Medicinal & Aromatic Plants
ISSN: 2167-0412

Reddy and Grace, Med Aromat Plants 2016, 5:2
DOI: 10.4172/2167-0412.1000231

Research Article Open Access

In vitro Evaluation of Antioxidant Activity of *Brugeiera Gymnorrhiza* and *Aegialitis Rotundifolia*

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Abstract

Mangroves have wide applications in folk medicine since ages due to the presence of several bioactive compounds. The current study was aimed at an evaluation of antioxidant activity of two mangrove species. Leaf extracts of *Brugeiera gymnorrhiza* and *Aegialitis rotundifolia* were prepared in Chloroform, Acetone, Ethyl acetate, and Methanol. The crude extracts were screened for antioxidant activity. The antioxidant activity of these extracts was done by DPPH and FRAP assay. Methanol infusions demonstrate the highest antioxidant activity. The present study reveals the potential of leaf extracts of *Brugeiera gymnorrhiza* and *Aegialitis rotundifolia* as an antioxidant agent.

Keywords: Antioxidant; DPPH; FRAP; Mangroves

Introduction

In recent years antioxidant research has gained much importance in the medicinal field as well as in the food industry. Research with important bioactive components present in many plants and food materials has received much attention. Free radicals or oxidative injury now appears to be the consequence of a number of human disorders and diseases. When a reactive molecule such as reactive oxygen, reactive nitrogen and reactive chlorine species contains one or more unpaired electrons, the molecule is termed as a free radical. The oxidation caused by reactive oxygen species (ROS) or free radicals are responsible for aging and causes cell membrane disintegration, membrane protein damage, DNA mutations and various other diseases. Antioxidants play an important role in the prevention of free radical induced diseases. By donating hydrogen atoms, the free radicals are converted to non-radicals [1,2]. Although the body posses various defense mechanisms, as enzymes and antioxidant nutrients which arrest damaging properties of ROS, continuous exposure to chemicals and contaminants may lead to an increase in the amount of free radicals in the body beyond its capacity to control them and cause irreversible oxidation. Therefore, antioxidants with free radical scavenging activities may have great relevance in the prevention and therapeutics of diseases in which oxidants or free radicals are implicated. In this respect, poly phenolic compounds, like flavonoids and phenolic compounds found in plants have been reported to have multiple biological effects, including antioxidant activity. It is generally assumed that frequent consumption of plant derived phyto chemicals present in vegetables, fruits, tea and herbs may contribute to shift the balance towards an adequate antioxidant status [3]. Thus interest in natural antioxidants, especially of plant origin has greatly increased in recent years.

Mangroves are the unique plant communities that inhabit the estuarine and intertidal zones of tropical and sub-tropical regions [4]. Mangroves are also biochemically unique, producing a wide array of novel natural products. Mangrove and mangrove associates contain biologically active anti diabetic, anti inflammatory and anti microbial compounds. Hence they have wide applications in folk medicine since ages due to their antioxidant, anti inflammatory and other medicinal properties [5]. Secondary metabolites like alkaloids, phenolics, steroids, and terpinoids have been characterized from mangroves and have toxicological, pharmacological, and ecological importance [6,7]. The mangrove plants have also been proved for their antioxidant and anti-microbial properties [8,9]. The potential of mangrove plants as a source of new bio-active principles is still unexplored. Further, there has been no detailed *in vitro* studies on antioxidant properties of leaves of the mangrove medicinal plants from Corangi reserve forest, Kakinada, East Godavari district, Andhra Pradesh, India. Hence the current study is aimed on the exploration of leaf extracts of two mangrove species, *Brugeiera gymnorrhiza* and *Aegialitis rotundifolia* in different organic solvents to screen for antioxidant activity by DPPH and FRAP assay methods.

Materials and Methods

Plant material

In the present study, the fresh leaves of *Brugeiera gymnorrhiza* and *Aegialitis rotundifolia* were collected from Corangi reserve forest, Kakinada, East Godavari district, Andhra Pradesh, India. Its ggeographical location is between 16°39' N longitude 17° N' longitude and 82°14' E latitude–82°23' E latitude. The collected leaves were taken to the laboratory and washed thoroughly with tap water to remove dust and dried in a shaded and well-ventilated place at room temperature. The dried leaves were ground to obtain coarse powder and subjected to solvent extraction.

Extraction

Different solvents in the increasing order of polarity were employed to prepare the extracts. Ethyl acetate, Acetone, Chloroform and Methanol were used to prepare the crude extracts of plants. The chopped material was first soaked for 12 Hrs in 500 ml of the respective solvent. The material was subjected to extraction by refluxing for 6 to 8 Hrs below the boiling point of the respective solvent. The extract was concentrated by evaporating at a reduced pressure using rotary evaporator. The concentrated extract was further dried at 37°C for 3 to 4 days in order to facilitate complete evaporation of the solvent.

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Received January 14, 2016; **Accepted** January 22, 2016; **Published** January 25, 2016

Citation: Reddy ARK, Grace JR (2016) *In vitro* Evaluation of Antioxidant Activity of *Brugeiera Gymnorrhiza* and *Aegialitis Rotundifolia*. Med Aromat Plants 5: 231. doi:10.4172/2167-0412.1000231

Methods for antioxidant analysis

The antioxidant activities were screened by DPPH and FRAP assay.

DPPH Activity: The antioxidant activity of the plant extracts and the standard was assessed on the basis of the radical scavenging effect of the stable 1, 1-diphenyl-2-picryl hydroxyl (DPPH) free radical activity method with minor modifications. The diluted working solutions of the test extracts were prepared in methanol. Ascorbic acid was used as reference compound(100 µg/ml solution). DPPH of 0.004% was prepared in methanol and a 3 ml of this solution was mixed with 1ml of sample solution and standard solution separately. The mixtures were kept in dark for 30 min at room temperature. After incubation, the absorbance was spectrophotometrically measured at 517 nm against a blank. The inhibition percentage was calculated using the following formula:

Percentage of Inhibition of DPPH activity=(A-B)/ Ax100

Where, A is the Absorbance of control and B is the absorbance of sample at 517 nm.

FRAP assay: The Frap Assay of Benzie and Strain method [10] was employed with minor changes. The FRAP method (Ferric reducing antioxidant power) is based on the reduction of complexes of 2, 4, 6-tripyridyl-S-triazine with ferric chloride.

FRAP reagent was prepared freshly by mixing 25 ml of acetate buffer (PH-3.6) with 2.5 ml of 10 mM 2, 4, 6 tri pyridyl triazine and 2.5 ml of 20 mM ferric chloride solution. The reagent was kept at 37°C before use.

Pocedure: Exactly, 300 µl of the leaf extract were dispensed into 2700 µl freshly prepared FRAP reagent and incubated at 37°C for 30 min. The absorbance was recorded at 593 nm against a blank. Standard curve was prepared with 100 µm FeSo4 solution. All readings were taken in triplicates and expressed as umFe^{2+} equivalents/100 gms.

Statistical analysis: Both the DPPH assay and FRAP assay were carried out in triplicates. The mean values were calculated from the triplicate values are as the mean ± SD. (n=3) of three parallel measurements.

Results and Discussion

DPPH free radical scavenging assay

In DPPH assay, DPPH radical has been used extensively as a stable free radical to determine the reducing substances or antioxidant activity of plant extracts [11,12]. The antioxidant activity of plant extract containing poly phenol compound is due to their capacity to be donors of hydrogen atoms or electrons and to capture the free radicals [13,14]. The purple coloured 1, 1diphenyl-2-picrylhhydrazyl (DPPH) will reduce to yellow coloured complex. This DPPH assay is considered most valuable procedure for the In vitro Evaluation of Antioxidant activity.

The results of DPPH free radical scavenging test confirmed that, in Brugeiera *gymnorrhiza* and *Aegialitis rotundifolia,* the Methanolic extracts showed significantly higher antioxidant activity than the other solvent extracts indicating the best scavenging activity of methanolic extract and this is followed by ethyl acetate, acetone and chloroform extracts (Figures 1 and 2). The percentage of inhibition of DPPH radical was given in Table 1. In this study, the antioxidant activity these two plant extracts were also measured by FRAP assay. FRAP assay measures the ability of antioxidant to reduce the 2, 4, 6 tripyridyl –s-tri azine complex Fe (III) to intensely blue coloured ferrous complex (FeII) in acidic medium. FRAP values are calculated by measuring the absorbance increase 593 nm and relating it to a ferrous ion standard. The reduction capacity of plant extract may serve as a significant indicator of its potential antioxidant activity. In the present study, the Methanolic extract showed highest anti oxidant activity and this is followed by ethyl acetate, acetone and chloroform extracts (Figures 3 and 4). The FRAP values for different plant extracts were given in Table 2. By observing the above results, the present study confirmed that the extraction solvent used in the preparation of samples has an

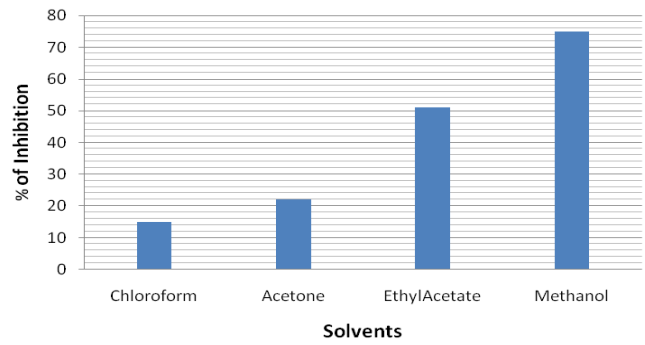

Figure 1: Antioxidant activity of leaf extracts of *Aegialitis rotundifolia* by DPPH Method.

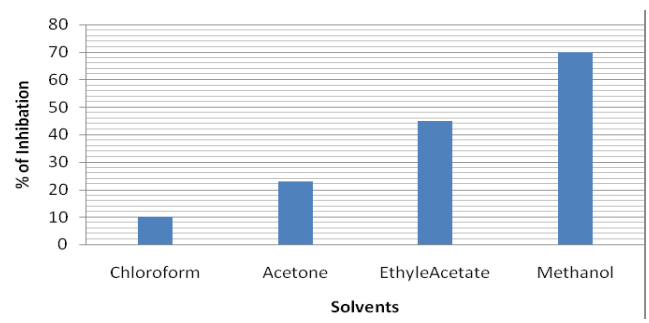

Figure 2: Antioxidant activity of leaf extracts of *Brugeiera gymnorrhiza* by DPPH method.

S.No	Plant Extract	% DPPH Inhibition (at con of 500 µg/ml)		Ascorbic acid Standard
		Brugeiera gymnorrhiza	*Aegialitis rotundifolia*	
1	Methanol extract	72 ± 1.63	75 ± 1.63	85.31 ± 0.05
2	Ethyl acetate extract	42 ± 1.68	56.33 ± 1.69	80.32 ± 0.07
3	Acetone extract	21 ± 1.24	20.33 ± 1.24	79.57 ± 0.08
4	Chloroform extract	9.66 ± 1.24	14 ± 0.8	78.56 ± 0.05

Results are expressed as mean ± SD (n=3) of the three parallel measurements.
Table 1: % of DPPH Inhibition Plant extracts of *Brugeiera gymnorrhiza* and *Aegialitis rotundifolia.*

S.No	Plant Extract	FRAP VALUE (µmol Fe2+ / g extract)	
		Brugeiera gymnorrhiza	*Aegialitis rotundifolia*
1	Methanol extract	1854 ± 3.1	2115 ± 3.7
2	Ethyl acetate extract	1548 ± 3.4	1890 ± 2.6
3	acetone extract	1257 ± 2.5	1241 ± 2.2
4	chloroform extract	658 ± 2.4	845 ± 3.2

Results are expressed as mean ± SD (n=3) of the three parallel measurements.
Table 2: FRAP Values of different plant extracts of *Brugeiera gymnorrhiza* and *Aegialitis rotundifolia.*

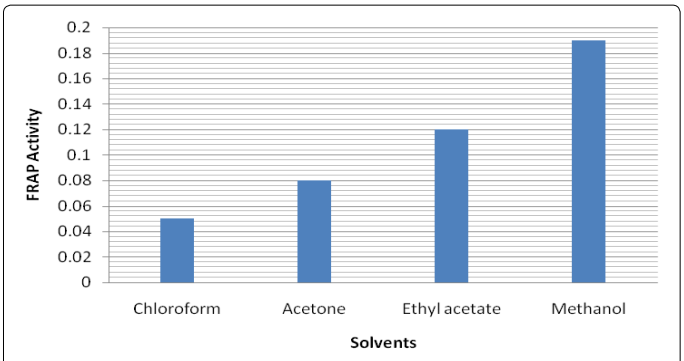

Figure 3: Antioxidant activity of *Brugeiera gymnorrhiza* by FRAP method.

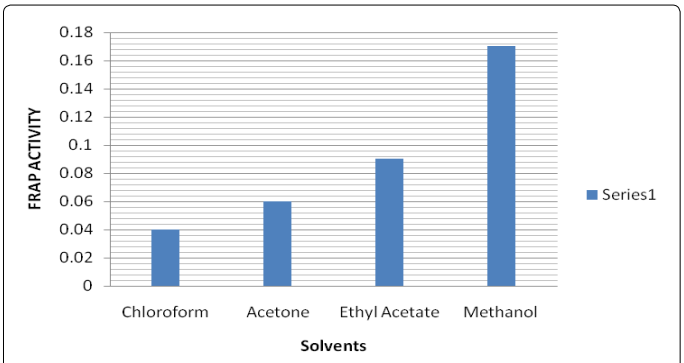

Figure 4: Antioxidant activity of *Aegialitis rotundifolia* by FRAP method.

important effect on the antioxidant activity. This result clearly showed that extraction of solvents significantly affected the total phenolic content of the prepared extracts.

Mangroves usually grow in estuarine swamps with high salinity, high temperature, low nutrients and high radiation. They survive in high environmental stress as they have unique properties to combat stress. Exposure to these stress situations results in production of reactive oxygen species in these plants. In order to reduce the adverse effects these ROS, the mangrove plants are known to produce antioxidant enzymes [15] and various defense compounds including poly phenols like tannins [16]. Free radicals or reactive oxygen species (ROS) are the primary cause of oxidative damage of biological molecules in the human body and are related to aging, cancer, heart diseases [17]. Antioxidants fight against these free radicals and protect the body from various diseases. The antioxidant properties of plant extracts are attributed to its secondary metabolites. The importance of secondary metabolites in plants as natural antioxidants and their uses as substitutes to synthetic antioxidants in food additives is well known [18,19]. Antioxidants in food play an important role as a health protecting factor [20]. From the results we found that the crude extracts were as effective as the positive control (Ascorbic Acid) except for the chloroform extracts. In the present study, the antioxidant activity of *Brugeiera* gymnorrhiza and *Aegialitis rotundifolia* were estimated by DPPH and FRAP assay in different solvents.

Conclusions

Based on the results, it may be concluded that the leaves of *Brugeiera gymnorrhiza* and Aegialitis *rotundifolia* contain phenolic compounds that contributed for their antioxidant properties. There is an increased interest for identifying alternative natural and safe sources of antioxidants and the search for natural antioxidants in the form of plant origin. The extracts of Brugeiera *gymnorrhiza* and Aegialitis *rotundifolia* had the ability of scavenging free radicals. These findings also showed that the antioxidant activity of extracts varies according to the method used and solvent used for extraction. Further investigations of identification of specific phytochemicals and bioactive principles that under lay the antioxidant properties of these plants will pave way for better recommendation of them as antioxidant sources to combat the oxidative injury.

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