Research Article

Phytochemical Evaluation and Antioxidant Activity of *Glycosmis mauritiana* (lam.) Tanaka Leaf Extract

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ABSTRACT

Medicinal plants can provide biologically active molecules and lead structures for the development of modified derivatives with enhanced activity and or reduced toxicity. Ensuring the safety, efficacy and quality of traditional medicine is a priority in as much as it must be respected, preserved and promoted as a branch of medicine. In plants, the medicinal value of the secondary metabolites is due to the presence of chemical substances that produce a definite physiological action on the human body. Research into the isolated plant constituents is of great importance for the development of bioactive substances from ethnic medicine. With the emergence of latest technologies and enhanced knowledge about the isolated plant constituents, characterization and analytical tools, lot of compounds are efficiently isolated from potential plants and have been of great contribution to the drug discovery from ethnic plants. The present investigation is aimed at investigating the phytochemical evaluation, quantification and antioxidant studies of a medicinal plant *Glycosmis mauritiana.* The phytochemical quantification was done for phenolics, tannins and flavanoids. The antioxidant screening was done by FRAP assay and DPPH free radical scavenging assay, using five different solvent extracts. Significant antioxidant activity of ethanolic extracts was recorded in *Glycosmis mauritiana* which can be used as a potent source of natural antioxidant.

Keywords: Antioxidants, DPPH, FRAP assay, *glycosmis mauritiana*, secondary metabolites.

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INTRODUCTION

About 80% of the world population relies on Traditional Medicines for their primary health care needs [1-3]. Attempts to document the plant wealth of India have continued since the landmark publication by [4]. Around 1700 species have been documented for their biological properties and drug action [5] and data is available for approximately 1200 species. Recently, interest has increased considerably in finding naturally occurring antioxidants for use in foods because of their potential in health promotion and disease prevention, and their high safety and consumer acceptability [6]. In Tamil Nadu, crushed leaves and barks of *Glycosmis mauritiana* (Lam.) Tanaka were externally applied on forehead to cure severe headache by the Irula tribes of the Nilgiris [7]; leaf paste was externally used for eczema and skin diseases by the Thottianaickan tribes of Semmalai hills [8] and Valaiyans tribes of Piranmalai hills [9]. Keeping the significances of Traditional Medicine in plant-based drug development *Glycosmis mauritiana* of family Rutaceae was selected for the present study.

MATERIALS AND METHODS

1. Collection of plant materials

The plant materials (leaves) of *Glycosmis mauritiana* (**Fig. 1**) were collected from the Madras Christian College Campus, Tambaram, Tamil Nadu and its botanical identity was confirmed at French Institute Herbarium, Pondicherry. The herbarium specimens were deposited in the Department of Botany, Kanchi Mamunivar Centre for Post Graduate Studies, Lawspet,

Pondicherry for further reference (Voucher no. DK & TE195).

2. Solvent extraction

The air dried powdered plant material (leaf) was extracted successively in soxhlet extractor with petroleum ether, chloroform, ethyl acetate ethanol and water. All the solvent extracts were evaporated to remove the final traces of the respective solvents. The extract recovery in different solvents was expressed as per cent of the plant sample dry matter.

Figure 1: Morphology of *Glycosmis mauritiana*

3. Preliminary phytochemical screening

Phytochemical screening of different solvent extracts was carried out following the methods of [10,11] for the presence of various classes of compounds such as alkaloids, flavanoids, tanins, phenolic compounds, sterols, saponins, resins, glycosides, carbohydrates, amino acids and proteins.

4. Quantitative estimation of phytochemicals

(i) Determination of total phenols, tannins and flavonoid

The total phenolic and tannin content were determined by following the method of [12]. Flavonoid content was determined by [13] method.

5. Pharmacological Studies

(i) *In vitro* antioxidant activity (Ferric Reducing Antioxidant Power (FRAP) assay and free radical scavenging activity on DPPH)

The FRAP activity was measured by using the [14] method and free radical scavenging activity was measured according to the method described by [15].

RESULTS

1. Preliminary phytochemical screening

Preliminary phytochemical screening of leaves revealed the presence of alkaloids, carbohydrates, flavonoids, phenols and tannins in all the tested extracts. Glycosides were present in all the extracts except water. Saponins were present in petroleum ether extract, steroids in ethylacetate and methanol extracts and proteins in ethylacetate and water extracts (**Table 1**).

Table 1: Preliminary phytochemical screening of various leaves extracts of *G. mauritiana*.

	Solvents				
Phytochemicals	**Petroleum ether**	**Chloroform**	**Ethyl acetate**	**Methanol**	**Water**
Alkaloids	+	+	+	+	+
Flavonoids	+	+	+	+	+
Saponins	+	+	-	-	-
Phenols	+	+	+	+	+
Steroids	-	-	+	+	-
Tannins	+	+	+	+	+
Carbohydrates	+	+	+	+	+
Protein	+	+	-	+	-
Glycosides	+	+	+	+	-

Note: + Present, - Absent

2. Quantitative estimation of phytochemicals

(i) Total flavonoids

Higher amount of total flavonoid content was recorded in leaves, as 13.41 ± 0.34 mg RE/g in petroleum ether followed by 12.99 ± 0.39 mg RE/g in ethylacetate, 12.13 ± 0.20 mg RE/g in methanol, 10.11 ± 0.44 mg RE/g in water and 8.44 ± 0.76 in chloroform (**Fig. 2**).

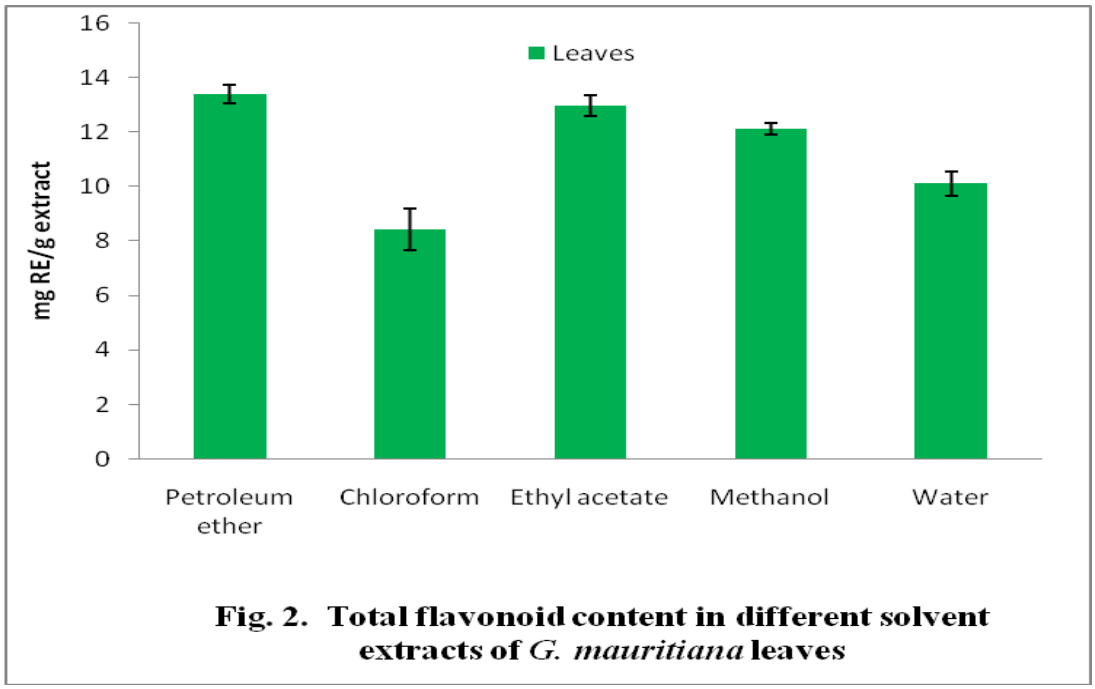

Fig. 2. Total flavonoid content in different solvent extracts of *G. mauritiana* leaves

(ii) Total phenols

In leaves, a high content of total phenolics was observed, as 226.03 ± 0.50 mg TAE/g in water followed by 122.04 ± 0.93 mg TAE/g in methanol, 87. 96 ± 0.54 mg TAE/g in ethylacetate, 47.64 ± 0.53 mg TAE/g in chloroform and 27.50 ± 0.83 mg TAE/g in petroleum ether (**Fig.3**).

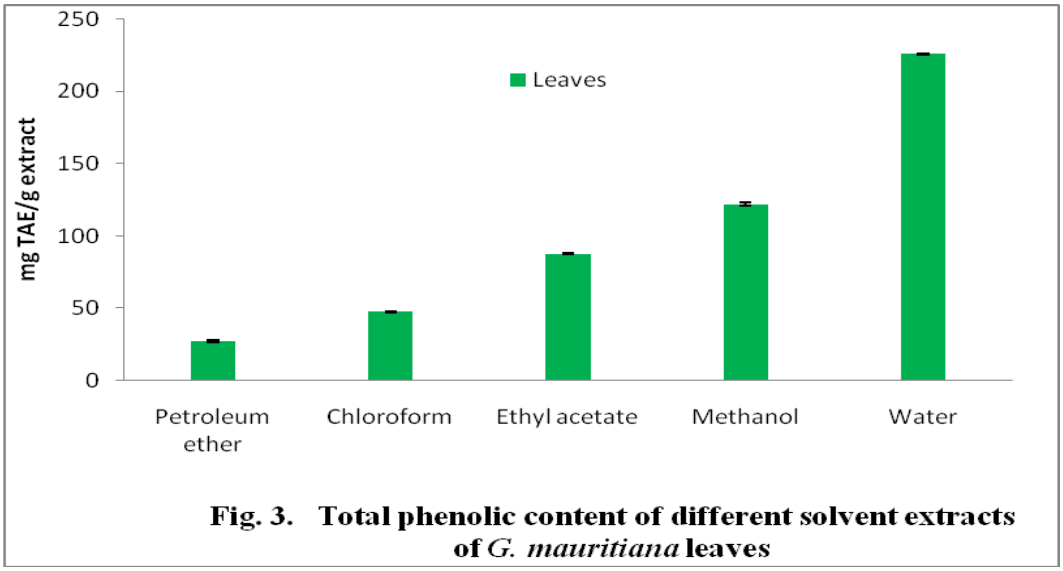

Fig. 3. Total phenolic content of different solvent extracts of *G. mauritiana* leaves

(iii) Total tannins

High tannin content was also observed in leaves, as 38.76 ± 0.44 mg TAE/g in water followed by 34.10 ± 0.83 mg TAE/g in methanol, 27.01 ± 0.41 mg TAE/g in ethylacetate, 17.88 ± 0.41 mg TAE/g in chloroform and 14.00 ± 0.63 mg TAE/g in petroleum ether (**Fig. 4**).

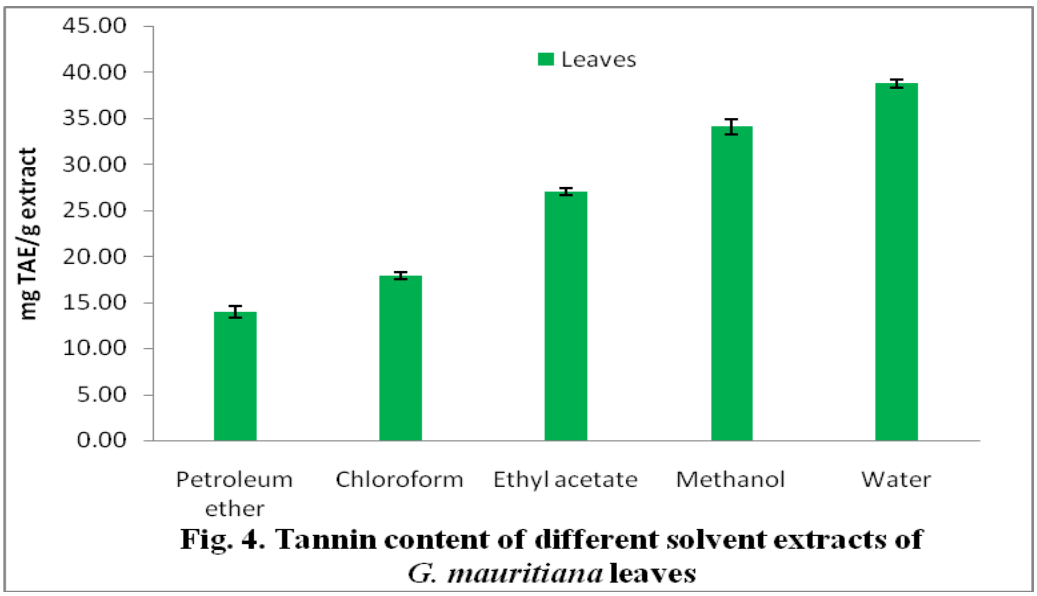

Fig. 4. Tannin content of different solvent extracts of *G. mauritiana* leaves

3. *In vitro* antioxidant activity

(i) FRAP Assay

In the FRAP assay, all the extracts showed concentration-dependent activity. In leaves, maximum value was observed in chloroform extract as 60.69 ± 0.89 followed by methanol extract (41.97 ± 0.27) and water extract (33.17 ± 0.38) while moderate value was observed in ethyl acetate extract (28.90 ± 0.47) and minimum value was observed in petroleum ether extract (12.19 ± 0.07) as shown in the (**Fig.5**).

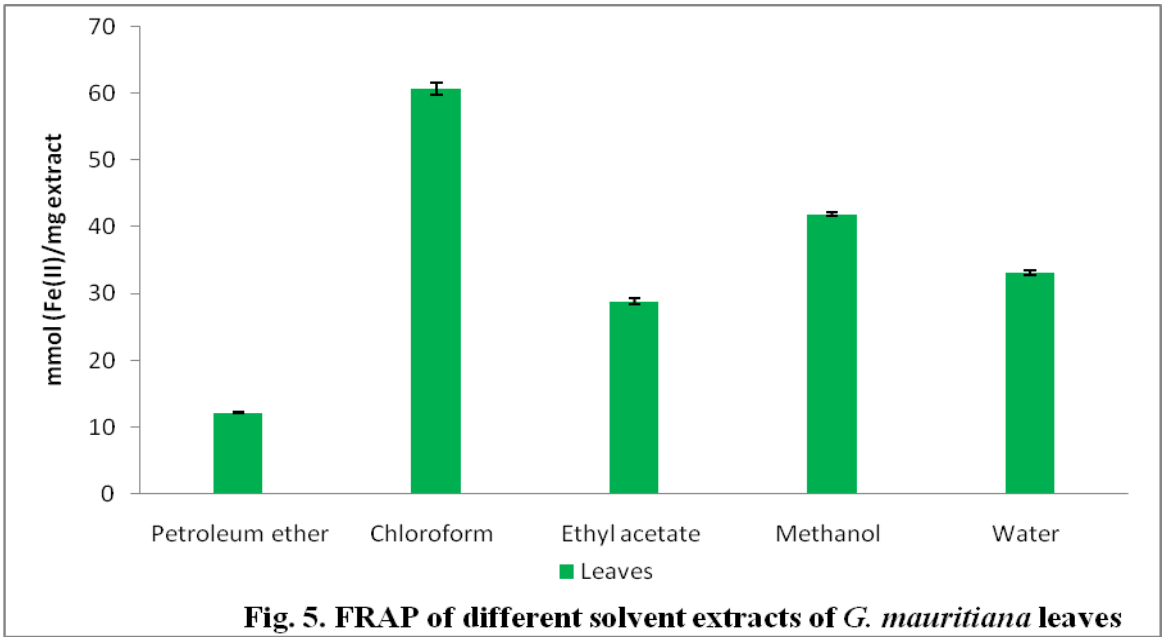

Fig. 5. FRAP of different solvent extracts of *G. mauritiana* leaves

(ii) DPPH scavenging activity

In DPPH scavenging activity, all the extracts showed concentration-dependent activity (**Fig.6**). In petroleum ether extract, the percentage of scavenging activity ranged from 10.63 ± 0.19 to 50.47 ± 0.24. Minimum and maximum activity was observed as 10.63 ± 0.19 at 40 µg and 50.47 ± 0.24 at 200 µg respectively, while moderate percentage activity was observed as 21.69 ± 0.39 at 80 µg, 31.47 ± 0.20 at 120 µg, 39.80 ± 0.29 at 160 µg concentrations.

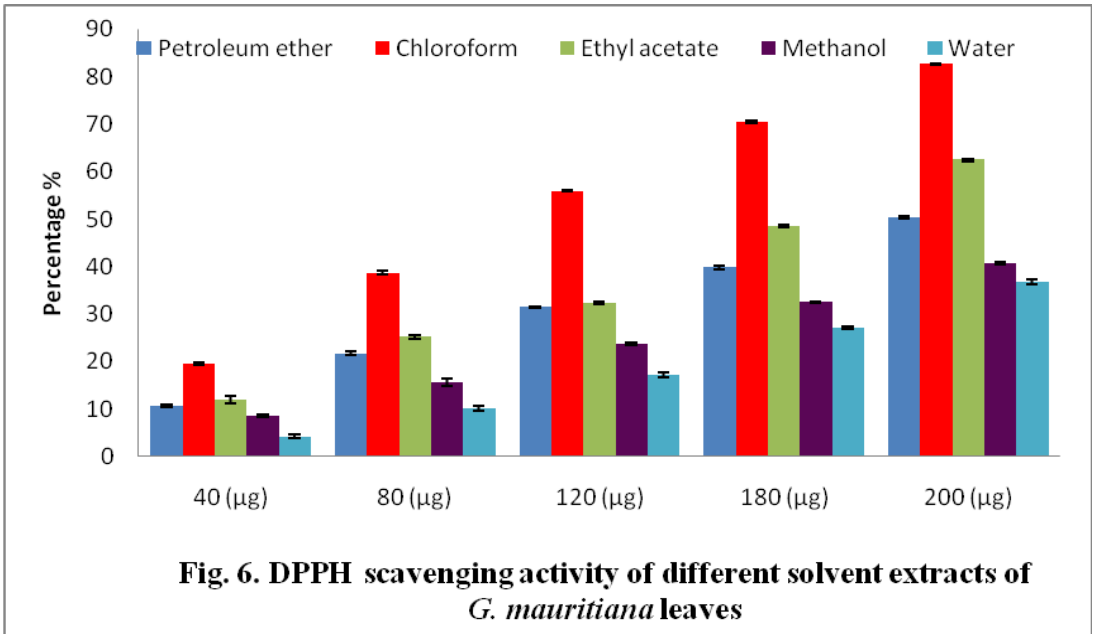

Fig. 6. DPPH scavenging activity of different solvent extracts of *G. mauritiana* leaves

In chloroform extract, the percentage of scavenging activity ranged from 19.54 ± 0.26 to 82.67 ± 0.15. Minimum and maximum activity was observed as 19.54 ± 0.26 at 40 µg and 82.67 ± 0.15 at 200 µg respectively, while moderate percentage activity was observed as 38.68 ± 0.36 at 80 µg, 55.93 ± 0.20 at 120 µg, 70.45 ± 0.33 at 160 µg concentrations. The percentage of scavenging activity in ethylacetate extract was ranged from 11.91 ± 0.84 to 62.48 ± 0.27. Minimum and maximum activity was observed as 11.91 ± 0.84 at 40 µg and 62.48 ± 0.27 at 200 µg respectively, while moderate percentage activity was observed as 25.21 ± 0.31 at 80 µg, 32.29 ± 0.31 at 120 µg, 48.42 ± 0.26 at 160 µg concentrations.

The percentage of scavenging activity in methanol extract was ranged from 8.59 ± 0.29 to 36.70 ± 0.52. Minimum and maximum activity was observed as 8.59 ± 0.29 at 40 µg and 6.70 ± 0.52 at 200 µg respectively, while moderate percentage activity was observed as 15.73 ± 0.78 at 80 µg, 23.83 ± 0.26 at 120 µg, 40.64 ± 0.27 at 160 µg concentrations. In water extract, the percentage of scavenging activity ranged from 4.26 ± 0.29 to 36.70 ± 0.52. Minimum and maximum activity was observed as 4.26 ± 0.29 at 40 µg and 36.70 ± 0.52 at 200 µg respectively, while moderate percentage activity was observed as 10.21 ± 0.49 at 80 µg, 17.21 ± 0.49 at 120 µg, 27.14 ± 0.34 at 160 µg concentrations.

DISCUSSION

Medicinal plants have invariably been a rich source of modern drug discoveries. Many drugs in use today were either obtained from plants or developed using their chemical structure as templates [16, 17]. They play an important role in health care dynamics throughout the world for health security. Plants have traditionally been used as a source of medicine in India by indigenous people of different ethnic groups inhabiting various terrains for the control of various ailments afflicting human and their domestic animals [18].The medicinal values of the plants are due to the bioactive constituents that produce definite physiological action on the human body [19]. The medicinal properties of plants could be based on the antioxidant, antimicrobial and antipyretic effects of the phytochemicals in them [20, 21]. In this context, an important medicinal plant *Glycosmis mauritiana* was chosen to investigate phytochemicals and antioxidant activity.

In plants, the medicinal value of the secondary metabolites is due to the presence of chemical substances that produce a definite physiological action on the human body [22-27]. Preliminary phytochemical screening of leaves of *G. mauritiana* revealed the presence of various classes of phytoconstituents such as alkaloids, carbohydrates, flavonoids,

glycosides, phenols, proteins, saponins, steroids and tannins in leaves (**Table 1**). Quantification of phytochemicals revealed the copious presence of alkaloids, flavonoids and total phenolic contents. However, the total content of phenols, flavonoids and tannins were found to be very much higher than the non-polar constituents like steroids. These reports indicate that total phenolics, flavonoid and tannin content are responsible for antioxidant activity of the plant as reported earlier by [28] and anti-hypertension activity by [29-30] Reported alkaloids, flavonoids and terpenoids from leaves and fruits and [31] reported alkaloids, carbohydrates, flavonoids, terpenoids and tannins from roots of allied species, *Glycosmis pentaphylla*.

Antioxidants are known to eliminate oxidative stress by scavenging free radicals and protect biological macromolecules from their toxic effect [32]. Therefore, in recent years evaluating plant antioxidant activity and their free radical quenching ability is considered to be an important task in pharmacological studies.

The FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex producing a colored ferrous tripyridyltriazine (Fe^{2+}-TPTZ) [33]. Generally, the reducing properties are associated with the presence of compounds which exert their action by breaking the free radical chain by donating a hydrogen atom [34]. Frap assay treats the antioxidants in the sample as a reductant in a redox-linked colorimetric reaction [35]. In the present study, the trend for ferric ion reducing activities of *G. mauritiana* leaf extract showed the increased absorbance, due to the formation of the Fe^{2+}-TPTZ complex with increasing concentration. In a related report, the water and ethanol extracts of sumac (*Rhus coriaria* L.) showed increased ferric reducing power with the increased concentration as standard antioxidants [36]. Hence they should be able to donate electrons to free radicals stable in the actual biological and food system.

The reactivity of ethanol extract of *G. mauritiana* of leaves was analyzed with the stable free radical, DPPH that picks up one electron in the presence of a free radical scavenger, the absorption decreases and the resulting discoloration is stoichiometrically related to the number of electrons gained [37]. In the present study, the ethanol extract exhibited concentration dependent scavenging activity comparable with BHT. Similar observation was also reported in various *Phyllanthus* species such as *P. amarus*, *P. maderaspatensis*, *P. urinaria* and *P. virgatus* [38], *Costus pictus* [39], *Maranta arundanacea* [40].

The ethanolic extracts of *G. mauritana* were found to be an effective scavenger of DPPH and also possessed a good reducing power and FRAP activity. Earlier reports on the antioxidant activity of *G. mauritana* are very scanty. Therefore, it is very interesting to work on antioxidant property in the present study. The high antioxidant activity of *G. mauritana* leaf enhanced the potential interest for improving the efficacy of different products as neutraceutical and pharmacological agents. The consumption of this plant may play a role in preventing human diseases in which free radicals are involved such as cancer, cardiovascular disease and aging. However, further investigations on phenols, flavonoids and their *in vivo* antioxidant activity, and the different antioxidant mechanism are warranted for drug development.

CONCLUSION

Medicinal plants have invariably been a rich source of modern drug discoveries. Medicinal plants can provide biologically active molecules and lead structures for the development of modified derivatives with enhanced activity and or reduced toxicity. In this context *G. mauritiana* was chosen to investigate phytochemicals, their antioxidant property in order to prove its traditional usages by different tribals of India for various ailments such as inflammatory, hepatic damage, analgesics and antioxidant activity *in vitro* and *in vivo* studies. The results of this study indicate that the leaves of *G. mauritiana* contain phytochemicals of potent pharmacological values. *G. mauritiana* is very rich in variety of secondary metabolites, which might be responsible for various pharmacological properties.

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