Quantitative determination of total Phenolic and Tannin contents of *Murraya koenigii* Twigs

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Abstract

The total phenolic and tannin content present in the ethanolic extracts of Murrayakoenigii twigs was estimated in this study. The ethanolic extracts of Murrayakoenigii twigs phenolic and tannin contents were measured using Folin-Ciocalteu's method. Folin-Ciocalteu's method. Works on the basis of oxidation and reduction reaction with single electron transfer with gallic acid and tannic acid taken as standards. The study revealed the presence of a significant amount of phenolic and tannin content in the ethanolic extracts of Murrayakoenigii twigs.

Keywords: Murrayakoenigii twigs, Tannin, Phenolic acids, Secondary metabolites, Total phenolic content.

Introduction

Murrayakoenigii also known as curry tree is a perennial shrub that is native to India and is also grown in Malaysia, Sri lanka, South Africa and other parts of the Asian subcontinent. The twigs and leaves of curry tree contain phytochemicals and other minerals that are essential for the diet and are also responsible for their medicinal properties. Which was used to treat various ailments since ancient times in India. [2] The twigs of the *Murrayakoenigii* tree are sometimes used as chewing sticks in Tamilnadu,India.The twigs of the curry tree is enriched with phytochemicals such as alkaloids, flavonoids, carbohydrates, amino acids and phenols and minerals such as calcium and magnesium.[6]

The presence of Phenolic acids in plants are responsible for their color, flavor and medicinal property. These Phenolic compounds also exhibit antioxidants, Anti-inflammatory activity, anticancer and antibacterial activity for the plant and the living beings that feed on them.[8][9] Thus, these biologically active molecules serve as readily available drugs to treat various ailments. The phenolic contents present in plants are very well known to play an essential role in providing protection against the free radicals generated in the living system. [3][4] Free radicals are said to cause various types of cancers such as oral cancer, breast cancer, lung cancer and other serious disorders in living beings. [5][10].Besides, the tannins and other phenolic acids present in plants are said to provide the plant a natural defense system from predators. Tannins are responsible for their aroma, flavour and colour of the plant which is also the reason for their exploitation for commercial purposes.

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Materials and methods

Sample preparation:
The plant sample is prepared by air drying the twigs for 3 days and was made in to fine powder. A conical flask was taken with the dried powder weighed and was added with Ethanol. The mixture is incubated at room temperature for two days following frequent agitation of the mixture. The supernatant was collected by filtering with Whatman no:1filter paper. The solvents were stored, evaporated and weighed for further analysis.

Phytochemical analysis:

Test for carbohydrates (Molisch's test): 2.0ml of the plant extract were taken and few drops of 5% of alpha naphthol solution was added to it. The mixture was shaken and added few drops conc.H2SO4 along the sides of the test tube. Appearance of violent ring at the junction of two layers of the solution indicates the presence of carbohydrates.

Test for alkaloids: To 2.0ml of the plant extract 1 or 2 drops of Mayer's reagent was added. Appearance of white creamy precipitate at the bottom of the test tube indicates the presence of alkaloids.

Test for phenols: To 2.0ml of the plant extract 5% ferric chloride was added and observed for the appearance of deep blue colour which indicates the presence of phenols.

Test for Phytosterol: To 1.0 ml of plant extract ,2.0m1 of chloroform and few drops of acetic anhydride and concentrated sulfuric acid was added. The solution changes to bluish green color precipitate which indicates the presence of phytosterols.

Test for flavonoids: To 2ml of the plant extract few drops of 10% ferric chloride was added. Appearance of green or blue colour indicates the presence of flavonoids.

Test for tannins: Ferric chloride test: To 1.0ml of the plant extract added few drops of 10%ferric. The formation of black colour precipitate will indicate the presence of tannins.

Test for Saponin: To 2.0m1 of the plant extract, 6.0m1 of water was added and the mixture was shaken vigorously. The formation of foam indicates the presence of saponins.

Test for amino acids: To 2.0m1 of the plant extract, few drops of ninhydrin reagent was added and observed for the development of violet or purple colour which indicates the presence of amino acids.

Test for Steroid: To 1.0m1 of the plant extracts 2.0ml of chloroform and 0.2m1 of concentrated sulfuric acid was added. The formation of red colour precipitate will indicate the presence of steroid.

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Test for cardiac glycosides: To 2.0ml of the plant extract few drops of concentrated sulphuric acid was added. Appearance of red color precipitate indicates the presence of cardiac glycosides.

Determination of total Phenolic content

Total Phenolic content: The total phenolic content was determined by using Folin-Ciocalteu assay. (Singleton et al.) A volume of 0.5 ml of the plant extract (100 µg/mL) was mixed with1.5ml of the 1:10 Folin-Ciocalteu reagent. The reaction was made to stand for 5 minutes and was added with 1.5ml of 7% of sodium carbonate solution. The solution is made up to 10ml with distilled water. The reaction mixture was incubated at room temperature for 30 mins. The appearance of blue color was measured at 765 nm using spectrophotometer with Gallic acid equivalent per gram of dry plant extract (GAE/g) used as standard for plotting the graph.

Total Tannin content: The total tannins contents were determined by the Folin-Ciocalteu method. To the 0.1 mL of the sample extract,0.5 mL of Folin-Ciocalteu reagent was added. To the mixture 1 ml of 35% sodium carbonate was added and made up to 10 ml with distilled water. The mixture was incubated at room temperature for 30 mins and the absorbance was measured at 700 nm with tannic acid equivalents per gram of dry plant extract (TAE/g) as standard at concentrations (100, 200, 300, 400, 500 µg/ ml). [7]

Results

Phytochemical constituents such as flavonoids, alkaloids, tannins are present in ethanol extracts.
Carbohydrates is present in both the chloroform and ethanol extracts.
amino acids and phytosterols were present in the chloroform extracts.

Table 1. Phytochemical Constituents Present in the Plant Sample

Phytochemicals	Ethanol	Chloroform
Alkaloids	+	-
Amino acids	-	+
Carbohydrates	+	+
Cardiac glycosides	-	-
Flavonoids	+	-
Phenols	+	-
Phytosterols	-	+
Saponins	-	-
Steroids	+	-
Tannins	+	-

(+) = Presence of phytochemicals
(-) = Absence of phytochemicals

Total Phenolic content:

Table 2 shows the total phenolic contents which were calculated using the following linear equation $y = 0.003x$, $R^2 = 0.97$ obtained from the calibration curve of Gallic acid standard. Percentage of yield of the twig extracts was found to be 0.34876mgGAe/g of sample.

Table 2. Comparison of Total Phenolic content of Sample at Five Different Concentrations with the Standard.

Gallic Acid (Standard)	Absorbance
100	0.15
200	0.72
300	1.15
400	1.55
500	1.98
***Murraya koenigii* twig extracts (1000 µg/ml)**	1.33

Total Tannin content:

Table 3 shows the total tannin contents which were calculated using the following linear equation $y = 0.001x$, $R^2 = 0.964$ obtained from the calibration curve of tannic acid standard. Percentage of yield of the twig extracts was found to be 1.386334746mgTAE/g of sample.

Table 3. Comparison of Total Tannin content of Sample at Five Different Concentrations with the Standard.

Tannic acid (Standard)	Absorbance
100	0.15
200	0.24
300	0.35
400	0.41
500	0.59
Murraya koenigii **twig extracts (1000 µg/ml)**	1.52

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