extracts, the absorbance of the activated HSCs was up-regulated to a level approximately 3.3-fold higher than those in blank, whereas the extracts were significantly down-regulated (anti-proliferation) in a concentration-dependent manner ($P < 0.05$ for 10 µg/mL; $P < 0.01$ for 20, 100, 200, and 500 µg/mL in both *S. grandiflora* leaves and flowers; in 50 µg/mL $P < 0.01$ for *S. grandiflora* flowers and $P < 0.001$ for *S. grandiflora* leaves compared with control) [Figure 1a and b]. Among the extracts tested, *S. grandiflora* leaves displayed the most potent inhibitory activity on HSCs proliferation after 48 h incubation. After testing, the inhibitory activity of the extracts on HSC proliferation, it is further observed that the cell morphology using a phase-contrast microscope. HSCs cultured in the absence of plant extracts exhibited flattened and membranous processes, representing myofibroblastic morphology. However, the morphology of HSCs treated with plant extracts was changed to slender cell shape at the tested concentrations.

Hydroxyproline content

To assess the effect of the extracts on ECM production, the hydroxyproline content was measured. Treatment with different extract concentrations significantly reduced the cell hydroxyproline content in dose-dependent manner compared to control. The hydroxyproline content was significantly reduced in *S. grandiflora* leaves ($P < 0.01$ for 10 µg/ml; $P < 0.001$ for 20, 50, 100, 200 and 500 µg/ml) and flowers ($P < 0.05$ for 50 µg/ml; 0.01 for 100 and 500 µg/ml; 10, 20 µg/ml; $P < 0.001$ for 200 µg/ml). DDB treatment showed results similar to the extracts ($P < 0.001$) [Figure 2a and b].

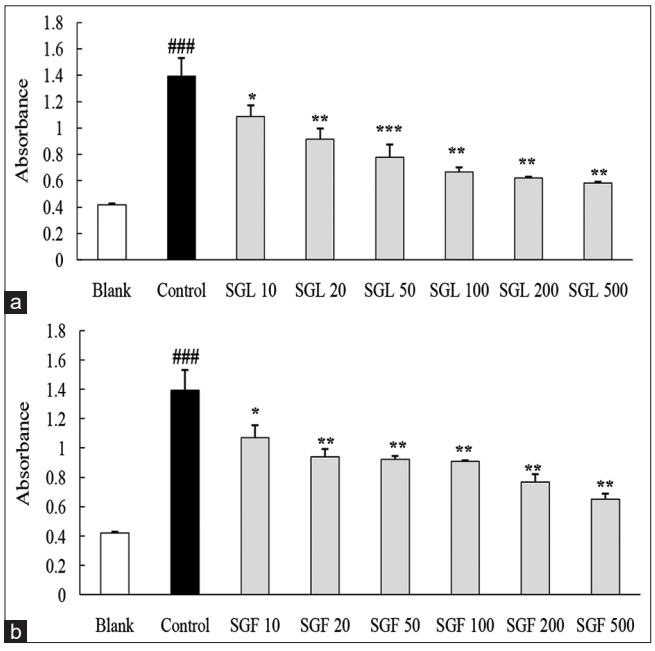

Figure 1: Effect of *Sesbania grandiflora* (*S. grandiflora*) on cell proliferation. Absorbance of (a) *S. grandiflora* leaf extract (*S. grandiflora* leaves) and (b) *S. grandiflora* flower extract (*S. grandiflora* flowers) on activated hepatic stellate cells treated at concentrations of 10, 20, 50, 100, 200 and 500 µg/ml. Data are expressed as the means ± SDs. ###$P < 0.001$ compared with blank. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$compared with the control group. SGL: *S. grandiflora* leaf extract, SGF: *S. grandiflora* flower extract

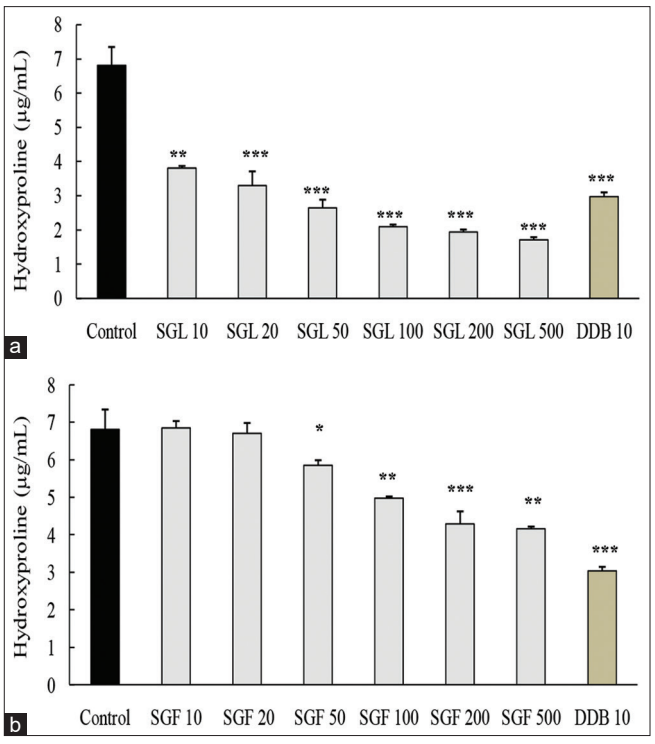

Figure 2: Effect of *Sesbania grandiflora* (*S. grandiflora*) on collagen accumulation. Collagen accumulations were determined by hydroxyproline content. Hydroxyproline content of hepatic stellate cell-T6 cells treated with (a) *S. grandiflora* leaf extract (*S. grandiflora* leaves) and (b) *S. grandiflora* flower extract (*S. grandiflora* flowers). Data are expressed as the means ± SDs. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$compared with the control group. SGL: *S. grandiflora* leaf extract, SGF: *S. grandiflora* flower extract, DDB: Dimethyl dimethoxy biphenyl dicarboxylate

Effects on fibrosis-related gene expression

Following the demonstration of the proliferation and hydroxyproline content, these plant extracts were further examined for the mRNA expression of two main fibrosis-related genes, TGF-β and α-SMA, in cultured activated rat HSCs. TGF-β and α-SMA gene expression levels were approximately 2.3 and 3.5-fold higher than those in the blank. The addition of extracts significantly downregulated TGF-β ($P < 0.05$ for 50, 100 μg/ml and $P < 0.01$ for 200, 500 μg/ml in *S. grandiflora* leaves; $P < 0.05$ for 50 μg/ml, $P < 0.01$ for 200 μg/ml and $P < 0.001$ for 100 and 500 μg/ml in SG flowers) [Figure 3a and b] and α-SMA ($P < 0.05$ for 50 μg/ml and $P < 0.01$ for 100 μg/ml and $P < 0.001$ for 200 and 500 μg/ml in SG leaves; $P < 0.05$ for 100 μg/ml, $P < 0.01$ for 200, 500 μg/ml in *S. grandiflora* flowers) [Figure 4a and b] as compared with control. DDB treatment also showed same results as that of the extracts ($P < 0.01$; $P < 0.001$).

Correlation between total phenolic/flavonoid contents and antioxidant activity

The antioxidant activities of plants containing polyphenolic compounds are mainly due to their ability to act as hydrogen donors, reducing agents, and radical scavengers.[28] The correlation between phenolic contents and antioxidant properties was determined by using Pearson correlation coefficient ® and the results are presented in Table 3. For all species, the correlation between TPCs and total flavonoids was higher with FRAP than with other methods. Sulaiman *et al.* reported the highest correlations between TPCs and FRAP with the extracts of *Coriandrum sativum*.[29] In the two extracts, the highest correlation was found between FRAP and total flavonoids ($r = 0.999$, $P < 0.01$) with the *S. grandiflora* leaf extracts. Meanwhile, the values of total flavonoids of *S. grandiflora* leaves were significantly correlated with the DPPH scavenging assay ($r = 0.998$, $P < 0.01$). The lowest correlation was found between TPCs and hydroxyl scavenging assay ($r = 0.902$, $P < 0.05$). In some methods, the lowest correlation was found between TPCs/flavonoids and free radical scavenging assays. These findings suggest that the activity of extracts on these methods might be attributed to the presence of nonphenolic compounds.

Table 3: Pearson's correlation coefficients of total phenolics, total flavonoids and antioxidant methods

Methods	*S. grandiflora* leaves		*S. grandiflora* flowers	
	Total phenols	Total flavonoids	Total phenols	Total flavonoids
DPPH	0.991**	0.998**	0.984**	0.979**
Superoxide	0.918**	0.933**	0.944**	0.940**
Hydroxyl	0.902*	0.913*	0.903*	0.905*
Nitric oxide	0.934**	0.956**	0.940**	0.935**
H_2O_2	0.914*	0.926**	0.946**	0.953**
FRAP	0.997**	0.999**	0.997**	0.995**
TAC	0.915*	0.942**	0.916**	0.915*
Iron chelating	0.967**	0.982**	0.989**	0.992**

*Significant at $P<0.05$, **Significant $P<0.01$. *S. grandiflora: Sesbania grandiflora*, DPPH: Diphenyl picrylhydrazyl, H_2O_2: Hydrogen peroxide, FRAP: Ferric reducing antioxidant power, TAC: Total antioxidant capacity

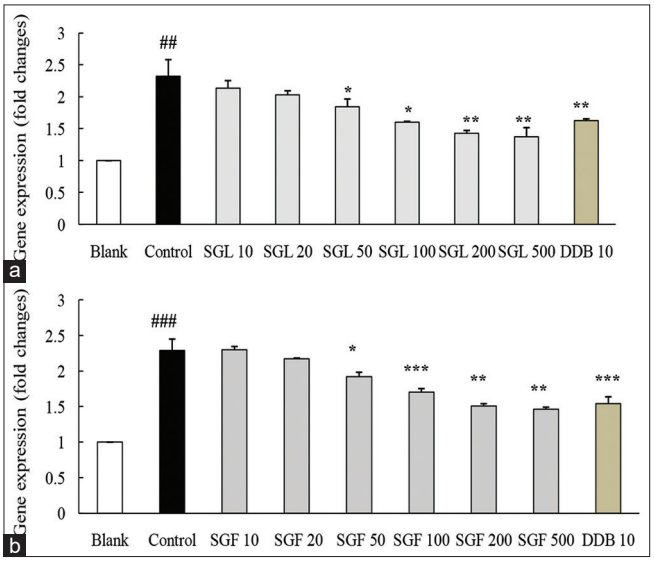

Figure 3: Gene expression levels of transforming growth factor β (TGF-β) in activated hepatic stellate cell-T6 cells. Expression of TGF-β treated with (a) *Sesbania grandiflora* (*S. grandiflora*) leaves extract (*S. grandiflora* leaves) and (b) *S. grandiflora* flower extract (*S. grandiflora* flowers). Data are expressed as the means ± SDs. ##$P < 0.01$,###$P < 0.001$ compared with blank. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with the control group. SGL: *S. grandiflora* leaf extract, SGF: *S. grandiflora* flower extract, DDB: Dimethyl dimethoxy biphenyl dicarboxylate

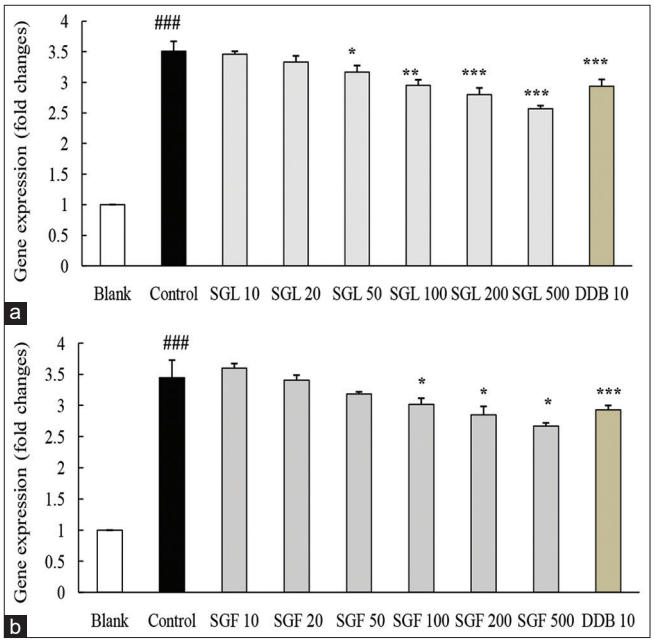

Figure 4: Gene expression levels of alpha smooth muscle actin (α SMA) in activated hepatic stellate cell-T6 cells. Expression of α SMA treated with (a) *Sesbania grandiflora* (*S. grandiflora*) leaf extract (*S. grandiflora* leaves) and (b) *S. grandiflora* flower extract (*S. grandiflora* flowers). Data are expressed as the means ± *SD*s. ###$P < 0.001$ compared with blank. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$compared with the control group. SGL: *S. grandiflora* leaf extract, SGF: *S. grandiflora* flower extract, DDB: Dimethyl dimethoxy biphenyl dicarboxylate

Discussion

Recently, several methods have been used to determine antioxidant activities. The chemical composition of extracts, often a mixture of dozens of compounds with different functional groups, polarity and chemical behavior, could lead to uneven results, depending on the test employed. Therefore, are liable approach for evaluating the antioxidant potential of extracts with multiple assays is desired. In this study, eight methods have been applied, DPPH, H_2O_2, superoxide anion, hydroxyl, nitric oxide radical scavenging activity, iron chelating activity, FRAP assay, and TAC assay. The concentrations of TPC and flavonoids were also determined for the tested extracts.

Two aqueous ethanol extracts of *S. grandiflora* leaves and flowers were tested for their antioxidant activity by measuring their ability to inhibit the free radicals. The two extracts showed different capacities to inhibit free radicals. At a given concentration, the inhibiting capacity for different extracts was in the order *S. grandiflora* leaves is more than *S. grandiflora* flowers. Antioxidants can exert their influence via various mechanisms, one of which is the scavenging of ROS and RNS. The free radical scavenging activity can be estimated by using many different testing methods, one of which is determining their efficiency to scavenge DPPH radicals. DPPH radical is a stable free radical and any molecule that can donate an electron or hydrogen to DPPH can react with it and thereby lightening or remove the DPPH color.[30] When the above extracts were tested for their DPPH scavenging ability, the *S. grandiflora* leaves showed higher and lower IC_{50} values than did the SG flowers, which suggested that all extracts contain compounds such as polyphenols that can donate electron/hydrogen easily, but to different extents.

Hydrogen peroxide (H_2O_2) is generated in large quantities during the inflammatory process, possibly due to the activation of mast cells, macrophages, eosinophils, and neutrophils, which generate superoxide radical, predominantly via NADPH oxidase.[31,32] Scavenging of superoxide radicals is important because it is one of the precursors of the singlet oxygen and hydroxyl radicals.[33] During oxidation reactions at the cellular level, superoxide radicals are normally formed first, and their effects can be magnified because they produce other kinds of cell-damaging free radicals and oxidizing agents.[34] Nitric oxide is an important bioregulatory molecule involved in several physiological processes such as neural signal transmission, immune response, control of vasodilatation, and blood pressure.[35] The hydroxyl radical is an extremely reactive free radical formed in biological systems, has been implicated as a highly damaging species in free radical pathology and is capable of damaging almost every molecule found in living cells.[36] This radical has the capacity to join nucleotides in DNA and can cause strand breakage which contributes to carcinogenesis, mutagenesis, and cytotoxicity.[37] The Fenton reaction generates hydroxyl radicals that degrade DNA deoxyribose, using Fe^{2+} salts as an important catalytic component. Oxygen radicals may attack DNA at either the sugar or the base, affording numerous products.[38] The results of free radical scavenging activity in all methods were similar to the DPPH assay, in the order, *S. grandiflora* leaves is more than *S. grandiflora* flowers. Overall, the results were in close agreement with the antioxidant activity of the methanol extract of *S. grandiflora* flowers.[39]

Antioxidant abilities in terms of mmol/g or µg/g of different standard equivalents. It were also confirmed through FRAP assay, that, the tested extracts were steadily increased with increasing sample concentration. In testing concentrations, all extracts were capable of reducing Fe^{3}+. Similar to other results obtained, the strongest reducing power was found in *S. grandiflora* leaves at a concentration of 10 mg/mL followed by *S. grandiflora* flowers. In TAC, the gallic acid was used as standard and the values obtained were expressed in µg/g of GAE. Among the extracts, *S. grandiflora* leaves showed a high level of TAC in terms of gallic acid equivalents. *S. grandiflora* flowers showed nearly equal capacity.

Activation of HSCs plays a major role in myofibroblast proliferation for the development of liver fibrosis. In this study, the effect of extracts on the proliferation of activated HSC–T6 cells was evaluated. The results showed that the growth of HSCs was inhibited by the extracts, which comprises the first evidence for an anti-proliferative effect of these extracts. Extracts decreased the proliferation of HSCs, as shown by the spectrophotometric determination of HSCs growth. Cell growth inhibition was significantly improved by increasing the extract concentrations. These data suggest that *S. grandiflora* leaf and flower extracts may play a key role as a negative regulator of liver fibrogenesis. The extracts directly inhibited the proliferation of HSCs, and induced cell death in a dose-dependent manner. As shown in Figure 2. the treatment of *S. grandiflora* leaf and flower extracts changed the cell morphology from flattened myofibroblastic membranous morphology, which is an indication of activation state, to slender shape, which is an indication of quiescent state. As compared with the antioxidant results, the HSC– T6 proliferation was in the same order of *S. grandiflora* leaves is more than *S. grandiflora* flowers.

Activated HSC-T6 cells were used to assess the anti-fibrotic activity in terms of measuring the hydroxyproline content. Hydroxyproline, the main component of the protein collagen, is produced by the hydroxylation of the amino acid proline by the enzyme prolyl hydroxylase following protein synthesis.[27] To evaluate ECM production, the hydroxyproline content was measured. The cell hydroxyproline content was higher in activated HSC-T6 cells but was significantly reversed or inversed in dose-dependent manner after the addition of the extracts.

In liver fibrosis, cytokines play a major role for activating the HSCs. These activated HSCs becoming the main source

for stimulating cytokines to induce collagen production. Among the cytokines, TGF-β1 is the most potent fibrogenic cytokine in liver fibrosis, and its expression is up-regulated during fibrogenesis which induces increased ECM synthesis.[40] The primary approach for treating liver fibrosis aims to disrupt or decrease TGF-β1 expression. α-SMA is a commonly used marker of myofibroblast formation and the TGF-β/α-SMA pathway is the most potent fibrogenic stimulus to HSCs, resulting in increased production of ECM.[41] *S. grandiflora* leaf and flower extracts were investigated for their inhibition of the development of hepatic fibrosis in terms of TGF-β 1, and α-SMA gene expression levels in cells either treated or nontreated with extracts. The results showed that both extracts markedly ameliorated the TGF-β1 and α-SMA mRNA gene expression levels in activated rat HSC's.

It is demonstrated that aqueous ethanol extract possesses prominent anti-proliferation and anti-fibrotic activity via inhibition of TGF-β gene expression in activated HSCs. In addition, the positive correlations between antioxidant assays and anti-fibrotic activity suggest its involvement in liver regeneration.

Conclusion

The results suggested that the *S. grandiflora* leaf extract possess more potent antioxidant capacity than *S. grandiflora* flower extracts. In addition, the anti-proliferative, anti-fibrotic, and mRNA gene expression data showed that *S. grandiflora* leaf extract remarkably inhibited or inversed liver fibrogenesis by down-regulating the TGF-β1 and α-SMA gene expression, possibly via the inhibition of the TGF-β/Smad pathway. Thus, by demonstrating the effectiveness of *S. grandiflora* in preventing the development of liver fibrosis, results will provide further insight into the design of new approaches to liver fibrosis.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Lee J, Koo N, Min DB. Reactive oxygen species, aging, and antioxidative nutraceuticals. Comp Rev Food Sci Food Saf 2004;3:21-33.
2. Mantena RK, Wijburg OL, Vindurampulle C, Bennett-Wood VR, Walduck A, Drummond GR. Reactive oxygen species are the major antibacterials against *Salmonella typhimurium* purine auxotrophs in the phagosome of RAW 264.7 cells. Cell Microbiol 2008;10:1058-73.
3. Sirkorski ZE. Chemical and Function Properties of Food Components. New York: CRC Press LLS; 2001. p. 245.
4. Bataller R, Brenner DA. Liver fibrosis. J Clin Invest 2005;115:209-18.
5. Friedman SL. Hepatic stellate cells: Protean, multifunctional, and enigmatic cells of the liver. Physiol Rev 2008;88:125-72.
6. Wu J, Zern MA. Hepatic stellate cells: A target for the treatment of liver fibrosis. J Gastroenterol 2000;35:665-72.
7. Atul N, Jadhav KK, Bhutani. Ayurveda and gynecological disorders. J Ethnopharmacol. 2005;97:151–9.
8. Gayatri S, Monisha S, Mythili K, Chitra K. Standardization and Quality Control Studies of Agastya Rasayanam–An Ayurvedic Drug for Asthma. Research J Pharm Tech. 2018; 11(6): 2271-75.
9. Joshi SG. Leguminoceae: Text Book of Medicinal Plants. New Delhi, India: Oxford Publishing; 2000. p. 130.
10. Keshava DV, Baghel MS, Harish CR, Mandip Goyal, Shukla V J. Pharmacognostical and Phytochemical evaluation of Agastya Rasayana Haritaki–A compound Ayurvedic formulation. J Ayurv Hol Med. 2014;2(5): 4–10.
11. Tamboli S. Antiinflammatory activity of *S. grandiflora*. Indian Drugs 1996;33:504-6.
12. Tamboli SA. Analgesic and antipyretic activity of *S. grandiflora*. Indian Drugs 2000;37:95-8.
13. Anonymous. The Useful Plants of India. New Delhi, India: Publications & Information Directorate, CSIR; 1986. p. 152.
14. Mannetje L, Jones RM. Plant Resources of South-East Asia. No. 4: Forages. Wageningen: Pudoc Scientific Publishers; 1992. p. 303.
15. Govindan S, Shanmugasundaram ER. Evaluation of the nutritive value of agathi (*Sesbania grandiflora*) leaf protein concentrates. Indian J Nutr Diet 1987;24:370-5.
16. Kim DO, Jeong SW, Lee CY. Antioxidant capacity of phenolic phytochemicals from various cultivars of plum. Food Chem 2003;81:321-6.
17. Park KS, Jung ST, Kang SG, Heo BG, Patricia AA, Fernando T. Antioxidants and proteins in ethylene-treated kiwi fruits. Food Chem 2008;107:640-8.
18. Brand-Williams W, Cuvelier ME, Berset C. Use of free radical method to evaluate antioxidant activity. LWT Food Sci Technol 1995;28:25-30.
19. Elizabeth K, Rao MN. Oxygen radical scavenging activity of curcumin. Int J Pharm 1990;58:237-40.
20. Smirnoff N, Cumbes QJ. Hydroxyl radical scavenging activity of compatible solutes. Phytochemistry 1989;28:1057-60.
21. Sreejayan, Rao MN. Nitric oxide scavenging by curcuminoids. J Pharm Pharmacol 1997;49:105-7.
22. Muller HE. Detection of hydrogen peroxide produced by microorganism on ABTS-peroxidase medium. Zentrabl Bakteriol Mikrobiol Hyg A 1985;259:151-4.
23. Dinis TC, Madeira VM, Almeida LM. Action of phenolic derivates (acetaminophen, salicylate, and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch Biochem Biophy 1994;315:161-9.
24. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Anal Biochem 1996;239:70-6.
25. Kambayashi Y, Binh NT, W Asakura H, Hibino Y, Hitomi Y, Nakamura, H. Efficient assay for total antioxidant capacity in human plasma using a 96-well microplate. J Clin Biochem Nutr 2009;44:46-51.
26. Guo CJ, Pan Q, Jiang B, Chen GU, Li DG. Effect of up regulated expression of microRNA-16 on biological properties of culture-activated hepatic stellate cells. Apoptosis 2009;14:1331-40.
27. Neuman RE, Logan MA. The determination of hydroxyproline. J Biol Chem 1950;184:299-306.
28. Nićiforović N, Mihailović V, Masković P, Solujić S, Stojković A, Pavlović Muratspahić D. Antioxidant activity of selected plant species; potential new sources of natural antioxidants. Food Chem Toxicol 2010;48:3125-30.
29. Sulaiman SF, Sajak AA, Ooi KL, Supriatno, Seowa EM. Effect of solvents in extracting polyphenols and antioxidants of selected raw vegetables. J Food Comp Anal 2011;24:506-15.
30. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature 1958;181:1199-201.
31. Barnes PJ. Reactive oxygen species and airway inflammation. Free Radic Biol Med 1990;9:235-43.
32. Hampton MB, Kettle AJ, Winterbourn CC. Inside the neutrophil phagosome: Oxidants, myeloperoxidase, and bacterial killing. Blood 1998;92:3007-17.
33. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 1974;47:469-74.
34. Aruoma O. Assessment of potential prooxidant and antioxidant actions.

J Am Oil Chem Soc 1996a; 73:1617-25.
35. Jagetia GC, Baliga MS, Malagi KJ, Kamath MS. The evaluation of the radioprotective effect of Triphala (an ayurvedic rejuvenating drug) in the mice exposed to γ- radiation. Phytomedicine 2002;9:99-108.
36. Hochestein P, Atallah AS. The nature of oxidant and antioxidant systems in the inhibition of mutation and cancer. Mut Res 1988;202:363-75.
37. Manian R, Anusuya N, Siddhuraju P, Manian S. The antioxidant activity and free radical scavenging potential of two different solvent extracts of *Camellia sinensis (L.) O. Kuntz*, *Ficus bengalensis L.* and *Ficus racemosa L.* Food Chem 2008;107:1000-7.
38. Rajeshwar Y, Kumar GP, Gupta M, Mazumder UK. Studies on *in vitro* antioxidant activities of methanol extract of *Mucuna pruriens (Fabaceae)* seeds. Eur Bull Drug Res 2005;13:31-9.
39. Loganayaki N, Suganya N, Manian S. Evaluation of edible flowers of agathi (*Sesbania grandiflora L. Fabaceae*) for *in vivo* anti-inflammatory and analgesic, and *in vitro* antioxidant potential. Food Sci Biotechnol 2012;21:509-17.
40. Shek FW, Benyon RC. How can transforming growth factor beta be targeted usefully to combat liver fibrosis? Eur J Gastroenterol Hepatol 2004;16:123-6.
41. Liu L, Li XM, Chen L, Feng Q, Xu L, Hu YY. The effect of gypenosideson TGF-β1/smad pathway in liver fibrosis induced by carbon tetrachloride in rats. J Integer Med 2013;1:1-6.