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RESEARCH ARTICLE

Phytochemical, Antioxidant potential and ftir analysis on the matured leaves of *Camellia Oleifera* abel

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ABSTRACT

Phytochemicals are physiologically active compounds and are derived from plants. Majority of the phytochemicals have been known to bear therapeutic activities like antibacterial, antioxidant, antifungal, antispasmodic, anticancer, hepatoprotective etc. The aim of this study is to evaluate the phytochemicals in the matured leaves of *Camellia oleifera* belonging to the family Theaceae. The phytochemicals were extracted from the dried matured leaves of *C. oleifera* using solvent extraction method. The preliminary phytochemical analysis showed the presence of alkaloids, tannins, saponins, phenols and flavonoids in the ethanolic extract. Further the antioxidant property of the leaf extract was carried out using standard procedure. The extract was subjected to FTIR analysis. The study shows that the ethanolic extracts possess secondary metabolites and antioxidant properties that are therapeutically valuable.

Keywords: *Camellia oleifera*, secondary metabolites, antioxidant, FTIR.

1. INTRODUCTION

Plant extracts have great potency and can be used for a variety of purposes. Approximately 80% of the world's population relies on traditional medicine for health care, and most therapies use plant extracts and their active compounds [1]. Medicinal plants are the richest bio-resources of folk medicines and traditional systems of medicine, and food supplements, pharmaceuticals industries and chemical entities for synthetic drugs [2].

India is the birth place of renewed system of indigenous medicine such as Siddha, Ayurvedha and Unani. Plants are a good source of various functionally active secondary metabolites and a good source of essential nutrients [3]. Recent studies have demonstrated the nutritional and nutraceutical efficacy of different plant tissues proving their commercial value [4, 5]. Several techniques are available to identify the phytochemical compounds in plant extracts. For example, Fourier transform infrared spectroscopy (FTIR) is a method used to identify the functional groups in gaseous, liquid and solid materials via infrared radiation beams [6]. It is possible to detect the minor changes in the primary and secondary metabolites in leaves by observing the IR spectra [7]. FTIR has been used to identify the complicated structures of plant secondary metabolites and in the characterization of bacterial, fungal and plant species [8]. This technique presents a rapid, inexpensive, and rather non-invasive method for obtaining chemical characteristics of a biological sample.

2. PLANT DESCRIPTION:

Kingdom: Plantae
Phylum : Tracheophyta
Order : Ericales
Family : Theaceae
Genus : Camellia L.
Species : *Camellia oleifera Abel*.

General Description:

Evergreen. Shrub reaching upto 5m in height. Leaves 3–7 cm in length, 1.2–3cm in width, broad, elliptic with a finely serrated margin. The flowers are dark pink, 5–8 petals and 5–7 cm in diameter.

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Figure 1. Habit of *Camellia oleifera* Abel.

3. MATERIALS AND METHODS

3.1. Collection of the plant samples

The matured leaves of *Camellia oleifera* belonging to the family Theaceae was collected from Kovillatty Village, Manjoor, Nilgiri district, Tamil Nadu, India during the month of June 2022, identified and confirmed by the Flora of the Presidency of Madras [9]. The collected materials were washed thoroughly with tap water to remove the sediment particles. The plant leaves were shade dried and powdered coarsely. The powder was stored in air tight container and used for further successive extraction.

3.2. Solvent extraction

The powdered plant sample was extracted with sequential solvent analysis (viz, Hexane, diethyl ether, ethanol and aqueous solutions) using cold maceration method for 8 to 10 hours in order to extract the non-polar and polar compounds. The solvent of respective extract was reduced at room temperature and stored under 4^0C for further use.

3.3. Phytochemical screening

Chemical tests were carried out using standard procedure to identify the preliminary phytochemical screening and the ethanolic extract were analyzed quantitatively. [10-15]

3.4. Determination of in vitro antioxidant activity

3.4.1. DPPH radical scavenging activity

The hydrogen donating capacity was assessed by using stable DPPH method. Briefly, a solution of 0.1Mm DPPH was prepared using methanol. The sample (50-250µg/mL) was mixed with 5.0 mL of DPPH solution. Reaction mixture was shaken incubated at 30^0 c for 20 minutes and the absorbance was measured at 517nm. Results were compared with the activity of rutin. Antioxidant activity of the extract were expressed as IC_{50}, the values were calculated from the linear regression of the percentage antioxidant activity versus concentration of the extracts. A lower IC50, values indicate greater antioxidant activity. DPPH dis-colouration of the sample was calculated using the formula.

DPPH radical scavenging activity (%) = [(control OD- sample OD)/control OD] x100.

3.4.2. $ABTS^+$ free radical scavenging assay

Antioxidant activity was performed using an improved ABTS+ method proposed by Siddhuraju and Manian. The ABTS radical cation was ABTS+ was generated by a reaction of 7mm ABTS+ and 2.45 mm potassium persulphate and the mixture was incubated for 12 – 16 hrs at room temperature in dark. Prior to assay, the solution was diluted in ethanol (about 1:89 v/v) and equilibrate to obtain an absorbance of 0.700± 0.02 at 734 nm. 10 µL/ml of the sample was added to 1.0ml of diluted ABTS+ solution. After 30 min of incubation, absorbance was read at 734nm. Trolox was used as reference material.

3.5. *FTIR analysis: Fourier Transform Infra-Red Spectroscopy Analysis*

The FTIR spectra of the fractions with the highest anti-inflammatory activity were carried out using FTIR-8400S spectrophotometer (Shidmazu model). FTIR analysis of the ethanolic extract was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks and their functional groups. The peak values of the FTIR were recorded. Each and every analysis was repeated twice and confirmed the spectrum. Fourier Transform Infra-Red (FTIR) is a tool used to identify the type of chemical compound in the sample. The sample was loaded in the FTIR with the scan range from 400-4000cm-1 with a resolution of 4cm-1 and the results were recorded.

4. RESULTS AND DISCUSSION

4.1. Qualitative estimation of secondary metabolites

Secondary metabolites are chemicals produced by plants that allow them to compete in their natural environment. In present investigation secondary metabolites were analyzed both qualitative and quantitatively. The present analysis of phytochemical screening revealed that *C. oleifera* contained flavonoids, phenols and tannins where

present. Whereas saponin and steroids were absent in the ethanolic extract.

4.2. Quantitative estimation of secondary metabolities

Flavonoids are a large class of natural aromatic compounds as there is reported to be the most common plants' phenolics [16]. The biological and oxidative properties of flavonoids are responsible for their anti-allergic, cardioprotective, anti-diabetic, anti-inflammatory, anti-oxidative activity, and free radical scavenging capacity [17]. The total flavonoid content of the extract was found to be 1.143±0.04mg RE /g in ethanolic extract and was expressed in Rutin equivalent.

Polyphenols are secondary plant metabolites that play a vital role in protecting plants from UV radiation and disease attacks [18]. The phenolic content in the extract of *C. oleifera* were expressed in gallic acid equivalence and were found to be 0.933± 0.03 mg GAE /g in ethanol .They carry strong natural antioxidants having key role in wide range of biological and pharmacological properties such as anti-inflammatory, anticancer, antimicrobial, anti-allergic, antiviral, antithrombotic, hepatoprotective [19] .Tannins are the main polyphenolics distributed widely in the range of 5 to 10% of dry vascular materials found mainly in bark, stems, seeds, roots, buds, and leaves [20]. The total tannin content of the extract was expressed in Tannic acid equivalents and was found to be 1.111±0.01mg GAE/g in ethanolic extract. In the past few years, tannins have also been studied for their potential effects against cancer through different mechanisms [21].

For FTIR, the analysis time was less than five minutes and it required a minute quantity of the sample [22]. The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infra-red radiation. The functional groups of the components were separated based on its peak ratio. As illustrated in (Figure 1), FTIR spectrum of ethanolic extract showed different peaks at 617.55, 628.99, 1057.85, 1383.78, 2344.42, 2927.66, 3305.05, 3362.24cm-1. It was confirmed the presence of functional groups such as Halo compound (C-Br streching), Halo compound (C-Br streching), sulfoxide (S=O streching), Phenol (O-H binding), Carbon dioxide (O=C=O), Aldehyde (C-H streching), Alkyne (C-H streching) and Aliphatic primary amine (N-H streching).

Table 1. Qualitative phytochemical analysis of ethanolic matured Leaf extract of *C. oleifera*

Constituents	Presence/ absence
Alkaloids	++
Flavonoids	+++
Phenols	+
Tannins	+
Saponins	-
Steroids	-

Table 2. Quantitative analysis of ethanolic Matured Leaf extract of *C. oleifera*

Constituents	Ethanolic extract mg/g ±SD
Flavonoids	1.668±0.02
Phenols	0.933±0.03
Tannins	1.111±0.01

Table 3. Antioxidant activity of ethanolic leaf extract of *C. oleifera*

ASSAY	IC_{50} value
DPPH	0.118±0.02
ABTS	0.156±0.03

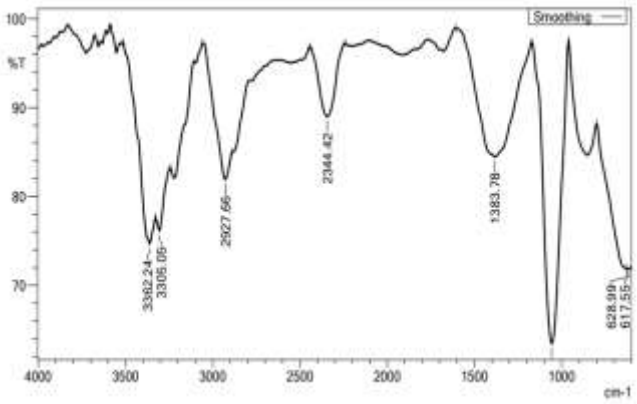

Figure 2. FTIR Spectrum of ethanolic Matured leaf extract of *C. oleifera*

4. CONCLUSION

The result of this study shows the presence of some phytochemicals such as alkaloids, flavonoids, phenols and tannins in ethanolic leaf extracts of *C. oleifera*. The flavonoids, phenols and tannins were analyzed quantitatively. The antioxidant activity revealed the highest inhibitory concentration in the ethanolic extract. The FTIR analysis confirmed the presence of various bioactive compounds. Thus it is concluded that the plant has various medicinal properties and can be used for further pharmacological studies.

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