Original Article

Assessment of Antioxidant Activity of Crude and Purified Bio-active Compound, Embelin in *Aegiceras corniculatum* (L.) Blanco: A Less-explored Mangrove Plant

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ABSTRACT

Context: Most of the Mangrove species are vital basically in terms of economic, environmental and medicinal point of view as several Mangrove species are used for preparation of many drug formulations. In this aspect, *Aegiceras corniculatum* is a unique black Mangrove plant. **Aim:** In this study antioxidant potency of different parts of *A. corniculatum* was evaluated through measuring the antioxidant parameters along with a comparative assessment between the crude extracts and the purified embelin isolates. **Settings and Design:** For this purpose, two different extraction procedures were opted along with two different solvent systems to confirm the viability of antioxidant values obtained from different plant parts of *Aegiceras corniculatum.* **Methods and Materials:** Antioxidant parameters like Phenol, Flavonoid, Total antioxidant content, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), Reducing power were quantified from all the extracted plant part samples. **Statistical analysis used:** The results of the non-enzymatic parameters of antioxidant activity of both the crude plant extracts and the purified embelin samples were analysed through Two Way Repetitive Measure ANOVA using GRAPHPAD PRISM software version 6.05. **Results:** From the results, crude extracts along with isolated compounds had shown better antioxidant potency particularly in fruit followed by root, stem bark and leaf. Optimization of extraction process validated Soxhlet method to be a superior one. **Conclusion:** Crude extracts were found to be superior to purified isolates in terms of antioxidant potency. All samples showed remarkable antioxidant potency hence can be used in medicinal industries.

Key words: *Aegiceras corniculatum,* Antioxidant, DPPH, FRAP, Reducing Power.

Key messages: Validation of antioxidant potency through non enzymatic parameters analysis for various plant parts of *Aegiceras corniculatum* paved a path towards use of vegetative parts also for drug formulation. However comparative appraisal of crude and purified embelin isolates helped in assessing their potent quality.

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INTRODUCTION

Substantial increase in the research pertaining to non-synthetic rather natural antioxidant bio molecules has been observed recently due to their natural origin, cost effectiveness, multifaceted activities and non or less toxicity.[1,2] The interest in intake of natural antioxidant compounds related to herbal medicine is getting popularized leading towards concentrated and elaborate research in inventing new compounds of antioxidant worth. Antioxidant molecules are capable of inhibiting the oxidation of other molecules by neutralizing free radical intermediates.[3,4] However, a number of synthetic anti-oxidants are having possible toxic and carcinogenic activities, while on the other hand for natural antioxidants, several health beneficial properties have been reported.

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The most fascinating bio-active compound in the benzoquinone group is embelin, mostly found in Myrsinaceae family.[4] Besides several medicinal properties, it has evident antioxidant activity.[5-11] However the alternative substitutive source of embelin has been found in *Aegiceras corniculatum*, a true mangrove plant[12-16] with enriched antioxidant values.[17,18]

The main goal of current work is to establish antioxidant activity of each and every part of *A. corniculatum* and to know the efficacy of crude extracts and the purified embelin isolates in terms of their antioxidant potency.

The present piece of work has been taken forward to calculate antioxidant potency of the different plant parts of *A. corniculatum* thus validating the plant as a source of natural antioxidants.

MATERIALS AND METHODS

Plant Material

Aegiceras corniculatum (L.) Blanco matured plant parts (Leaves, Stem Bark, Roots and Fruits) were collected during the month of July, 2015 from Bhitarkanika mangrove forest areas of the Odisha coast, India (20°18'-20°32'N and 86°41'-86°48'E). The sample species was compared with herbarium specimens present in the institutional herbarium (bearing voucher specimen no 4618) and also verified through the reference book "The Flora of Odisha".[19]

Pre-treatment of Samples

All the plant parts (Leaf, stem bark, fruit and root samples) were collected and shade dried and pulverizedinto fine powder.[20]

Sample Extraction

Extraction-1

Powdered samples (leaf, stem bark, fruit and root) were extracted with methanol and chloroform separately for 16-18 hr using Soxhlet apparatus.[15] Filtrates were collected, condensed and kept as stock solution.

Extraction-2

Powdered samples (leaf, stem bark, fruit and root) were extracted with methanol and chloroform separately at 60°C for a period of 12-14 hr through water bath.[21] Filtrates were collected, condensed and kept as stock solution.

Total Phenol Content (TPC)

Phenol content was estimated following the method of Swain and Hills with slight modification.[22,23] Phenol content values were expressed as mg GAE/gm dry wt.

Total Flavonoid Content (TFC)

Total Flavonoid content (TFC) was measured by the Aluminium Chloride method.[24] Quercetin was used as standard and the values were expressed as mg QE/gm dry wt.

DPPH Free Radical Scavenging Activity

DPPH free radical scavenging activity of the extracted and purified samples was measured following protocols of Brand-Williams.[25] Ascorbic acid was used as standard. Radical Scavenging Activity of DPPH was calculated and expressed as % scavenging activity.[26]

IC_{50} value in DPPH Assay

The IC_{50} values of each extracted and purified samples were determined graphically. The IC_{50} was defined as the concentration in µg of dry sample/ml that inhibits the formation of DPPH radicals by 50%.[27]

Phosphomolybdenum Reduction Assay

The total antioxidant content of the extracted and purified samples was evaluated using the phosphomolybdenum method.[28] The antioxidant content values were expressed as mg AAE/gm dry wt.

Ferric Reducing Antioxidant Power (FRAP) Assay

FRAP assay is used for measuring the total antioxidant capacity.[29] The FRAP values were expressed in mMol ascorbic acid equivalent (AAE)/gm dry wt.

Reducing Power Activity

The reducing power of the sample was determined using potassium ferricyanide and ferric chloride.[30,31] Ascorbic acid was used as standard and the reducing power values were expressed as IC_{50}.

Statistical Interpretation

In present study, the results of the non-enzymatic parameters of antioxidant activity of both the crude plant extracts and the purified embelin samples were analysed through Two Way Repetitive Measure ANOVA using GRAPHPAD PRISM software version 6.05. All the data are expressed as Mean ± SD. All the percentile values were first converted into angular transformation value before statistical analysis. The variations in results were analysed at 99.9% significant level.

RESULTS

Total Phenol Content (TPC)

The total phenol content was assessed from different plant parts of both the crude extracts and purified isolates and was expressed in terms of mg GAE/gm

dry wt. In case of *A. corniculatum* samples, TPC content in crude extracts was found to be in a range of 4.75-23.27 mg GAE/gm dry wt. and in purified isolates 0.78-14.35 mg GAE/gm dry wt. The fruit part showed highest total phenol content followed by root, stem bark and finally by leaf parts. The solvent systems used along with the methods involved for extraction had also played a crucial role in phenol content variations. When both crude extracts and purified isolates were compared, the crude extracts were found to be superior to the purified isolates. In a experiment similar type of difference in total phenol content of crude and partition extract of *Dendrobium sabin* flower was observed.[32] In another study, crude extracts of *Enhydra fluctuans* Lour showed higher efficacy than the fractionated isolates.[33] In the multiple comparison analysis, the row factor i.e. the plant parts extracts was found to be highly significant with P value < 0.0001, where as the column factor i.e. the crude and purified isolates were found to be significant at P value 0.0003 (Table 1).

Total Flavonoid Content (TFC)

In case of *A. corniculatum* samples, TFC content in crude extracts was found to be in a range of 12.10-52.07 mg QE/gm dry wt. and in purified isolates 2.29-12.87 mg QE/gm dry wt. Fruit part showed highest total Flavonoid content followed by root, stem bark and finally by leaf parts. The solvent systems used along with the methods involved for extraction had also played a crucial role in phenol content variations. When both crude extracts and purified isolates were compared, the crude extracts were found to be superior to the purified isolates. This data was validated by another experiment of antioxidant study in sweet potato leaf crude and fractionated extracts.[34] In the multiple comparison analysis, both the row factor i.e. the plant parts extracted through different processes with various solvent systems and column factor i.e. the crude and purified isolates were found to be highly significant with P value < 0.0001 (Table 2).

Total Antioxidant Content (TAC)

TAC content in the crude extracts was found to be in a range of 0.54-2.1 mg AAE/gm dry wt. /ml and in purified isolates 0.13-0.41 mg AAE/gm dry wt. /ml. The fruit part showed highest total antioxidant content in terms of non-enzymatic evaluation followed by root, stem bark and finally by leaf parts. Our result was being supported by findings of other work where similar pattern of total antioxidant content in different plant parts of *Cucumis melo* was seen.[35] In the multiple comparison analysis, the row factor i.e. the plant parts extracted through different processes was found to be highly significant with P value < 0.0001, and the column factor i.e. the crude and purified isolates were found to be significant at P value 0.0003 (Table 3).

Table 1: Total phenol content (mg GAE/g dry wt.) of crude extracts and purified embelin compounds of *A. corniculatum*.

Plant Parts	Extraction Process	Solvents Used	TPC Content (mg GAE/gm dry wt.)	
			Crude	Pure
FRUIT	Soxhlet	Methanol	21.4 ± 1.021 (10)	11.63 ± 0.462 (10)
		Chloroform	23.27 ± 2.003 (10)	14.35 ± 2.631 (10)
	Water bath	Methanol	18.36 ± 2.661 (10)	4.53 ± 0.092 (10)
		Chloroform	20.92 ± 2.481 (10)	7.79 ± 0.805 (10)
LEAF	Soxhlet	Methanol	6.61 ± 0.185 (10)	1.12 ± 0.161 (10)
		Chloroform	7.76 ± 0.081 (10)	1.33 ± 0.244 (10)
	Water bath	Methanol	4.75 ± 0.244 (10)	0.78 ± 0.129 (10)
		Chloroform	5.97 ± 0.881 (10)	0.88 ± 0.081 (10)
STEM BARK	Soxhlet	Methanol	8.32 ± 2.541 (10)	1.44 ± 0.161 (10)
		Chloroform	9.33 ± 0.562 (10)	1.87 ± 0.244 (10)
	Water bath	Methanol	8.19 ± 2.313 (10)	1.28 ± 0.161 (10)
		Chloroform	8.59 ± 1.554 (10)	1.6 ± 0.161 (10)
ROOT	Soxhlet	Methanol	14.35 ± 0.185 (10)	2.29 ± 0.333 (10)
		Chloroform	15.15 ± 0.514 (10)	2.99 ± 0.369 (10)
	Water bath	Methanol	10.24 ± 0.321 (10)	2.03 ± 0.333 (10)
		Chloroform	11.15 ± 0.647 (10)	2.88 ± 0.847 (10)

NB- Data are expressed as Mean ± SD, (where n=10)

Table 2: Total flavonoid content (mg QE/g dry wt.) of crude extracts and purified embelin compounds of *A. corniculatum.*

Plant Parts	Extraction Process	Solvents Used	TFC Content (mg QE/gm dry wt.)	
			Crude	Pure
FRUIT	Soxhlet	Methanol	50.72 ± 0.561 (10)	11.84 ± 0.197 (10)
		Chloroform	52.07 ± 0.649 (10)	12.87 ± 0.374 (10)
	Water bath	Methanol	39.0 ± 0.657 (10)	10.43 ± 0.218 (10)
		Chloroform	48.94 ± 0.191 (10)	11.12 ± 0.225 (10)
LEAF	Soxhlet	Methanol	18.89 ± 0.261 (10)	4.16 ± 0.056 (10)
		Chloroform	19.88 ± 0.441 (10)	4.37 ± 0.268 (10)
	Water bath	Methanol	12.10 ± 0.471 (10)	2.29 ± 0.036 (10)
		Chloroform	17.95 ± 0.611 (10)	3.35 ± 0.191 (10)
STEM BARK	Soxhlet	Methanol	21.79 ± 0.574 (10)	7.92 ± 0.046 (10)
		Chloroform	23.76 ± 0.191 (10)	8.11 ± 0.051 (10)
	Water bath	Methanol	20.47 ± 0.322 (10)	6.97 ± 0.105 (10)
		Chloroform	20.91 ± 0.381 (10)	7.74 ± 0.141 (10)
ROOT	Soxhlet	Methanol	38.29 ± 0.835 (10)	9.51 ± 0.174 (10)
		Chloroform	38.49 ± 0.304 (10)	9.89 ± 0.156 (10)
	Water bath	Methanol	24.91 ± 0.768 (10)	8.3 ± 0.147 (10)
		Chloroform	26.08 ± 0.706 (10)	8.75 ± 0.173 (10)

NB- Data are expressed as Mean ± SD, (where *n*=10)

Table 3: TAC content (mg AAE/gm dry wt./ml) in crude extracts and purified embelin compounds of *A. corniculatum.*

Plant Parts	Extraction Process	Solvents Used	TAC Content (mg AAE/gm dry wt./ml)	
			Crude	Pure
FRUIT	Soxhlet	Methanol	2.09 ± 0.017 (10)	0.39 ± 0.084 (10)
		Chloroform	2.1 ± 0.042 (10)	0.41 ± 0.013 (10)
	Water bath	Methanol	1.54 ± 0.022 (10)	0.37 ± 0.117 (10)
		Chloroform	1.73 ± 0.232 (10)	0.38 ± 0.021 (10)
LEAF	Soxhlet	Methanol	0.55 ± 0.042 (10)	0.13 ± 0.011 (10)
		Chloroform	0.56 ± 0.042 (10)	0.15 ± 0.011 (10)
	Water bath	Methanol	0.54 ± 0.125 (10)	0.13 ± 0.046 (10)
		Chloroform	0.543 ± 0.006 (10)	0.14 ± 0.066 (10)
STEM BARK	Soxhlet	Methanol	0.69 ± 0 .061 (10)	0.23 ± 0.121 (10)
		Chloroform	0.73 ± 0.011 (10)	0.25 ± 0.011 (10)
	Water bath	Methanol	0.673 ± 0.023 (10)	0.24 ± 0.052 (10)
		Chloroform	0.69 ± 0.057 (10)	0.23 ± 0.023 (10)
ROOT	Soxhlet	Methanol	1.01 ± 0.117 (10)	0.33 ± 0.117 (10)
		Chloroform	1.02 ± 0.053 (10)	0.35 ± 0.094 (10)
	Water bath	Methanol	0.93 ± 0.011 (10)	0.31 ± 0.011 (10)
		Chloroform	0.96 ± 0.043 (10)	0.32 ± 0.022 (10)

NB- Data are expressed as Mean ± SD, (where *n*=10)

Percentage of Radical Scavenging Activity (RSA)

The radical-scavenging activity in the crude extracts was found to be in a range of 39.13-65.18% dry wt. and in the purified isolates in a range of 51.6-72.16% dry wt. The fruit part showed highest radical-scavenging activity followed by root, stem bark and finally by leaf parts. This data was supported by similar kind of work where radical scavenging activities of leaf, stem bark, root, flower and fruit of *Blighia unijugata* Baker was measured.[36] In the multiple comparison analysis, the row factor i.e. the plant parts extracted through different processes with various solvent systems was found to be highly significant with *P* value < 0.0001, where as the column factor i.e. the crude and purified isolates were found to be significant at *P* value 0.0003 (Figure 1).

DPPH Radical Scavenging Activity (IC_{50})

The DPPH radical-scavenging activity in the crude extracts was found to be in a range of 12-40 µg of dry wt. /ml. and in the purified isolates in a range of 33-100 µg of dry wt. /ml. The fruit part showed highest radical-scavenging activity followed by root, stem bark and finally by leaf parts. Our result was being supported by findings of other work where similar pattern of DPPH radical scavenging activity in different plant parts of *Cucumis melo* was seen.[35] The following sequence of scavenging activity in the plant parts was also partly supported by antioxidant activity study of methanolic extracts from different parts of *Morus alba*.[37] In the multiple comparison analysis, the row factor i.e. the plant parts extracted through different processes with various solvent systems was found to be highly significant with *P* value < 0.0001, whereas the column factor i.e. the crude and purified isolates were found to be significant at *P* value 0.0003 (Figure 2).

Ferric Reducing Antioxidant Power (FRAP)

The FRAP activity in the crude extracts was found to be in a range of 1.4-7.1 mg AAE/gm dry wt. and in purified isolates in a range of 0.1-0.46 mg AAE/gm dry wt. The fruit part showed highest radical-scavenging activity followed by root, stem bark and finally by leaf parts. Our result was being supported by findings of other work where similar pattern of DPPH radical scavenging activity in different plant parts of *Cucumis melo* was seen.[35] All data were analysed statistically at 99.9% interval level through two ways RM ANOVA along with Sidak's multiple comparisons test. In the multiple comparison analysis, the row factor i.e. the plant parts extracted through different processes with various solvent systems was found to be highly significant with *P* value < 0.0001, whereas the column factor i.e. the crude and purified isolates were found to be significant at *P* value 0.0002 (Figure 3).

Reducing Power Activity

In case of *A. corniculatum* samples, reducing power activity in the crude extracts was found to be in a range of 40-66µg of dry wt. /ml. and in purified isolates in a range of 61-85µg of dry wt. /ml. The fruit part showed effective reducing power activity followed by root, stem bark and finally by leaf parts. This work was validated by another study in which antioxidant activity of different plant parts of *Sida cordifolia* were assessed.[38] In the multiple

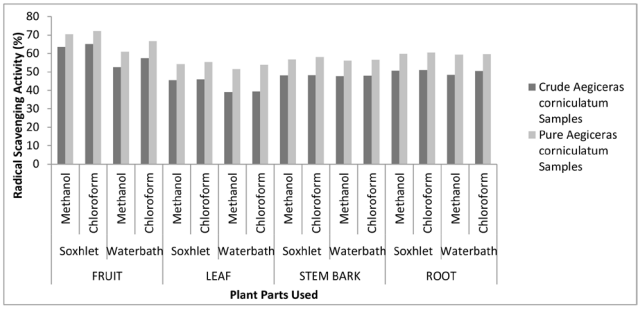

Figure 1: Percentage of Radical Scavenging Activity of Crude extracts and Purified Embelin Compounds of *A. corniculatum*.

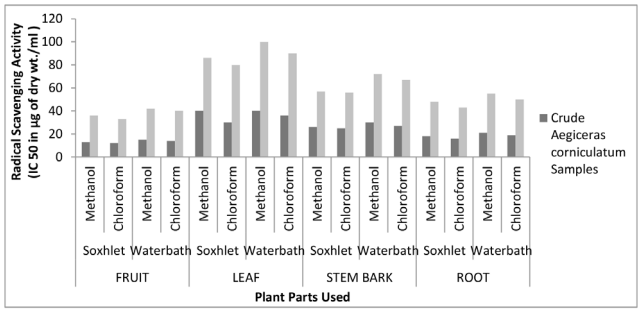

Figure 2: DPPH Radical Scavenging Activity (IC_{50} Value) of Crude extracts and Purified Embelin Compounds of *A. corniculatum*.

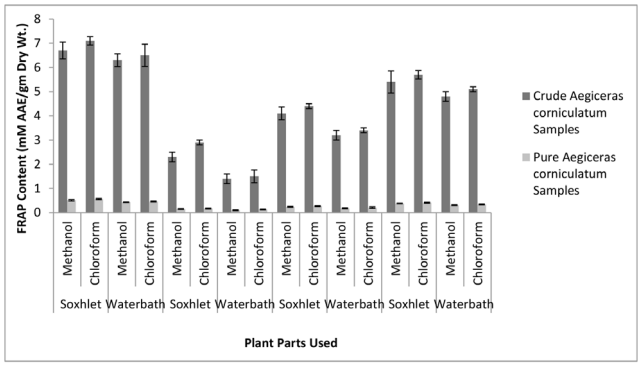

Figure 3: Ferric Reducing Antioxidant Power Activity (mM AAE/g dry wt.) of Crude extracts and Purified Embelin Compounds of *A. corniculatum*.

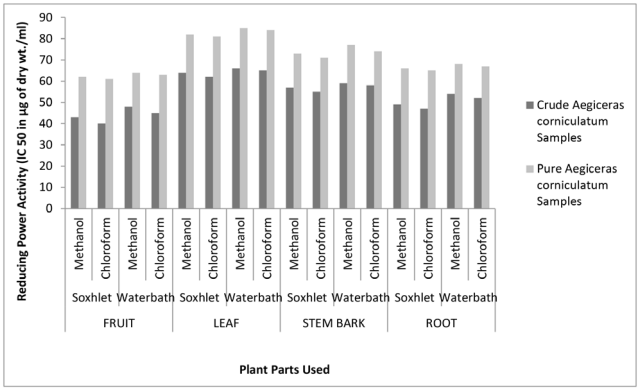

Figure 4: Reducing Power Activity (IC_{50} in µg of dry wt./ml) of Crude extracts and Purified Embelin Compounds of *A. corniculatum.*

comparison analysis, the row factor i.e. the plant parts extracted through different processes with various solvent systems was found to be highly significant with *P* value < 0.0001, whereas the column factor i.e. the crude and purified isolates were found to be significant at *P* value 0.0003 (Figure 4).

DISCUSSION

Embelin is one of the biologically active benzoquinone derivatives that can be used in several medicament purposes. This is one of the vital secondary metabolites, capable of inhibiting the harmful effects of free radicals.[39] Due to high sensitivity and versatile medicinal applications of embelin compound; alternative source of its occurrence has been in a verge of search. *A. corniculatum* is a least concerned special group of Mangrove plant having embelin as the bio-active compound.

The antioxidant activity of all the plant parts was being validated by several other references. The antioxidant activity of *A. corniculatum* has been quantified by several other authors. The antioxidant activity in fruit parts of *A. corniculatum* have been evaluated by measuring TPC content and DPPH free radical scavenging activity.[40] In most of the cases only the fruit parts were used as target sample to evaluate the antioxidant activity. However no concrete evidences regarding the antioxidant study in other plant parts besides the fruits are available. In our study we have focused our concern on the evaluation of the antioxidant activity in all the plant parts of the selected plant to find out the most potent source for natural antioxidant activity.

However utilization of crude plant extracts as antioxidants instead of using the eluted pure compounds is a favourable alternative from an economic and time saving point of view. In some cases these crude extracts have also been proved to be superior to that of the synthetic compounds as the other bio constituents present in the crude extracts may act synergistically to produce higher antioxidant potency.[41] Furthermore comparative analysis of crude extracts with purified embelin compounds for antioxidant activity was an interesting area to find out the more potent source as antioxidant compound. This fact of superiority of crude extracts over purified compounds in terms of antioxidant activity was supported by several other researchers.[42-44] Basically the more phenolic compounds present in the plant sample attributes to more antioxidant activity.[35,37] The antioxidant potency of fruit parts in several other plants were also found to be superior than compared to leaf parts, which supported our study.[35,37,45] In several studies this pattern may change that depends mostly upon presence of phenolic content in the plant parts.

CONCLUSION

The present piece of work gives a detailed view on the antioxidant activity of all the plant parts of *A. corniculatum* keeping in view on its non-enzymatic antioxidant activity. The crude extracts and embelin isolates had shown better antioxidant potency particularly in the fruit parts and lowest in leaf parts. When both the crude extracts and purified isolates were compared for antioxidant potency, the crude extracts were found to be superior to that of the purified isolates. Hence both the crude formulation and the purified compound can be used in drug formulation industries after further elaborate studies.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

DPPH: 2-diphenyl-1-picrylhydrazyl; **FRAP:** Ferric Reducing Antioxidant Power; **AAE:** Ascorbic Acid Equivalent; **GAE:** Gallic Acid Equivalent; **QE:** Quercetin Equivalent; **TPC:** Total Phenol Content; **TFC:** Total Flavonoid Content; **TAC:** Total Antioxidant Content.

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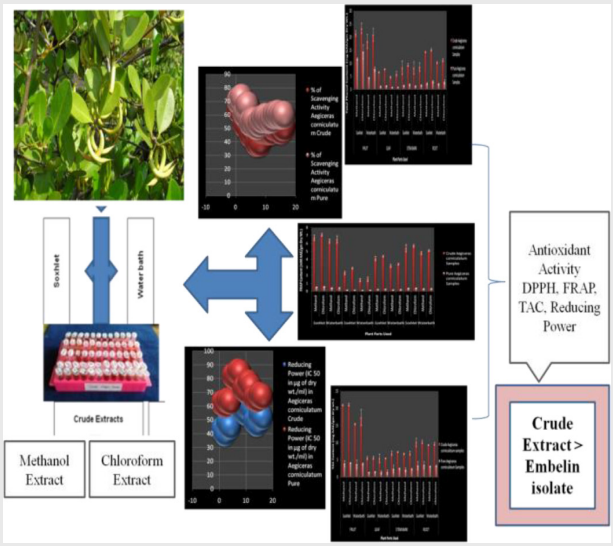
PICTORIAL ABSTRACT



SUMMARY

Aegiceras corniculatum is a specialised black mangrove plant mostly found mostly in coastal areas. This plant has a myriad array of medicinal potency and some of them are endowed due to its high antioxidant capacity. Though qualitative assessment of antioxidant properties of A. corniculatum has been carried out in a scattered manner, our aim is to have an elaborate study regarding the comparative appraisal of antioxidant properties of each plant parts of this specialized medicinal plant along with its comparison with the isolated purified embelin compounds. All the vegetative and reproductive plant parts are being assessed for quantification of its radical scavenging activities through parameters like TPC, TFC, TAC, DPPH, FRAP, Reducing power. Both crude extracts and purified embelin isolates have got higher antioxidant capacity.

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Dr. Manisha Mohapatra, has done her Ph.D. from Utkal University in Botany (Medicinal Plant Biochemistry). She works as a lecturer and a vivid Academic Scientific Editor in reputed Institution. Dr. Mohapatra has got expertise on handling several sophisticated instruments including HPLC, UHPLC, HPTLC, Atomic Absorption spectroscopy and Spectralyzer. She has several papers published in peer reviewed national and international journals and book chapter to her credit. Her interest areas include bio-active compound analysis, Natural product biochemistry, antioxidant potency study and Clinical study of biomolecules.

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