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Phytometabolite Profiling Of *Combretum Indicum (L.*) Defilipps And Its Characterization Studies

Sidharth. K[1], K. Narayanasamy[2]*, Sruthy Mohan[3], K. Baskaran[4], Nirmala Devi. N[5], R. Ragunathan[6]

[1],[4,5] Department of Biochemistry, Sree Narayana Guru College, K.G. Chavadi, Coimbatore.
[2],[3] Department of Biochemistry, School of Life sciences, Nehru Arts and Science College, Coimbatore.*
[6]Director of Center for Bioscience and Nano Science research, Echanari, Coimbatore.

***Corresponding Author*:** Dr. K. Narayanasamy *MSc, MPhil, Ph.D.,
Associate Professor and Head, Department of Biochemistry,
School of Life sciences, Nehru Arts and Science College, Coimbatore.
Mail id: harikns.2005@gmail.com.Phone.No: +919361441583

Abstract:

Combretum indicum (L.) DeFilipps., also known as *Quisqualis indica*, belongs to the combretaceae family. Literature suggests the hardy nature and predominant presence of medically important phytometabolites within these plants. This study aimed to identify the phytochemical constituents of the *Combretum indicum* flower extracts. Flower extracts were prepared using Aqueous, ethanol, n-Hexane and petroleum ether as the solvents of choice. Qualitative phytochemical analysis indicated the presence of phytometabolites including alkaloids, flavonoids, terpenoids, phenols, quinines, steroids, saponins etc. Among these solvents ethanolic flower extract showed better results. The quantitative analysis of flower extract indicated the presence good quantity of flavonoids and phenols respectively. Anti-oxidant activity was tested using DPPH scavenging assay. Characterization of phytochemicals was done by using UV-Visible spectrophotometry, FTIR analysis and thin layer chromatography.

Key words: Combretum indicum, phytochemical analysis, Total phenol, Total flavonoids, DPPH Assay, UV-Visible spectrophotometry, FTIR, TLC.

INTRODUCTION

Plants are the sources of various potent drugs. Medicinal compounds or medications derived from plants have many benefits include minimal side effects, cost- efficiency, accessibility and physiological effectiveness [1]. Plants synthesize lower molecular weight organic compounds which possess various biological activities [2, 3]. These organic compounds are secondary metabolites. Secondary metabolites are plant specific and are produced as a part of plant defence system against pests and pathogens [4, 5].

Microbial pathogens are continuously evolving; hence, advanced remedial methods need to be introduced [6]. Continued research and analysis of current pathogens are crucial in order to develop efficient, naturally derived, plant drug-delivery methods [7].
Combretum indicum (L.) DeFilipps., also known as *Quisqualis indica*, belongs to the combretaceae family. Literature suggests the hardy nature and predominant presence of medically important phytometabolites within these plants [8-10]. *Combretum indicum* or Rangoon creeper is a vine with red flower clusters which is native to tropical Asia. This creeper can reach from 2.5 meters to up to 8 meters. The leaves are elliptical with an acuminate tip and a rounded base. They grow from 7 to 15 centimetres and their arrangement is opposite. The flowers change their colour with age; this is a strategy to attract more pollinators. The flowers are fragrant and tubular and their colour varies from white to pink to red. The 30-35 mm long fruit is ellipsoidal and has five prominent wings. Rangoon creeper is found in thickets or secondary forests of the Philippines, India, Pakistan and Malaysia.

Figure 1: *Combretum indicum*

The fruit, leaves, and roots of *Combretum indicum* have been used in folk medicine for the treatment of worms, toothache, head ache, pain, fever and rheumatism [11]. Previous studies on *Combretum indicum* proved that its different parts possessed many bioactivities such as killing worms, antibacterial, antifungal, anti-inflammatory, antioxidant, anticancer, acetylcholinesterase inhibition and reducing blood lipid level [12-19]. This species was known to the presence of steroids, terpenoids, flavonoids and polyphenol [20-23]. The seeds of *combretum indicum* and related plants contain the chemical quisqualicacid, which is an agonist for the AMPA receptor, a kind of glutamate receptor in the brain. The chemical is linked to excitotoxicity (cell death) [24, 25]. The seeds mainly used for treating round and pin worms.

MATERIALS AND METHODS

Collection of flower sample:

Flowers that appeared healthy were collected from different branches of *Combretum indicum* from Ottapalam, Palakkad, Kerala. The plant authentication was done in Botanical Survey of India, Southern regional Centre and Coimbatore with reference no: BSI/SRC/5/23/2023/Tech-638.

Preparation of flower extract:

Extraction is the crucial first step in the analysis of medicinal plants, because it is necessary to extract the desired bioactive components from the plant materials for further separation and characterization. Water is the most polar solvent and is used in the extraction of a wide range of polar compounds, which dissolves a wide range of substances. The powdered sample was dissolved in water, ethanol, n-Hexane and Ethyl acetate (10% of extract- 10g in 100ml distilled water, ethanol, n-hexane and ethyl acetate) and incubated in orbital shaker at 60-70rpm, 40^0C for 24 hrs. After incubation the mixture was filtered and used for further study.

Phytochemical analysis (Qualitative):

The extracts were subjected to preliminary phytochemical tests to detect the presence of bioactive compounds.

Phytochemical analysis (Quantitative): Total phenol:

Total phenolic content of the extract was studied with the Folin–Ciocalteau reagent method with modifications. To 0.5 ml of each extract, 2.5 ml of 0.2 N Folin–Ciocalteau reagents was added, mixed by

gentle shaking, and kept for 10 minutes in room temperature. to the mixture, 2 ml of Na_2CO_3 (20%, w/v) was added and incubated at 30^0C for 20 min. Three replicates were maintained per each experimental procedure. The absorbance of the sample was recorded at 765 nm using a UV/vis spectrophotometer (Labtronics LT291, Microprocessor). The phenolic content of the extract was estimated from the standard curve of Gallic acid and the results were expressed in Gallic acid equivalent (GAE)/g (gallic acid standard was prepared in methanol at the concentration of 20, 40, 60, 80, and 100 µg ml-1) of extract [26].

Total flavonoids:
Total flavonoids content was determined by spectrophotometrically using the method of aluminium chloride assay. 1ml of the extract was dissolved with 0.1 ml of 10% aluminium chloride solution and 0.1ml of sodium potassium tartarate followed by 2.8ml of distilled water. After adding the reagents, the tubes were incubated in room temperature for 30minutes and the measurement was taken in the nanometer of 415 using spectrophotometer (ELICO SL 159). Blank was maintained without adding the sample and standard quercetin was used to calculate the mg/g of the flavonoids content [27].

DPPH scavenging assay:
Free radical scavenging ability of the extracts was tested by DPPH radical scavenging assay, the hydrogen atom donating ability of the plant extract was determined by the decolorization of methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH produces violet/purple color in methanol solution and fades to shades of yellow color in the presence of antioxidants. A solution of 0.1 mM DPPH in methanol was prepared, and 0.5 mL of this solution was mixed with different concentration of sample (250, 500,750, and 1000µL) along with 50Mm tris HCl solution. The reaction mixture was vortexed thoroughly and left in the dark at RT for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. A control was prepared using 0.1 ml of respective vehicle in the place of plant extract/ ascorbic acid.
Percentage DPPH radical scavenging activity was calculated by the following equation:
% DPPH radical scavenging activity = (A0− A1)/A0×100
where A0 is the absorbance of the control, and A1 is the absorbance of the extractives/standard [28,29].
Characterization of the compounds:

UV-Visible spectral analysis:
UV-visible study of the ethanolic extract using a UV-visible spectrophotometer (UV-Visible spectrophotometer, LT291) with a slit width of 100nm, using a 0.5nm absorbance at room temperature. The extract was examined under visible and UV light in the wavelength ranging from 200-800nm for proximate analysis. For UV-VIS spectrophotometer analysis, the extract was centrifuged at 3000 rpm for 10 min and filtered through Whatman No. 1 filter paper the final extract was taken.

FTIR Analysis:
Fourier Transform Infrared (FTIR) was used to identify the characteristic functional groups in the extract. A small quantity of the extract was kept in the disc and was placed in a sample cup of a diffuse reflectance accessory. The IR spectrum was obtained using Shimadzu, infrared spectrometer. The sample was scanned from 4000 to 400 cm-1. The peak values of the FTIR were recorded and analyzed. It provides the information about the structure of a molecule could frequently be obtained from its absorption spectrum.

Thin layer chromatography (TLC):
TLC is also used to identify the compound in a mixture when the Rf of a compound is compared with the Rf of a known compound. The extracts were added as spot using capillary tubes (20µl) on the one end of the thin layer plate at above 1 cm. Plate was allowed it for air dry, then it was placed in a beaker containing solvent of formic acid: ethyl acetate: acetic acid and water in the ratio of 1:10:1:2.5. The samples were allowed to run towards the other end of the plate. The sheet was removed and allowed it to air dry and incubated in iodine chamber for 5 to 10 minutes [30, 31].

RESULTS AND DISCUSSION

Phytochemical analysis (Qualitative):
Preliminary phytochemical tests indicated the presence of multiple phyto compounds (Table 1). The polarity of compound differs; hence a variety of organic solvents was utilized to extract a maximum number of compounds present. The following compounds were detected within the crude flower extracts, namely:

alkaloids, flavonoids, phenols, terpenoids, proteins, quinines, steroids, saponins. The Aqueous and ethanolic flower extracts showed the highest number of phytometabolites present within this species.

Table 1: Phytochemical analysis of *Combretum indicum* flower extracts

Test	Aqueous	Ethanol	n-Hexane	Ethyl acetate
Alkaloids (Mayers test)	+	+++	-	+
Flavonoids (Lead acetate test)	+	+	+	-
Phenols (Ferric trichloride test)	++	+++	+	-
Terpenoids	-	++	-	+
Sugar (Fehling's test)	-	-	-	-
Proteins (Folin's Lowry's method)	+	+	-	-
Quinines	+++	++	+	-
Steroids (H2SO4 test)	-	+	+	-
Saponins (Foam test)	++	+	-	-

Intensity of reaction: (+++) Intense colour change, (++) Moderate colour change (+) Mild colour change, (-) Negative reaction

Total phenol and Total flavonoids:

In this study, the total phenolic and flavonoid contents of flower extracts of ***C. indicum*** were tested. The results of total phenolic content were expressed as gallic acid equivalents using the equation of a standard curve y = 0.0042x + 0.0234, R2 = 0.9901, as shown in Fig.2. The ethanol extract showed the highest phenolic content. Ramirez and co-workers (2019) found the same profile of ethanol extract with high phenolics from the epicarp and leaves. In contrast, Aqueous, n-Hexane and ethyl acetate extract, as shown in Table 1, ethanolic flower extract had the highest phenolic contents. The flavonoid contents were expressed as quercetin equivalent using the equation y = 0.0337x: R2 = 0.9973, as shown in Fig.2. The ethanol, aqueous, n-hexane flower extract displayed the highest flavonoids. It was noticed that flavonoid compounds were accumulated in polar solvents and lower in nonpolar solvents.

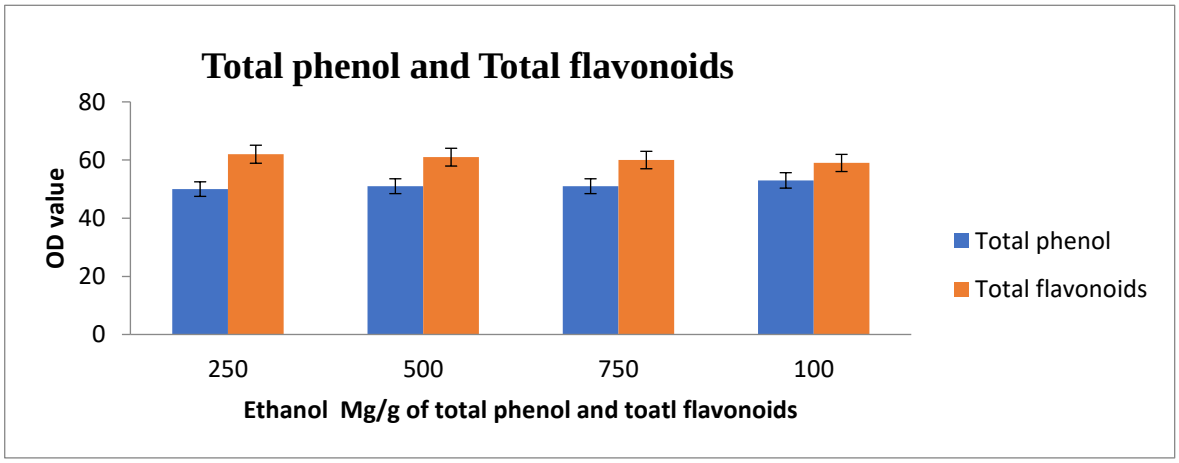

Figure 2: Total phenol and Total flavonoids of ethanolic *C. indicum* flower extract

2,2-Diphenyl-1-picrylhydrazyl (DPPH) activity:

DPPH assay is an easy, rapid, and sensitive way to survey the antioxidant activity of a specific compound or plant extract. The antioxidant effect is proportional to the disappearance of purple color of DPPH in test samples. Thus, antioxidant molecules can quench DPPH free radicals by providing hydrogen atoms or by electron donation and a colorless stable 2,2-Diphenyl-1-picrylhydrazyl is formed and as a result of which the absorbance of the solution is decreased. The **IC50 value** is **102.7µg/ml.**

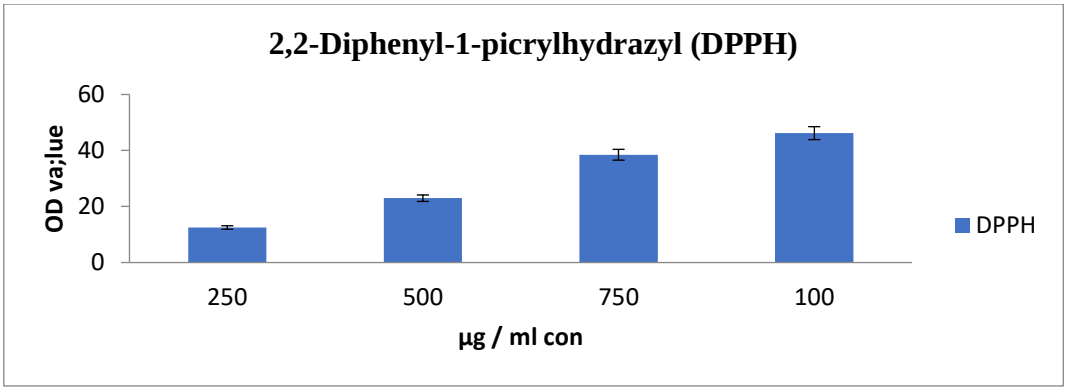

Figure 3: DPPH of ethanolic *C. indicum* flower extract

UV-Visible spectrophotometry:

The UV-VIS analysis performed for identification of phytoconstituents in ethanolic flower extract of *Combretum indicum*. The profile showed the peaks at 245nm, 290nm, 295nm, 300nm, 360nm, 395nm and 665nm with the absorption 2.491, 2.302, 2.291, 2.298, 2.313, 1.576, 1.583, 0.397 respectively. The spectrum for *C. indicum* extract shows peaks at 245nm and 360nm. This confirms the presence of alkaloids, flavonoids, phenolic compounds and Tannins.

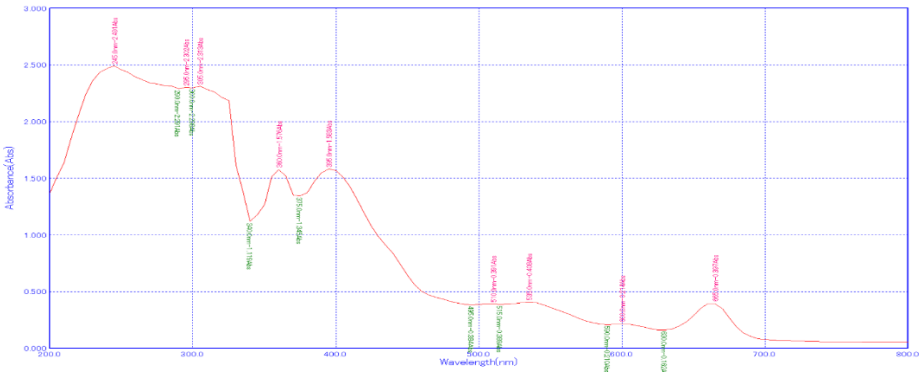

Figure 4: UV-VIS spectra of ethanolic *C. indicum* flower extract

Fourier Transform Infrared Spectroscopy (FTIR):

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The band at 3444.82 is due to N-H (strong) stretch. The band at 2925.80 represents weak C-H stretch. The band at 2359.87 & 2342.43 represents C=C conjugated. The band at 1620.50 indicate the presence of C=C weak alkene group. The band at 1399.87 is due to medium CH3 bend. The band at1338.84 represents strong NO2 stretch, 1248.04 represents strong C-O-C stretch, 1169.13 represents strong C-OH stretch. The band 668.98, 575.92, 509.19 is due to the presence of strong C-Cl, strong C-Br, and strong C-l respectively.

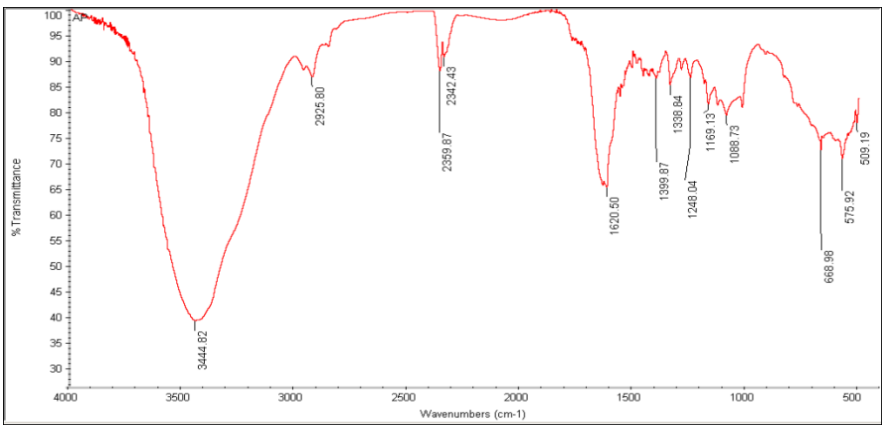

Figure 5: FTIR spectra of ethanolic *C. indicum* flower extract

Thin layer chromatography:
Different compound classes were noted by the appearance of bands that resulted from the upward movement of mobile solution on the plate. Compounds found closer to the baseline are more polar (primary metabolites) than those compounds which have traveled further up. TLC was performed with all extracts in which ethanolic extract showed the best result. Spot 4 shows highest R_f vale of 0.636, while the spot 1 shows lowest R_f value of 0.18, thus indicating their possible polarity and distance travelled within the plate. As compounds moved up the plate, colorless bands were formed.

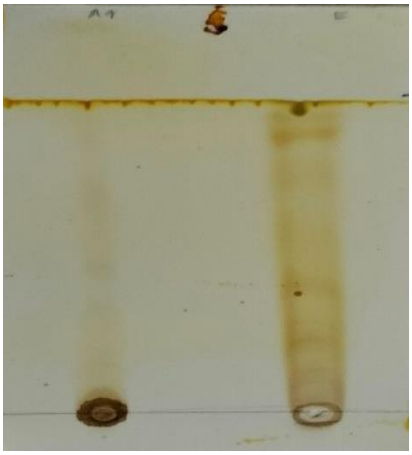

Figure 6: Thin layer chromatograph showing the separation of the ethanolic flower extract of *C. indicum*.

CONCLUSION

The term herbal drugs denote plants or plant parts that have been converted into phytopharmaceuticals by means of simple processes involving harvesting, drying and storage. The *Quisqualis indica* Linn plants are widely used either directly as folk remedies or indirectly as pharmaceutical preparation of modern medicine. By considering ethnomedicinal background and phytochemical screening it has been concluded that this plant contains some important phytochemical constituents showing various pharmacological activities. *Combretum indicum* flower extract also showed significant anti-oxidant activity.

CONFLICT OF INTEREST:

We declare that we have no conflict of interest.

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