Research in Pharmacy 2022, 12: 1-4
doi: 10.25081/rip.2022.v12.7740
http://updatepublishing.com/journal/index.php/rip

Research Article

ISSN: 2231-539X

Evaluation of antioxidant activity of *Impatiens walleriana* & *Sauropus androgynus* extracts – A combination study

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ABSTRACT

"Oxidative stress" underlies a number of ailments & lipid peroxidation (LPO) also has economic implications/safety concerns as it contributes to spoilage of food products/cosmetics and pharmaceuticals. Antioxidants seem to hold solutions for such problems. Culinary herbs based antioxidants are in demand be it food, cosmetic or pharma industry. In pursuit of finding such safe antioxidants present evaluation employing a combined hydroalcoholic extract of two nutritious plants namely *Sauropus androgynus* & *Impatiens walleriana* is carried out using three *in-vitro* assays. FRAP assay results seem promising with the extract under investigation showing ferric reducing ability between 73-88% compared to standard showing 59-84%. Antioxidant effect in nitric oxide scavenging assay is averaged at 20% in comparison to the standard for which it is 70%. Results of hydroxyl radical scavenging assay range between 31-72% compared to standard wherein effect is between 79-88%. Ascorbic acid has been used as standard in the assays. Findings from all the three *in vitro* assays reveal antioxidant potential of the combined extract. Further additional studies to determine mechanisms underlying antioxidant activity along with finding active principles contributing to the same are recommended.

KEYWORDS: Antioxidant, *Sauropus androgynus*, *Impatiens walleriana*, *In-vitro*

Received: June 07, 2022
Revised: July 05, 2022
Accepted: July 06, 2022
Published: July 15, 2022

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INTRODUCTION

LPO reactions adversely affect living beings by damaging cell components be it DNA/proteins/lipids and nonliving entities like food/feed/cosmetics. In humans LPO could lead to development of diseases like Diabetes mellitus, Cancer, Atherosclerosis, Hypertension, etc., (Haider & Ullah, 2019; Badusha *et al.*, 2020; Felix *et al.*, 2020). LPO can cause development of rancid smell affecting appearance & shelf life of food/feed/cosmetics leading to economic losses (Boligon *et al.*, 2014). Oxidative damage can result from exposure to heat, light, oxidizing chemicals (Haider & Ullah, 2019; Badusha *et al.*, 2020; Felix *et al.*, 2020). As reactive oxygen species (ROS) are ubiquitous in biological tissues their production and removal are in a state of balance under normal physiological conditions but when imbalance sets in it causes a state of "Oxidative stress" ultimately resulting in tissue damage and disease (Keser *et al.*, 2012). Antioxidants are entities that protect against such oxidative damage and retard the progress of chronic diseases and are also in demand for use in food industry as food additives to protect against oxidative degradation of foods (Gulcin *et al.*, 2010).

Natural antioxidant defenses in our body can be insufficient & therefore intake of antioxidants is recommended (Boligon *et al.*, 2014). Antioxidant containing foods are in demand as the market for healthy foods is undergoing expansion. Also natural antioxidants are being used as additives in product formulations (Felix *et al.*, 2020). Herbs contain antioxidants such as Vitamin C, Vitamin E, carotenoids, flavonoids, tannins, etc., and are of considerable interest as synthetic antioxidants like BHA, BHT are getting restricted owing to their toxicity & DNA damage inducing abilities (Dluya *et al.*, 2017; Ruskin *et al.*, 2017). Traditionally tea, wine, fruits, vegetables, spices, etc., have been investigated in the process of finding novel antioxidants (Dluya *et a.l*, 2017). Epidemiological studies have shown that use of plant antioxidants is correlated with a reduction in chronic diseases (Nishaa *et al.*, 2012). Plants therefore constitute main source of natural antioxidant molecules and their antioxidant ability is associated with molecules such as flavonoids and phenols (Sylvie *et al.*, 2014). Phenolics particularly have redox properties that allow them to react as reducing agent, an electron donor, an oxygen quencher or chelate metal (Sylvie *et al.*, 2014).

Sauropus androgynus (Family- Euphorbiaceae) commonly known as star gooseberry is a tropical shrub grown as a nutritious leafy vegetable found in Malaysia, India, Indonesia and Thailand. *S. androgynus* leaves are commonly known as sweet leaves. Traditionally leaves of *S. androgynus* have been used to treat genito-urinary diseases & CVS disorders. A traditional Indonesian preparation "Jamu" restores uterus & abdomen to original size post giving birth also relieves fatigue. *S. androgynus* leaves are also used to reduce body weight, improve vision & treat skin problems (Khoo *et al.*, 2015).

Impatiens walleriana Hook.f. (Family-Balsaminaceae) commonly known as "Bizzy-Lizzie" is an ornamental herb native to east Africa & is naturalized in many parts of the world. Flowers are edible and have sweet taste. They can be added to salads or mixed into fancy drinks. Leaves & roots are dried, pounded, mixed with water and this juice is drank as abortifacient (Lim, 2014). This eatable garden plant is traditionally used for antimicrobial, anti-anaphylactic, anti-inflammatory, anti-dermatitic, anti-tumor and anthelmintic properties (Haider & Ullah, 2019).

Both these edible plants are rich in protein & mineral content. Protein content of dry & fresh samples of *S. androgynus* leaf are ranged between 4.2% & 29.15%. *S. androgynus* leaves also have high fibre & carbohydrate content. Major vitamins present in the leaves include Vitamin A, B1, B2 & C. *S. androgynus* leaves contain α-tocopherol & are rich in minerals like sodium, potassium, calcium, phosphorus, iron, zinc, manganese, magnesium, cobalt (Khoo *et al.*, 2015). *I. walleriana* edible flowers have 4.60 g/kg crude protein content apart from minerals like sodium, potassium, calcium, phosphorus, iron, zinc, manganese, magnesium, molybdenum (Boligon *et al.*, 2014). Both these plants contain flavonoids & phenolics. Antioxidant activity of leaves of *S. androgynus* & leaves, flowers of *I. walleriana* has been reported (Lim, 2014; Khoo *et al.*, 2015; Haider & Ullah, 2019).

In the present study an attempt has been made to evaluate antioxidant potential of combined hydroalcoholic extract of *S. androgynus* & *I. walleriana* whole plant excluding roots as both these plants are nutritious, edible and are safe in moderation (Rop *et al.*, 2012; Khoo *et al.*, 2015).

MATERIALS AND METHODS

Collection of Plant and Extraction

The fresh plants of *I. walleriana* were collected from local garden Belgaum. The fresh plants of *S. androgynus* were collected from the Rani chennamma college of Pharmacy campus, Belgaum. Authentication was carried out at Regional medical research centre (RMRC), Belgaum & specimens were deposited in RMRC herbarium with accession number 1341 & 1501 for *I. walleriana* & *S. androgynus* respectively. The fresh plants were first washed under running tap water and root portions were removed followed by drying in shade at room temperature. Dried plant material was ground to coarse powder and stored in airtight bottle.

Extraction was done using 50% hydroalcoholic solvent by maceration for 72 hrs. Both the plants were extracted separately using magnetic stirrer and yield calculated.

Antioxidant activity

Nitric oxide scavenging assay

2 ml of 10 mm sodium nitroprusside in 0.5ml phosphate buffer saline (pH 7.4) was mixed with 0.5ml of extract at various concentrations & the mixture was incubated at 25°C for 150 mins. Then 0.5 ml was taken out & added to 1.0ml sulphanilamide solution (0.33% in 20% glacial acetic acid) & incubated at room temperature for 5 mins. Finally 1.0ml naphthylethylenediamine dihydrochloride (0.1% w/v) was mixed and maintained at room temperature for 30 mins. The absorbance was measured at 546nm. A typical blank solution was made without plant extract/standard. The % inhibition shown in Figure 1 was calculated as follows:

$$\% \text{ inhibition} = (1\text{-}A1/A0) \times 100$$

where
A_1 = Absorbance of the extract or standard
A_0 = Absorbance of the control (Shahriar *et al.*, 2012).

FRAP assay

A mixture of 2.5ml of 0.2M phosphate buffer (pH 6.6) & 2.5ml of 1% w/v potassium ferricyanide was added to 1.0ml of combined extract & ascorbic acid (1.0mg/ml) prepared in distilled water. Mixture was then incubated at 50^{0}C for 20mins followed by addition of 2.5ml TCA (10% w/v) Then centrifugation was done at 3000rpm for 10mins. 2.5ml of supernatant was collected & mixed with 0.5ml $FeCl_3$ (0.1 % w/v) & 2.5ml distilled water. Absorbance was measured spectrophotometrically at 700nm against blank. A high reducing power (Figure 2) was indicated by an increased absorbance

$$\% \text{ reduction ability} = (A_0\text{-}A_1/A_0) \times 100$$

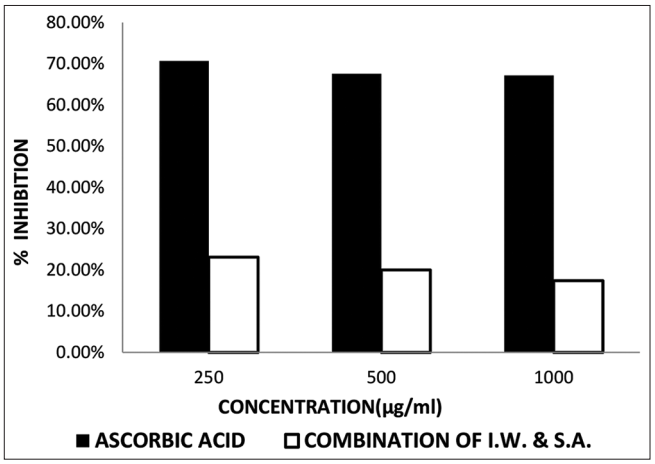

Figure 1: Nitric oxide scavenging assay. % Inhibition of combined extract of *I. walleriana* and *S. androgynus*

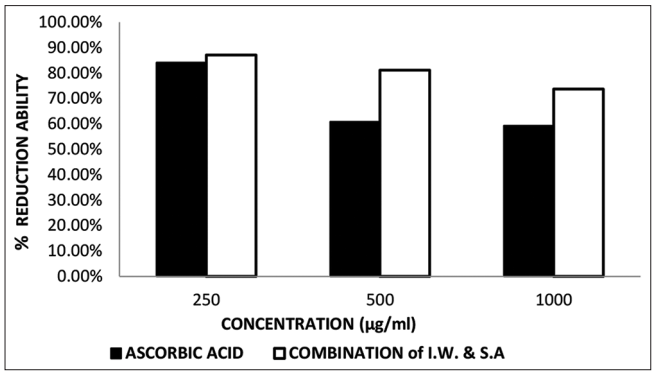

Figure 2: Ferric reducing activity power (FRAP) assay. % Reduction ability of combined extract of *I. walleriana* and *S. androgynus*

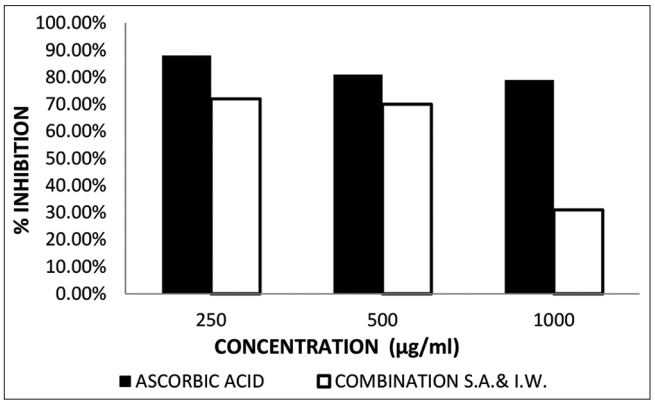

Figure 3: Hydroxyl radical scavenging assay. % Inhibition of combined extract of *I. walleriana* and *S. androgynus*

where
A_0 = absorbance of control
A_1 is the absorbance of the sample (Idamokoro *et al.*, 2017).

Hydroxyl radical scavenging assay

1.5 ml of extract was combined with 60µl of $FeCl_3$ (1 mmol/l), 90µl of 1, 10-phenanthroline (1mmol/l), 2.4 ml of 0.2mol/l phosphate buffer (pH 7.8) & 150µl of H_2O_2 (0.17mol/l). The mixture was homogenized and incubated at room temperature for 5mins. Absorbance (Figure 3) was read at 560nm against blank.

% of hydroxyl radical scavenging activity = [OD control- OD sample/OD control] × 100

where OD is the optical density (Sylvie *et al.*, 2014).

RESULTS

Percentage yield of *I. walleriana* and *S. androgynus* extract

- Weight of dried *S. androgynus* sample: 50 gm
- Weight of dried *I. walleriana* sample:50 gm
- Weight of *S. androgynus* extract:9.20 gm
- Weight of *I. walleriana* extract:12.28 gm

$$\% \text{ Yield} = W_1/W_2 \times 100$$

Where
W_1 = Weight of the extract after extraction
W_2 = Weight of plant powder.

Yield
I. walleriana: 24.56%
S. androgynus: 18.40%.

DISCUSSION

LPO adversely affects living beings & has economic impacts affecting shelf life of a number of food products, cosmetics, etc., (Felix *et al.*, 2020). Though natural anti-oxidant defense mechanisms exist they are not sufficient to protect against oxidative damage hence dietary intake of antioxidants is recommended. Also synthetic antioxidants are being replaced with plant based products in foods, pharmaceuticals owing to toxic nature of synthetic antioxidants (Sylvie *et al.*, 2014).

Food antioxidants such as ascorbic acid, carotenoids, flavonoids & other phenolic compounds can play an important role as physiological & dietary antioxidants contributing to body's natural resistance against oxidative damage (Gulci *et al.*, 2010). Plants contribute a valuable source of antioxidants with their antioxidant properties commonly associated with molecules such as phenols & flavonoids (Sylvie *et al.*, 2014). Phenols basically act as antioxidants owing to their redox properties that allows them to behave as hydrogen donor/reducing agent/ metal chelator or quencher of singlet oxygen. Superoxides used by our immune system also serve as precursors to active free radicals & flavonoids are known to scavenge these superoxide anion radicals (Gulcin *et al.*, 2010). Phenolics & flavonoids contribute to antioxidant activity by stabilizing lipid oxidation (Sylvi *et al.*, 2014).

S. androgynus & *I. walleriana* are both nutritious, rich in minerals, safe when used in moderation & both have culinary uses. Moreover antioxidant activity of leaves of *S. androgynus* & *I. walleriana* including the presence of phenolics is established by earlier researchers using hexane, chloroform like solvents. In the present study an attempt has been made to evaluate antioxidant activity of combined hydroalcoholic extracts of *S. androgynus* & *I. walleriana* using whole plant except root portion. Plant phenolics that are principally natural antioxidants are derived from not only leaves but also from parts like barks, seeds & nuts. Flavonoids are potent water soluble antioxidants. In the present study ethanol-water blend is employed as solvent since such high polarity ones can maximally extract phenolic compounds (Wong-Paz *et al.*, 2015). The amount of phenolic compounds extracted from plants is also a function of solvent used & that could be a reason for disparity between results obtained in assessment of antioxidant activity (Idamokora *et al.*, 2017). Also difference could be attributed to unequal distribution of phenolics in different parts of the plant. Several scavenging compounds from different plant parts could be contributing in synergistic manner to the observed antioxidant activity.

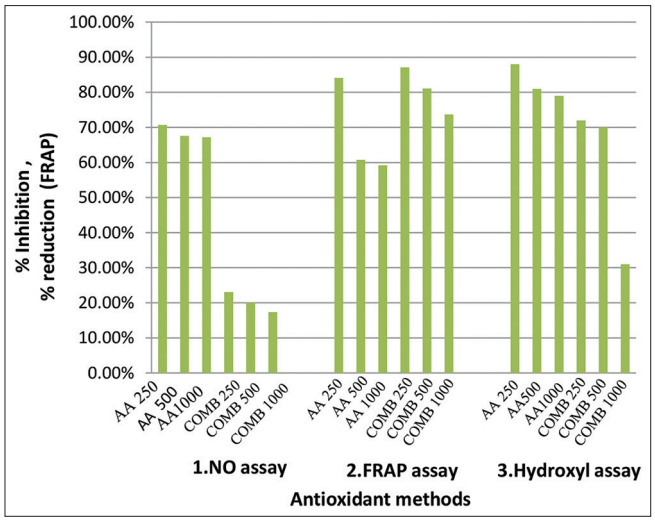

Figure 4: Comparison of the three *in vitro* assays

Methods to determine antioxidant activity have their own applications as well as limitations. In the present study three *in vitro* methods namely, nitric oxide radical scavenging activity, Ferric reducing antioxidant power (FRAP) assay & hydroxyl radical scavenging capacity assay have been carried out (Figure 4). These methods involve simple spectrophotometric determinations. The assays differ from each other in terms of reactions, conditions as well as in the expression of results. Also when a single method is used, results may still differ owing to solvent used, pH, time of reaction & standard used making comparison of data from various studies rather difficult. Current findings establish antioxidant activity based on all the three assays carried out. More antioxidant activity has been observed in FRAP assay & HOradical scavenging assay compared to NO scavenging assay. Results of FRAP assay are promising to an extent that observed antioxidant activity is more than the standard, ascorbic acid used in the study. However a dose dependent increase in antioxidant activity has not been observed. A high reducing power of the combined plant extracts observed in the present study is indicated by an increased absorbance.

The reducing power activity of compounds is a significant indicator of antioxidant activity. Ability of the extract to reduce Fe^{3+} could be most likely due to reducing agents such as phenol groups & the number or/position of HO· group on these (Sylvie *et al.*, 2014). Current study results do provide scientific evidence adding to the existing knowledge about antioxidant activity of the culinary plants. Further research in the form of additional assays, *in-vivo* studies & findings to support exact mechanism of action, isolation & characterization of active principles is recommended.

CONCLUSION

Findings reveal antioxidant potential of the combined extract as antioxidant activity is revealed in all the three assay methods used in the study. Results are promising enough to take the study further using additional antioxidant determining technique, finding the specific constituents responsible for the observed effects and supporting with proper application of statistics to analyze observed result.

ACKNOWLEDGEMENT

Authors immensely thank Dr. Harsha Hegde, Scientist RMRC for plant authentication & students of final B. Pharm for assisting in carry out the assays.

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