Phytochemical analysis, Antioxidant activity and Anti-inflammatory activity of *Murraya koenigii* Twigs

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Abstract

In this study the phytochemical composition of Murrayakoenigii twigs in the ethanol and chloroform extracts were identified. We also evaluated the invitro-antioxidant activity by DPPH assay, FRAP assay,H2O2 assay and anti-inflammatory activity by protein denaturation assay of ethanolic extracts. The phytochemical screening revealed the presence of alkaloids, flavonoids, phenols, amino acids, carbohydrates, tannins, steroids and phytosterols in the ethanol and chloroform extracts. The ethanolic extracts of Murrayakoenigii twigs also express significant amount of antioxidant and anti inflammatory activity.

Keywords: Phytochemical analysis, invitro-Antioxidant activity, DPPH assay, FRAP assay, H2O2 assay, Anti inflammatory activity, BSA Denaturation assay, Protein Denaturation assay, medicinal plants, *Murrayakoeinigii* twigs, natural chewing sticks.

Introduction

Natural chewing sticks have been used in different cultures across the world [1].*Murrayakoenigii* twigs are said to be used as natural chewing sticks in Tamilnadu, India. Various studies on the usage of natural chewing sticks have shown their effective nature in reducing bacterial colonies in teeth and help in maintaining good oral hygiene[2]. The oral cavity is said to be damaged by the generation of free radicals by the intake of certain foods and substances through the mouth such as nicotine, drugs, alcohol and teeth whitening products. Free radicals are substances that can cause risk of cancer and various other ailments in the body [11]. Plants contain bioactive compounds as a part of natural defence mechanism these substances can act as antioxidants that destroys the free radicals. Frequent application of natural chewing sticks can help us to maintain the balanced concentration of antioxidants to scavenge the free radicals in the mouth and help in maintaining good oral hygiene [3].

Inflammation in the mouth can be induced by microbes such as bacteria and viruses, autoimmune diseases, gum diseases and various other external factors like drugs, tobacco, chemicals in the food which can cause allergic reactions resulting in ulcers and sores on the mouth [10]. It has been necessary to find out the effective alternative resources from the nature. Identifying natural chewing sticks can help in making various formulations for preventing gum diseases and inflammation in the oral cavity. Phytochemicals present in the plants can be assessed for their anti inflammatory activity and can be utilized to prevent oral inflammation. These phytochemicals can interfere with the local inflammation and help in preventing the spread of inflammation.

Materials and methods

Sample preparation:
The plant sample is prepared by air drying the twigs for 72 hours in sunlight and was made in to fine powder. Two conical flasks were taken with the dried powder of sufficient grams and one of the conical flask containing was added with Ethanol and other conical flask with chloroform. 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 and solvents were stored for further analysis.

Phytochemical analysis:

Test for carbohydrates (Molisch's test): To 2.0ml of the plant extract few drops of 5% of alpha naphthol solution was added and shaken. To the mixture 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 1-2 drops of mayer's reagent (mercuric chloride-1.36g and potassium iodide -5.0g dissolved in 100ml of distilled water) was added. Appearance of white creamy precipitate indicates the presence of alkaloid.

Test for phenols: To 2.0ml of the plant extract 5% ferric chloride was added. Appearance of deep blue or black colour indicates the presence of phenols.

Test for Phytosterol: (Liebermann's test) To 1.0 ml of plant extract ,2.0m1 of chloroform and few drops of acetic anhydride and concentrated sulfuric acid was added. The formation of bluish green color precipitate 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 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.

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

In-vitro anti oxidant activity

DPPH scavenging activity: The DPPH scavenging activity of plant samples was measured according to the procedure described by (Braca *et al.*,)[8]. DPPH solution was made by taking 2mg of DPPH in 50ml of ethanol. From the stock 20,40,60,80,100 µg/ml of plant extracts were taken and made up to 3ml with ethanol. The tubes are then treated with 1ml of DPPH solution and incubated for 30 minutes in dark.The antioxidants present in the sample gets reduced and the solution turns in to violet colour which is measured colorimetrically at 517nm. A graph was plotted with concentration vs percentage of inhibition. Ascorbic acid was taken as positive standard reference.

$$\text{Scavenging activity \%} = \frac{\text{Absorbance517 (control)} - \text{Absorbance517 (sample)}}{\text{Absorbance517 (control)}} \times 100$$

Reducing power assay: In this method the antioxidants present in the plant extracts on reaction with potassium ferricyanide, trichloroacetic acid and ferric chloride forms a blue colour complex. The plant extracts of 20µl,40µl,60µl,80µl,100µl were taken and made up to 1ml with the solvent. 2.5ml of 0.2m phosphate buffer of ph. 6.6 and 2.5 ml of (1%) potassium ferricyanide were added and incubated at 50°c for 20 min. After incubation ,2.5ml of 10% trichloroacetic acid were added to all the test tubes and centrifuged at 1000 rpm for 10 mins. The supernatant was collected . 2.5ml of distilled water and 0.5ml of 0.1% of ferric chloride was added and measured at 700nm. A graph was plotted with ascorbic acid as positive standard. The increase in absorbance is equal to the presence of increased antioxidants present in the sample.

$$\text{Percentage (\%) of inhibition} = \frac{\text{Absorbance(control)} - \text{Absorbance(sample)}}{\text{Absorbance (control)}} \text{ X } 100$$

H2O2 scavenging assay:25-400 µg/mL of the sample extracts was taken and made up to 0.4ml with 50mM phosphate buffer saline (pH-7.4) and 0.6ml of 2mM hydrogen peroxide was added to it. The reaction mixture was vortexed and incubated for 10 minutes. The absorbance was read at 230 nm. Ascorbic acid was taken as positive control. The H2O2 scavenging activity of the extracts was determined by,

$$\text{Percentage (\%) of inhibition} = \frac{100 - \text{Absorbance(control)} - \text{Absorbance(sample)}}{\text{Absorbance (control)}} \text{ X } 100$$

In-vitro anti-inflammatory activity

Inhibition of Protein Denaturation assay: Determination of anti-inflammatory activity by this method was proposed by methods of Mizushima and kobayashi and sakat et al. [17]. Followed with minor modifications. The plant extracts of 100,200,300,400,500µl were taken and 0.45 ml of aqueous solution of 1% BSA was added to it. The pH of the solution was adjusted to 6.3 by adding a small amount of HCL to the mixture. The samples are then incubated at 37 ºc for 20 min and are cooled. 2.5ml phosphate buffer saline (ph. 6.3) was added to each tube and Turbidity was measured at 660 nm. The percentage inhibition of protein denaturation was calculated using the formula,

$$\text{Percentage (\%) Anti-Denaturation activity} = \frac{\text{Absorbance(control)} - \text{Absorbance(sample)}}{\text{Absorbance (control)}} \text{ X } 100$$

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	+	-
Aminoacids	-	+
Carbohydrates	+	+
Cardiac glycosides	-	-
Flavonoids	+	-
Phenols	+	-
Phytosterols	-	+
Saponins	-	-
Steroids	+	-
Tannins	+	-

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

DPPH radical scavenging activity:

Figure1 shows the in vitro antioxidant activity of the plant sample at different concentrations. The percentage inhibition of DPPH increases with increasing concentration confirming the antioxidant activity of the plants.

Table 2. Comparison of Free Radical Scavenging Capacity of Sample at Five Different Concentrations with the Standard.

Concentration (µl)	% Inhibition of Standard (Ascorbic acid)	% Inhibition of Sample
S-1	15 %	10 %
S-2	38 %	15 %
S-3	56 %	41%
S-4	73 %	73 %
S-5	86 %	173.4%

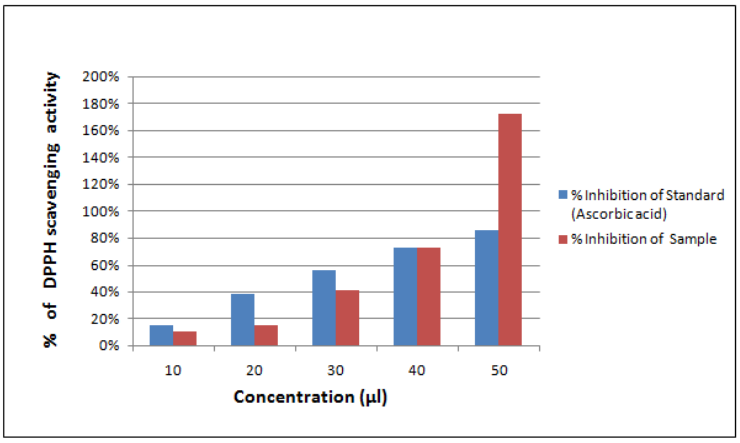

Figure 1. Percentage Inhibition of the Plant Extracts in Five Different Concentrations.

Reducing power assay:

From the results of the study the reducing power of the sample was confirmed. Figure shows the in vitro antioxidant power of the plant sample at different concentrations. The percentage inhibition of plant sample increases with increasing concentration. Ascorbic acid was taken as standard.

Table 3. Comparison of Reducing Power Capacity of Sample at Five Different Concentrations with the Standard.

Concentration (µl)	% Inhibition of Standard (Ascorbic acid)	% Inhibition of Sample
S-1	33 %	17.3
S-2	44 %	74.43
S-3	56 %	83.45
S-4	73 %	95.48
S-5	86 %	125

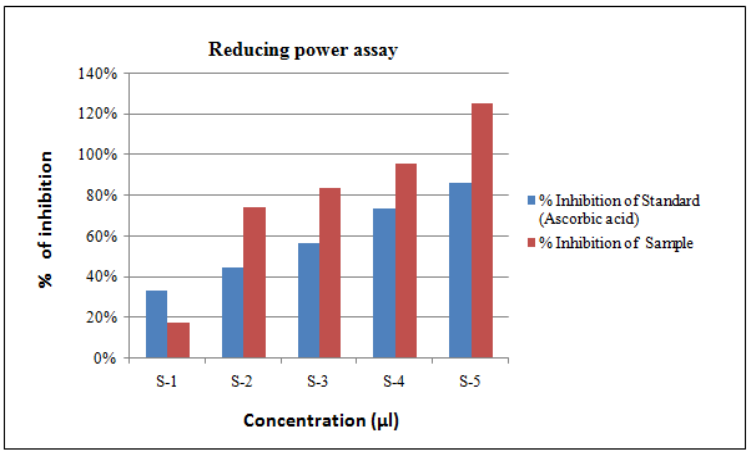

Figure 2. Percentage Inhibition of the Plant Extracts in Five Different Concentrations

H2O2 scavenging assay:

The hydrogen peroxide scavenging activity of the sample was confirmed with the percentage inhibition of plant sample increases with increasing concentration. Ascorbic acid was taken as standard.

Table 4. Comparison of H2O2 Scavenging Capacity of the Sample at Five Different Concentrations with the Standard.

Concentration (µl)	% Inhibition of Standard (Ascorbic acid)	% Inhibition of Sample
S-1	35 %	31 %
S-2	48 %	47 %
S-3	62 %	65%
S-4	83 %	80 %
S-5	91%	87%

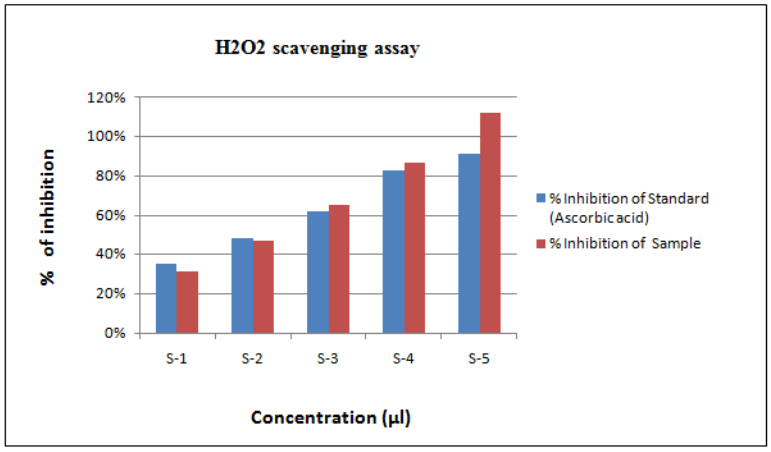

Figure 3. Percentage Inhibition of the Plant Extracts in Five Different Concentrations

Inhibition of Protein Denaturation

From the results of the study the anti inflammatory activity of the sample was confirmed. The anti inflammatory activity of the plant sample at different concentrations increases with increasing concentration.

Table 5. Comparison of Anti Denaturation Activity of Sample at Five Different Concentrations with the Standard.

Concentration (µl)	% of Anti Denaturation activity of Standard (Ascorbic acid)	% of Anti Denaturation activity of Sample
S-1	33 %	32 %
S-2	42 %	45 %
S-3	55 %	73%
S-4	82 %	85 %
S-5	95%	114%

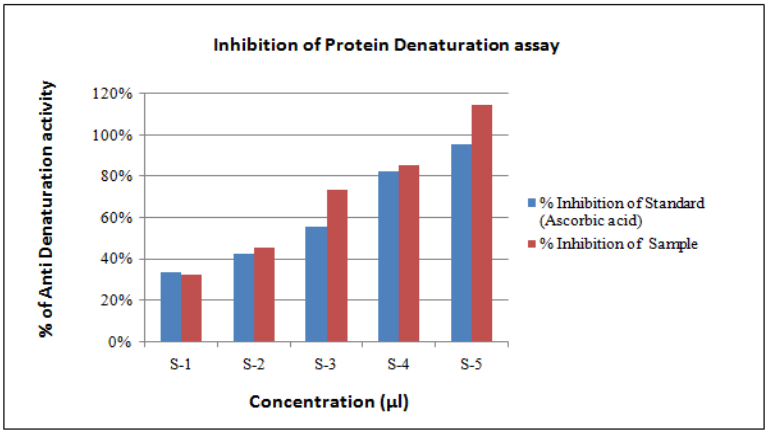

Figure 4. Percentage of Anti Denaturation activity of the Plant Extracts in Various Different Concentrations

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