Phytochemical and antioxidant studies on dried leaves of *Crotalaria gajureliana* Gholave, Madhav & Gosavi

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ABSTRACT

Crotalaria gajureliana is a new plant species and its biological activity and phytochemical composition are unknown. Leaves are a renewable plant part, meaning they can be harvested without harming the plant or threatening its survival. The current study is an evaluation of the phytochemical profile, total phenolic content (TPC), total flavonoid content (TFC), along with antioxidant activity of dried leaves of Crotalaria gajureliana by successive solvent extraction. Analysis began with standard qualitative tests to confirm the presence of these phytoconstituents. The phytochemical analysis revealed that the extracts contained flavonoids, alkaloids, tannins, glycosides, and phenolic compounds. The methanol extract exhibited the highest levels of total phenolic and flavonoid content, followed by the aqueous extract, and then the ethanol extract. DPPH antioxidant activity here shows significant free radicals scavenging, where aqueous extract showed the highest inhibition percentage which can be explained by the higher levels of total phenolic and Flavonoid compounds.

Keywords: Antioxidant activity, *Crotalaria gajureliana*, phytochemicals, successive extraction, total flavonoid content, total phenolic content

INTRODUCTION

Many plants or plant-based materials are getting a lot of attention in the field of modern medicine for the development and extraction of possible therapeutic candidates for the treatment of many diseases (Mirihagalla & Fernando, 2021). *Crotalaria gajureliana* Gholave, Madhav & Gosavi, *Synonym: Phatakadi,* is a recently discovered plant of the genus Crotalaria in the Fabaceae plant family (subfamily: Papilionoideae). The plant is so far known to occur in two locations in Maharashtra, India: Nandur-Madhyameshwar and Chamar Leni (Gholave *et al.,* 2021). This herb, thrives in open grassland areas (Khot *et al*., 2023), whose phytochemical and pharmacological properties have not yet been fully investigated. *Crotalaria gajureliana* is a plant that has never been documented in the literature, and the primary goal of this effort is the first scientific analysis of this plant.

For herbal medication compositions to be safe, effective, and of high quality, medicinal plant standardization is essential. Extraction of bioactive chemicals varies by successive extraction using solvents with different polarities, whereas first phytochemical screening provides information about the chemical components that may have pharmacological effects. *Crotalaria gajureliana* leaves will be subjected to DPPH (1, 1-Diphenyl-2, Picryl-Hydrazyl) free radicals to assess their free radical scavenging activity, TPC, TFC, in addition to successive extraction yield. This will help *Crotalaria gajureliana* 's scientific validity as well as its possible uses in herbal medicine. Future pharmacological and phytochemical studies on this newly

discovered plant species will be built upon the results.

MATERIALS AND METHODS

The study was made at Pharmacognosy PG Laboratory, Department of Pharmacognosy, Mahatma Gandhi Vidyamandir's Pharmacy College, Nashik, Maharashtra, India 402003.The leaves of *Crotalaria gajureliana* was collected from Chamar Leni, Nashik District, India, in August 2024. The plant was identified by Dr. Avinash Gholave, a botanist at Department of Botany, K.V.N. Naik Arts, Commerce and Science College, Nashik, India. The voucher sample (SRV-02) was held as a future reference in the specimen section of the Department Museum. After collection, the leaves were dried under shade and used for further research (Figure 1).

Every chemical, solvent, and reagent employed in the investigation was of analytical grade. The Soxhlet apparatus, water bath, electronic balance and UV-Spectrophotometer were among the equipment utilised. Using a Soxhlet apparatus with polarity-increasing solvents pet ether to be followed by ethyl acetate, methanol, ethanol, followed by water, along with dried leaves of *Crotalaria gajureliana* were extracted one after the other. The effective extraction of various plant phytoconstituents based on their solubility in various solvents was guaranteed by this approach. Upon completion, the extract was concentrated by solvent evaporation and the dried residue was weighed to determine the extraction values. The % yield of extract was evaluated by employing following formula: Weight of Extract (g) × Weight of Leaf Powder (g) × 100 = Percentage Yield (%).The standard procedure outlined by Khandelwal was used to conduct the preliminary screening (Khandelwal, 2016).

Total phenol content: *Crotalaria gajureliana*'s total phenol content was assessed by employing modified version of the (Dewanto *et al*. 2002) technique. 0.25mL of Folin Ciocalteu reagent was mixed with a diluted extract in aliquots of 0.5,1,1.5,2, and 2.5ml at concentrations of 1mg/ml. Elucidation was shaken thoroughly after distilled water was added to reduce its final amount to 3ml. A produced blank was compared to the solution's 765 nm reading following incubation and dark storage. Plant part's TPC had been expressed in milligrammes of gallic acid equivalents per gramme of dry weight. Complete sample was analysed in 3 replicates.

Total Flavonoid Content: Using an aluminium chloride colorimetric technique, the flavonoid content of the *Crotalaria gajureliana* extract was estimated as a percentage (Mervat *et al.*, 2009). After adding 3 ml of methanol to 0.5 ml of an extract with different concentrations (0.5, 1, 1.5, 2, and 2.5ml of 1mg/ml), the mixture was shaken vigorously. Next, 2.8ml of distilled water was added, along with 0.1ml of potassium acetate, and then 0.1ml of 10% AlCl3 was added to test solution while it was being shaken. After the solution remained for half an hour, absorbance was examined at 415nm. Flavonoid concentration in the test samples was determined and reported as equivalent to quercetin (QE) per gram of sample. All samples were analyzed in three separate trials.

Free radical scavenging activity: The inhibition percentage of test substance was assessed for DPPH free radical scavenging activity. Test tubes were set up containing 1 ml of each concentration: 20,40,60,80, and 100µg/ml. After combining 1.5ml of each concentration with 1.5ml of 0.1% methanolic DPPH, mixture was kept in dark for 30min. Following this period, the samples were examined for color changes from purple to yellow, and absorbance was recorded at 510nm by employing colorimeter. Additionally, each test was performed in triplicates (Baliyan *et al.*, 2022). The radical scavenging activity was evaluated by employing given formula: DPPH radical scavenging activity (%) x 100 = (Absorbance

of control-Absorbance of test sample)/ (Absorbance of control)**.** Each test sample's IC50 value was determined.

RESULTS AND DISCUSSION

Yield of Extraction

Different yields were obtained by successively extracting the dried leaves using solvents with increasing polarity. The lowest yield, 0.1%, was obtained from the ethyl acetate extract, whereas 1.6% was obtained from the petroleum ether extract. 1.5% and 4.2% were obtained from the methanol and ethanol extracts, respectively (Table 1). The aqueous extract, which made up 20.8% of the total extracts, produced the highest yield. The significant yield in the water extract indicates a larger concentration of polar components in the plant material, according to these results.

Preliminary phytochemical screening

Preliminary phytochemical screening of various solvent extracts of *Crotalaria gajureliana* leaves showed the presence of various plant constituents (Table 2). Petroleum ether extract showed the presence of fixed oils and oils. Ethyl acetate extract was shown to contain fixed oils, fats, steroids and acidic compounds. Methanol extract was shown to contain flavonoids (flavanes), tannins, phenolic compounds and alkaloids. It was found that the ethanol extract contained alkaloids, tannins, phenolic chemicals, and flavonoids (chalcones, aurones, and flavanes). It was discovered that the aqueous extracts contained alkaloids, tannins, phenolic compounds, flavonoids (flavanes), and cardiac glycosides (cardenolides and deoxysugars). These findings show the various phytochemical components in various solvent extracts, suggesting potential pharmacological action. Many of the bioactive properties of plant extracts are due to phytochemicals, which are secondary plant metabolites. (MacDonald *et al.,* 2022).

Total phenolic content

The linear calibration curve of gallic acid, whose equation is y = 0.3448x + 0.5064 R^2 = 0.9911, was used to determine the phenolic content (Figure 2). The obtained results showed that the solvent employed for extraction affects the amount of phenolic content in the dried leaves of *Crotalaria gajureliana*. At 26.46 mg/g, the methanolic extract had the highest phenolic content, while the aqueous extract came in second with 20.65 mg/g. The phenolic concentration of the ethanolic extract was 11.14 mg/g, which was a rather low amount. According to these results, the best solvent to extract the phenolic compounds from *Crotalaria gajureliana*'s dried leaves is methanol. As phenolic compounds have been known for being antioxidants, they can scavenge the harmful free radicals that are produced within cells by oxidising the substance's phenolic group. Since harming free radicals and non-communicable diseases (NCDs) are strongly correlated, this feature is attributed to the potential to fight against NCDs (Bulugahapitiya *et al.,* 2020).

Total flavonoid content

The linear calibration curve of quercetin, whose equation is y = 0.3448x + 0.5064 R^2 = 0.9911, was used to determine the flavonoid content (Figure 3). Total phenolic and flavonoid content has been presented in Table 3. According to the results, the content of flavonoids in the dried leaves of *Crotalaria gajureliana*, measured in quercetin equivalents (QE/g of extract), varies depending on the solvent used. With 36.51 QE/g, the methanolic extract had the highest flavonoid content, followed by the aqueous extract (12.44 QE/g), while the ethanolic extract (7.24 QE/g) had the lowest. According to these results, the best solvent to extract the flavonoid compounds from plant material is methanol. Various aromatic and medicinal plants are abundant in phytochemicals, which are known for their antioxidant properties which include phenolic compounds, flavonoids, sterols, tannins, and essential oils (Almi *et al.,* 2022).

Free radical scavenging activity

The antioxidant profile of compounds Methanol, Ethanol along with aqueous extracts was assessed by evaluating percent of inhibition against DPPH reagent via test tube method. The compound Methanol, Ethanol and Aqueous extracts exhibited good antioxidant activity against DPPH scavenging reagent and however concentration rises, antioxidant activity of compound also rises as compared to the standard ascorbic acid. Using methanol, ethanol, and aqueous solvents, the DPPH free radical scavenging activity of leaf extracts from *Crotalaria gajureliana* was assessed at different doses (20–100 µg/ml). The findings show that the antioxidant activity of all extracts increases in a concentration-dependent manner. Following the methanolic extract at 55.95% and the ethanolic extract at 51.81%, the aqueous extract demonstrated the highest scavenging capability among them, reaching 62.17% at 100 µg/ml. This finding is supported by the IC50 values, which reveal that the aqueous extract has the highest antioxidant activity with the lowest IC50 value of 67.92 µg/ml. In addition, the IC50 values of the methanolic and ethanolic extracts were 80.84 µg/ml and 98.06 µg/ml, respectively. According to these results, *Crotalaria gajureliana* leaf aqueous extract has the strongest free radical scavenging ability among all extracts. The results shown in (Figure 4) indicate the Percentage inhibition of DPPH for different extracts of *Crotalaria gajureliana* Gholave, Madhav & Gosavi leaves. Table No. 4 shows the results for Free radical scavenging activity and Table No. 5 shows the results for IC 50 value of different extracts. Phenolics and flavonoids, directly contribute to the antioxidant capacity of plants, according to (Abou Zeid *et al*., 2014; Ali *et al.*, 2023)

CONCLUSION

The extractability of bioactive compounds varies as per the polarity of the solvent used for extraction. Significant bioactive components, including flavonoids, tannins, alkaloids, glycosides, and phenolics, were detected by phytochemical screening. Due to highest antioxidant activity in DPPH analysis, highest TPC and TFC of the solvents were in methanol extract. Ethanol and aqueous extracts also exhibited principal bioactive features. These results guarantee that the leaves of *Crotalaria gajureliana* can be an effective natural source of antioxidants, validating the potential of pharmacological investigations. The function of these bioactive substances in scavenging free radicals is demonstrated by the link seen between antioxidant activity and phenolic and flavonoid levels.

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CONFLICT OF INTEREST STATEMENT

The authors affirm that none of their known financial conflicts or personal connections might have influenced the research presented in this paper.

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Table 1: Extractive Values by successive extraction of leaves of *Crotalaria gajureliana*

Sr. No.	Solvent	Percentage yield
1	Petroleum ether	1.6%
2	Ethyl acetate	0.1%
3	Methanol	1.5%
4	Ethanol	4.2%
5	Water	20.8%

Table 2: Preliminary phytochemical screening of different extracts of *Crotalaria gajureliana*

Sr. No.	Test	Extracts				
		Petroleum Ether	Ethyl acetate	Methanol	Ethanol	Water
1	Carbohydrates	-	-	-	-	-
2	Proteins	-	-	-	-	-
4	Fats & Oils	+	+	+	+	-
5	Terpenoids	-	-	-	-	-
6	Steroids	-	-	-	-	-
7	Triterpenoids	-	+	-	-	-
8	Glycosides	-	-	-	-	+
9	Cardiac glycosides	-	-	-	-	+
10	Saponins	-	-	-	-	-
11	Flavonoids	-	-	+	+	+
12	Tannins & Phenolic Compounds	-	-	+	+	+
13	Alkaloids			+	+	+

Table 3: Phenol and Flavonoid content of Methanolic, Ethanolic and Aqueous extracts of *Crotalaria gajureliana*

Sr. no.	Sample	Total phenolic content of *Crotalaria gajureliana* leaves (mg GAE/g of extract)	Total flavonoid content of *Crotalaria gajureliana* leaves (QE/g of extract)
1	Methanolic extract	26.46	36.51
2	Ethanolic Extract	11.14	12.44
3	Aqueous extract	20.65	07.24

Table 4: *Crotalaria gajureliana* leaves DPPH scavenging activity in various solvent extracts.

Concentration (µg/ml)	Methanol	Ethanol	Aqueous
20	20.72%	10.88%	16.58%
40	31.60%	20.72%	23.31%
60	37.30%	30.56%	45.07%
80	49.74%	33.16%	57.51%
100	55.95%	51.81%	62.17%

Table 5: IC 50 value of different extracts.

Extract	IC 50 value (µg/ml)
Methanol	80.84
Ethanol	98.06
Aqueous	67.92

Figure 1: Dried leaves of *Crotalaria gajureliana* Gholave, Madhav & Gosavi

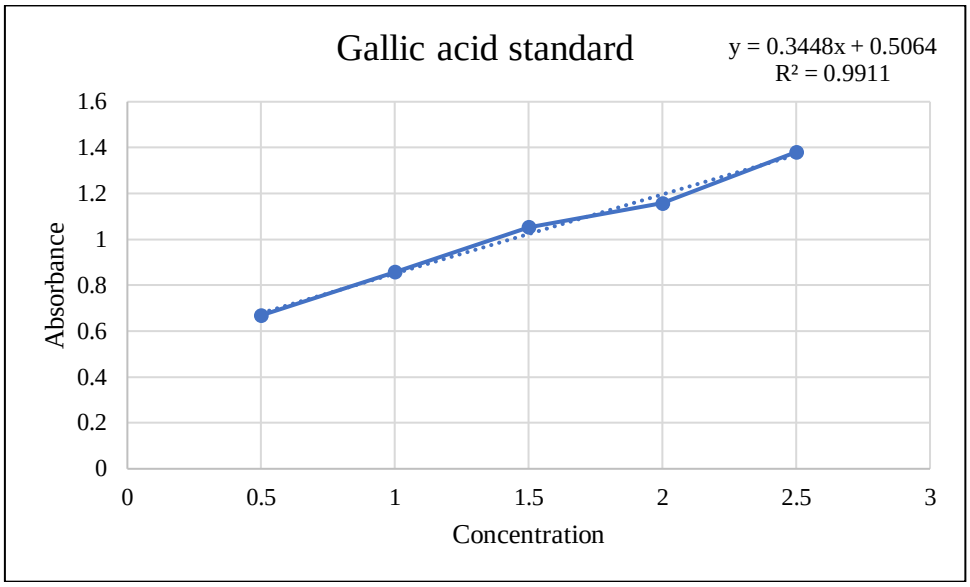

Figure 2: Evaluation curve of standard Gallic acid against absorbance measured at 765nm

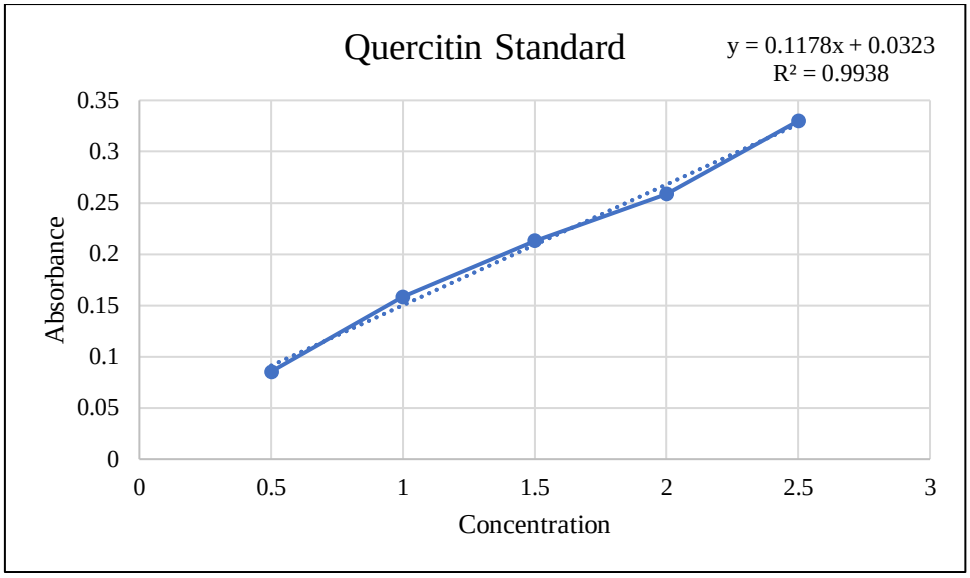

Figure 3: Evaluation curve of standard Quercetin against absorbance measured at 510nm

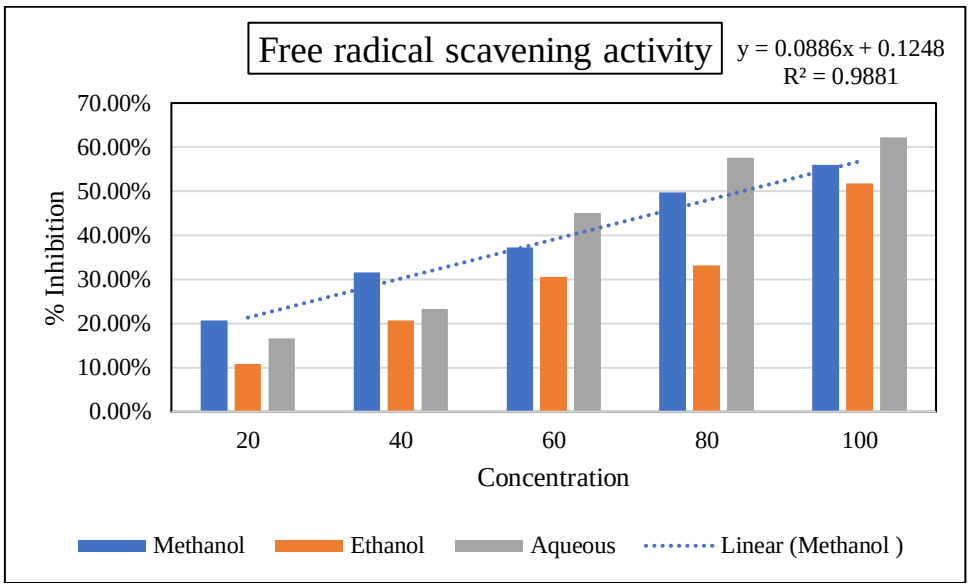

Figure 4: Percentage inhibition of DPPH for different extracts of *Crotalaria gajureliana* Gholave, Madhav & Gosavi leaves