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PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT ACTIVITY OF GRANGEA MADERASPATANA (L) POIR.

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ABSTRACT

This study contributes to a better understanding of Grangea maderaspatana (L) Poir. a species of the Asteraceae family, as a pharmaceutical plant for its phytochemicals. Extracts from leaves analysed for phytochemicals. Methanol and hexane were used to prepare the extracts from the leaf. Spectrophotometric techniques were used to determine the total phenolics, total flavonoids, total tannin and total antioxidants contents. The phytochemical screening was also carried out by means of thin layer chromatography.

Keywords: Grangea maderaspatana (L) Poir., phytochemicals, TPC, TFC, TTC, total antioxidant

INTRODUCTION

Grangea is a small plant genus found in Africa and Asia's tropical and subtropical regions. Grangea maderaspatana (L) Poir. Belongs to Compositae (Asteraceae) family and grows in damp areas of India, notably Bengal. G. maderaspatana (L.) Poir. (Phayaa Mutti) is one of the most common medicinal plants used in traditional Thai medicine in various therapeutic approaches, including ingestion of the whole plant to stimulate digestion, reduce pain and inflammation (Chaturvedi D, 2011). Plant steroidal components include Hardwicke acid, the corresponding 1, 2-dehydro-derivative acetylenic compounds (Iyer C. et al.,), aura amide (Singh P. and Jain S.), clerodane diterpenes (Krishna v. et al.,), eudesmanolides (Ruangrungsi et al., 1998), Penta and hexa methoxy flavones (Krishna V. et al.,). The root is an appetiser, astringent to the intestines, diuretic, anthelmintic, beneficial in griping, chest and lung problems, headache, rheumatism in the knee joint, piles, muscular discomfort, illnesses of the spleen and liver, ear, mouth, and nose problems, and it reduces sweating (Nadkarni A., 1976). Therefore, the facility has recorded. Further, plant has reported to have analgesic, oestrogenic and anti-implantation activities (Ahmed et al., 2001).

Plants are known for producing bioactive compounds. These are the substances The most known secondary metabolites include tannins, flavonoids, alkaloids, sterols, and terpenes. Because of their numerous biological properties, specifically their antioxidant capabilities, these various classes of molecules are increasingly sought by the food, pharmaceutical, and cosmetics industries (Issiaka et al., 2019). Medicinal plants have become a global topic, with an effect on global health. Herbal medicine has played an important part in the maintenance of the global healthcare system. Asteraceae was the most common family of therapeutic plants described (Ullah et al., 2020). The interest in these natural compounds with antioxidant characteristics stems mostly from the fact that synthetic antioxidants are being questioned due to their toxicity risk. In higher plants, these secondary metabolites can be found in different organs (leaves, flowers, roots, stems) at varying levels.

Despite the numerous properties attributed to this plant, few studies on the phytochemicals and antioxidant capabilities of extracts of the various organs of this plant adapted in Burkina Faso have been published to our knowledge. The current study aims to carry out a phytochemical study and an evaluation of the antioxidant activities of extracts of leaves of G. maderaspatana.

METHOD

1. Collection of plant Material

The leaves of Grangea maderaspatana (L.) Poir. plant was collected in February 2023 from the Kheralu taluka, Mehsana district of Gujarat state, India. The collected plant was

authenticated by Department of Botany, Bioinformatics and Climate Change Impacts Management, Gujarat University, Ahmedabad. After collection, the plant material washed with tap water to remove dust and debris. The washed plant material was dried under shade and grind to make powder.

2. Extraction

The extraction was carried out using the cold extraction method. 10gm of powder material was placed in a conical flask with 100ml of Methanol and Hexane respectively and leave it for 24 hours, at room temperature. The solution was filtered using Whatman filter paper number 1 and allow solvent to become evaporate from the filtrate.

3. Phytochemical screening

Phytochemical examinations were carried out for both the extracts as per the standard method.

3.1 Alkaloids

a) Mayer's test

Two ml of Mayer's reagent are added along with one ml plant extract. Formation of a yellow colour and white creamy precipitate indicates the presence of alkaloids.

b) Dragendroff's test

Two ml of Dragendroff's reagent are added along with one ml plant extract. Formation of an orange-red precipitate indicates the presence of alkaloids.

c) Hager's test

One ml of Hager's reagent is added along with one ml plant extract. Formation of a Yellow precipitate indicates the presence of alkaloids.

3.2 Carbohydrates

a) Molish's test

One ml of Molish's reagent is added along with one ml plant extract. Formation of a violet ring junction indicates the presence of Carbohydrates.

b) Fehling's test

One ml of Fehling solution A and B are added along with one ml plant extract and it should boil in a water bath for two minutes. Formation of red precipitate indicates the presence of Carbohydrates.

c) Benedict's test

One ml of Benedict's reagent is added along with one ml plant extract. Formation of a red-orange precipitate indicates the presence of Carbohydrates.

3.3 Flavonoids

a) Lead acetate test

One ml **extract** treated with three ml of 10% lead acetate solution. Formation of a yellow colour and bulky white precipitate indicates the presence of flavonoids.

b) Alkaline reagent test

Few ml **e**xtract treated with few drops of sodium hydroxide (NAOH) solution. Formation of a yellow colour turn in colourless indicates the presence of flavonoids.

c) H_2SO_4 test

Few ml extract treated with few drops of sulfuric acid. Formation of a orange precipitate indicates the presence of flavonoids.

3.4 Phenols

a) Ferric chloride test

Few ml extract treated with few drops of 5% ferric chloride solution. Formation of a Bluish black and blue-green precipitate indicates the presence of phenols.

b) Alkaline reagent test

Few ml **extract** treated with few drops of sodium hydroxide solution. Formation of a yellowish- red precipitate indicates the presence of phenols.

3.5 Tannins

a) Gelatine test

Few ml **extract** treated with few drops of 1% gelatine solution containing sodium chloride. Formation of a white precipitate indicates the presence of tannins.

b) Lead acetate test

Few ml extract treated with 1 ml of 10 % lead acetate solution. Formation of a white precipitate indicates the presence of tannins.

3.6 Glycosides

a) Borntrager's test

one ml extract treated with few drops of ferric chloride solution and boiling it for 5 minutes. Formation of the rose-pink colour indicates the presence of glycosides.

b) Ferric acid test

one ml extract treated with 1 ml glacial acetic acid and add 1-2 ml of concentrated H_2SO_4. Formation of a Violet to blue-green indicates the presence of glycosides.

3.7 Protein

a) Ninhydrin test

Two ml extract treated with two drop of ninhydrin reagent. Formation of a purple colour indicates the presence of protein.

b) Xanthoproteic test

One ml **extract** treated with few drops of nitric acid. Formation of a yellow coloured indicates the presence of protein.

3.8 Saponins

a) Forth test

50mg extract diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 to 2 cm layer of foam indicates the presence of saponins.

b) Foam test

0.5 gm extract diluted with distilled water to 2ml and this was shaken in graduated cylinder for 10 minutes. Formation of foam indicates the presence of saponins.

3.9 Diterpens

a) Copper acetate test

one ml **extract** treated with 3-4 drops of copper acetate solution. Formation of a green colour indicates the presence of diterpens.

3.10 Steroids

a) Libermann–sterol test

One ml extract treated with one ml acetic acid and add one drop concentrated sulfuric acid. Formation of a red, violet, blue, or green indicates the presence of steroids.

b) Salkowaski's test

two ml extract shake with chloroform and add sulfuric acid. Formation of a red coloration indicates the presence of steroids.

4. Total Phenolic Content

The total phenolic content was determined using the Folin-Ciocalteu reagent method (Singleton et al., 1965) (Khaled Tawaha and Alali, et al., 2007). In this method, 1 ml of Folin-Ciocalteu reagent (FCR) were added to 1 ml of diluted methanolic extract. Then 1 ml of a 20% sodium carbonate solution (Na_2CO_3) was added and the total volume was made up to 10 ml with double distilled water. Absorbances were recorded at 765 nm using a UV spectrophotometer after incubation for 30 minutes. The results, determined from the equation of a calibration curve established from the gallic acid taken as reference, were expressed in mg of Gallic Acid Equivalent per gram of extract (mg GAE/ g).

5. Total flavonoid content

Total flavonoid content was measured using an aluminium chloride colorimetry assay (Kamtekar et al., 2014). In this method, 4 ml of distilled water were added to 1 ml of Diluted methanolic extract, followed by add 0.3 ml of the 5% sodium nitrite solution ($NaNO_2$). After that, 0.3ml of 10 % aluminium chloride solution was added. The mixture was allowed to stand at room temperature for 6 min. Then, 2 ml of a 1M sodium hydroxide solution were

added and the total volume was made up to 10 ml with double distilled water. The absorbance was measured at 510 nm using UV spectrophotometer. A calibration curve was established using quercetin as a reference according to the same procedure as the sample. Flavonoid contents of the extracts, expressed in mg of Quercetin Equivalent per gram of extract.

6. Total Tannin Content

Total Tannins were determined using the peri and Pompei method. TTC activity is the primary method for determining the amount of tannin content in a sample. 0.3 ml of folin-denis reagent were added to 1 ml of diluted methanolic extract. Then 1 ml of a 7.5% sodium carbonate is added and the total volume was made up to 10 ml with double distilled water. Absorbances were recorded at 750 nm was measured using a UV spectrophotometer after incubation for 30 minutes. The results, determined from the equation of a calibration curve established from the tannic acid taken as reference, were expressed in mg of Tannic Acid Equivalent per gram of extract (mg GAE/ g).

7. Total antioxidant content

7.1 DPPH assay

Antioxidant content were determined by DPPH assay (Germano et al., 2002). Stock methanolic solution of DPPH 4 mg was first prepared in methanol. Then 2 ml DPPH solution was added to the produced series of extracts with various concentrations (100 µg/ml - 1000 µg/ml). The resulted mixture was shaken and then stored at room temperature and protected from light. The absorbances were read at 517 nm using a, 30 minutes after incubation. The ascorbic acid standard curve was used to obtain the results. Using absorbance data, the radical scavenging activity was calculated using the equation below. This formula is derived by leh and duh,1994.

% Inhibition = [{Abs control – Abs sample}/Abs control] × 100

7.2 ABTS assay

The ABTS method described by Miller and Rice-Evans. Plant extract and standard (0-200 µg/ml) concentration was placed in a test tube and diluted up to 1 ml with methanol. 3 ml of ABTS reagent was mixed and incubated at room temperature for 30 minutes. At 730 nm, the absorbance was measured using a UV spectrophotometer (Opitz et al., 2014). The Trolox standard curve was used to obtain the results were represented as a percentage of ABTS scavenging using the following formula.

% Inhibition = (Abs control – Abs sample) / (Abs control) × 100

8. Thin Layer Chromatographic Characterization

Thin layer chromatography was used for the qualitative estimation of the phytochemicals. Both the extract was analysed by TLC methods. This technique uses glass or aluminium plates coated with silica gel G- 60 (1-1.5mm thick layers). 50µl aliquot of solution of the leaf extract were applied separately to each plate. Leaves extract was run in 11 different solvent system, which was considered as mobile phase ethyl acetate: hexane (10%: 90%), ethyl acetate: hexane (20%:80%), methanol: chloroform (70%:30%), methanol: acetone (90%:10%) give the best separation the finalisation of the solvent system develops the TLC plated and derivatization of P- Anisaldehyde reagent. After spraying the TLC plates observed under the UV short wavelength (245nm) and UV long wavelength (365nm) and the Rf value of spots were calculated.

RESULTS AND DISCUSSION

Phytochemical screening

Pharmacological activities are possessed by phytochemical elements such as alkaloids, flavonoids, carbohydrates, protein, diterpen, tannins, phenols, saponins, steroids, and glycosides. In methanol and hexane extracts, preliminary phytochemical screening of Grangea maderaspatana (L.) Poir shows the presence of phytochemical result as per table 1.

Table 1: Phytochemical screening of leaves of Grangea maderaspatana (L) Poir.

Sr. no.	phytochemicals	Test	Methanol	Hexane
1	Alkaloids	mayer's test	-	-

		Dragendorff's	-	-
		Hager's test	-	-
2	carbohydrates	Molisch's test	-	+
		Fehling's test	+	-
		Benedict's test	-	-
3	Flavonoids	lead acetate test	+	-
		Alkaline reagent test	-	-
		H2So4 test	-	+
4	phenols	Ferric chloride test	+	-
		Alkaline reagent test	-	-
5	Tannins	Gelatine Test	-	-
		lead acetate test	+	-
6	Glycoside	Borntrager's test	-	-
		Ferric chloride test	+	-
7	protein	Ninhydrin test	-	-
		Xanthoproteic test	-	-
8	Saponins	Forth test	-	+
		Foam test	-	+
9	Diterpenes	Copper acetate test	+	-
10	steroids	Liebermann sterol test	+	-
		Salkowski's test	-	+

Total phenolic compound

The procedure of determining the amount of phenolic content in samples is known as total phenolic content. Phenolic compounds found in plants have antioxidant properties that increase their ability to act as antioxidants. The solvents used for extraction have a direct impact on the overall phenolic content of plant extracts and their yields. The phenolic content of Grangea maderaspatana (L) Poir. leaves were determined using gallic acid as a standard. The standard calibration equation for total phenol concentration was found to be y = 20.155x - 0.1495 (R^2 = 0.91). Using the following calibration equation, the phenol content of a leaf sample of Grangea maderaspatana (L) Poir. was calculated and found to be 0.016±0.0004 mg GAE/g**.**

Total flavonoid compound

The procedure of determining the quantity of flavonoid content in a sample is known as total flavonoid content. The flavonoid content was tested using Quercetin as a control. The standard calibration equation for total flavonoid concentration was found to be y = 0.88x + 0.056 (R^2 = 0.9928). Using the following calibration equation, the flavonoid content of a leaf sample of Grangea maderaspatana (L) Poir. was calculated and found to be 0.042±0.010 mg QAE/g.

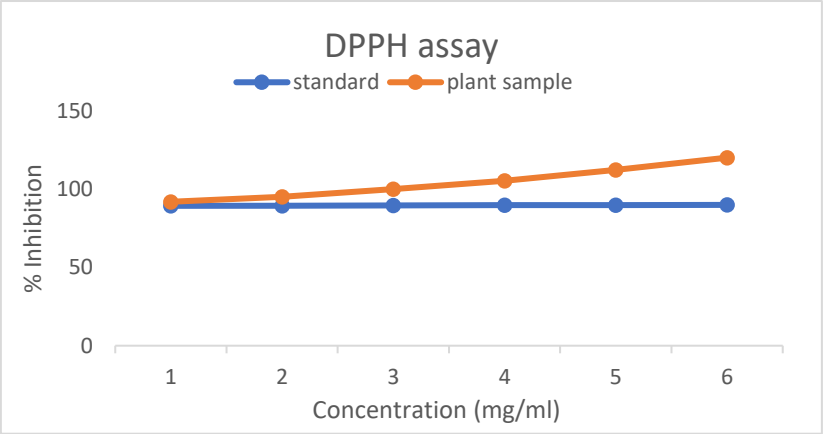
Total Tannin Compound

The procedure of determining the quantity of tannin content in a sample is known as total tannin content. The tannin content was tested using tannic acid as a control. The standard calibration equation for total tannin concentration was found to be y = 8.545x - 0.0433 (R^2 = 0.9969). Using the following calibration equation, the tannin content of a leaf sample of Grangea maderaspatana (L) Poir. was calculated and found to be 0.016±0.00034 mg GAE/g.

DPPH (2,2- diphenyl-1-picryl-hydrazyl-hydrate) assay

Ascorbic acid was utilized as a standard equivalent for this experiment in terms of antioxidant activity. The free radical scavenging activity of Grangea maderaspatana (L) Poir. methanolic extract was determined by the DPPH radical scavenging method and ascorbic acid as a reference. Free radicals are harmful by-products of oxygen metabolism that cause oxidative stress in living tissue or cells. The inhibiting activity was determined by using sample extracts Optical Density (OD). The standard calibration equation for DPPH was found to be y = 1.6714x + 89.007 (R^2 = 0.9364). Using the following calibration equation, the DPPH content of a leaf

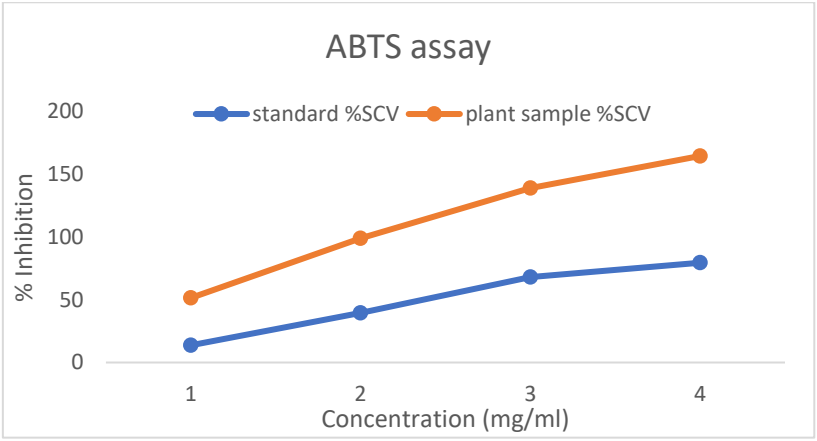
sample of Grangea maderaspatana (L) Poir. show highest radical scavenging activity at 0.6 mg/ml concentration.



Graph 1: Standard curve of DPPH assay

(ABTS) 2,2'-Azino-bis (3-Ethylbenzothiazolin-6-sulfonic Acid) / (TEAC) Trolox Equivalent Antioxidant Capacity Assay

The total antioxidant capacity assay can be used to determine the capacity of antioxidants through the formation of 2,2'-Azino-bis (3-Ethylbenzothiazolin-6-sulfonic Acid). In ABTS assay Troxol used as a standard. The standard calibration equation for ABTS was found to be y = 0.2637x + 29.889 (R^2 = 0.8707). Using the following calibration equation, the ABTS content of a leaf sample of Grangea maderaspatana (L) Poir. show highest radical scavenging activity at 200 mg/ml concentration.



Graph 2: Standard curve of ABTS assay

Thin Layer Chromatography (TLC)

Table 2: methanolic leaves extract solvent system, total bands and Rf values.

Sr no.	Solvent system	Total band	Rf value
1	ethyl acetate: hexane (1:9)	7	0.87,0.83,0.77,0.58,0.387,0.161,0.096
2	ethyl acetate: hexane (2:8)	8	0.94,0.91,0.88,0.85,0.82,0.705,0.44,0.32
3	methanol: chloroform (7:3)	4	0.95,0.92,0.9,0.82
4	methanol: acetone (9:1)	5	0.85,0.87,0.92, 0.95,0.975

CONCLUSION

Presented study concludes that, Methanolic and Hexane extract of leaves shows the presence of various phytochemicals and this study successfully identify the quantity of total phenolic compound, total tannin compound and total flavonoid compound. TLC values also shows the presence of various phytochemicals. The methanol extract of leaves of the Grangea maderaspatana (L) Poir. showed strong antioxidant activity as compared to standard. Because of the plant's antioxidant function, more research may be done to study its usage in many diseases where free radicals play a role in their pathology. More research will be required to analyse the extracts and its fractions in vivo capabilities in various animal models.

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