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Determination of secondary metabolites content in the leaves and roots of Conocarpus lancifolius and Cassia glauca

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Abstract

This research study compared the levels of compounds, like phenolic, flavonoid, alkaloid, tannin, glycoside and saponine in both the leaves and roots of *Cassia Glauca* and *Conocarpus Lancifolius* collected in September 2023 locally from the local gardens in Baghdad, The aqueous plant extract was prepared using the traditional method. The leaves *C. lancifolius* exhibited concentrations of phenolic content (235.4 mg/g) total flavonoid content (108.9 mg/g) total tannin content (55.7 %) and glycoside content (1.9 mg/g) than *C. glauca* which had higher levels of total alkaloid content (2.44 mg/g). While the roots *C. glauca* showed levels of phenolic content (62.5 mg/g) and total tannin content (9.58 %) compared to *C. lancifolius* where higher amounts of total saponine content were observed (0.25%). In summary it was noted that *C. lancifolius* leaves generally displayed concentrations, across categories when compared to those of *C. glauca*.

Keywords: *C. lancifolius*, *C. glauca*, Phytochemical Screening.

Introduction

Species of the *Conocarpus* genus of the Combretaceae family are well-known for their resilience in harsh conditions and their many applications in urban landscaping and ecological restoration. *Conocarpus* species, particularly *Conocarpus erectus* and *Conocarpus lancifolius*, are native to tropical and subtropical coastal regions and are very tolerant to saline soils, arid environments, and intense heat. Therefore, widespread use in arid regions and coastal cities worldwide, where they serve to stabilize soil, improve air quality, and encourage greenery in urban environments [1].

Research suggests that *Conocarpus's* leaves and bark have antibacterial, antifungal, and antioxidant properties, which might make it beneficial for medicine and environment. *Conocarpus* is becoming more and more involved in desert greening and phytoremediation initiatives as low-maintenance and sustainable plants gain appeal. However, its rapid growth and extensive root system have raised concerns about its potential to infiltrate and damage urban infrastructure, particularly in areas where it is not native [2]. *Conocarpus* was brought to Iraq as part of initiatives to combat desertification and green urban areas. It soon gained popularity in Iraqi cities for street and park landscaping because of its fast growth, drought tolerance, and minimal care requirements. Although it

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is still preferred for its shade and dust-control qualities, its large root system may sometimes provide problems for urban infrastructure, such as water pipes [3].

Cassia glauca is a tiny tree or deciduous shrub, a member of the Fabaceae family [4], it is also known by the common names Indian Senna, Tora, and Sickle pod [5]. South and Southeast Asia is where it originated [6]. The plant blooms in racemes of yellow flowers during the monsoon season and has glaucous-looking compound leaves. The cylindrical pods contain the seeds [7]. *Cassia glauca* has long been used in a variety of ancient medical systems, such as Ayurveda [8], where the seeds and leaves are used for detoxifying and cure constipation [9]. Secondary metabolites compounds are produced in different parts of the plant, and the diversity of compounds and their synthesis varies according to different plant species. However, some compounds are produced in all types of plant tissues, while some compounds are produced in special tissues, cells or organs. So studies have tended to find and study the effectiveness of secondary metabolites compounds produced by plants. Therefore, it is necessary to stop at these plants, including the *Cassia* and *Conocarpus* plant which is known for its ability to grow in different environments and under environmental stress and currently used in many countries, and know the compounds that they form and the role that these compounds play, whether for the growth and reproduction of the plant or its role in controlling pathogens, which many studies have proven its effective and important role in inhibiting the growth of pathogens [10]. This study aims quantitative estimation of the secondary metabolites in the leaves and roots of the *Conocarpus Lancifolius* and *Cassia glauca* for the purpose of determining the part of the plant in which the highest quantities of active substances are available, which are considered as an element for biological control in the future.

Materials and Methods

Plant Collection

In September 2023, leaves and roots of *C. glauca* and *C. lancifolius* were gathered locally from Baghdad gardens. Keeping the environment in mind, such as avoiding plants that have had pesticide treatment. The plants were botanically classified as Senna sulfurea and Conocarpus L. at the Department of Biology, College of Science for Girls, University of Baghdad.

Preparation of Aqueous Plant Extract:

The aqueous plant extract for each of the leaves and roots of *C. glauca* and C. *lancifolius* was prepare using the conventional procedure [11], which involves carefully washing the plant parts with water to remove surface contaminants and then using dry air for three to seven days to dry them out. After 50 grammes of the plant materials were ground up, they were put into a 500 ml glass beaker, contains 250 milliliter of water that has been deionized. After 24 hrs, the mixture was shaken and swirled at 45^0C. The extracts were then filtered using Whatman No. 1 filter paper and stored in an air tight container at 4^0C for future inspections.

Phytochemical Screening

The confirmatory quantitative phytochemical screening of plant extracts was performed to identify the main classes of compounds (tannins, saponins, flavonoids, alkaloids, phenols, and glycosides) present in the extracts following standard protocols, at Biotechnology Research Center – Al-Nahrain University.

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Determination of Total Phenolic Compounds (T.P.C.)

Using a conventional Folin-Ciocalteu reagent, the extract's total phenolic component content was ascertained [12]. The reaction mixture included 1.5 ml of 20% sodium carbonate, 500 µl of the Folin-Ciocalteu reagent (Merck, Germany), and 100 µl of the extract. Following mixing on a vortex mixer, the sample was diluted with distilled water until it reached a final volume of 10 milliliters. Using the calibration curve created using gallic acid (Sigma-Aldrich, Germany), the absorbance at 765 nm was measured after the two-hour process and used to assess the phenolic content (Figure 1, A). Gallic acid equivalent (GAE) milligrams per gramme of dry weight was used to represent the overall quantity of phenolic compounds.

Total Flavonoid Content (T.F.C.)

The aluminum chloride colorimetric technique was used to assess the crude extract's total flavonoid content [13]. The procedure was as follows: 50µL of crude extract (1 mg/ml ethanol) was built up to 1 ml with methanol, combined with 4 ml of distilled water and 0.3 ml of 5% $NaNO_2$ solution; after 5 minutes of incubation, 0.3 ml of 10% $AlCl_3$ solution was added, and the combination was let to stand for 1 minute. After that, 2 ml of a 1 mol/l NaOH solution was added, then double-distilled water was added to get the mixture's final volume down to 10 ml. After 15 minutes of standing, the mixture's absorbance at 510 nm was measured. A calibration curve was used to determine the total flavonoid content, which was then represented as mg rutin equivalent per g dry weight (Figure 1, B).

Total alkaloid content (T.A.C.)

Qualitative estimation (test for alkaloids)

Dragendroff's approach was used to confirm the presence of alkaloids [14]. When two drops of Dragon Drops were applied to a portion of the extract that had been dissolved in diluted HCl, a crystalline precipitate formed, indicating the presence of alkaloids. A further quantitative analysis was performed on the sample that showed positive alkaloids.

Separation of alkaloid

A part of the extract residue was dissolved in 2N HCl and then faltered. 1ml of this solution was transferred to a separatory funnel and washed with 10ml chloroform. The pH of this solution was adjusted to neutral with 0.1N NaOH. Then 5ml of Bromocresol Green (BCG) solution and 5ml of phosphate buffer were added to this solution [15].

Standard carve of Atropine

Atropine standard solution aliquots (0.4, 0.6, 0.8, and 1) that had been precisely measured were moved to various reparatory funnels [15]. Next, 5 ml of BCG solution and 5 ml of pH 4.7 phosphate buffer were obtained, and the mixture was agitated with extract using 1, 2, 3, and 4 ml of chloroform. Following collection in a 10-milliliter volumetric flask, the extracts were diluted with chloroform to optimize the solution. In comparison to the blank made as before but without atropine (Figure 1, C), the absorbance of the complex in chloroform was measured at 470 nm in the spectrum using a UV Spectrophotometer (SHIMADZU UV-1800).

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Determination of total Tannins content (T.T.C.)

Ferric chloride was added to the extracts. The presence of tannins is indicated by a dark-green solution. Five milliliters of 1% gelatin solution were combined with one milliliter of extract and two milliliters of 2% sodium chloride, which were then filtered. When tannin is present, a precipitate forms, and absorption measurement at = 540 nm [16].

Total glycosides content (T.G.C.)

A 10% of each extracts was combined with 10 mL of newly made Baljet's reagent (95 mL of 1% picric acid + 5 mL of 10% NaOH) to determine the glycosides. An hour later, the mixture was diluted with 20 milliliters of distilled water, and a Shimadzu UV/VIS spectrophotometer model 1600A (Kyoto, Japan) was used to measure the absorbance at 495 nm. Ten milliliters of securidaside at various concentrations (12.5–100 mg/L) were prepared to create the standard curve. Securidaside was separated from the extract of the sample. Securidaside milligrams per gramme of dried extracts were used to express the total glycosides from triple duplicates [17 18], (Figure 1, D).

Total saponins content (T.S.C.)

0.5g of the extract was shaken with water in a test tube. The presence of saponins was shown by a steady foaming [19].

Quantitative Determination of Saponin

The twofold extraction gravimetric technique was used to ascertain the samples' saponin content [19]. In a flask, 50ml of a 20% aqueous ethanol solution was combined with 5g of the powdered material. After 90 minutes of heating at 55ºC in a water bath, the mixture was filtered using Whatman filter paper (No 42). After extracting the residue using 50 milliliters of 20% ethanol, the two extracts were mixed, reduced to approximately 40 milliliters at 90^0C, and then moved to a separating funnel where 40 milliliters of diethyl ether were added and forcefully shaken. Partitioning and reextraction were carried out frequently until the aqueous layer's colour became apparent. 60 milliliters of regular butanol were used to extract the saponins. After being cleaned with a 5% aqueous sodium chloride (NaCl) solution, the mixed extracts were dried out in an evaporation plate that had been previously weighed. It was dried in an oven at 60^0C, cooled in a desiccator, and then weighed again. To get an average, the procedure was carried out twice more. The difference was used to compute the saponin content, which was then expressed as a percentage of the original sample:

$$\textbf{Percentage of saponins} = \left(\frac{W_2 - W_1}{Wt.of\ sample}\right) \times 100$$

Where; W_1= weight of evaporating dish
W_2 = weight of evaporating dish + sample

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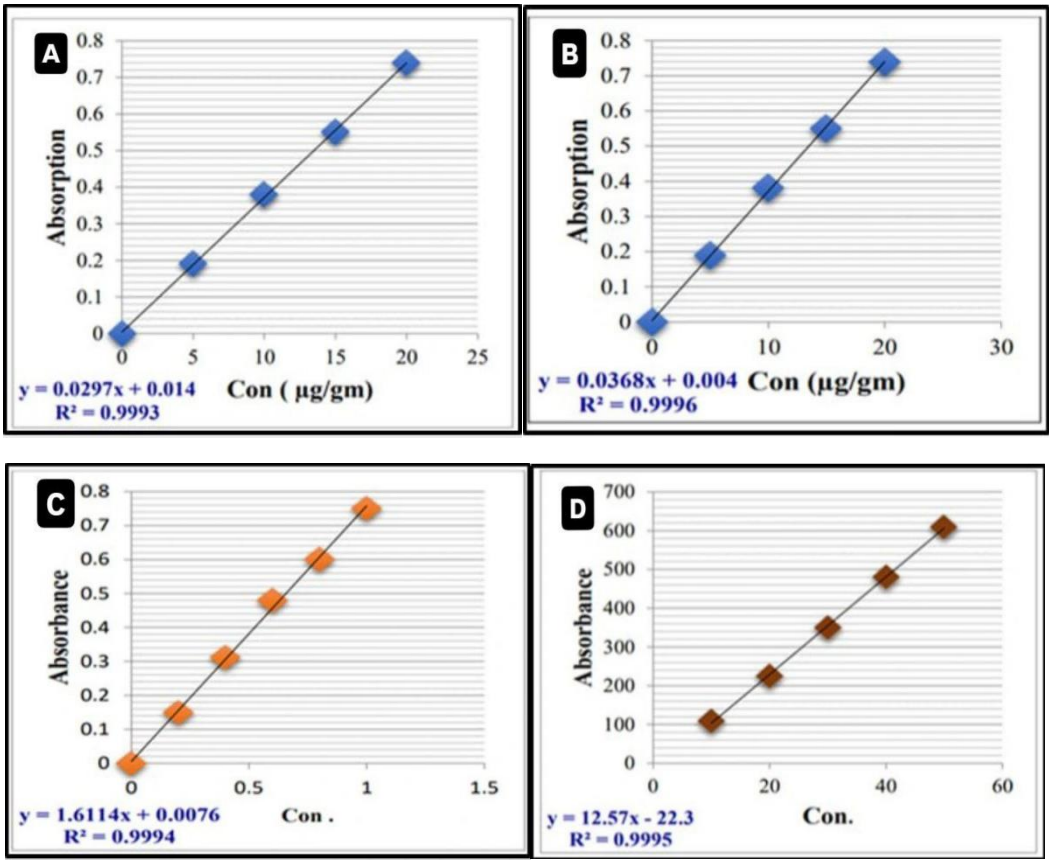

Figure (1). Profile of standard curves: (A) Gallic acid, (B) Rutin, (C) Atropine, and (D) Securidaside.

Statistical Analysis

The statistical program IBM SPSS Statistics Base version 24 was used to analyze the data according to the factorial experiment. The experimental design was completely randomized blocks design Graphpad Prism version 10 and compared the significant differences between means at (p=0.05).

Result and Discussion

The leaves of the *C. glauca* plant are more enriched with active compounds compared to its roots. Secondary metabolites, such as phenolic, flavonoids, alkaloid, tannins, and saponine, are produced in higher concentrations. While the roots contain secondary metabolites as well, the chemical profile and abundance in the leaves make them a more potent source of bioactive compounds. The results presented in Table 1 show the total active compounds found in both the leaves and roots of *C. glauca*. Previous studies were also conducted [20] on secondary metabolites using assays on *Cassia* and analysis of various plant components such as alkaloids, tannins, saponins, phenolic flavonoids, flavonoids and glycosides was carried out. Extracts of the leaves were prepared and phytochemical examination showed the presence of all the above mentioned chemical components.

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Table (1): The content of different secondary metabolites in roots and leaves of C. glauca.

Name	Leaves	Root
T.P.C. (*mg/gm*)	215.8	62.5
T.F.C. (*mg/gm*)	66.9	16.9
T.A.C. (*mg/gm*)	2.44	0.21
T.T.C. %	45.9	9.58
T.G.C. (*mg/gm*)	1.08	0
T.S.C. %	0.18	0

The leaves of the Conocarpus plant are superior to its roots in terms of secondary metabolite content. Leaves are directly exposed to environmental factors such as sunlight, herbivores, and pathogens, which stimulates the production of secondary metabolites like flavonoids, tannins, alkaloids, saponine, and phenolic compounds. Table 2 displays the total active compounds identified in the leaves and roots of *C. lancifolius*. Which was confirmed by previous studies [21] the data showed that the extracts contained phenolic compounds, flavonoids, and tannins, and were free of alkaloids. The data reveal that the leaves and fruits of *C. lancifolius* are the most important plant parts from which to extract these compounds. This study draws attention to the unordinary use of *Conocarpus spp.* as a source of natural food additive.

Table (2): The content of different secondary metabolites in roots and leaves of *C. lancifolius.*

Name	Leaves	Root
T.P.C. (*mg/gm*)	235.4	30.2
T.F.C. (*mg/gm*)	108.9	6.5
T.A.C. (*mg/gm*)	2.1	0.19
T.T.C. %	55.7	0.24
T.G.C. (*mg/gm*)	1.9	0
T.S.C. %	1.4	0.25

Where *C.lancifolius* leaves have higher phenolic, flavonoid, tannin, glycoside, and saponin contents compared to *C. glauca* leaves. *C. glauca* leaves have higher alkaloid contents compared to *C. lancifolius* leaves. . While *C. lancifolius* root have higher saponin contents compared to *C. glauca* root. *C. glauca* root have higher phenolic, flavonoid, tannin, glycoside, and alkaloid contents compared to *C. lancifolius* root (Fig.5). From the result we exclude roots due to their low values.

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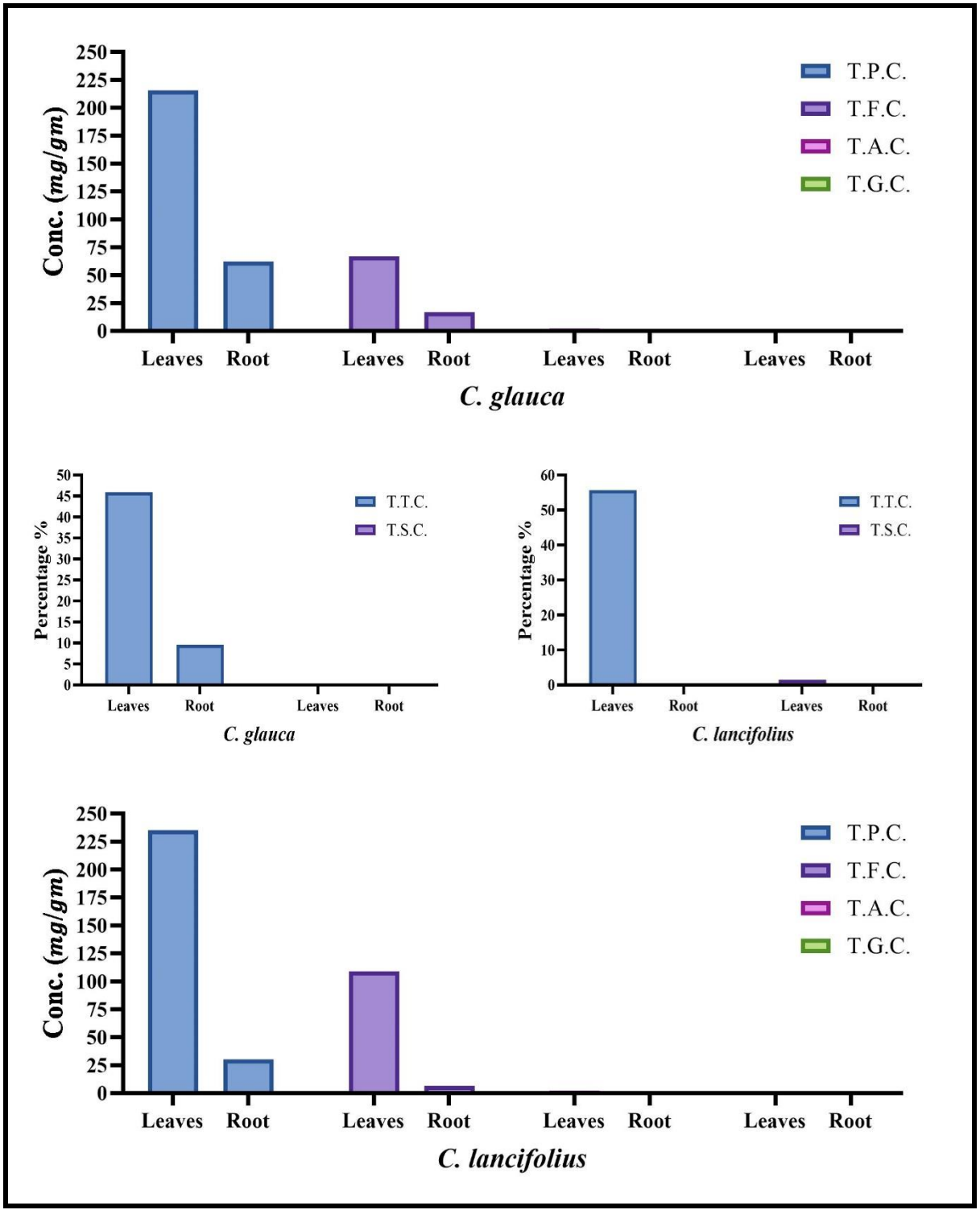

Figure (2). The comparison content of main secondary metabolites between *C. glauca* and *C. Lancifolius*

Conclusion

From this study we conclude the *C. lancifolius* plant contains secondary metabolites in higher concentrations compared to the *C. glauca* plant. Secondary metabolites such as flavonoids, phenols, and tannins are critical for both plants' defense mechanisms and medicinal properties. However, studies suggest that *C. lancifolius* leaves, in particular, exhibit stronger bioactive potential due to their higher levels of these compounds. This makes *C. lancifolius* a more effective source for pharmacological applications, including antioxidant, antimicrobial, and anti-inflammatory uses, compared to *C. glauca*.

Which was confirmed by previous studies[22 23], which aimed to demonstrate the effectiveness of the *conocarpus* plant and its importance in several fields, including its ability to grow and survive and resist the environmental conditions to which it is exposed, and its properties in protecting plants from pathogens, which is due to its containment of secondary metabolites compounds (derivatives of primary metabolic compounds), in addition to the statement of the positive effect of Conocarpus plant in reducing the plant diseases, Analysis of the leaf extracts showed the presence of phenolics, terpenoids, and alkaloids. Phenolic compounds were predominant in the extracts and may have played a major role alone and/or collectively with other groups.

Author's Contribution Statement

Taqwa A. Qader Abdel Dayem: Conducted some experiments, data rearrangement and drafted the initial manuscript.

Enas H. AL-Ani: Contributed to the conception and design of the study, and conducted some characteristics of the products.

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