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Evaluation of antioxidant activities and quantitative estimation of phytochemicals in *Kandelia candel* (L.) Druce

Jasna T K*[1], Khaleel K M[2]

[1]Research Scholar, Department of PG Studies and Research in Botany, Sir Syed College, Kerala, India
[2]Department of PG Studies and Research in Botany, Sir Syed College, Taliparamba, Kerala, India

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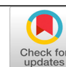

ABSTRACT

Kandelia candel, a mangrove belonging to the family Rhizophoraceae, the whole plant is reported to have anti-diabetic properties and was studied for the evaluation of *in vitro* antioxidant and antidiabetic study. Total Phenolic Content (TPC), Total Flavonoid Content (TFC) and Total Alkaloid Content were quantitatively estimated by using standard protocols. The study evaluated the antioxidant activity of the extract and the method was employed for the evaluation of antioxidant activity are DPPH radical scavenging assay, reducing power assay and Phosphomolybdenum assay. Potent antioxidant activities were shown by the acetonitrile extract and water extract of leaf and stem in all the assays. Acetonitrile extract of bark showing highest DPPH scavenging activity (IC_{50} = 31.84 g/ml). Plant extract showing poor, reducing power. The better reduction of molybdenum was shown by acetonitrile extract of bark followed by leaf (617.33 $\pm$ 4.44). Chloroform, acetonitrile and water extract of leaf and bark of *Kandelia candel,* were evaluated for antidiabetic activity by alpha-amylase inhibition assay, Water and acetonitrile extract show good inhibition. Highest TPC and TFC were obtained for the acetonitrile extract of bark (237.6 $\pm$ 1.06 and 1277.19 $\pm$ 3.41). These findings show that the polyphenolic constituents in the extracts are responsible for free radical scavenging capacity. The results of this study indicate that this plant is medicinal with prominent antioxidant activities.

*Corresponding Author

Name: Jasna T K
Phone: +91- 9633435902
Email: Jasnathaikandy@gmail.com

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INTRODUCTION

Mangroves are seen in a stressful environment with the condition like high salinity, high solar radiation during low tide and low nutrition. By these conditions, reactive oxygen species produces in mangrove plants. They are rich in polyphenol compounds which act as antioxidants by a struggle with the oxidative stress [1]. Mangrove protecting the adjacent land as a barrier against tidal waves and sea storm [2]. Flavonoid and other polyphenolic group have antioxidant property and this free radical scavenging is mediated by the hydroxyl groups present in them. Study shows that mangrove root extract has antioxidant activity and it is by the presence of flavonoid and polyphenol compounds [3]. Mangroves produce several secondary metabolites. 80% of the carbon is utilized for the production of naturally occurring phenolics and the rest is assumed for photosynthesis [4]. Molecular oxygen produces as a result of photosynthesis. Reactive oxygen species such as superoxide, hydroxyl and peroxyl radical produces as a consequence of this normal process.

During several metabolic processes, reactive oxygen species produced in cells and these are an essential part of metabolism. Due to abiotic stress, free radicals produce excessively and these leads to oxidative stress and various disorders. The cytotoxic activity of reactive oxygen species leads to the development of a detoxification mechanism in plants. There are various enzymatic and nonenzymatic antioxidants to scavenge the reactive oxygen species and ultimately avoid the oxidative damage. Antioxidants can inhibit the synthesis of free radicals and inactivate them. Radical scavenging ability, prevention of chain reaction, reductive capacity and chelation of metals are the various factors attributed to the antioxidant capacity of compounds [5].

Phenolic compounds reported possessing various biological activities. They are commonly found in plants with strong antioxidant activity [6]. The phenolic extract shows antioxidant activity and it is by the radical scavenging ability by the hydroxyl groups [7]. Antioxidant activity of flavonoid include suppression of reactive oxygen species, the direction of ros scavenging and protection of antioxidant defenses [8]. The antioxidant present in plants shows resistance against oxidative damage either constitutively or by inducing [9].

MATERIALS AND METHODS

Plant Collection

Kandelia candel (L) Druce collected from Pazhayangadi, Kannur district, Kerala was taken for the study. For the removal of dust and soil particles, leaf and bark of the plants were washed with tap water followed with distilled water. The plants cut into pieces and were shade dried at room temperature for 15 days then coarsely powdered and used for extraction.

Preparation of Extract

The powder (50 gm) was extracted with petroleum ether, chloroform and acetonitrile in a soxhlet apparatus in increasing order of their polarity. Finally, the dried powder was macerated using water with constant stirring for 48 hours using the orbital shaker and the extract was filtered. The extracts were concentrated, dried and stored at -20 ºC in the deep freezer (RQV- 300; plus, REMI electro technik Ltd., Thane, Maharashtra, India) for further analysis.

Quantification assays

Quantification of total phenolics

The total phenolics of the different plant extracts were determined according to the method described by Makkar (2003) [10]. Results were expressed as Gallic acid equivalents.

Quantification of total flavonoids

The flavonoid contents of all the extracts were quantified according to the method described by Zhishen et al. (1999) [11]. Results were expressed in Rutin equivalents.

Quantification of total alkaloid

Alkaloid determination using Harborne (1973) [12] method.

In vitro antioxidant assays

DPPH scavenging activity

The antioxidant activity of the extract was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH, according to the method of Braca et al. (2001) [13]. Radical scavenging activity of the samples was expressed as IC_{50}, which is the concentration of the sample required to inhibit 50% of DPPH concentration.

Phosphomolybdenum assay

The antioxidant activity of samples was evaluated by the green phosphomolybdenum complex formation according to the method of Prieto et al. (1999) [14]. Ascorbic acid was used as the reference standard and the results were expressed as milligrams of ascorbic acid equivalents per gram extract.

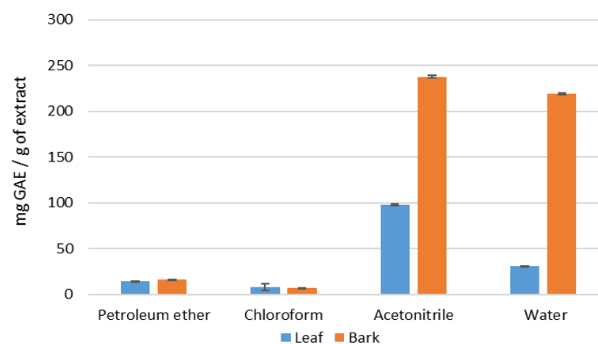

Figure 1: Total phenolic content of *Kandelia candel.*

Reducing power assay

The reducing power of sample extracts was determined according to the method of Oyaizu (1986) [15].

Evaluation of antidiabetic activity by using *invitro* assay

Alpha-amylase inhibitory assay

The Alpha-amylase inhibitory assay for different solvent extracts of *Kandelia candel* were evaluated according to a previously described method by Malik and Singh et al. (1980) [16] with slight modification.

Statistical analysis

Measurements were recorded in triplicates for all the analysis. Results were calculated as the mean ± SD for each sample. One-way ANOVA followed by student's t-test was performed using GraphPad Prism version 7.02 for Windows (Graphpad Software, La Jolla California, USA) $p < 0.05$ were considered significant. IC_{50} values were determined using a nonlinear regression method.

Table 1: Total phenolic content of *K.candel* (mg GAE/g extract).

Solvents	Leaf	Bark
Petroleum ether	14.35±0.35	16.31±0.25
Chloroform	7.88±3.90	6.75±0.090
Acetonitrile	97.87±0.62	237.6±1.06
Water	30.57±0.65	219.02±0.82

Values are mean of triplicate determination (n=3) ± standard deviation, GAE-Gallic Acid Equivalents, * statistically significant at P<0.05.

RESULTS AND DISCUSSION

Quantification of Total Phenolics

Total phenolic contents of the various extract were determined using Folin-Ciocalteu's method and represented in terms of gallic acid equivalent. The total phenolics of different extract of leaf and bark of *K. candel* were analyzed (Table 1, Figure 1). The phenol content in the extracts ranged from 14.35 to 237.6 μg of GAE/ g. The results revealed that the phenol content of different extract of *K. candel* varied significantly. It ranged from 14.35 to 97.87 μg of GAE/ g extract in the leaf extract. Among the different parts analysed bark extract obtained by acetonitrile (237.6 μg of GAE/ g) showed better phenolic content, while petroleum ether extracts showed lower concentration.

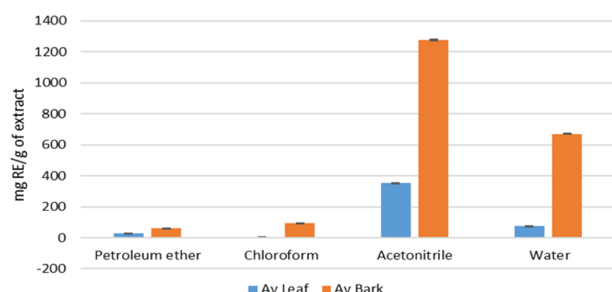

Figure 2: Total flavonoid content of *K.candel.*

Quantification of Total Flavonoids

The flavonoid content of different extract in the selected plants was analyzed. Present investigation revealed the presence of a broad range of flavonoid content with high significance in bark extracts of *K. candel* (Figure 2). Total flavonoid content was determined from standard (+)-rutin regression curve and expressed as (+)-rutin equivalents per gram of extracts. Total flavonoid content of leaf and bark of *K. candel* is shown in Table 2. Flavonoid content of leaf extracts of *K. candel* varied from 28.39 to 352.90 mg RE/g of extracts. The acetonitrile extract of bark (1277.19 mg RE/g extract) was found to be having an appreciable amount of flavonoids followed by water extract of bark (669.84 mg RE/g).

Table 2: Total flavonoid content of *K.candel* (mg RE/g extract).

Solvents	Leaf	Bark
Petroleum ether	28.39±1.27	59.35±1.21
Chloroform	0.96±1.27	92.39±2.40
Acetonitrile	352.90±1.54	1277.19±3.41
Water	75.23±0.73	669.84±2.98

Values are mean of triplicate determination (n=3) ± standard deviation, RE-Rutin Equivalents *statistically significant at P<0.05.

Quantification of total alkaloid

The total alkaloid content of leaf and bark of *K. candel* has been estimated and results are given in Table 3. The alkaloid content was comparatively more in the bark of *K. candel* and it is 0.086 mg / 1g powder.

Table 3: Total alkaloid content (mg/g powder).

Plant	Plant part	Total alkaloid
Kandelia candel	Leaf	0.085±1.06
	Bark	0.086±1.34

In vitro antioxidant assay

Phosphomolybdenum assay

The phosphomolybdenum assay is a reduction based analysis in which the reduction of Mo (VI) to Mo (V) occurs. The reduction of molybdenum occurs in the presence of the antioxidant compound. The green phosphomolybdenum complex is formed at high temperature and in acidic pH. The phosphomolybdenum antioxidant capacity of different solvent extracts of leaf and bark of *K. candel* were analyzed and are shown in Table 4. The better reduction of molybdenum was shown by acetonitrile extract of bark followed by leaf.

Reducing power assay

The property of reducing power is generally related

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to the occurrence of reductones. The reductones present in the plant extract is responsible for antioxidant activity. Antioxidant potential was estimated according to their reducing capabilities and results are represented in figures. Among all the extracts of different parts, the reducing power of acetonitrile extract of bark was found to be higher. Reducing the power of all the extract of *K. candel* were evaluated. The reducing power of petroleum ether, chloroform, acetonitrile and water extract of leaf and bark shown in Figures 3 and 4. Ascorbic acid is used as a standard to evaluate the reducing activity.

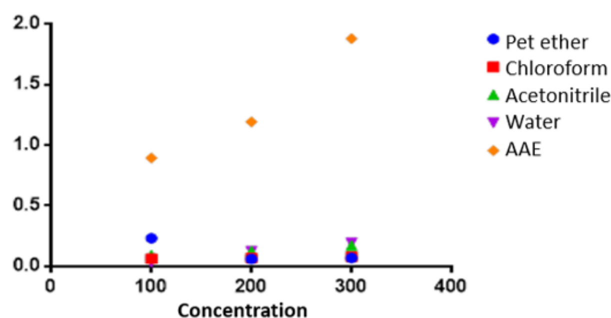

Figure 3: Reducing power of *K.candel* leaf.

DPPH scavenging activity

The radical scavenging activity of the stable 1, 1, -diphenyl-2-picryl- hydrazyl (DPPH) is the basis for the evaluation of antioxidant activity of plant extract as well as the standard [13]. DPPH radical scavenging activities of different extract of leaf and bark of *K. candel* in Tables 5 and 6.

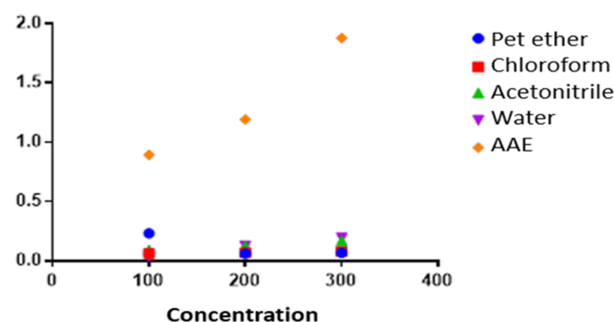

Figure 4: Reducing power of *K.candel* bark.

The lower value of IC_{50} indicates the higher antioxidant activity of extracts. Among the different part studied, bark extracts showed appreciable activity compared to leaf. The significant activities were shown by acetonitrile and water extract of bark. The radical scavenging activities of the plant extract are highest when it is in higher concentration. Radical scavenging activity of leaf of *K. candel* was tested and water extract shows the lowest IC_{50} (128.23) value among other extracts. *K. candel* bark has good inhibition activity compared to leaf Figures 5 and 6.

Among the extracts, acetonitrile and water extract of bark showed the best activity, i.e., IC_{50} = 31.84 g/ml and IC_{50} = 35.56 g/ml respectively Table 6.

Table 4: Phosphomolybdenum assay of *K.candel* (mg AAE/g extract).

Solvents	Leaf	Bark
Petroleum ether	562.33±2.51	40.33±0.57
Chloroform	42.33±2.51	64.33±4.04
Acetonitrile	495.66±4.69	617.33±4.44
Water	417.66±3.29	322.66±2.51

Values are mean of triplicate determination (n=3) ± standard deviation, AAE-Ascorbic Acid Equivalents, *statistically significant at P<0.05.

***In vitro* α-amylase inhibition**

In the present study, chloroform, acetonitrile and water extract of leaf and bark of *Kandelia candel,* were evaluated for antidiabetic activity by using standard *in vitro* technique, alpha-amylase inhibition assay. Alpha-amylase is the enzyme involved in the metabolism of carbohydrates, i.e. hydrolysis of starch and disaccharides to glucose. In the alpha-amylase inhibitory assay, the antidiabetic activity of the known concentration (500μg) of different solvent extracts of *Kandelia candel*, were evaluated along with acarbose as a standard. Among three solvent extracts, water extract showed very good antidiabetic activity with an inhibitory percentage of 50.96 Table 7. In the case of *K. candel* bark, acetonitrile extract showed inhibition (50.32) comparable to the standard, as shown in Table 8.

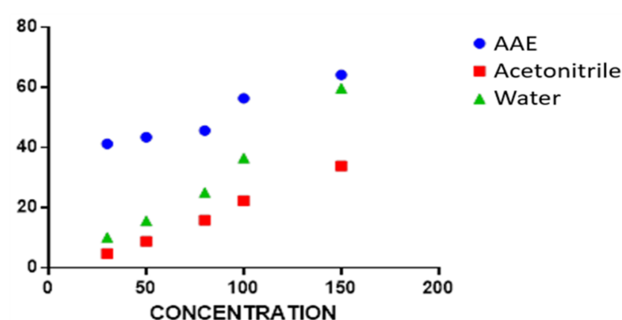

Figure 5: DPPH scavenging activity of *K.candel* leaf.

Lignin, tannin and melanin are the polyphenols that make plant tissues and phenols are monomeric components. Health benefits of plant are attributed by antioxidant [17]. Phenols have scavenging ability so it can act as terminators of free radicals. Redox property of phenolics allows them to act as hydrogen donors, reducing agent and singlet oxygen quenchers [18].

Table 5: DPPH scavenging activity of *K.candel* leaf.

Plant part	Solvent	Concentration	Inhibition (%)	IC_{50}
Leaf	Acetonitrile	30	4.70±0.28	187.06
		50	8.78±0.53	
		60	11.18±0.54	
		80	15.85±1.02	
		100	22.27±1.12	
	Water	30	10.09±0.92	128.23
		50	15.60±0.88	
		80	25±0.86	
		100	36.48±1.21	
		150	59.72±0.83	

Table 6: DPPH scavenging activity of *K.candel* bark.

Plant part	Solvent	Concentration	Inhibition (%)	IC_{50}
Bark	Acetonitrile	30	43.57±0.60	31.84
		50	79.73±2.77	
		80	89.97±0.96	
		100	91.53±0.32	
		150	93.65±0.70	
	Water	30	45.56±0.52	35.56
		50	73.92±0.39	
		80	93.49±0.68	
		100	94.35±0.080	
		150	95.05±0.40	

A linear correlation exists between antioxidant activity and phenol content, that is extract with high phenolic content show high antioxidant activity [19].

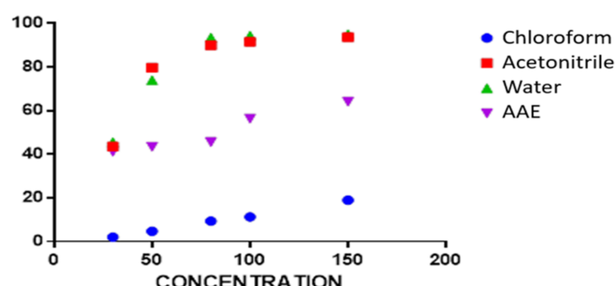

Figure 6: DPPH scavenging activity of *K.candel* bark.

Flavonoids are low molecular weight component which includes flavones, anthocyanidins, flavonoids and chalcones. These compounds containing A and B ring and their alkoxylation and hydroxylation pattern vary extensively and it is very important for the antioxidant activity. Flavonoids possess biological activities such as anti-inflammatory, anticarcinogn etc. these may be by antioxidant properties. Flavonoids can reduce free radicals and also scavenge free radicals. Reduction of Mo (VI) to Mo (v) by the extract and green color phosphate complex formed at acidic pH. This model, assay the water-soluble and fat-soluble antioxidant capacity.

Table 7: *Invitro* α-amylase inhibition activity of *K.candel* leaf.

Samples	Concentration (μg/mL)	Inhibition %
Chloroform	500	9.45±0.34
Acetonitrile	500	33.33±0.18
Water	500	50.96±0.44
Acarbose	500	70.87±0.65

A number of electron or radicals donates or quenched by the antioxidant molecule can be measured by this assay [20]. DPPH is mainly used to evaluate the free radical scavenging activity of antioxidant [15]. It is stable free radical that can accept hydrogen or electron to become a diamagnetic molecule [21]. The antioxidants present in the extract can reduce the stable DPPH radical to

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di-phenyl-picryl-hydrazine, which is yellow colored. The property of reducing power is generally related to the occurrence of reductones.

Table 8: *Invitro* α-amylase inhibition activity of *K.candel* bark.

Samples	Concentration (μg/mL)	Inhibition %
Chloroform	500	11.06±0.72
Acetonitrile	500	50.32±0.37
Water	500	32.11±0.49
Acarbose	500	70.87±0.65

The reductones present in the plant extract is responsible for antioxidant activity. Reducing power is associated with antioxidant activity by the compounds present in the sample. The compound with electron donating capacity have reducing power ability and they can reduce the oxidized intermediate and also act as good antioxidant. Reduction of ferric to ferrous is by the activity of the reducers present in the plant extracts and color change occurs from green to blue. The iron reduction is an important mechanism in the phenolic antioxidant activity [22].

CONCLUSIONS

From the result, it can be concluded that the acetonitrile and water extract of leaf and bark of *Kandelia candel* is the good source of an antioxidant molecule. The antioxidant molecule present in the extract could be useful in treating the disease due to oxidative stress. They could be used to synthesize drug for the treatment of various diseases.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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