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Phytochemical Screening and Antioxidant Activity Determination of Different Solvent Extracts of Dillenia Pentogyna and Crateva Religiosa Important Plants

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Abstract
The aim of present study was to evaluate the phytochemical analysis of two medicinal plants such as Dillenia pentogyna and Crateva religiosa. Crude leaves extracts were prepared by using four different solvents such as acetone, ethyl acetate, methanol and water. Further leaf extract used to evaluate the antioxidant activity, qualitative and quantitative phytochemical analysis. Qualitative phytochemical analysis of among these two plants confirms the presence of various phytochemicals like alkaloids, flavonoids, tannins, phenol, coumarine, terpenoid, saponin and steroid in their different solvent leaf extracts. Quantitative analysis for such compounds like, phenol, flavonoid and tannin. The results of the present study revealed that the phytochemical constituents in the leaves extracts are mainly responsible medicinal properties.

Keywords: Phytochemicals, Antioxidant activity and Leaf extracts

INTRODUCTION

Plants are used medicinally in different countries and are a source of many potent and powerful drugs. According to World Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Medicinal plants are the well sources of drugs of folk system of medicine, recent medicines, nutraceuticals, food supplements, pharmaceuticals intermediates and chemical entities for artificial drugs (Hammer et al., 1999).

Besides, phenolic compounds and flavonoids are also widely distributed in plants which have been reported to antioxidant, free radical scavanging activities, anti inflammatory and anticarcinogenic (Miller., 1996). While the flavonoids are a group of polyphenolic compounds with known properties which possess many medicinal activities similar to anti ulcer, anti mature, anti microbial activity, anti-

seditious, anti-diabetic properties (Shohaib, et al., 2011).

Dillinea pentagyna is being used traditionally for its many medicinal properties, especially for tuberculosis, fistula, sores, carbuncle, neuralgia, pleurisy and pneumonia. Barks and leaves are used for the treatment of diarrhoea and dysentery in Rema-Kalenga. Bark is used for blood dysentery in Khagrachari (Yusuf et al. 2009). The tribal and folk communities use the various part of it for the treatment of their different ailments and diseases are leaves used to bone fracture, piles and bark is used to delivery and diabeties and dysentery (Dubey., et al 2009.).

Crataeva religiosa (Hook and Frost) is native to Australia much of Southest asia and several South Pacific islands, belongs to the family capparidaceae. The treatment is healthy known for its various pharmacological properties like diuretic, antiinflammatory, laxative, antioxidant, antioxaluric, hepatoprotectant, lithonotriptic, antirehumatic, antiperiodic, antimycotic, contraceptive, antipyretic, antilithitic, antihelminthic, rubifacient and vasicant properties (Udaysing et al., 2011).

Materials and Methods

Plant materials

Plants were collected from natural population growing in the Courtallam forest Area, Tirunelveli district, Tamil Nadu, India. The plant sample was carried to the Botany Research Laboratory; Voucher specimen of the plant was deposited in the Botany research laboratory V.H.N.S.N.College (Autonomous) for further references.

Preparation of leaves extracts

In order to perform phytochemical analysis, thirty grams of dry ground plant leaves of the two plants was extracting with polar aqueous, methanol, acetone and ethyl acetate solvents. 30g of the dried leaves sample was taken in a conical flask and 200 ml of methanol was added. The conical flask was kept on mechanical shaker for 24 hours, after that the extract was filtered through what man No: 1 filter paper in the glass beaker and the are allowed drying in incubated in oven at 64.7ºC. The dried extract was recovered and stored in Refrigerator for further analysis.

Qualitative phytochemical analysis

The dried leaves extracts were subjected to qualitative phytochemical screening for the identification of various classes of active chemical constituents.

Test for Alkaloids (Mayers' Test)

To 1 ml of leaves extract, 6 drops of Mayer's reagent was added. The formation of yellowish creamish precipitate indicated the presence of alkaloids (Edeoga et al., 2005; Harbone, 1973).

Test for Saponins (Foam Test)

1ml of leaves extract was mixed with 5ml distilled water. The contents were heated in a boiling water bath. Frothing indicated the presence of saponins (Edeoga et al., 2005; Harbone, 1973).

Test for Tannins (Braymers Test)

1ml of the leaves extract was added mixed with 2 ml of water. To this 2 drops of 5% ferric chloride solution was added. Appearance of dirty green precipitate indicated the presence of tannins (Edeoga et al., 2005; Harbone, 1973).

Test for Steroids (Salkowski Test)

To 2ml of the extract, 2 ml of chloroform was added and followed by concentrated sulphuric acid. Formation of reddish brown ring at the junction showd the presence of steroids. (Yadav et al., 2014).

Test for Terpenoids

2ml of the extract was added with 2 ml of acetic acid. Then concentrated sulphuric acid was added. Deep red color development showed the presence of steroids (Yadav et al., 2014).

Test for Coumarins

Take 2 ml of the extract and add 3 ml of 10% sodium hydroxide. Formation of yellow coloration indicates the presence of Coumarins (Yadav et al., 2014).

Test for Catachin

2 ml of alcoholic extract solution was treated with few drops of Echrilich reagent and few drops of concentrated HCL. The pink color formation indicates the presence of catachin (Yadav et al., 2014).

Test for Phenols

1 ml of the extract was treated with 3% ferric chloride. The appearance of deep blue color, then it shows the presence of phenol (Kokate, 2000; Harborne, 1999).

Test for Flavonoids

1ml of extract was added with 1 ml of sulphuric acid. Orange colour formation conformed the presence of flavonoids (Kokate, 2000; Harborne, 1999).

Test for Quinone

1 ml of extract was treated with 5 ml of HCL. Formation of yellow color precipitate indicated the presence of quinone (Kokate, 2000; Harborne, 1999).

Quantitative phytochemical analysis

Estimation of total phenol content

The amount of total phenol was determined using the Folin–Ciocalteu reagent method of Lister and Wilson, (2001). A standard curve was prepared by using gallic acid. Different concentrations of gallic acid were prepared in 80% methanol, and their absorbance was recorded at 760 nm. 100 µl of sample was dissolved in 500 µl (1/10 dilution) of Folin–Ciocalteu reagent and 1ml of distilled water. The contents were mixed and incubated at room temperature for 1 min. After 1 min, 1.5ml of 20% sodium carbonate solution was added. The final mixture was shaken and incubated for 2 h in the dark at room temperature. The absorbance of all samples was measured at 760 nm using a UV–Vis spectrophotometer (Model. U.2800, Hitachi). The results were expressed in mg gallic acid equivalents (GAE) per milligram of dry weight of the plant. The amount of phenol in plant extracts was calculated by the following formula:

$X = (A.\ m_o) / (A_o.m)$

Where X is the phenol content, mg/mg plant extract in GAE, A is the absorbance of plant extract, A_o is the absorption of standard gallic acid solution, m is the weight of plant extract, and m_o is the weight of gallic acid in the solution.

Estimation of total flavonoid content

The flavonoid content in the extracts were determined spectrophotometrically by the method of Quettier-Deleu et al., (2000). This method was based on the formation of a complex, flavonoid–aluminium, with the absorbance maximum at 430 nm. Rutin was used as standard to make the calibration curve. 1 ml of diluted sample was separately mixed with 1 ml of 2% aluminum chloride methanolic solution. After incubation at room temperature for 15 min, the absorbance of the reaction mixture was measured at 430 nm in a UV–Vis spectrophotometer (Model. U.2800, Hitachi). The flavonoid content was expressed in mg per mg of rutin equivalent (RE). The amount of flavonoid in plant extracts in RE was calculated by

the following formula:

$X = (A. m_o) / (A_o.m)$

Where X is the flavonoid content, mg/mg plant extract in RE, A is the absorbance of plant extract solution, A_o is the absorption of standard Rutin acid solution, m is the weight of plant extract, mg and m_o is the weight of Rutin acid in the solution.

Free-Radical Scavenging Ability (DPPH-assay)

The scavenging ability of methanol extract on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free- radicals was estimated according to the method of Shimada et al., (1992). This method depends on the reduction of purple DPPH to yellow coloured diphenyl picryl hydrazine. 2 ml of various concentrations (10-100µg/ml) of test sample was mixed with 0.5 ml of 0.1 mM DPPH in methanol. An equal amount of methanol and DPPH served as control. The mixture was shaken vigorously and then steadily kept for 30 min at room temperature in dark. The absorbance of the resulting solution was measured at 517 nm against the blank using UV–Vis spectrophotometer. The experiment was performed in triplicates. The DPPH radical scavenging activity was calculated by the following equation:

$\%$ DPPH radical scavenging activity $= (A_o - A1)/ A_o \times 100\%$

Where A_o is the absorbance of the control reaction and A1 is the absorbance of the sample of the tested extracts. Percentage of radical activity was plotted against the corresponding antioxidant substance concentration to obtain the IC_{50} value, which is defined as the amount of antioxidant substance required to scavenge the 50% of free radicals present in the assay system. IC_{50} values are inversely proportional to the antioxidant potential.

Result and Discussion

In the present study, phytochemical screening was performed with methanol, ethyl acetate, acetone and aqueous extracts of the leaf of two plants. These two plants collected from Western Ghats. Phenol, steroid and terpenoid in two plants investigated, while coumarine are present only in Crataeva religiosa. Similarly alkaloid, phenol, flavonoid, quionone, tannin and terpenoid give their presence in two plants of methanol extracts Crataeva religiosa and Dillenia pentagyna. Tannin and terpenoids are presented in methanol, acetone and ethyl acetate extract except aqueous extract of investigated plants. Catachine was absent in two plant crude extract. Aqueous extract of Crataeva religiosa extracts shows negative results all the phytochemical. In fact, some effects of phenolic and flavonoid compounds include anti-inflammatory, antispasmodic, antiulcer, antidepressant, antidiabetic, cytotoxicity and antitumor, antimicrobial, and antioxidant properties. Additionally, steroids derived from medicinal plants are known to possess antibacterial and insecticidal properties (Bhatti et al., 2022).

Quantitative estimation of crude phytochemicals from these two plants is given in table The total phenolic content of Dillenia pentagyna in different solvent extracts such as Methanolic extract, Acetone extract and Ethyl acetate extract are 0.274 mg/mg GAE, 0.234 mg/mg GAE and 0.170 mg/mg GAE respectively followed by total flavonoid content in methanol (0.158 mg/mg RE), acetone extract (0.151 mg/mg RE) and ethyl acetate extract (0.150 mg/mg RE) and total tannin content of methanol extract (1.805 mg/mg RAE), acetone extract (1.948 mg/mg RE) and ethyl acetate extract (1.136). Crataeva religiosa showed the total phenolic content of C. religiosa in different solvent extracts such as Methanolic extract, Acetone extract and Ethyl acetate extract are 0.227mg/mg GAE, 0.168mg/mg and 0.101mg/mg GAE respectively followed by total flavonoid content in methanol extract (0.738 mg/mg RE), acetone extract (0.290 mg/mg RE) and ethyl acetate extract (0.245 mg/mg RAE) and total tannin

content in methanol extract (1.53 mg/mg TAE), acetone extract (1.874 mg/mg TAE) and ethyl acetate extract (1.500 mg/mg TAE). The high amounts of phenolic and flavonoid compounds in this plant could increase its biological properties compared to other studied medicinal plants. The antioxidant activity should not be concluded on the basis of a single method (Munteanu et al., 2021).

10-100µl of leaf extracts (methanol, ethanol, ethyl acetate and aqueous extracts of leaves of Dillenia pentagyna and Crataeva religiosa were estimated for free radical scavenging activity using Diphenyl-2-picryl hydrazyl (DPPH) assay. The samples observed for its bleaching from purple to yellow and pale pink were considered as strong positive and weak positive respectively. Among the five different extracts of Dillenia pentagyna and Crataeva religiosa, the methanolic leaf extract of Dillenia pentagyna and methanolic leaf extract of Crataeva religiosa recorded the most effective DPPH radical scavenging activity (84.83 %) and (83.58 %) values being close to synthetic antioxidant (AA) as positive control (92.96 %). In each case, methanolic leaf extracts recorded higher percentage of free radical scavenging activity. This antioxidant activity observed in the studied medicinal plants could be attributed to the presence of phenolic compounds such as phenolic acids and flavonoids. These phenolic compounds act as antioxidants by hydrogen-donating properties of their phenolic group hydroxyls (Pereira et al., 2009).

Conclusion:

The current investigations of phytochemicals in the leaves different solvent extract of Dillenia pentogyna and Crateva religiosa might be responsible for its therapeutic and antioxidant effect and the respond of this research also supports the number of biological activities and provide protection against free radical

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