Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(5): 2080-2084
Received: 16-07-2018
Accepted: 18-08-2018

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In vitro antioxidant activity of *Brassica oleracea*, *Aristolochia bracteolata* leaves extract

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Abstract
The chosen medicinal plant namely as *Brassica oleracea* and *Aristolochia bracteolata* leaves. Hence, the antioxidant activity of *Brassica oleracea* and *Aristolochia bracteolata* leaves were not evaluated. The aim of the study was to investigate the free radical scavenging activity of *Brassica oleracea* and *Aristolochia bracteolata* leaves through DPPH, total antioxidant assay, superoxide, metal chelation and iron reducing power activity at different concentrations (20, 40, 60 and 80µg/ml). Throughout the studies leaves extract showed marked antioxidant activity. The antioxidant activity was found to be concentration dependent and may be attributed to the presence of bioflavonoids content in the *Brassica oleracea* and *Aristolochia bracteolata* leaves. Overall, the *Brassica oleracea* and *Aristolochia bracteolata* leaves extract is a source of natural antioxidants which might be helpful in preventing the progress of various oxidative stress mediated diseases including aging. Among the two plants, the activity of *Aristolochia bracteolata* is near to standard as ascorbic acid.

Keywords: Antioxidant activity, *Brassica oleracea* and *Aristolochia bracteolata*

Introduction
The recent abundant evidence suggesting the involvement of oxidative stress in the pathogenesis of various disorders and diseases has attracted much attention of the scientists and general public to the role of natural antioxidants in the maintenance of human health and prevention and treatment of diseases [1]. Plant and its products are rich sources of a phytochemicals and have been found to possess a variety of biological activities including antioxidant potential [2]. The majority of the active antioxidant constituents are flavonoids, isoflavones, flavones, anthrocyanins, coumarins, lignans, catechins, and isocatechins. In addition to the above compounds found in natural foods, vitamins C and E, beta-carotene, and tocopherol are known to possess antioxidant potential [3]. With this background and abundant source of unique active components harbored in plants. Therefore, the present study were to investigate the free radical scavenging activity of *Brassica oleracea* and *Aristolochia bracteolata* leaves through the free radical scavenging such as DPPH scavenging, nitric oxide, superoxide anion radical scavenging, metal chelation, reducing power activity and total antioxidant assay.

Materials and Methods
Plant materials and Preparation of alcoholic extract
The fully mature *Brassica oleracea and Aristolochia bracteolata* leaves were collected in October 2015 from the weekly market, Muthur, Tirupur District, South Tamil Nadu, India. The healthy fresh leaves collected thoroughly washed with distilled water and kept in shade at room temperature about one week to dry. Then made into powder with the help of a Pulveriser and sieved. The Dried powdered samples were Soxhlet extracted with ethanol until the solvent was colourless. The extracts were filtered and concentrated under reduced pressure in a rotary evaporator. The dried extracts were kept in the refrigerator at 4°C until use.

In vitro antioxidant activity
In vitro antioxidant activity of *Brassica oleracea* and *Aristolochia bracteolata* leaves investigated by the following method. DPPH radical-scavenging activity was determined by the method of Shimada, *et al.*, [4]. The antioxidant activity of the extracts was evaluated by the phosphomolybdenum method according to the procedure of Prieto *et al.*, [5]. The superoxide anion radicals scavenging activity was measured by the method of Liu *et al.*, [6]. The Fe^{3+} reducing power of the extract was determined by the method of Oyaizu [7]. The chelating activity of the extracts for ferrous ions Fe^{2+} was measured according to the method of Dinis *et al.*, [8].

Statistical analysis

The percentage mortality observed was corrected using Abbott's formula. Statistical analysis of the experimental data was performed using the MS EXCEL 2003 to find out the $IC_{50.}$ The plant concentration for 50% inhibition (IC_{50}) was determined by plotting percentage inhibition with respect to control against treatment concentration.

Results and Discussion

Free radicals implicated more than 100 diseases including cardiovascular diseases, cancers, neurodegenerative diseases, Alzheimer's disease and inflammatory diseases. Natural and synthetic antioxidants are beneficial to free radical mediated diseases. Synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), have been widely used as antioxidants in the food industry and may be responsible for liver damage and carcinogenesis but observed the side effects for long term use. For this reason, interest in the use of natural antioxidants has increased. The phenolic compounds in plants act as antioxidants due to their redox properties, allowing them to act as reducing agents, hydrogen donors, free radical quenchers and metal chelators. With this background and abundant source of unique active components harbored in plants [9]. The aim of the study was to investigate the free radical scavenging activity of *Brassica oleracea* and *Aristolochia bracteolata* leaves through DPPH, total antioxidant assay, superoxide, metal chelation and iron reducing power activity at different concentrations (20, 40, 60 and 80µg/ml).

DPPH radical scavenging activity

DPPH radical scavenging activity of *Brassica oleracea*, *Aristolochia bracteolata* extract and standard as ascorbic acid are presented in Fig 4.11. The half inhibition concentration (IC_{50}) of *Brassica oleracea*, *Aristolochia bracteolata* extract and ascorbic acid were 52.20, 37.18µg/ml-1 and 34.89µg/ml-1 respectively. The *Aristolochia bracteolata* extract exhibited a significant dose dependent inhibition of DPPH activity (Table 1 and Fig 1). The potential of L-ascorbic acid to scavenge DPPH radical is directly proportional to the concentration. The DPPH assay activity *Aristolochia bracteolata* is near to standard as ascorbic acid

Table 1: *In vitro* Antioxidant Activity of DPPH

Samples	Concentrations (µg/ml)				IC_{50} value
	20	40	60	80	
Brassica oleracea	22.27±1.55	37.27±2.60	59.55±4.17	73.18±5.12	52.20
Aristolochia bracteolata	29.54±2.06	57.27±4	74.09±5.19	89.54±6.26	37.18
Standard as Ascorbic acid	25.6±2.04	61.26±4.90	88.98±7.11	99.34±7.94	34.89

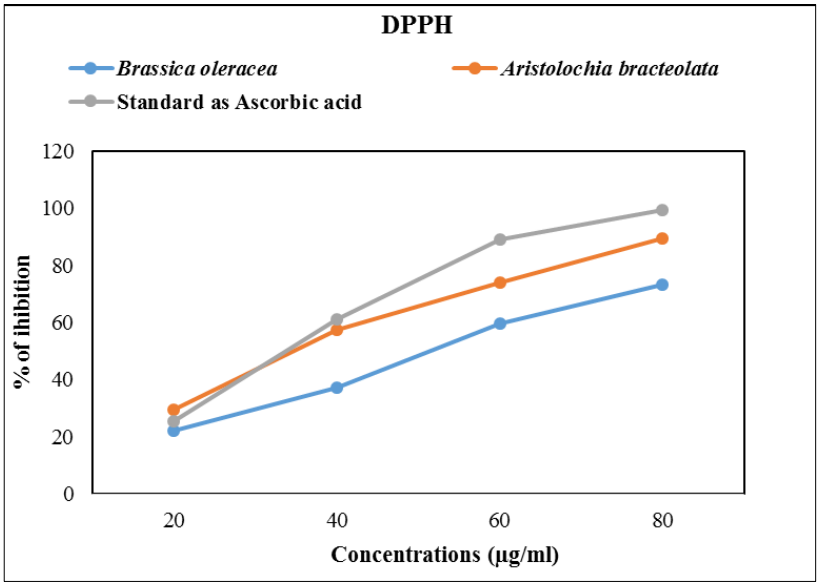

Fig 1: Antioxidant Activity of DPPH

Total antioxidant activity

The extract of the *Brassica oleracea* and *Aristolochia bracteolata* its total antioxidant capacity are given in Fig. 2. The study reveals that the antioxidant activity of the extract is in the increasing trend with the increasing concentration of the *Brassica oleracea*, *Aristolochia bracteolata* extract (Table 2). The half inhibition concentration (IC_{50}) of *Brassica oleracea*, *Aristolochia bracteolata* extract and ascorbic acid were 48.17, 38.49µg/ml^{-1} and 42.38 µg/ml^{-1} respectively**.** The IC_{50} value of *Aristolochia bracteolata* is near standard as ascorbic acid.

Table 2: *In vitro* Total antioxidant assay

Samples	Concentrations (µg/ml)				IC_{50} value
	20	40	60	80	
Brassica oleracea	23.43±1.64	41.25±2.89	63.75±4.46	78.43±5.49	48.17
Aristolochia bracteolata	33.43±2.34	51.25±3.58	69.68±4.88	86.56±6.05	38.49
Standard as Ascorbic acid	22.35± 1.80	51.23± 4.09	72.54± 5.80	86.35± 6.91	42.38

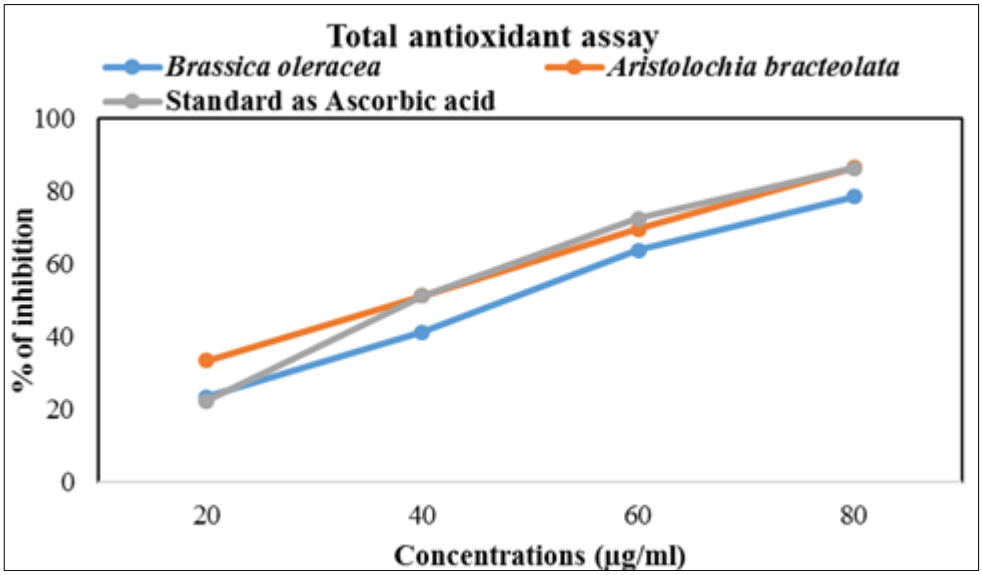

Fig 2: Total antioxidant assay

Superoxide scavenging activity

The superoxide anion radical scavenging activities of the extract from *Brassica oleracea* and *Aristolochia bracteolata* assayed by the PMS-NADH system was shown in Fig 3. The superoxide scavenging activity of *Evolvulus alsinoides* was increased markedly with the increase of concentrations (Table 3). The half inhibition concentration (IC_{50}) of *Brassica oleracea*, *Aristolochia bracteolata* extract and ascorbic acid were 49.59, 41.52µg/ml-1 and 31.60µg/ml-1 respectively**.**

Table 3: Superoxide radical scavenging

Samples	Concentration (µg/ml)				IC_{50} value
	20	40	60	80	
Brassica oleracea	23.57±1.65	41.07±2.87	62.50±4.38	74.28±5.20	49.59
Aristolochia bracteolata	29.28±2.05	52.85±3.70	66.07±4.62	79.64±5.57	41.52
Standard as Ascorbic acid	31.25±2.50	64.23±5.13	89.54±7.16	98.51±7.88	31.60

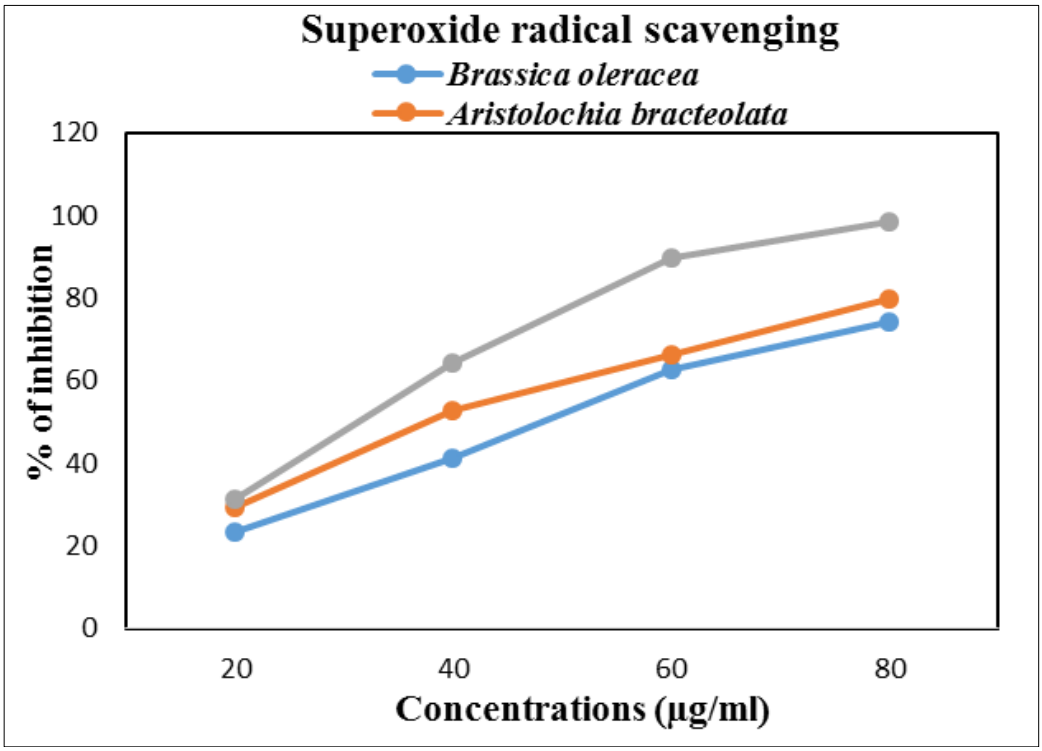

Fig 3: Antioxidant Activity of Superoxide radical scavenging

Fe^{2+} chelating activity

The formation of the ferrozine– Fe^{2+} complex is interrupted in the presence of *Brassica oleracea* and *Aristolochia bracteolata* extract indicating that have chelating activity with an IC_{50} of 53.81 µg/ml and ascorbic acid was 40.13 µg/ml respectively (Fig. 4, Table 4). The IC_{50} value of standard as ascorbic acid was 30.93 µg/ml

Table 4: Fe^{2+} chelating activity

Samples	Concentrations (µg/ml)				IC_{50} value
	20	40	60	80	
Brassica oleracea	22.30±1.56	33.84±2.37	53.84±3.76	76.15±5.33	53.81
Aristolochia bracteolata	31.92±2.23	48.84±3.42	69.23±4.85	86.15±6.03	40.13
Standard as Ascorbic acid	35.23±2.81	65.21±5.28	78.51±6.28	98.65±7.89	30.93

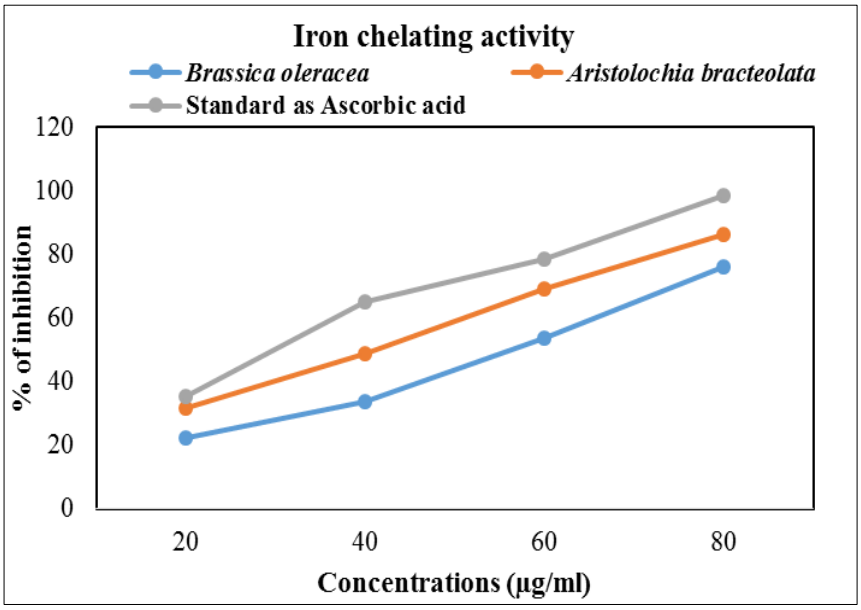

Fig.4: Antioxidant Activity of Fe^{2+} chelating activity

Reducing power activity

Fig. 5 depicts the reductive effect of *Brassica oleracea and Aristolochia bracteolata* extract. Similar to the antioxidant activity, the reducing power *Brassica oleracea* and *Aristolochia bracteolata* extract increased with increasing dosage (Table 5). All the doses showed significant activities near to the control exhibited greater reducing power, indicating that *Brassica oleracea and Aristolochia bracteolata extract* consist of hydrophilic polyphenolic compounds that cause the greater reducing power.

Table 5: Reducing power assay

Samples	Concentrations (µg/ml)			
	20	40	60	80
Brassica oleracea	0.23±0.02	0.41±0.03	0.67±0.05	0.78±0.05
Aristolochia bracteolata	0.32±0.02	0.53±0.04	0.72±0.05	0.85±0.06
Standard as Ascorbic acid	0.41±0.03	0.71±0.05	0.89±0.07	0.98±0.08

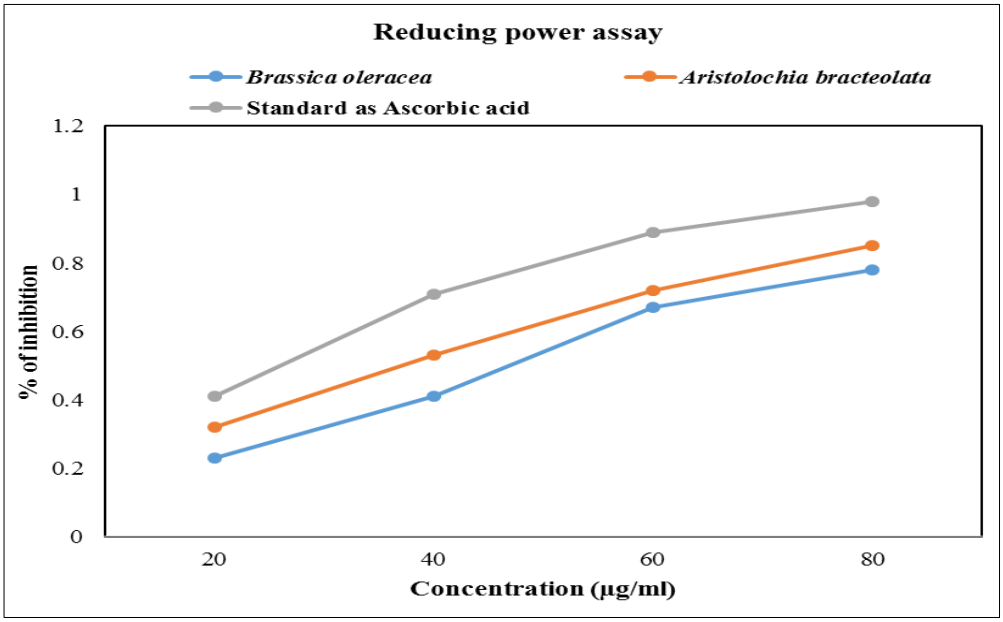

Fig 5: Antioxidant Activity of Reducing power assay

Conclusion

The extract of *Brassica oleracea* and *Aristolochia bracteolata* leaves were screened for *in vitro* antioxidant activity by DPPH, Total antioxidant activity, oxygen radical scavenging such as superoxide and metal chelation, iron reducing power activity at different concentrations (20µg/ml to 80µg/ml). The antioxidant activity was found to be concentration dependent and may be attributed to the presence of bioflavonoids content in *Brassica oleracea and Aristolochia bracteolata* leaves. Among the two plants, *Aristolochia bracteolata* leaves possess considerable antioxidant activity than *Brassica oleracea* leaves.

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