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ANTIOXIDANT POTENTIAL OF S. ASOCA AND P. GRANATUM LEAVES

Swati Kabra* and Chahat Sisodiya

Department of Biotechnology, Sir Padampat Singhania University, Bhatewar, Udaipur-Rajasthan- 313601.

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***Corresponding Author**
Swati Kabra
Department of Biotechnology, Sir Padampat Singhania University, Bhatewar, Udaipur-Rajasthan- 313601.

ABSTRACT

The present study is aimed to investigate antioxidant potential of methanolic leaf extracts of *P. granatum* & *S.asoca*. To determine the free radical scavenging activity, 2, 2-Diphenyl picrylhydrazyl assay was used. Reducing power ability of the above plant extracts was also determined. As per this study, leaves of *S. asoca* and *P.granatum* have significant free radical scavenging activity. The minimum concentration of the crude leaf extract to show this kind of activity was found to be 60µg/mL and 100µg/mL in *S.asoca* & *P.granatum*, respectively. Both the extracts had potent antioxidant activity. Both the extracts had also shown high reducing potential with increasing concentration of extracts. Antioxidant activities of extracts might be due to presence of various phytochemicals. Preliminary studies to check the presence of phytochemicals in above leaf extracts was also performed before checking antioxidant potential. Thus the results suggests that the methanolic leaf extracts of both the plants could provide antioxidants and these antioxidants can be further explored so that they can be helpful in free radical induced diseases.

KEYWORDS: *P.granatum*, *S.asoca*, radical scavenging, 2-Diphenyl picrylhydrazyl, reducing potential, antioxidant.

INTRODUCTION

Plants are the supply source of medicines from very early times, and India is known for a rich tradition of ethano-botanical medicine-based remedial practices primarily involving plants. India accounts for sizeable biodiversity on the planet & various traditional treatment systems of medicine such as Ayurveda, Unani, Siddha, etc. have been extensively practised by locals

since ancient times. Close to 25,000 effective plant-based drug formulations have been reported in traditional Indian folk medicine.[1]

Plants have many chemical compounds such as tannins, alkaloids, flavanoids, phenolics which make them medicinally important. Such compounds present in plants are known as phytochemicals and they can be used in treatment of various diseases. One medicinal property of plant which is output of above phytochemicals is anti-oxidation.

Oxidation is a chemical reaction that can produce free radicals, leading to chain reactions that may damage cells. Human health is affected by free radicals. Free radicals such as Reactive oxygen species (ROS) are small, highly reactive, oxygen-containing molecules generated in the cells as a result of reactions.[2] In low concentration they are not harmful but in excess they increase oxidative stress. Oxidative stress can cause various diseases. They can cause oxidative damage to lipids, proteins, DNA leading to many chronic diseases such as atherosclerosis, cancer, diabetics, rheumatoid arthritis, post-ischemic perfusion injury, myocardial infarction, cardiovascular diseases, chronic inflammation, stroke and septic shock, aging and other degenerative diseases in humans. [3,4]

To prevent above effects there is a requirement of antioxidants. An antioxidant is a molecule that inhibits the oxidation of other molecules. Majority of antioxidants are the natural attributes of plant and they occur especially in phenolics structures. Phenolics compounds are secondary metabolites that possess an aromatic ring. They are reported to have variety of activities. One of the biological activities they exhibit is antioxidant potential. In plants various phenolics structures are there such as flavanoids, tannins etc. Plant phenolics in many studies have been reported to induce antioxidant activity.

Owing to the vast treasure of medicinal wealth that can be found in our motherland we have devoted our time to study two of these plants, namely *P.granatum and S.asoca.* In this study, leaves of *P. granatum and S.asoca* were checked for their antioxidant properties.

Saraca asoca is very important plant and it belongs to the family Caesalpinaceae. It is distributed in evergreen forests of India up to an elevation of about 750 meters. It is cultivated in many gardens because of its decorative orange red flowers and evergreen beautiful foliage. The leaves are parpinnate with 6 to 12 leaflets, oblong and rigidly sum-coriaceous. It is used as a drug for the treatment of several female disorders and have

stimulating effect on endometrial and the ovarian tissue.[5] It has great medicinal value and is used to treat painful conditions , improve complexion of the body, improve digestion and assimilation, alleviate excessive thirst, kill infectious agents etc.[6]

Punica granatum or pomegranate is a member of family Punicaceae, and is mainly found in Iran. Its chemical components possess various pharmacological and toxicological properties including antioxidant, anti-inflammatory, anti-cancer and anti-angiogenesis activities.[7] The leaves are shiny and about 7.6 cm long. It has also been used as traditional medicine in many countries for the treatment of dysentery, diarrhoea etc. Its leaves can induce apoptosis, cell cycle arrest and impairing cell migration and invasion & is helpful in lung cancer.[8]

MATERIALS AND METHODS

Materials: Methanol, acetone, ferric chloride, potassium ferricyanide, disodium hydrogen phosphate, sulphuric acid, chloroform, lead acetate, aluminium chloride, acetic anhydride, Fehling's Reagent, ,2,2-Diphenyl picrylhydrazyl (DPPH), trichloroacetic acid.

Methods:

Collection of plant material & extraction

The fresh leaves of 10 year old plants of *Saraca asoca* and *Punica granatum* were selected and collected from Sir Padampat Singhania University campus, Udaipur. The collected leaves were washed with distilled water. The leaves were air-dried at room temperature for 7-10 days, then oven-dried at 40˚C to remove the residual moisture and finally the dried leaves were pulverised into fine powder. After that approximately 20 gms of the powdered samples were taken for the extraction purpose using acetone and methanol as solvents and maceration and decoction extraction techniques. The prepared extracts of both plants were then used for this study.

Qualitative phytochemical analysis of crude extracts

The total of four extracts was obtained. Two from the powdered leaves of *Saraca asoca* (methanolic & acetone extracts) and other two from *Punica granatum* **(**methanolic & acetone extracts). All extracts were subjected to phytochemical tests to determine the presence of secondary metabolites such as phenols, tannins, tri-terpenoids, steroids, glycosides, saponins, alkaloids and flavonoids. For carrying out preliminary phytochemical determination, aluminum chloride test for flavanoids, ferric chloride test for phenolics & tannins, acetic

anhydride test for steroids, Salkowski test for triterpenoids, frothing test for saponins, and Fehling's test for glycosides were done using standard procedures.[9]

Antioxidant activity Assay/Radical Scavenging Activity

Various concentrations of methanolic plant extracts (20, 40, 60, 80 & 100 µg/ml, 2.5 ml) were mixed with 0.1mM of 0.5 ml methanolic solution of 2, 2-Diphenyl picrylhydrazyl (DPPH).The mixture was shaken vigorously and incubated in dark for 30 min at room temperature. Then the absorbance was measured at 517 nm in a spectrophotometer.[10] The percentage of inhibition of DPPH free radical activity was calculated using the equation:

$$Percentage\ in\square ibition = \frac{Ac - As}{Ac} x\ 100$$

Where, 'Ac' is the absorbance of control, and 'As' is the absorbance of solution containing sample extracts. All assays were carried out in triplicates and results are expressed as mean value.

Ascorbic acid was used as a standard. (As)

Control was prepared as above without plant extracts.

Reducing power assay

Various concentrations of the methanolic plant extracts (0.2, 0.4, 0.6, 0.8, 1.0 mg/ml) were mixed with phosphate buffer (2.5 ml, 0.2M, pH 6.6) and 2.5 ml of 1 % potassium ferricyanide, then the mixture was incubated at 50º C for 20 minutes. 2.5 ml of trichloroacetic acid (100g/l) was added to the mixture, and then centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and 0.5 ml FeCl3 (1g/l) and absorbance was measured at 700nm in UV-Visible Spectrophotometer. Phosphate buffer is used as blank solution. Increased absorbance of the reaction mixture indicates stronger reducing power.[10]

RESULTS AND DISCUSSION

Phytochemical Qualitative Analysis

Preliminary phytochemical analysis conducted on the *Saraca asoca* and *Punica granatum* leaves extracts revealed the presence of constituents such as flavonoids, saponins, phenols, tannins, glycosides and steroids. Table 1 shows the presence of various active constituents.

Table 1: Preliminary phytochemical screening of methanol & acetone extracts of *Saraca asoca* and *Punica granatum*.

S. No.	Test	*Saraca asoca*		*Punica granatum*	
		Methanolic extract	Acetone extract	Methanolic extract	Acetone extract
1	Phenols & Tannins	+	+	+	+
2	Flavonoids	+	+	+	+
3	Saponins	+	-	+	-
4	Glycosides	+	+	-	-
5	Steroids	+	+	+	+
6	Phenolic compounds	+	-	+	+
7	Triterpenoids	+	+	+	+

(+ *indicates presence*) (- *indicates absence*)

Among two solvent systems, most of the phytochemicals were found in methanolic extracts so methanolic extracts of both the plants *Saraca asoca and Punica granatum* were used further for determination of antioxidant potential

Antioxidant activity Assay/Radical Scavenging Activity

DPPH is a stable nitrogen-centered free radical commonly used for testing radical scavenging activity of the compound or plant extracts. When the stable DPPH radical accepts an electron from the antioxidant compound, the violet color of the DPPH radical was reduced to yellow colored diphenylpicrylhydrazine radical which was measured colorimetrically. Substances which are able to perform this reaction can be considered as antioxidants and therefore can act as radical scavengers.[11] Fig 1 and 2 represents antioxidant activity in methanolic extracts of *S. asoca (S Ac)* and *P. granatum (P gr)* respectively compared to control ascorbic acid (As). The violet color of DPPH disappears when an antioxidant is present in the medium. Thus, antioxidants molecules can quench DPPH free radicals and convert them to a colourless product, resulting in a decrease in absorbance at 517 nm. The DPPH free radical scavenging assay showed potent inhibitory capacity of methanolic extracts of *S. asoca* and *P. granatum* when compared with ascorbic acid.

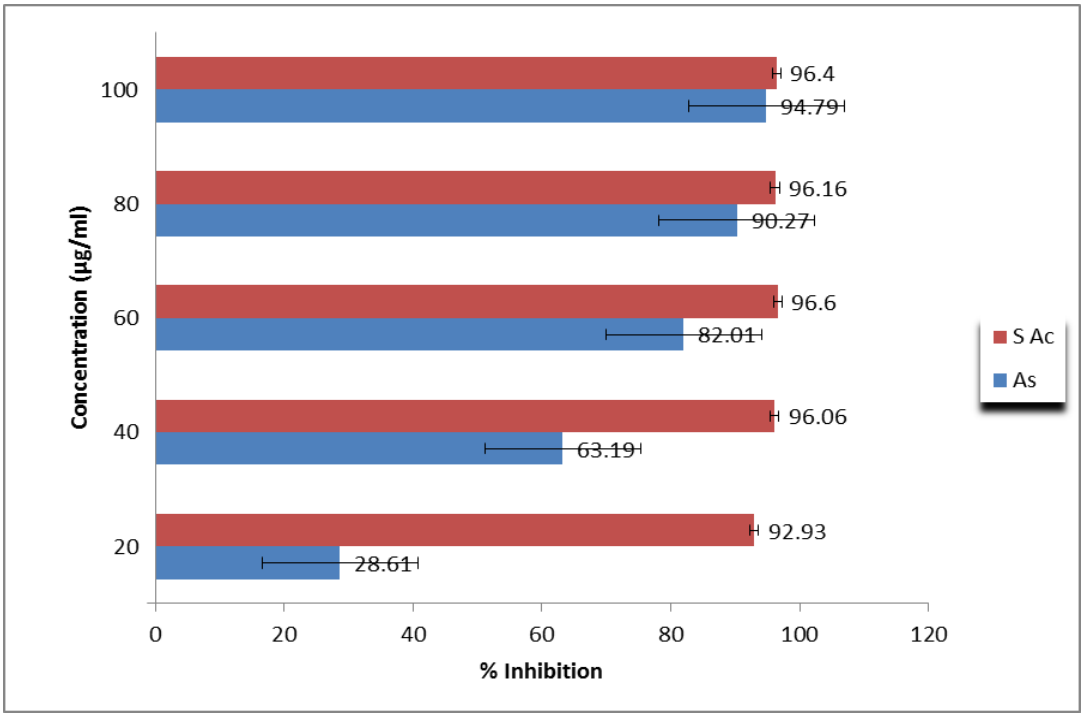

Fig. 1: DPPH free radical scavenging assay of *S. asoca* methanolic leaf extract.

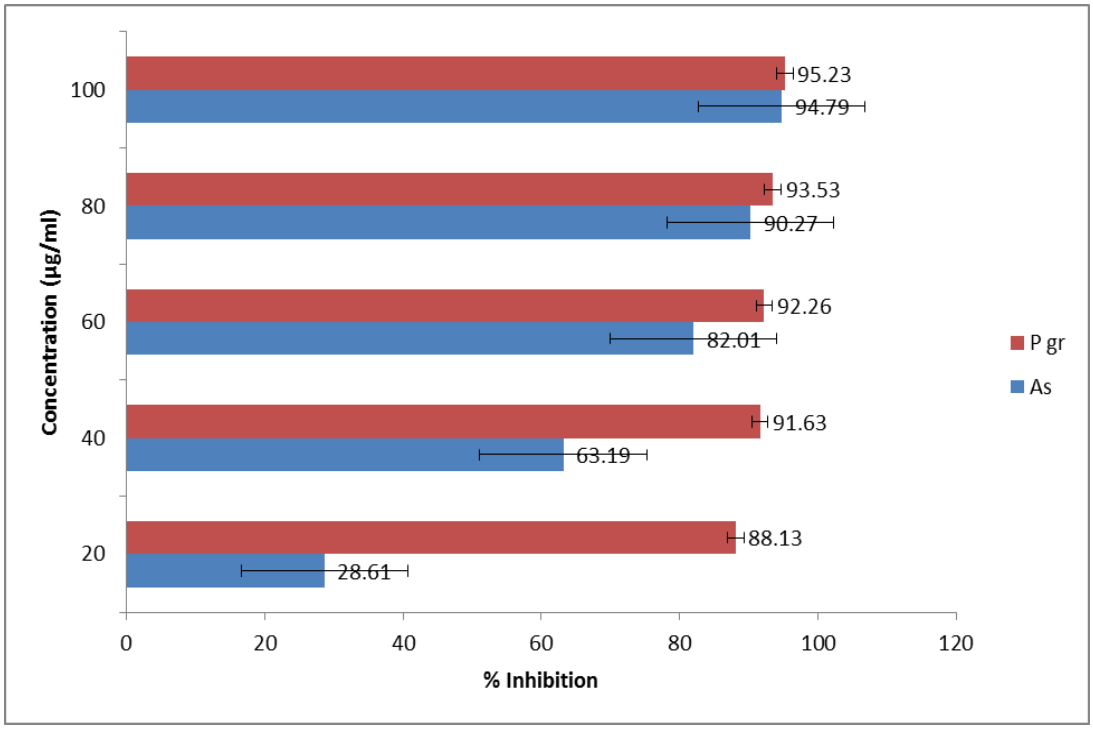

Fig 2: DPPH free radical scavenging assay of *P. granatum* methanolic leaf extract.

Reducing power assay

Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants.[12] In this assay, the yellow colour of the test solution changes to

various shades of green and blue depending on the reducing power of each compound. Presence of reducers causes the conversion of the Fe3+/ferricyanide complex used in this method to the ferrous form. With the increase in concentration of methanolic extracts of both the plants absorbance has increased (Table 2). Higher absorbance of the reaction mixture indicates higher reductive potential.

Table 2: Reducing ability shown by methanolic extracts of *S. asoca* and *P. granatum*.

S. No.	Concentration (mg/ml)	Absorbance at 700nm	
		Saraca asoca	*Punica granatum*
1	0.0	1.505	1.505
2	0.2	1.953	1.713
3	0.4	2.376	1.889
4	0.6	2.690	2.009
5	0.8	2.778	2.157
6	1.0	2.922	2.277

CONCLUSIONS

Saraca asoca is highly regarded as universal panacea in the ayurvedic medicine and *Punica granatum is* having wide-ranging potential therapeutic properties. Preliminary phytochemical analysis conducted on the *Saraca asoca* and *Punica granatum* leaves extracts revealed the presence of constituents such as flavonoids, saponins, phenols, tannins, glycosides and steroids which are known to exhibit medicinal as well as physiological activities. The antioxidant activity of leaf extracts of *S. asoca* and *P.granatum* is comparable to ascorbic acid and hence both plants leaf extracts can act as radical scavengers. The reducing power is also associated with the antioxidant activity and methanolic extracts of both the plants showed increase in absorbance with increase in concentration of crude extract, which indicate high reducing potential. Reactive oxygen species can cause variety of diseases including cancer and free radical induced diseases can be cured/ treated with the help of antioxidants. As in-vitro assaying of the extracts of *P. granatum and S. asoca* revealed significant antioxidant properties, they can be further investigated for in-vivo studies.

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