Phytochemical Analysis of Medicinal Mangrove Plant Species Ceriops decandra

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Abstract: Ceriops decandra is very potential mangrove plant species. Traditionally used in the treatment of several diseases (Inflammation diabetics, wound). The present study was carried out to investigate qualitative and quantitative phytochemical profile of leaves of C. decandra. The leaves powder was successively extracted with ethanol and n-butanol solvents. Results preliminary phytochemical analysis for protein, coumarin, phenols, flavonoids, saponins, glycosides, alkaloids, terpenoids and tannins were presence in the both solvent extracts. Whereas, steroids, flavoindoids, coumarin and tannins were deficient in C. decandra. Also, Carbohydrates, were absent in only n-butanol extract. In the case of quantitative phytochemical analysis studies highly observed in total alkaloids and followed by total phenol, total antioxidant, total flavonoids. It can be concluded that the species is effective in anti-oxidant anticancer and anti-microbial potential effects.

Keywords: Qualitative • Quantitative • C. decandra • Phytochemical compounds • Extraction • Antioxidant activity

INTRODUCTION

Plants contains various phytochemical molecules such as vitamins, terpenoids, phenolic acid, lignins, stilbenes, tannins, flavonoids, quinones, cumarins, alkaloids, amines, betalains and other metabolites which are rich in antioxidant activity [1, 2]. Studies have shown that many of these antioxidant compounds possess anti-inflammatory, anti-atherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial and antiviral activities. In recent years, secondary plant metabolites (Phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents [3]. Thus, it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infection [4]. They are formed as natural products of the normal metabolism of oxygen and have important roles in cell signaling and homeostasis. However, during times of environmental stress, reactive oxygen species levels can dramatically increase that resulting in significant damage to cell structure [5]. Natural antioxidant present in plant origin protects against these radicals and are therefore important tools in obtaining and preserving good health [6]. Potentially active components from fruits, herbs, roots and leaves have been studied extensively in order to avoid oxidative cellular events [7].

Coastal vegetation has been traditionally used in fisher-folk medicine [8, 9] and an under-explored source of anticancer drugs [10]. The mangrove plants Ceriops decandra has been used traditionally and scientifically for its biological activities such as antiviral, antibacterial, antioxidant and chemo preventative potential [11]. Mangrove like C. decandra has been proved to be a potential source of black tea to have the activities. In recent years, secondary plant metabolites aflavins and the arubigins which resemble constituents (Phytochemicals), previously with unknown of the commercial tea plant. The present investigation is undertaken to determine the phytoconstituents present in the extracts by qualitative and quantitative analysis in the extracts of ethanol and n-butanol from mangrove plant, C. decandra.

MATERIALS AND METHODS

Chemicals: Chemical reagents such as Nitro blue tetrazolium (NBT), was purchased from Sigma, USA, Gallic acid (Standard solution) (LobaChemie, Mumbai), Sodium carbonate (Hi Media, Mumbai), Sodium Nitroprusside (10 mM) solution and Trichloro Acetic Acid (TCA) (S.D. Fine Chemicals, Mumbai). All other reagents used were of analytical grade.
Collection of Plant Material: Leaves of the mangrove plant, Ceriops decandra was collected from the Pichavaram mangrove forest (Lat.11° 27’N; Long.79° 47’ E), Southeast coast of Tamil Nadu, India. After that the dried specimen was identified (AUOCAS0072) and its halotype has been deposited to herbarium at C.A.S. in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai. The fresh leaves washed in distilled water and air dried at room temperature 37°C. The dried leaves were made powder form using electrical blender and stored at 4°C for further extraction.

Preparation of Extracts: One kg of powdered material of C. decandra was soaked in 4 L of Ethanol and n-butanol for 24 hrs. at 25°C. The extraction was repeated thrice to obtain a sizable quantity of extract, after that the extract were pooled, filtered using Whatmann No. 1 paper and concentrated by using rotary evaporator (Buchi Rotavapor R-124). Finally, the resultant residues of crude extracts were kept at 4°C for further investigation.

Qualitative Phytochemical Analysis: The ethanolic and n-butanol extracts of C. decandra were screened for the presence of phytochemicals such as proteins, carbohydrates, phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids, alkaloids, total phenolic content, total flavonoid and total antioxidant by following the standard methods.

Test for Proteins
Millon’s Test: The ethanolic and n-butanol extracts of C. decandra were mixed with 2 mL of Millon’s reagent, white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein [12, 13].

Ninhydrin Test: The ethanolicand n-butanol extracts of C. decandra were boiled with 2 mL of 0.2% solution of Ninhydrin. Whereas, the pink colour appeared confirmed the presence of amino acids and proteins[12, 13].

Test for Carbohydrates
Fehling’s Test: Equal volume of Fehling A and Fehling B reagents were mixed together with 2 mL of C. decandra extracts and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars[12, 13].

Benedict’s Test: The ethanolic and n-butanol extracts of C. decandra were mixed with 2 mL of Benedict’s reagent and boiled. Finally, a reddish brown precipitate was formed, which is indicated the presence of the carbohydrates [12, 13].

Molisch’s Test: The ethanolic and n-butanol extract of C. decandra were mixed with 2 mL of Molisch’s reagent and the mixture was shaken properly. After that, 2 mL of concentrated H₂SO₄ was poured carefully along the side of test tube. Appearance of a violet ring at the interphase that is concluded and it is confirmed the presence of the carbohydrate [12, 13].

Iodine Test: The ethanolic and n-butanol extract of C. decandra were mixed with 2 mL of Iodine solution. Finally, a dark blue or purple coloration is appeared and it is indicated the presence of carbohydrate [12, 13].

Test for Phenols and Tannins: The crude extracts ethanolic and n-butanol of C. decandra were mixed with 2 mL of 2% solution of FeCl₃. A blue-green or black coloration is appeared and it is indicated the presence of phenols and tannins [12, 13].

Test for Flavonoids
Shinoda Test: The ethanol and n-butanol extract of C. decandra were mixed with few fragments of magnesium ribbon and added concentrated HCl in drop wise. Pink scarlet colour is appeared after few minutes which indicated the presence of flavonoids [12, 13].

Alkaline Reagent Test: The crude ethanol and n-butanol extracts of C. decandra were mixed with 2 mL of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated and confirmed the presence of flavonoids [12, 13].

Test for Carbohydrates
Fehling’s Test: Equal volume of Fehling A and Fehling B reagents were mixed together with 2 mL of C. decandra extracts and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars[12, 13].

Benedict’s Test: The ethanolic and n-butanol extracts of C. decandra were mixed with 2 mL of Benedict’s reagent and boiled. Finally, a reddish brown precipitate was formed, which is indicated the presence of the carbohydrates [12, 13].
Salkowski’s Test: The crude ethanol and n-butanol extracts of C. decandra were mixed with 2 mL of chloroform. Then added a 2 mL of conc. H$_2$SO$_4$ and shaken gently. Whereas, a reddish brown colour appeared indicated and confirmed the presence of steroidal ring, i.e., glycone portion of the glycoside [12, 13].

Keller-Kilani Test: The crude ethanol and n-butanol extract of C. decandra were mixed with 2 mL of glacial acetic acid containing 1-2 drops of 2 % solution of FeCl$_3$. The mixture was then poured into another test tube containing 2 mL of concentrated H$_2$SO$_4$. A brown ring presence at the interphase indicated and confirmed the presence of cardiac glycosides [12, 13].

Test for Steroids: The crude ethanol and n-butanol extracts were mixed with 2 mL of chloroform and added concentrated H$_2$SO$_4$ in sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids. Another test was performed by mixing crude extracts with 2 mL of chloroform. Then 2 mL of each of concentrated H$_2$SO$_4$ and acetic acid were poured into the mixture. The development of a greenish colouration indicated and confirmed the presence of steroids [12, 13].

Test for Terpenoids: The crude ethanol and n-butanol extracts of C. decandra were dissolved in 2 mL of chloroform and evaporated to dryness. To this, 2 mL of concentrated H$_2$SO$_4$ was added and heated for about 2 min. A grayish colour indicated the presence of terpenoids [12, 13].

Test for Alkaloids: The crude ethanol and n-butanol extracts were mixed with 2 mL of 1 % HCl and heated gently. Mayer’s and Wagner’s reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids [12, 13].

Quantitative Phytochemical Analysis: Determination of Total Phenol: The amount of phenol in the ethanolic and n-butanol extracts C. decandra were determined by Folin-Ciocalteu reagent, according to the method using gallic acid as a standard phenolic compound [14]. One mL of test solution containing 1.0 g ethanol and n-butanol extract in a volumetric flask was diluted with 46 mL of distilled water in methanol. 1.0 mL of Folin-Ciocalteau reagent was added and mixed thoroughly. After three minutes 3.0 mL of 2% sodium carbonate was added and the mixture was allowed to stand for 3 h with intermittent shaking. The absorbance of the blue colour that developed was read at 760 nm. The concentration of total phenols was expressed as mg/g of dry extract [15]. All determinations were performed in triplicate. The concentration of total content of phenolic compounds in plant extracts was determined under g of gallic acid equivalent (GAE) was calculated by the following formula

\[
C \frac{1}{4} c \_ V=m;
\]

where: C – total content of phenolic compounds, mg/g plant extract in GAE

V - The volume of extract, mL
m - The weight of pure plant extract

Determination of Alkaloids: A total of 200ml of 20% acetic acid was added to 5g of C. decandra powder taken in a separate 250ml beaker and covered to stand for 4h. This mixture containing solution was filtered and the volume was reduced to one quarter using water bath. To this sample, concentrated ammonium hydroxide was added drop wise until the precipitate was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed [16].

The percentage of total alkaloids (%) = Weight of residue×100/ Weight of sample taken.

Total Flavonoid Content: Aluminum chloride colorimetric method was used with some modifications to determine the flavonoid content. One mL of plant extracts were mixed with 3 mL of methanol, 0.2 mL of 10% aluminum chloride, 0.2 mL of 1M potassium acetate and 5.6mL of distilled water and remains at room temperature for 30 min. The absorbance was measured at 420 nm. Quercetin was used as standard (1mg/mL). All the tests were performed in triplicates. Flavonoid content were determined from the standard curve and expressed as quercetin equivalent (mg/g of extracted compound) [17].

Total Antioxidant: Total antioxidant activities of ethanolic extracts and n-butanol of C. decandra were determined according to the method of Prieto et al. [18]. Briefly, 0.03 mL crude extract were mixed with 3.0 mL reagents solution 0.6 M Sulphuric acid, 28mM sodium phosphate and 4mM ammonium molydate. Reaction mixture was incubated at 95°C for few minutes in water bath.
Absorbance of all the extracts mixture was measured at 695 nm. Total antioxidant activities are expressed as the number of equivalent of ascorbic acid in milligram per gram extract.

**RESULTS**

Phytochemical analysis plays a major resource for information on analytical and instrumental methodology in plant sciences. A preliminary study was done to identify the active constituents from *C. decandra*. The phytochemical characteristics of ethanolic and n-butanol extracts of *C. decandra* tested and summarized in Table 1. From the Table 1, it could be observed that protein, coumarin, phenols, flavonoids, saponins, glycosides, alkaloids, terpenoids and tannins were presence in the two different solvent extracts. Whereas, the steroids, flavonoids, coumarin and tannins were deficient in *C. decandra*. Also, carbohydrates, were absent in only n-butanol extract only.

**Determination of Total Phenolics Content:** Total phenolics content of *C. decandra* ethanolic extract were varying widely between 5.22mg GAE/10 g extract and in the case of n- butanol extract exhibited 4.7mg GAE/10g

Table 1: Phytochemical constituents of different solvent extract of mangrove species *C. decandra*

<table>
<thead>
<tr>
<th>Phytochemical constituents</th>
<th>Ethanol</th>
<th>n-butanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Coumarin</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 2: Quantitative Analysis of *C.decandra* extract

<table>
<thead>
<tr>
<th>S.No</th>
<th>Phyto chemical Assay</th>
<th>Ethanol extract mg/10g of extract</th>
<th>n-butanol extract mg/10g of extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Total Alkaloids</td>
<td>5.62</td>
<td>5.0</td>
</tr>
<tr>
<td>2</td>
<td>Total Phenolics</td>
<td>5.22</td>
<td>4.7</td>
</tr>
<tr>
<td>3</td>
<td>Total flavonoids</td>
<td>2.9</td>
<td>2.3</td>
</tr>
<tr>
<td>4</td>
<td>Total Antioxidant</td>
<td>4.6</td>
<td>4.2</td>
</tr>
</tbody>
</table>
Determination of Alkaloids: The gravimetric analysis for total alkaloid contents in *C. decandra* leaf extract exhibited that higher alkaloid contents were present in leaf powder in ethanol extract (5.62mg/10mg extract) and n ethanol extract (5.0mg/10mg extract).

Total Flavonoids Content: The total flavonoids content were observed in ethanolic extract of *C. decandra* 2.6mg RE/10g extract in the case of n-butanol extract 2.3mg RE/10g.

Total Antioxidant: The Total antioxidant capacity observed in ethanolic extract of *C. decandra* at range of 476 mg/μmol Trolox equivalent/g extract. The n-butanol extract exhibited total antioxidant capacity observed in 410mg/μmol Trolox equivalent/g extract respectively.

**DISCUSSION**

Preliminary qualitative phytochemical analysis made for the leaf of *C. decandra* revealed the presence of protein, coumarin, phenols, flavonoids, saponins, glycosides, alkaloids, terpenoids and tannins were presence in the two different solvent extracts. Whereas, the steroids, flavonoids, coumarin and tannins were deficient in *C. decandra*. Carbohydrates, were also absent in only n-butanol extract. These secondary metabolites are reported to have many biological and the rapeucic properties [19, 20] so this species is expected to have many medicinal uses. The extraction yield calculated both ethanol and n-butanol extract showed that ethanol extract registered higher percentage of yield. It may be due to high polarity of ethanol solvent which can draw high variety of plant constituents than the other solvents [21].

Generally, majority of the secondary metabolites studied and ascorbic acid in leaf of *C. decandra* have present with higher amount in ethanolic extract than that of the other extract n-butanol. The biological property, antioxidant activity was determined to be effective through various as says for the leaf of *C. decandra*. The presence of phenolic compounds (Total phenol, flavonoids, alkaloids and total antioxidants) provides pharmacological activities like anti-cancer [22, 23] anti-oxidant [23, 24] antimicrobial [25, 26] wound-healing [27] and anti-inflammatory [28, 29] that may suggest an association to the species here investigated.

The healing properties of medicinal plants are possible due to the presence of various phytochemical constituents such as phenolics, flavonoids, alkaloids, terpenoids and phytosterols etc. Phytochemical analysis of plant extracts revealed the presence of constituents which are known to show medicinal properties in addition to physiological activity [30, 31]. Several classes of polyphenolic compounds such as phenolics, flavonoids and tannins contribute to plant defense mechanism in resisting pathogenic microorganisms [32].

In the present study, phytochemical analysis showed that extracts both ethanol and n-butanol from *C. decandra* leaves contain most of principles. Such phptochemical are an indicative for the antimicrobial and antioxidant activities. Therefore, the beneficial medicinal effects of plant materials may result from the combinations of antimicrobial , antioxidants and other secondary products present in plant, as well as phytochemical [33].Such secondary products play and important role in a plants defense through cytotoxicity towards microbial pathogen and this could prove the usefulness of these secondary products as antimicrobial medicines for humans [16].

Mangrove plants are biochemically unique, producing a wide array of novel natural products. Mangrove possesses novel agrochemical products, compounds of medicinal values and biologically active compounds [9]. For a long period of time in history, plants have been valuable and indispensable sources of natural products for the health of human beings and they have a great potential for producing new drugs [34]. Mangrove and mangroves associates contain biologically active antiviral, antibacterial and antifungal compounds. Extracts from different mangrove plants are active against human and plant pathogens. Similar result of phytochemical screening of flower extract of this species were obtained by Shanmoga priya [35], differing only in the Alkaloids presence and the result may be related to the parts of the leaf.

**CONCLUSION**

This work is a successful attempt of phytochemical characterization and antimicrobial efficiency of mangrove plant *C. decandra*. In recent years screening of mangrove plants for a variety of biological activities, further attention should be paid to develop the novel drugs from natural product.

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**REFERENCES**


