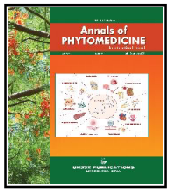
Annals of Phytomedicine 13(2): 703-713, 2024

DOI: http://dx.doi.org/10.54085/ap.2024.13.2.72

Annals of Phytomedicine: An International Journal
http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Original Article : Open Access

Evaluation of phytochemical, antioxidant, antimicrobial, and anti-inflammatory properties of *Gnidia glauca* (Fres.) Gilg: A potential therapeutic plant from the Western Ghats

Neharani*, K.P. Ramesha*, B. R. Nuthan*, K. K. Sampath Kumar and S. Satish***

**Department of Studies in Microbiology, University of Mysore, Manasagangotri, Mysuru-570006, Karnataka, India*
*** Department of Pre-University College, Davangere-577002, Karnataka, India*

Article Info

Article history
Received 1 November 2024
Revised 18 December 2024
Accepted 19 December 2024
Published Online 30 December 2024

Keywords
Antimicrobial
Antioxidants
Gnidia glauca (Fres.) Gilg
DPPH
FRAP

Abstract

Plants are sessile organisms and to protect themselves against exogenous constraints, they synthesize an array of secondary metabolites which have important physiological and ecological effects. The present study investigates the extraction, phytochemical composition, antioxidant, antimicrobial, and anti-inflammatory activities of *Gnidia glauca* (Fres.) Gilg leaf and stem extracts using different solvents of varying polarity. The highest extraction yield was observed in methanol leaf extracts (9.6%), followed by methanol stem extracts (9.2%), while the lowest yield was in stem n-hexane extracts (3.6%). Phytochemical analysis revealed the presence of several bioactive compounds, including phenols, flavonoids, carbohydrates, tannins, terpenoids, steroids, glycosides, and saponins, with methanol extracts demonstrating the richest variety and concentration of phytochemicals, especially in leaf material. Antioxidant activities were evaluated using the DPPH and FRAP assays, showing strong radical scavenging potential in methanol and ethyl acetate leaf extracts, with IC_{50} values of 47.73 µg/ml and 49.00 µg/ml, respectively. Antimicrobial activity was assessed against bacterial and fungal pathogens, with methanol and ethyl acetate leaf extracts exhibiting significant antibacterial activity, particularly against P. and Salmonella typhi. The methanol extract also demonstrated antifungal activity against *C. albicans* and *E. floccosum*. Furthermore, the methanol leaf extract showed considerable anti-inflammatory effects by inhibiting LPS-induced TNF-α release by 52.13%. This study offers an opportunity to conserve and explore this potent medicinal plant for further pharmaceutical usage.

1. Introduction

Herbal medicine is a wonderful remedy for most human disorders and the most common reason for using traditional medicine in most countries is that more affordable (Ansari and Ahmad, 2019). The majority of plant-derived herbal products are used as nutraceuticals and 80% of modern-day drugs are obtained from natural resources and used for various medicinal purposes all over the world (Bharathi *et al.,* 2021). *G. glaucoma* is a shrub or small-sized tree belonging to the family Thymelaeaceae commonly called the Daphne family. The synonyms of *G. glauca* include *G. glauca* and *G. avolkensii*. It is widely distributed in India, Sri Lanka and Africa; in India it is found in Karnataka, Kerala, and Tamil Nadu state. Belagavi, Chikkamagaluru, Dakshina Kannada, Hassan, Shivamogga, and Uttara Kannada are the major districts of Karnataka where the diversity of *G. glauca* is found. Here glauca means blue-green or glaucous, concerning the colour of the leaves (Ghosh *et al.,* 2013). The tree grown up to 6 m tall, the leaves are alternate, mostly towards the ends of branches, narrowly elliptic to obovate, 32-80 mm long, glaucous, hairless, midrib prominent beneath; petiole is 1-3 mm long (Arika *et al.*, 2019). Inflorescences in terminal, densely 20-50 flowered globose heads, 20-50 mm in diameter. Flowers are pentamerous, orange to golden yellow, fading to brown, and found on the edges of montane forests, in wooded grassland and along streams (Figure 1):

All parts of the *G. glauca* plant exhibit medicinal properties and it is used as traditional phytomedicine for treating sore throat, abdominal pain, wounds, burns, and snake bites. Leaves have been applied to treat contusions, swellings, backaches, and joint aches (Ghosh *et al.*, 2012). It is considered as a powerful vesicant. It also has agrochemical applications as a molluscicide, insecticide, piscicide, and even larvicidal agents. It has been shown that several *Gnidia* species possess remarkable antineoplastic activity. It is used as an antiviral agent against rabies in Ethiopia. Its antidiabetic properties are not known and decoction of the boiled root is drunk as a treatment of indigestion. The root powder is used in the treatment of rabies and bark and leaves are used for the treatment of blisters, swelling, and contusions (Sharma and Alam, 2022; Valleti *et al.,* 2024).

This research serves as a foundation for advancing our understanding of the active compounds present in *G. glauca*. The findings highlight the phytochemical, anti-microbial activity, antioxidant, and anti-inflammatory activities the plant extracts. Additionally, the study underscores the need for further investigation into the biochemical mechanisms underlying these biological activities and explores the potential pharmacological applications of *G. glauca* extracts.

Corresponding author: Dr. S. Satish
Professor, Department of Studies in Microbiology, University of Mysore, Manasagangotri, Mysuru-570006, Karnataka, India
E-mail: satish.micro@gmail.com
Tel.: +91-821-2419734

Figure 1: Morphology of *G. glauca*: (a) Habit, (b) Leaves and (c) Inflorescence.

2. Materials and Methods

2.1 Collection of plant material and preparation of extracts

Healthy stem and leaf materials of *G. glauca* were collected from Virajpete Taluk, Kodagu District, located in the Western Ghats of Karnataka, India (Latitude: 12° 25' 37" N; Longitude: 75° 44' 51" E). The plant material was authenticated by Dr. K. K. Sampath Kumar, a taxonomist, and assigned the Voucher Specimen Number UOMBOT24GG07. Approximately 50 g each of dried stem and leaf powder were subjected to successive extraction in a Soxhlet apparatus using n-hexane, ethyl acetate, and methanol, based on their polarity index. The resulting extracts were stored in airtight containers for further analysis.

2.2 Preliminary phytochemical analysis

The extracts of different solvents were subjected to investigation of secondary metabolites present in them. The stem and leaf extracts of *G. glauca* were subjected to a comparative qualitative phytochemical analysis following the protocol outlined by Harborne (1973).

2.3 *In vitro* antioxidant activities

2.3.1 DPPH radical scavenging assay

The leaf extracts of *G. glauca* were subjected to DPPH (2, 2-diphenyl-1-picrylhydrazyl radical) radical scavenging activity by using the standard protocol of described by Rajakumar (1994) and Anvitha *et al*. (2023) with ascorbic acid as standard. Briefly, 80 ml of DPPH solution was prepared, and various concentrations of the test solution were added, with the final volume adjusted to 240 ml using HPLC-grade methanol. The test samples were tested at concentrations of 3.125, 12.5, 50, and 100 µg/ml, with reference standards, quercetin, at 0.3125, 0.625, 1.25, 2.5, 5, and 10 µg/ml. The reaction mixture was mixed and incubated at 25°C for 15 min. Absorbance was measured at 510 nm using a spectrophotometer. A control reaction was conducted without the test sample. Per cent inhibition and IC_{50} values were calculated using GraphPad Prism software (version 5.0) and nonlinear regression analysis with the following formula:

Per cent inhibition =

$$\frac{\text{Optical density of control} - \text{Optical density of sample}}{\text{Optical density of control}} \times 100$$

2.3.2 Ferric reducing antioxidant power (FRAP) assay

Different concentrations of test samples and a reference standard of 0.7 ml were added to 2.3 ml of working frap reagent to give a final volume of 3 ml, and then this mixture was kept in incubation for 30 min, in the dark at 37°C. Absorbance was measured at 593 nm against

blank ascorbic acid was used as the reference standard excluding samples. A stronger absorbance will indicate increased reducing power. Results were expressed in mM of ascorbic acid equivalents per gram extract.

2.4 Determination of *in vitro* antimicrobial activity

The bacterial strains *Bacillus subtilis*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhi*, as well as fungal strains such as *Candida albicans* and *Epidermophyton floccosum*, were obtained from the Microbial Type Culture Collection (MTCC), Chandigarh, India. The antimicrobial evaluation was carried out using the protocol, followed Krishnarao and Rajeshwari (2023) with some modifications.

2.4.1 Antibacterial activity (disc diffusion method)

Sterilized 6 mm filter paper discs were prepared, and 50 µl of *G. glauca* leaf extract (100 µg/disc) was loaded onto the sterile discs. Gentamicin (10 µg/disc) was used as a positive control. A uniform bacterial lawn inoculum was prepared on Mueller-Hinton Agar (MHA) plates using sterile cotton swabs. The extract-loaded discs, along with the positive control discs, were placed equidistantly on the inoculated agar plates. The plates were then incubated at 37°C for 24 h. After incubation, the zones of inhibition were measured in millimeters (mm) using a ruler or calipers.

2.4.2 Antifungal activity (well diffusion method)

Wells (6 mm in diameter) were made in Sabouraud Dextrose Agar (SDA) plates, and fungal strains were evenly spread on the plates using sterile cotton swabs. 50 µl of *G. glauca* leaf extract (100 µg/disc) was loaded onto the wells, and nystatin (10 µg/disc) was used as a positive control. The plates were incubated at 26 ± 2°C for 72 h. After incubation, the clear zone of inhibition around the wells was measured in millimeters (mm) to assess the antifungal activity.

2.4.3 Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) determination process is a systematic method to identify the lowest concentration of an antimicrobial agent that inhibits visible growth of microorganisms (Pragyandip *et al*., 2023). The procedure begins with preparing plant extracts at a stock concentration of 50 mg/ml. Microbial suspensions are then standardized to a 0.5 McFarland standard (approximately 10v CFU/ml) to ensure consistency. In a sterile 96-well microplate, 100 µl (5 mg) of the extract is added to the first row, and two-fold serial dilutions are performed down the rows to achieve concentrations ranging from 5 mg/ml to 0.0024 mg/ml. A volume of 100 µl of microbial suspension is added to each well.

Positive controls (*e.g.*, gentamicin for bacteria and nystatin for fungi) are included in designated wells. The plate is incubated at 37°C for 24 h for bacterial strains or at 26 ± 2°C for 72 h for fungal strains. After incubation, 10 µl of 2,3,5-triphenyl tetrazolium chloride (TTC) solution (2 mg/ml) is added to each well and incubated for an additional 30 min. The color change is observed as an indicator of microbial growth, with red formazan indicating active growth and no color change indicating inhibition. The absorbance is measured at 624 nm using an ELISA plate reader (LabTech 4000, Japan: CLSI, 2017). The MIC is identified as the lowest concentration at which no color change is observed, indicating complete inhibition of microbial growth. The procedure is repeated in triplicate to ensure reproducibility and reliability of results.

2.5 Evaluation of anti-inflammatory effects against LPS induced TNF-α release

Raw-264.7, a promonocytic cell line was obtained from the ATCC. Cells were cultured in DMEM supplemented with 10% inactivated FBS and penicillin (100 IU/ml) in a humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were aspirated from the 80% confluence culture flask and centrifuged at 1500 rpm for 5 min. The cell pellet was then re-suspended in 1 ml of DMEM and 1 x 10^5 cells/well were seeded to 96 well microtiterplates and incubated for 24 h. After incubation to determine the cytokine (TNF-Á) release, raw cells were pretreated for 2 h with various concentrations of test samples followed by LPS (1 µg/ml) stimulation for an additional 24 h and dexamethasone served as a positive control. Post incubation, the cell supernatant is aspirated from each of the wells into sterile micro centrifuge tubes and centrifuged at 1000 rpm for 2-3 min to settle any cells, if present. The cell supernatant is used for evaluation of the presence of TNF-α using ELISA and experiments were carried out in duplicates.

2.5.1 Evaluation of TNF-α release

Standards of various two-fold dilution series from 450 pg/ml (top standard) to 3.5 pg/ml and samples of 100 µl/well were added to the designated wells in the ELISA plate. The assay diluent (1X) was the zero standard (0 pg/ml) and the plate was sealed and incubated at 37°C for 2 h. After incubation, the plate was washed four times with wash buffer (1X) and the residual buffer was blotted by firmly tapping the plate upside down on an absorbent paper. Diluted Avidin-HRP of 100 µl solution was added to each well; the plate was sealed and incubated at 37°C for 30 min, followed by washing plates with wash buffer (1X). For this final wash, wells were soaked in wash buffer for 30 sec. for each wash. TMB substrate of 100 µl solution was added to each well and incubated in the dark for 30 min at 37°C. The reaction was stopped by adding 100 µl of stop solution to each well. Absorbance was recorded at 450 nm after adding the stop solution and the percentage of inhibition was calculated by using the following formula:

$$\text{Per cent inhibition} = \frac{\text{OD of control} - \text{OD of sample}}{\text{OD of control}} \times 100$$

2.6 Statistical Analysis

Each experiment was performed in triplicates and the data is represented as mean ± SE using analysis of variance (ANOVA) (IBM software SPSS Inc. 16.0 Ver.) Significant variations for each treatment were determined by F values ($p \leq 0.05$) after Tukey's HSD test.

3. Results

3.1 Phytochemical analysis

The percentage yield was determined in stem and leaf extracts by using various solvents based on their polarity.The extraction yield was higher in leaf methanol thanin stem methanol extracts (9.6% and 9.2%) and the lowest percentage yield was noticed in stemn-hexane extract (3.6%). Overall, the yield was higher in leaf material thanin stem and these variations may be due to the lack of chlorophyll and other associated components in stem.

Each extract underwent a comparative phytochemical analysis, which revealed the presence of various phytochemical constituents, as summarized in Table 1. A qualitative phytochemical study was conducted on different extracts, methanol, ethyl acetate, and n-hexane,of *G. glauca* leaves and stem. Methanol extracts, particularly from leaves, demonstrated the highest concentration and variety of phytochemicals, including phenols, flavonoids, carbohydrates, tannins, terpenoids, steroids, glycosides, and saponins.

Phenolic compounds were predominantly present in stem methanol extracts. Flavonoids were detected in leaf and stem methanol extracts through concentrated HCl testing. Carbohydrates were found consistently across all solvents, with methanol extracts yielding the most robust responses. Tannins were identified in methanol extracts, with leaf methanol extract showing a stronger presence. terpenoidswere observed in all solvent extracts, with the highest concentration in ethyl acetate stem extracts. Steroids were detected only in methanol stem extracts, while glycosides and saponins were found predominantly in methanol extracts of both leaves and stems.

The results highlight methanol as the most effective solvent for extracting diverse secondary metabolites from *G. glauca*, making it suitable for further investigations. Notably, the methanol leaf extracts yielded the best results (Table 1).

Table 1: Preliminary phytochemical evaluation of *G. glauca* leaf and stem extracts

	Phytochemical tests	Meth.		Eta.		Hex.	
		Leaf	Stem	L	S	L	S
Phenol	$FeCl_3$	-	++	-	-	-	-
Flavonoids	1. Conc. HCl	+	+	-	-	-	-
	2. NaOH	-	-	-	-	-	-
	3. Magnesium reduction	-	-	-	-	-	-
Carbohydrates	1. Fehling's test	+	+	+	+	+	+
	2. Molish test	+	++	+	++	+	++
	3. Benedict's test	+	+	-	-	-	-
	4. Barfoed's test	-	-	-	-	-	-
Tannins	1. $FeCl_3$	++	-	-	-	-	-
	2. Potassium dichromate	+	++	-	-	-	-
Terpenoids	Salkowski test	+	+	+	++	++	+
Steroids	1. Lieberman-Burchard test	-	+	-	-	-	-
	2. H_2SO_4	-	+	-	-	-	-
Glycosides	Keller-Kiliani test	+	+	-	-	-	-
Saponins	Foam test	+	++	-	-	-	-

Note:"+"= Positive with less amount, "++"= Positive with more amount, "-"= Negative, Hex = Hexane, Eta: Ethyl acetate, Meth: Methanol, "S"= Stem, "L"= leaf.

3.2 *In vitro* antioxidant activities

3.2.1 DPPH radical scavenging assay

The DPPH assay was performed to check the total percentage of antioxidant properties in *G. glauca* leaf methanol and ethyl acetate extracts. All the tested extracts showed the significant radical scavenging antioxidant capacity ($p \leq 0.05$) in a concentration's dependent manner. Investigations on the methanol and ethyl acetate extracts of *G. glauca* at various concentrations are presented in Table 2. The results revealed a significant dose-dependent inhibition across concentrations of 3.16, 10, 31.62, and 100 µg/ml. The methanol extract showed % inhibition values of 10%, 30%, 60%, and 90%, while the ethyl acetate extract exhibited inhibition percentages of 20%, 40%, 60%, and 85%.

The leaf methanol and ethyl acetate extracts exhibited the highest percentage of radical scavenging activity of 84.8% and 84.6% at maximum concentrations of 100 µg/ml (IC_{50} of 47.73 µg/ml and 49.00 µg/ml) and it was depicted in Figures 3 a and b. The standard quercetin showed 89.17% (IC_{50}: 1.519 µg/ml) (Figure 4). Hence, the results of this assay clearly indicates that the plant possesses significant antioxidant properties.

Table 2: *In vitro* antioxidant activity of *G. glauca* leaf methanol and ethyl acetate extracts

Concentration (µg/ml)	Methanol extract	Ethyl acetate extract
	Per cent inhibition	Per cent inhibition
3.16	20.4	9.8
12.5	40.7	31.5
50	60.3	59.5
100	84.8	84.6
Quercetin@ 10	89.17%	

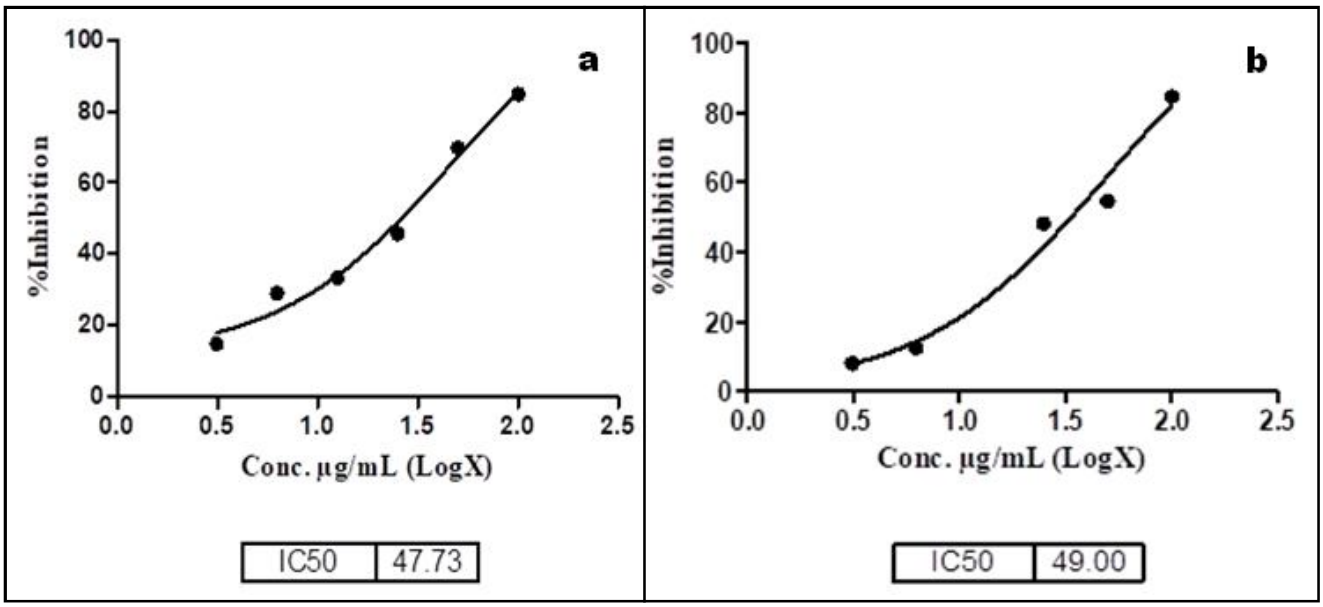


Figure 3: DPPH activity of *G. glauca* leaf extracts. (a) Methanol extracts, (b) Ethyl acetate extract.

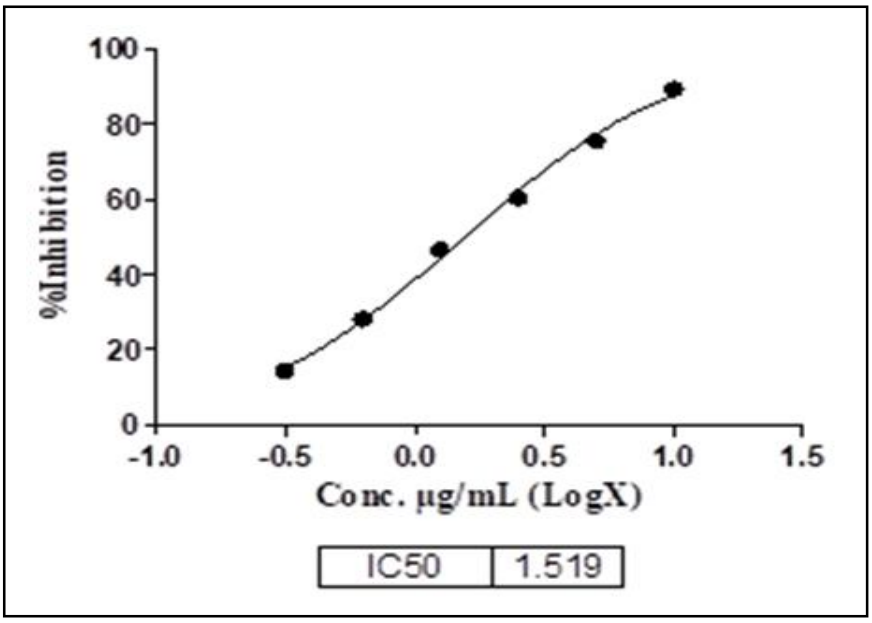


Figure 4: DPPH activity of standard quercetin.

3.2.2 Ferric reducing antioxidant power (FRAP) assay

This study investigates the antioxidant potential of *G. glauca* leaf extracts prepared using methanol (GGM) and ethyl acetate (GGE) as solvents, along with standard ascorbic acid, through the ferric reducing antioxidant power (FRAP) assay. The extracts demonstrated the ability to reduce $Fe^{3}z$ to $Fe^{2}z$, indicating strong antioxidant activity. At higher concentrations, the methanol extract (GGM) exhibited a FRAP value of 357.91 mM EAA/g, while the ethyl acetate extract (GGE) showed 259.54 mM EAA/g (Figure 5). Standard ascorbic acid displayed 1.144 % activity at 20 µM concentration (Figure 6). These findings highlight the potential of *G. glauca* leaf extracts, particularly the methanol extract, as a rich source of natural antioxidants, suggesting their applicability in pharmaceutical and nutraceutical formulations.

3.3 Antimicrobial activity and minimum inhibitory concentrations (MIC)

This study evaluates the antimicrobial activity of *G. glauca* leaf extracts prepared using methanol (GGM), ethyl acetate (GGE), and hexane (GGH) against selected bacterial and fungal pathogens. The antimicrobial potential was assessed through the zone of inhibition (mm) and minimum inhibitory concentrations (MIC, mg/ml), with standard antibiotics nystatin and gentamicin serving as controls.

The results revealed no inhibitory activity against Gram-positive bacteria (*Bacillus subtilis, Listeria monocytogenes,* and *Staphylococcus aureus*) for any extract. However, Gram-negative bacteria such as *Pseudomonas aeruginosa* exhibited zones of inhibition ranging from

15-17 mm and MIC values of 1.25-2.5 mg/ml. *Salmonella typhi* showed moderate inhibition with GGE (12 mm) and GGM (13 mm), with MIC values of 2.5 mg/ml as shown in Figures 7 a and b. Among fungal strains, methanol extract (GGM) demonstrated significant activity against *Candida albicans* (20 mm, MIC = 4.6 mg/ml) and *Epidermophyton floccosum* (18 mm, MIC = 3.4 mg/ml) (Figure 8). In contrast, GGE and GGH displayed no antifungal effects. Hence, the results indicated that methanol and ethyl acetate leaf extractshad significant antibacterial activity, but antifungal activity was observed only in methanol extract.

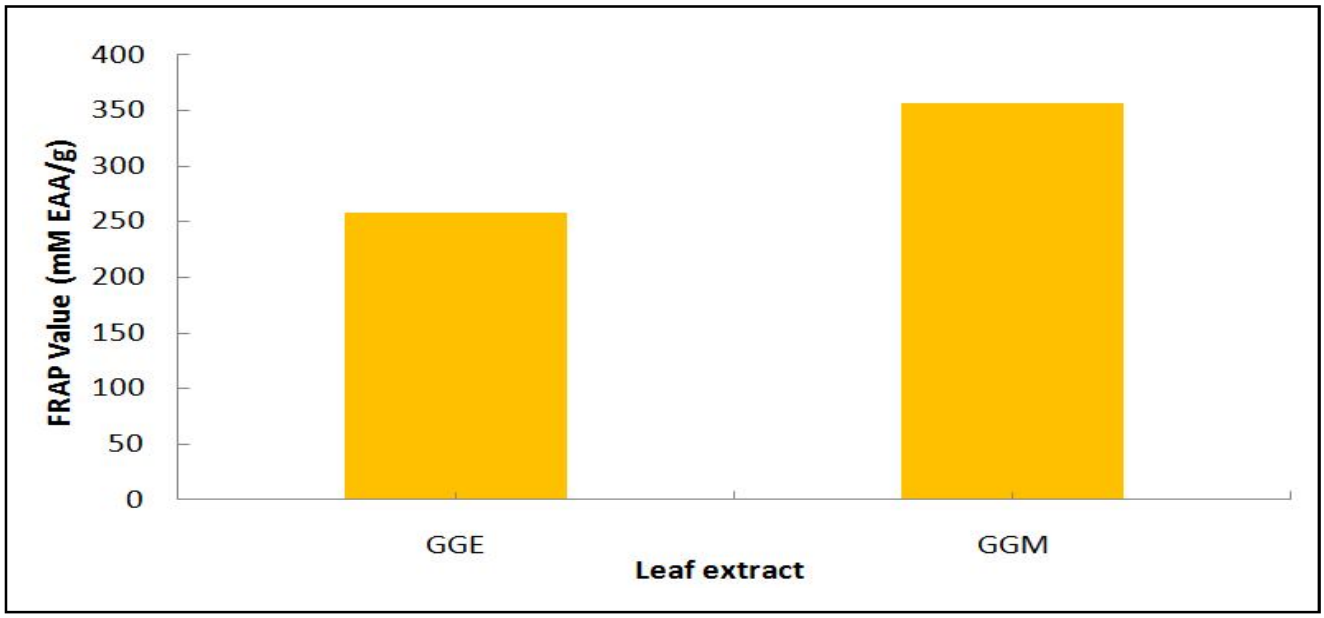


Figure 5: FRAP assay of methanol and ethyl acetate assay of *G. glauca* leaf (GGE- *G. glauca* Ethyl acetate, GGM- *G. glauca* Methanol).

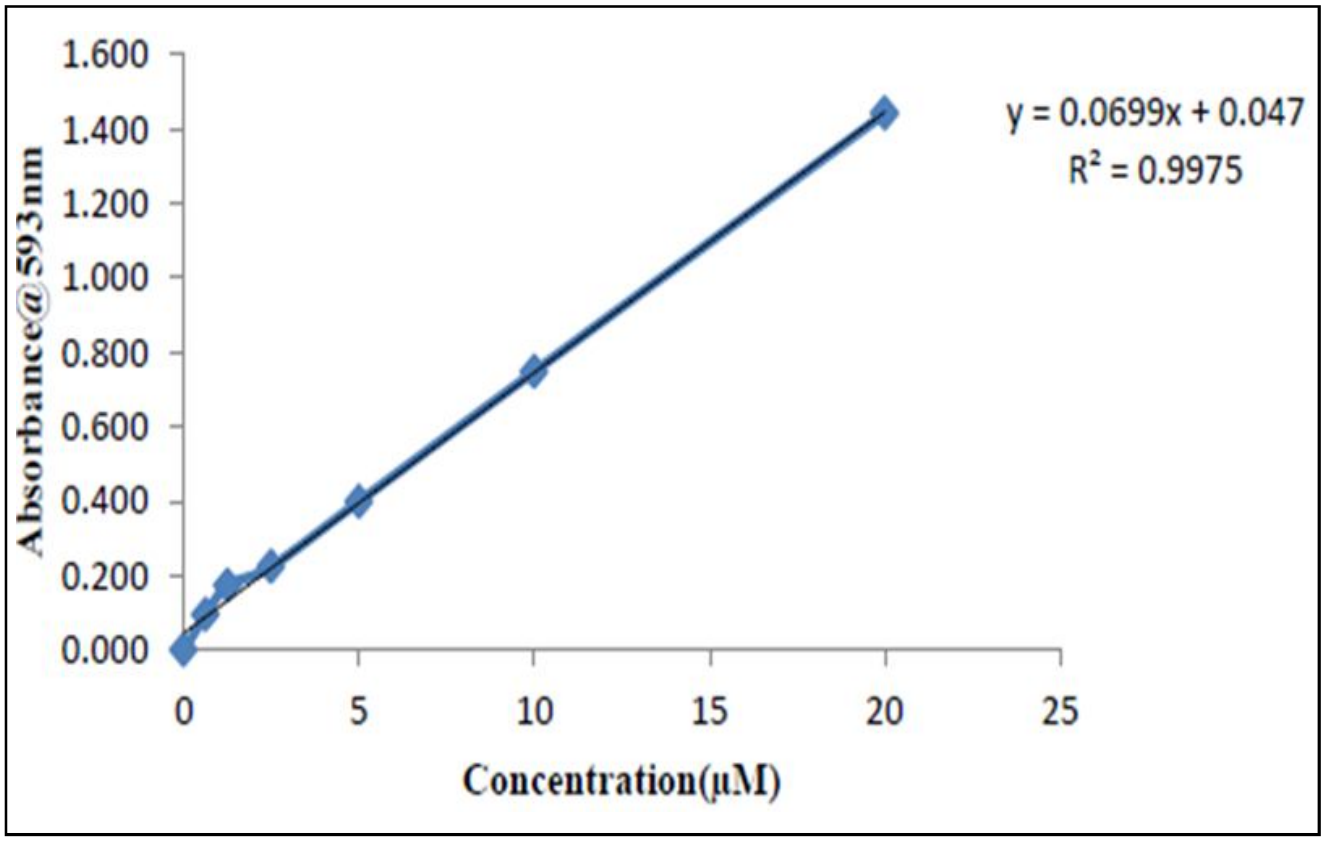


Figure 6: FRAP assay of standard ascorbic acid.

Table 3: Antimicrobial screening of *G. glauca* leaf various solvent extracts

		Zone of inhibition (mm) and minimum inhibitory concentrations (MIC) (mg/ ml)				
Pathogens		**GGE (100 µg/disc)**	**GGH (100 µg/disc)**	**GGM (100 µg/disc)**	**Nystatin (10 µg/well)**	**Gentamicin (10 µg/disc)**
Gram-positive bacteria	*B. subtilis*	00	00	00	—	25 (0.156)
	L.monocytognes	00	00	00	—	25 (0.156)
	S. aureus	00	00	00	—	26 (0.039)
Gram-negative bacteria	*E. coli*	00	00	00	—	25 (0.039)
	P.aeruginosa	16 (1.25)	15 (2.5)	17 (1.25)	—	22 (0.156)
	S. typhi	12 (2.5)	00	13 (2.5)	—	24 (0.156)
Fungi	*C. albicans*	00	00	20 (4.6)	15 (0.156)	—
	E. floccosum	00	00	18 (3.4)	17 (0.156)	—

Note: GGM- Methanol extract; GGH: Hexane extract; GGE: Ethyl acetate extract. Values are represented as mean ± SE for three independent replicates. $p \leq 0.05$ according to Tukey's HSD was considered significant.

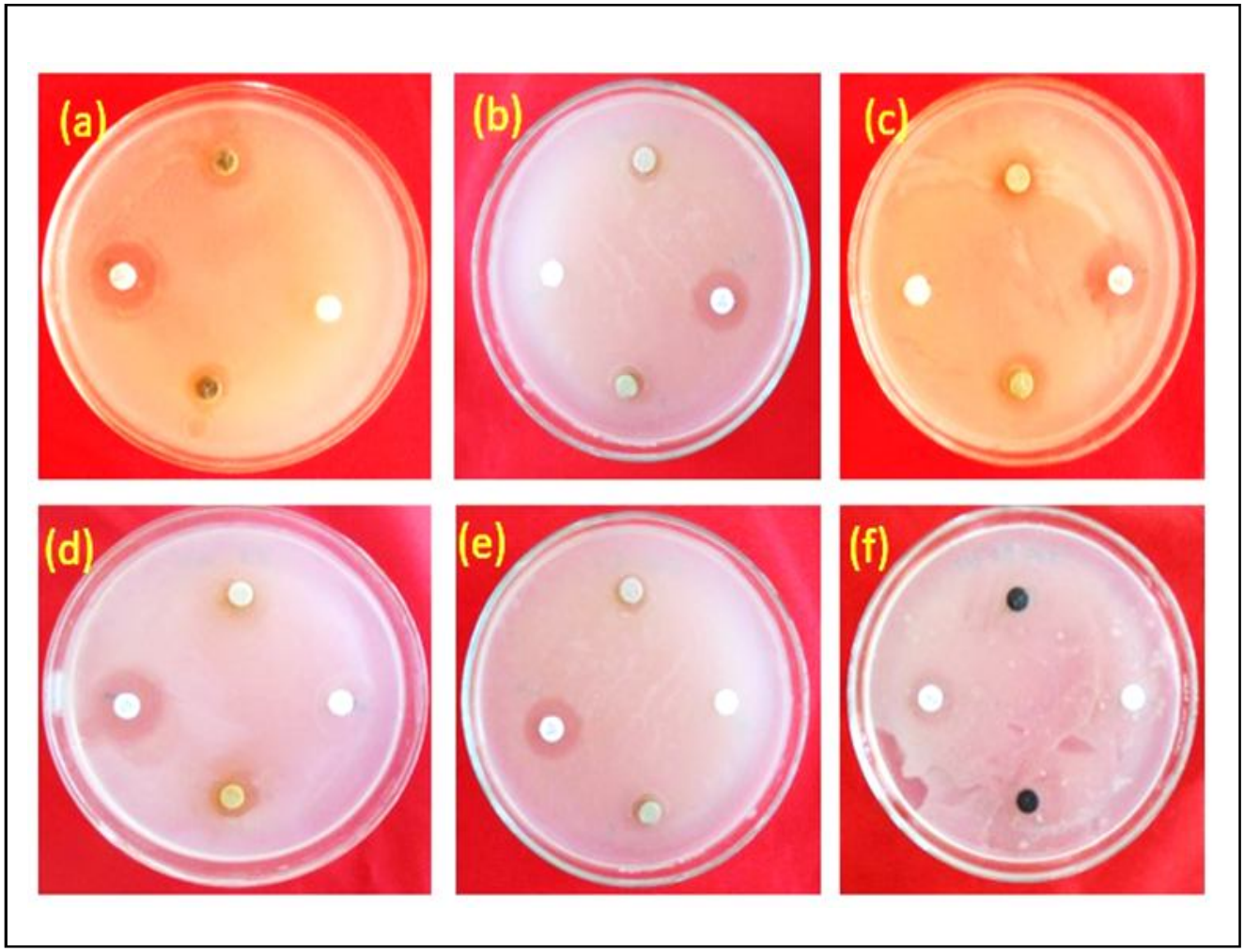


Figure 7: Antibacterial screening of *G. glauca* leaf extracts. (a) *P. aeruginosa*, (b) *S. typhi*, (c) *B. subtilis*, (d) *L. monocytogenes* (e) *S. aureus*, and (f) *E. coli*.

In line with our findings, the methanolic leaf extract of *D. sissoo* exhibited significant antimicrobial and antifungal activity. This effect may be attributed to bioactive compounds associated with antioxidant activity, derived from secondary metabolism. These compounds play a crucial role in plant defense, adaptation, and ecological interactions, often increasing under stress conditions (Krishnarao and Rajeshwari, 2023).

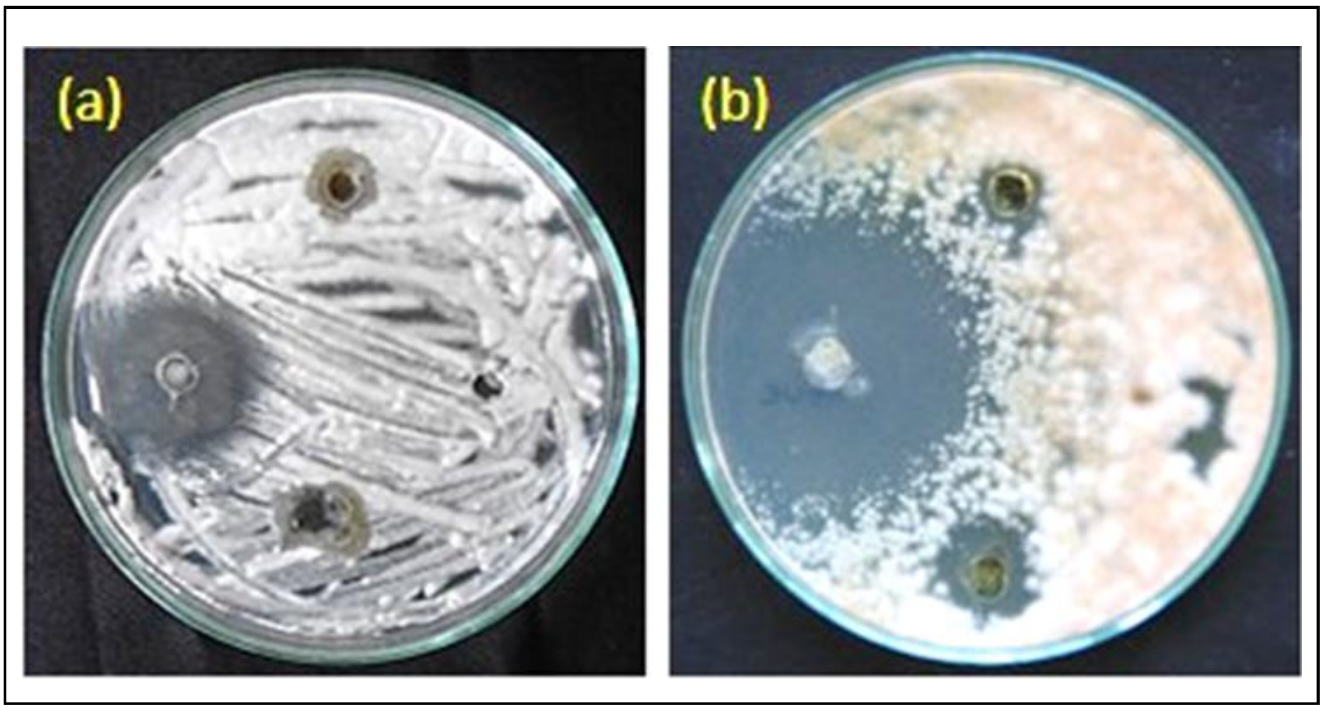


Figure 8: Antifungal screening of *G. glauca* leaf extracts. (a) *E. floccosum*, (b) *C. albicans.*

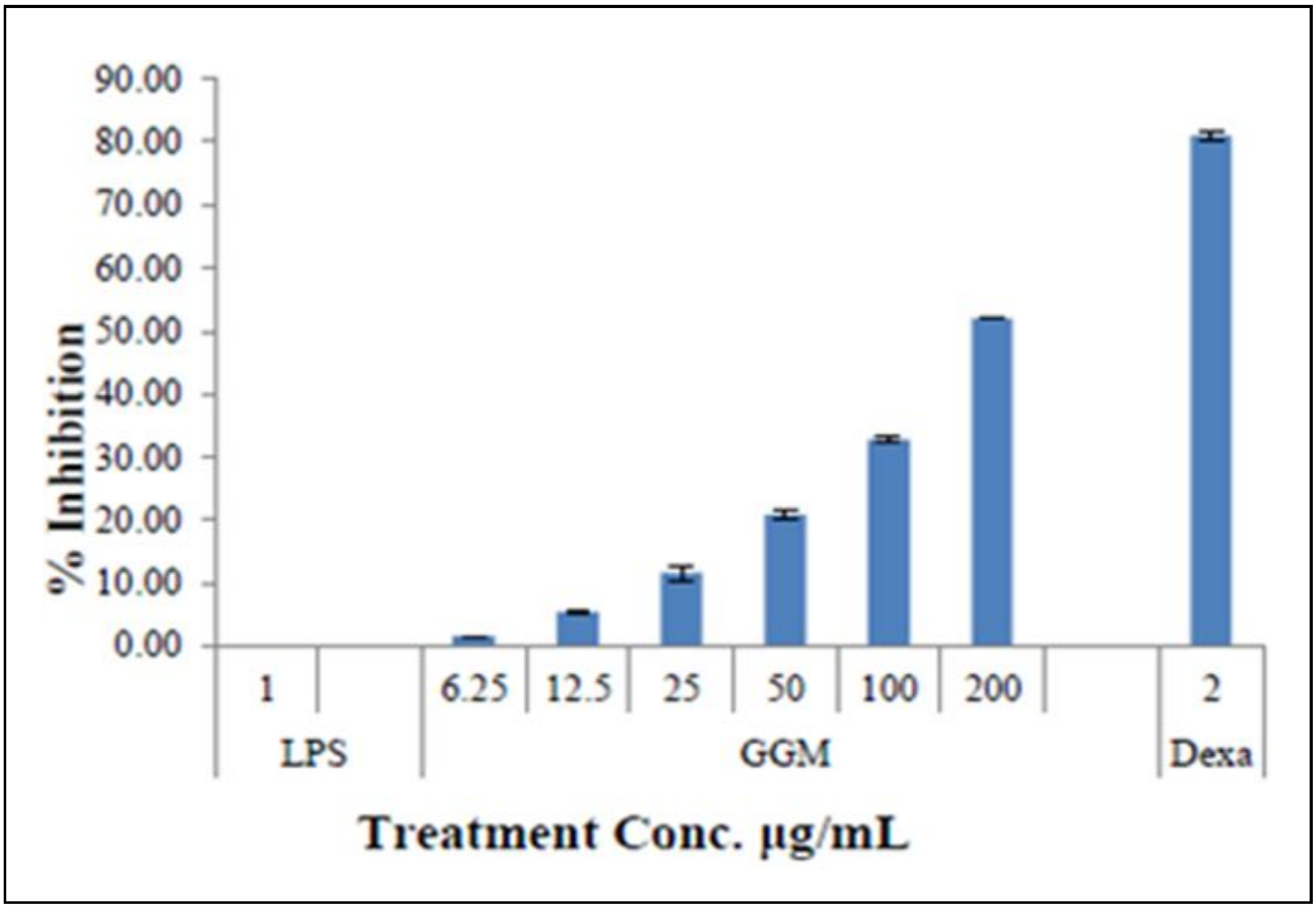


Figure 9: Inhibition of LPS induced TNF-α in Raw 264.7 cells treated with test compounds.

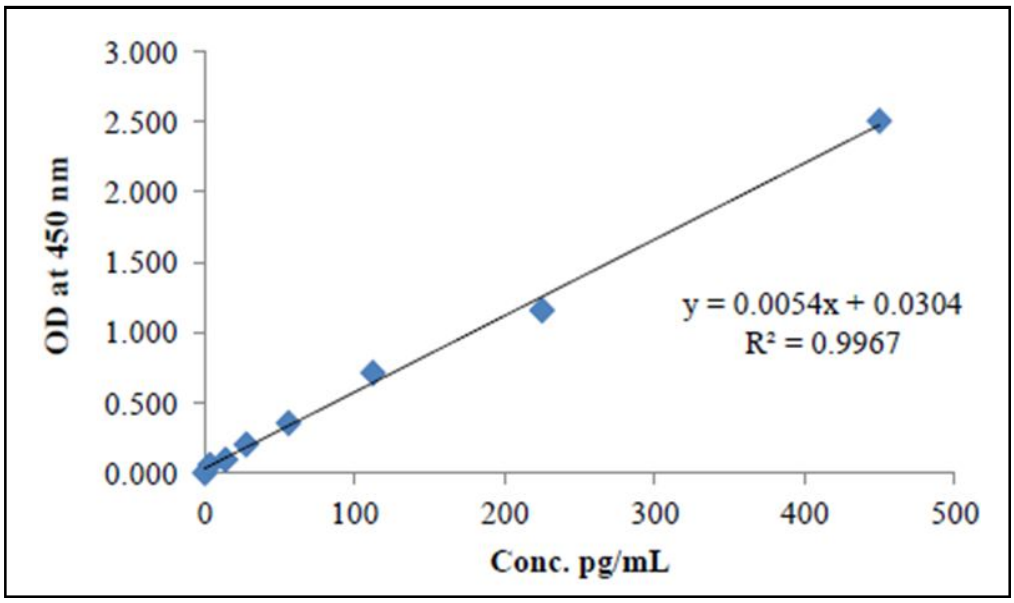


Figure 10: TNF-α standard plot.

3.4 Evaluation of anti-inflammatory effects against LPS induced TNF-α release

Various concentrations of *G. glauca* leaf methanol extract were tested on Raw-264.7 cells to assess their inhibitory effects on LPS-induced TNF-α release. TNF-α production was stimulated by treating the cells with 1 µg/ml LPS for 24 h, followed by treatment with different concentrations of the test compounds. The results indicated that the methanol extract inhibited LPS-induced TNF-α release by more than 50%, reaching up to 52.13% at the highest concentration of 200 µg/ml (Figure 9). In comparison, the positive control, dexamethasone (2 µg/ml), exhibited 81.34% inhibition. Additionally, *G. glauca* leaf methanol extract demonstrated 2.507% inhibition at a TNF-α concentration of 450 pg/ml, with an R^2 value of 0.9967 (Figure 10).

4. Discussion

Nowadays plants are being extensively used for various purposes in our day-to-day life due to the diversity of the phytochemicals varies between the plant species (Arif *et al.,* 2022). Therefore, the extractions yield determination revealed that the highest yield was obtained in the leaf compared to the stem between the solvents. The obtained results are in substantiated with the earlier reports of Felhi *et al.* (2017) wherein, the percentage yield was determined by using different solvents in *Ecballium elaterium* in leaf and fruits, respectively (14% in diethyl ether and 11% in methanol). The profuse phytochemicals in any of the plants often plays a significant role in their way. Some secondary metabolites act as a defense system against invading prey or some microbes. The secondary metabolites such as tannins, carbohydrates, flavonoids, phenol, saponins, glycosides, terpenoidsand steroids were present in both extracts which have demonstrated various therapeutic applications due to antioxidant activities. Phytochemical analysis of different solvent extracts reveals significant results in leaf methanol extract. Earlier, Do *et al.,* (2014) reported similar observations in methanol, ethanol, and aqueous extracts of *Limnophila aromatica*.

G. glauca leaf hexane, ethyl acetate, and methanol extracts were subjected to determine the antimicrobial efficacy. Leaf methanol extracts exhibit better results than compared hexane and ethyl acetate extracts. These results were similar to the report of Radfar *et al.* (2012) wherein, the *Trianthema decandra* root extracts using chloroform and ethanol showed significant antibacterial activity at 3.12-12.50 µg/ml. Out of three solvent extracts, methanol extract exhibited significant results by inhibiting the growth of selected microbes compared to hexane and ethyl acetate extracts. Reports indicate that Gram-positive microorganisms are more susceptible than Gram-negative microorganisms, primarily due to differences in their cell wall composition (Pragyandip *et al.*, 2023). A contrasting result was published by Muthukrishnan *et al.*(2018) where they demonstrated the effective inhibition of leaf methanolic extract compared to ethyl acetate extract. Minimum inhibitory concentration is a measure of concentration of drugs or antimicrobial entity that stops the growth of pathogens in 24 h of incubation.

The antioxidant properties of *G. glauca* leaf extracts were evaluated using DPPH and FRAP assays. The DPPH scavenging assay indicated the highest antioxidant potential in leaf methanol extract which may be attributed to the production of antioxidant substances such as phenolics, saponins, and other associated polyphenols dissolved in ethanol. Every organism has its self-defense mechanism for its self-protection from its surroundings; likewise, plants also have their self-defense system by producing some chemical substances within them. These chemical constituents are nothing but secondary metabolites, sometimes they act as harmful and some are beneficial to mankind. Hence, we tested *G. glauca* leaf extracts for their anti-inflammatory properties. Shrilakshmi *et al.* (2022) reported similar study and conducted *in vitro* anti-inflammatory assay by using leaf extracts of *Callicar patomentosa* wherein, the methanolic extracts exhibited higher anti-inflammatory effects against tested cell lines.

5. Conclusion

The current study revealed the presence of promising phytochemicals in methanol, ethyl acetate and hexane extracts of the stem and leaf of *G. glauca*. The antimicrobial test revealed that the leaf methanol extract inhibited the growth of tested pathogens. Similarly, all the

extracts showed significant antioxidants in dose-dependent wages in the DPPH and FRAP radicle scavenging assay. The *in vitro* antioxidant assays, DPPH and FRAP, confirmed the strong radical scavenging and ferric reducing potential of the leaf methanol extract, indicating its significant antioxidant properties. The anti-inflammatory test showed that the leaf methanol extract showed moderate anti-inflammatory activity. Therefore, we believe that the research work presented here paves the way for initiating the production of high-valued biomolecules using biotechnological approaches.

Acknowledgments

The first author is thankful to the University of Mysore and the Department of Microbiology for providing the laboratory facility.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

Reference

Ansari, M. H. R. and Ahmad, S. (2019). Herbs that heal: Natural remedies for health promotion and longevity. Ann. Phytomed., **8**(1):7-18.

Anvitha, N. R.; Vinuthashree, R. and Shobha Jagannath (2023). Phytochemical screening, antioxidant and anti-inflammatory activity of vulnerable tree species *Diospyroscandolleana* Wight. Ann. Phytomed., **12**(1):529-537.

Arif, S.; Sharma, A. and Islam, M. H. (2022). Plant derived secondary metabolites as multiple signalling pathways inhibitors against cancer. Ann. of Phytomed, **11**(1):189-200.

Arika, W.; Kibiti, C. M.; Njagi, J. M. and Ngugi, M. P. (2019). *In vitro* antioxidant properties of dichloromethanolic leaf extract of *Gnidia glauca* (Fresen) as a promising antiobesity drug. Journal of Evidence-Based Integrative Medicine, **24**(2019):1-7.

Arjun, H.B,; Yasuhiro T,; Ketut, A.; Kiyoshi M.; Katsumichi.M.; Dejair, M.; Alfredo A.G.H. and Shigetoshi, K. (2000), Phytochemical analysis, antioxidant and antihemolytic activity of hibiscus and amla. Journal of Ethnopharmacology, **72**:239-246.

Benzie,; Iris, F.F.; John, J. and Strain. (1996). "The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: T he FRAP assay. Analytical Biochemistry, **239**(1):70-76.

Bharathi, G. S.; Lalitha, V.; Sethumathi, P. P.; Sivakumar, T.; Selvi, P. P.; Kavitha, M. and Kumar, R. N. (2021). Plants and their derivatives as a potential source in the treatment of alcohol withdrawal syndrome and other treatment strategies: A review. Ann. Phytomed., **10**(2):36-43.

Bhawana Sharma and Afroz Alam (2022). Phytochemical profiling, antioxidant potential and antimicrobial activities of *Dalbergiasissoo* Roxb. Ann. Phytomed., **11**(1):383-388.

Buritis, M. and Bucar, F. (1999). Antioxidant activity of *Nigella sativa* essential oil. Phytotherapy Research, **14**:323-328.

Burits, M., and Bucar, F. (2000). Antioxidant activity of *Nigella sativa* essential oil. Phytotherapy Research, **14**(5):323-328.

Crouch, S. P. M.; Kozlowski, R.; Slater, K. J. and Fletcher, J. (1993). The use of ATP bioluminescence as a measure of cell proliferation and cytotoxicity. Journal of Immunology Methods, **160**(1):81-88.

De Zwart, L, L.; Meerman, J.H.N.; Commandeur, J.N.M. and Vermeulen, N.P.E. (1999). Biomarkers of free radical damage applications in experimental animals and humans. Free Radical Biology and Medicine, **26**(12): 202-226.

Denizot, F. and Lang, R. (1986). Rapid colorimetric assay for cell growth and survival: Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. Journal of Immunology Methods, **89**(2):271-277.

Ghosh, S.; Ahire, M.; Patil, S.; Jabgunde, A.; BhatDusane, M.; Joshi, B. N. and Chopade, B. A. (2012). Antidiabetic activity of *Gnidia glauca* and *Dioscorea bulbifera*: Potent amylase and glucosidase inhibitors. Evidence Based Complementary and Alternative Medicine, **3**(1): 929051.

Ghosh, S.; Derle, A.; Ahire, M.; More, P.; Jagtap, S.; Phadatare, S. D. and Chopade, B. A. (2013). Phytochemical analysis and free radical scavenging activity of medicinal plants *Gnidia glauca* and *Dioscorea bulbifera*. PloS one, **8**(12):82529.

Gonzalez, R.J. and Tarloff, J.B. (2001).Evaluation of hepatic sub-cellular Fractions for alamar blue and MTT reductase activity. Toxicology, **15**:259-9.

Halliwell, B. and Gutteridge, J.M.C. (1989). Free radicals in biology and medicine. Clarendon Press, Oxford, 2nd edn. **pp**:416-419.

Halliwell, B. Gutteridge, J.M.C. and Aruoma, O.I. (1987). The deoxyribose method: A simple test tube assay-determination of rate constants for reaction of hydroxyl radicals. Analytical Biochemistry, **165**: 215-219.

Hattori, N.; Sakakibara, T.; Kajiyama, N.; Igarashi, T.; Maeda, M. and Murakami, S. (2003). Enhanced microbial biomass assay using mutant luciferase resistant to benzalkonium chloride. Analytical Biochemistry. **319**(2):287-295.

Hemant, R.; Jadhav and Bhutani.; K.K. (2002). Antioxidant properties of Indian Medicinal plants. Phytotherapy Research, **16**:771-773.

Henkelman, S.; Rakhorst, G.; Blanton, J. and Van Oeveren, W.(2009) Standardization of incubation conditions for hemolysis testing of biomaterials. Material Science and Engineering C-Biomimetic and Supramolecular Systems, **29**:1650-1654.

Ibrahim, I. H.; Sallam, S. M.; Omar, H. and Rizk, M. (2006). Oxidative hemolysis of erythrocytes induced by various vitamins. International Journal of Biomedical Science, **2**(3):95-298.

Kangas, L.; Grönroos, M. and Nieminen, A. L. (1984). Bioluminescence of cellular ATP: A new method for evaluating cytotoxic agents *in vitro*. Medical Biology. **62**(6):338-343.

Krishnarao, K. L. and Rajeshwari, T. R. (2023). Phytochemical investigation and evaluation of the antioxidant, antibacterial and antifungal activities of *Stephaniajaponica* L. leaves extract. Ann. Phytomed., **12**(1):477-485.

Lundin, A.; Hanson, M.; Persson, J. and Pousette, A. (1986). Estimation of biomass in growing cell lines by adenosine triphosphate assay. In: Methods in enzymology. Academic Press, **33**:27-42.

Martín Aragón, S.; Basabe, B.; Benedí, J. M. and Villar, A. M. (1998). The antioxidant action of *Vaccinium myrtillus* L. Phytotherapy Research: International Journal of Pharmacology and Toxicology, **12**(1):104-106.

Mebrahtom Gebrelibanos (2012) *In vitro* erythrocyte hemolysis inhibition properties of *Sennasingueana* extracts. Momona Ethiopian Journal of Science, **4**(2):16-28.

Mehboob Hoque, M. H.; Sandeep Dave, S. D.; Pawan Gupta, P. G. and Mohammed Saleemuddin, M. S. (2013). Oleic acid may be the key contributor in the BAMLET-induced erythrocyte hemolysis and tumoricidal action. PloS One, **8**(9):68390.

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. The Journal of Immunological Methods, **65**(1):55-63.

Pragyandip, P. D.; Sushil Kumar, Anuradha Mishra and SajalSrivastava (2023). Antimicrobial activity of *Haldina cordifolia* (Roxb.) Ridsdale and *Thevetiaperuviana* (Pers.) Schum. leaf extract against multidrug resistant microbes. Ann. Phytomed., **12**(1):431-439.

Rajakumar, D.V. (1994). Biochemical & Pharmacological studies on the antioxidant properties of Dehydrozingerone and its analogs, University of Mangalore (unpublished Ph. D thesis).

Shrilakshmi, G.; Kumar, H. and Jagannath, S. (2022). Anti-inflammatory activity of leaf and leaf derived callus extracts of *Callicarpato mentosa* L.: An endemic medicinal plant of Western Ghats, Karnataka, India on carrageenan-induced rat paw edema. Ann. Phytomed., **11**(1):346-350.

Slater, T.F. (1984). Free radical mechanisms in tissue injury. Biochemical Journal. **222:**1-15.

Valleti, P. V.; Kumar, V.; Ramayanam, P. K.; Gopalappa, R.; Vijendra Dittekoppa, P.; Cm, A. and Al-Farraj, S. (2024). Multifaceted anticancer potential of *Gnidia glauca* (Fresen.) Gilg leaf alkaloids: Impact on multiple cellular targets. ACS omega, **9**(8):9615-9624.

Vani, T.; Rajini, M.; Sarkar, S. and Shishoo, C. J. (1997), Antioxidant properties of the Ayurvedic formulation-Triphala and its constituents. International Journal of Pharmaceutics, **35**(5):313-317.

Citation **Neharani, K.P. Ramesha, B. R. Nuthan, K. K. Sampath Kumar and S. Satish (2024). Evaluation of phytochemical, antioxidant, antimicrobial, and Anti-inflammatory properties of *Gnidia glauca* (Fres.) Gilg: A potential therapeutic plant from the Western Ghats. Ann. Phytomed., 13(2):703-713. http://dx.doi.org/10.54085/ap.2024.13.2.72.**