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Development and Validation of an HPTLC Method for Qualitative and Quantitative Estimation of Quercetin in *Glinus oppositifolius* (L.)

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ABSTRACT

Glinus oppositifolius (L.) is a perennial herb used in Indian folk medicine as a stomachic, aperients, antiseptic, uterine stimulant, and to promote menses and lochia. The reported pharmacological activities of this plant are immunomodulatory, hepatoprotectivite, anthelmintic, anti-hyperglycemic etc. activities. To an HPTLC densitometric method was developed and validated for the qualitative and quantitative estimation quercetin in *Glinus oppositifolius* (L.) available from West Bengal. The shad-dried leaves of *Glinus oppositifolius* (L.) were extracted with Methanol. HPTLC analysis was carried out on aluminum-backed silica gel 60 F254 plates with Ethyl acetate-Toluene–Formic acid 5:4:0.2 (v/v/v) as mobile phase. The HPTLC densitometric method was developed and validated as per ICH guidelines for estimation of quercetin. Total Flavonoid Content (TFC) is 102.95 ± 3.85 mg QE/gm. In HPTLC analysis, *G. oppositifolius* ethanolic extract showed a maximum of 8 well-resolved peaks at R_f 0.005, 0.098, 0.266, 0.466, 0.655, 0.724, 0.776 and 0.827. Well separated and compact spots (R_f) of quercetin (0.81±0.06) were detected. The regression equation obtained was y = 0.0002x + 0.0019, with a correlation coefficient (R^2) of 0.9852. The linearity range (μg/spot) 20-100. Quercetin content was found to be 0.25 ± 0.0047 mg of quercetin /100gm sample.The developed method was fond precise, robust an accurate and was successfully used for the detection and quantification of quercetin in *Glinus oppositifolius* (L.) and the quantities of quercetin was 0.25 ± 0.0047 mg of quercetin /100gm sample.

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INTRODUCTION

Herbal medicines have been used to treat ailments since time immemorial. The fact that many contemporary medicines are derived from higher plants is evidence that plant-derived natural products play an important role in modern day drug discovery. A majority of recent research focuses on the phytochemical investigation of plants with ethnopharmacological evidence. India has one of the most ancient, diverse, and rich cultural traditions associated with the utilization of medicinal plants. Herbs, being easily available to humans, have been extensively researched for their medicinal properties. Some medicinal plants utilised in Ayurvedic formulations have been investigated thoroughly, while

others remain unexplored [1]. One such less explored herb is *Glinus oppositifolius* (L.), commonly known as Indian carpetweed, which is freely available in West Bengal and its neighbouring regions, and is used as a vegetable.

Glinus oppositifolius (L.) August to December is a profusely branched, annual/ perennial herb, commonly found within the tropics in areas of low elevation. The plant is slender spreading, ascending or almost prostrate with stems up to 40 cm long [2]. The plant generally grows close to the ground in open areas, lake shores and river banks. The leaves are usually arranged oppositely in an unequal whorl, in sets of 4-5 leaves. Each leaf is 0.5 to 1.5 cm in size. The shape of the leaves is mostly oblanceolate or linear lanceolate, but sometimes spathulate or rounded leaves are also seen – varying from one region to another. They are green in colour with a sub-acute or acute leaf apex. The leaves have an unpleasant odour and are bitter in taste [3, 4]. The root of *Glinus oppositifolius* (L.) is a typical taproot. There is a primary and secondary root system for the tap. There are a number of rootlets, each varying from 0.1 to 0.4 cm thick [4]. Flowers are white and arranged in cluster form in axillary fashion. Around 3 to 6 flowers are attached with node [3].

Ethnomedicinal Uses of *Glinus oppositifolius*

Glinus oppositifolius (L.) is used by several different communities as ethnomedicine, like in Taiwan, Mali, Bangladesh and mostly the southern parts of India. In addition to its portions (usually leaves) being eaten as vegetables, it is useful as an anti-diabetic medication. The plant also has some nutritional benefit because it is commonly used as a dietary staple, particularly in South India.

In the Salem district of Tamil Nadu, India, folk healers treat poisonous animal bites using the leaves of the *Glinus oppositifolius* (L.) plant [5]. The tribes of Maharashtra's Nandurbar district utilise the whole plant extract as a carminative, stomachic, and tonic [6]. According to the Narikorava tribe of Tamil Nadu, one of the significant applications for this herb is as a tonic for new mothers to alleviate postpartum weakness [7]. For liver diseases, it can be used as a bitter tonic [8]. In Maldah district of West Bengal, the root paste of this plant is given orally to treat dysentery [9]. In traditional Mali (West Africa) medicine, dried stems and leaves are ground into a fine powder, mixed with food, and used to alleviate gastrointestinal pain and jaundice [10]. In Thailand, the whole plant aqueous extract of *Glinus oppositifolius* (L.) has been traditionally used as expectorant and antipyretic [11]. In Bangladesh, *Glinus oppositifolius* (L.) extracts have long been traditionally used for the treatment of inflammation, joint pain, fever, diarrhoea, and skin disorders [12]. Whole plants are used in Bangladesh's southern district Noakhali to treat earaches, skin conditions, gastritis, and appetite loss [13]. According to folklore in Philippines, the plant has anti-diabetic and antimicrobial effects [14]. There have been reports of a variety of phytochemical compounds from *G. oppositifolius* (L.) in recent years, but there hasn't yet been a thorough investigation using a purified chemical compound from the plant. Aim of the present study is to HPTLC densitometric method was developed and validated for the qualitative and quantitative estimation quercetin in *Glinus oppositifolius* (L.) available from West Bengal.

MATERIALS AND METHODS

Collection of Plant Material

The whole plant of *Glinus oppositifolius* (L.), locally known as 'Gima shaak', was procured from a local market in Kolkata, By the Central National Herbarium (CNH), Botanical Survey of India, Shibpur, West Bengal, the plant was identified.

Chemicals

Standard quercetin was obtained from Loba Chemie Pvt. Ltd. All chemicals and reagents used for preliminary phytochemical analysis were of analytical grade. The solvents used were all of HPLC standard.

Preparations of Plant Extract for Detection and Quantification of Quercetin in Methanolic Extract of *Glinus oppositifolius* (L.)

The leaves of *Glinus oppositifolius* (L.) were air-dried, coarsely ground up, and then extracted thoroughly by macerating with methanol for seven days. Using a rotary vacuum evaporator, the solvent was evaporated to dryness under decreased pressure, and then each of the leftovers was individually dissolved in methanol in 50 ml volumetric flasks.

Preliminary Phytochemical Analysis

The plant extracts of *Glinus oppositifolius* (L.) were assessed qualitatively for their phytochemical content using standard methods [15–17]. The extracts were tested for the presence of flavonoids, glycosides, alkaloids, terpenoids, tannins, phenols and saponins (Table 1).

Determination of Total Flavonoid Content

Total flavonoid content of methanolic extract of *Glinus oppositifolius* (L.) was determined by aluminum chloride colorimetric method. The reaction mixture contained 1ml of solution of extracts in the concen-

tration of 1mg/ml and 1ml of 2% aluminum chloride solution dissolved in water. At room temperature, the sample was incubated for one hour. At 415 nm, the absorbance was measured. The same procedure was repeated for the solution of quercetin (standard) and the calibration line was constructed. Based on the measured absorbance, the calibration line; then the content of flavonoids in the extract was expressed in terms of quercetin equivalent.

Solvent Selection

The development of an appropriate TLC method for the measurement of quercetin in the methanolic extract of G. oppositifolius involved screening a number of different solvent systems. For these goals, movable phases were tested:

Toluene: Ethyl acetate: Formic acid (5:4:0.2v/v/v)

Ethyl acetate: Toluene: Formic acid (5:4:0.2v/v/v)

HPTLC Fingerprinting

HPTLC studies were carried out following the method of Adhikari et al. [18].

Sample Preparation

The standard and the methanolic extract were dissolved in 1ml of chromatographic grade methanol, which is used for sample application on HPTLC plate's pre-coated silica gel 60F $_{254}$ aluminum sheets.

Developing Solvent System

A number of solvent system were tried for extract, but the satisfactory resolution was obtained in the solvent Ethyl acetate: Toluene: Formic acid (5:4:0.2 v/v/v).

Sample Application

Samples were applied on pre-coated silica gel 60F $_{254}$ aluminum sheets with the help of Linomat 5 applicator attached to CAMAG mark HPTLC system.

Development of Spots

After the application of sample, the chromatogram was developed in Twin trough glass chamber 10*10 cm saturated with the solvent Ethyl acetate: Toluene: Formic acid (5:4:0.2 v/v/v) for 20 min.

Detection of Spots

The air-dried plates were viewed in white light, UV λ 254nm and UV λ 366nm with and without staining with 10% H_2SO_4 solution. The chromatogram was scanned by Densitometry TLC Scanner 4. The R_f value and fingerprint data were recorded.

Method Validation

Validation studies ensure the suitability and reproducibility of the method in analyzing the desired analyte. The method was validating for linearity, Limit of Detection (LOD), Limit of Quantification (LOQ), specificity and precision (repeatability) as per the ICH guidelines [19].

Linearity

Dilutions of standard in the range of 20-100 ng per band were analyzed in triplicate to prepare five-point linear calibrations. The plates were created, scanned, and given a quantitative assessment. Peak area and concentration were plotted to produce calibration curves. By averaging the results of the regression analysis performed on these plots, linearity was established.

Precision

Two levels of precision were assessed in accordance with ICH recommendations. Repeatability was determined as intraday precision whereas intermediate precision was determined by carrying out interday variation for the determination of quercetin levels of 20, 40, 60, 80 and 100 ng per band in triplicates.

Robustness

The suggested TLC densitometric method's robustness was assessed in order to assess the impact of tiny, intentional alterations to the chromatographic conditions during the detection of quercetin. The polarity of the mobile phase was changed to assess robustness.

LOD and LOQ

The values for the signal-to-noise ratios for the limits of detection (LOD) and quantification (LOQ) were found to be 3:1 and 10:1, respectively.

Specificity

The specificity of the method was ascertained by analyzing the standard quercetin and extract. By contrasting the R_f values and spectra of the spot with those of the standard, the presence of quercetin in the spot in the sample was verified. By comparing the spectra at three different levels, namely the peak start, peak apex, and peak end positions of the spot, the peak purity of quercetin was determined.

RESULTS

Phytochemicals Screening

Table 1 displays all of the phytochemical analysis' finding. In the study tannins, glycosides, flavonoids and terpenes were determined in methanolic extract of *Glinus oppositifolius* (L.).

Total Flavonoid Contents

Five concentrations of standard Quercetin (20 μg/ml to 100 μg/ml) were used to prepare the standard

Table 1: Phytochemical Analysis

Secondary metabolite	Phytochemical Test	Ethanol Extract
Alkaloid	Dragendorff's Test	-
	Hager's Test	+
Glycoside	Salkowski test	+
	Liebermann test	+
	Keller- Kilani test	+
Flavonoid	Alkaline reagent test	+
	Shinoda test	+
Tannin	Lead acetate test	-
	Ferric Chloride test	+
Terpenoid	Chloroform test	+
Saponin	Frothing Test	+

Table 2: Linear Regression Data for the Calibration Curve of Quercetin

Linear Regression Parameter	Data
Linearity range (μg/ spot)	20-100
Regression equation	y = 0.0002x + 0.0019
Correlation coefficient	0.9852
Slope	0.00019005
Intercept	0.001859
SE of intercept	0.00089
SD of intercept	0.00199

Table 3: Recovery Study for Proposed Method (n=3)

Excess drug added to analyte (%)	Concentration found (μg±SD)	% recovery	%RSD
50	50.37±0.03	100.75	0.06
100	91.29±1.8	91.29	1.97
150	154.59±1.89	103.06	1.22

Table 4: Precision of the Proposed Method (n=3)

	Repeatability (Intra-day precision)			Repeatability (Intra-day precision)		
Conc.(μg/ml)	Area±SD	Std. error	%RSD	Area±SD	Std. error	%RSD
100	0.025±0.00025	0.00014	0.98	0.037±0.00034	0.0002	0.92
100	0.021±0.00028	0.00016	1.33	0.038±0.00034	0.0002	0.88
100	0.024±0.00032	0.00018	1.31	0.039±0.00040	0.00023	1.01

Table 5: Robustness of the Propose HPTLC Method (n=3)

Con (μg/ml)	Original Mobile Phase	Used Mobile Phase		Area±SD	%RSD	R_f
100	5:4:0.2	5:4:0.3	+0.1	0.03727±0.00048	1.30	0.85
		5:4:0.2	0	0.03916±0.0028	0.90	0.81
		5:4:0.1	-0.1	0.06089±0.00066	1.08	0.82

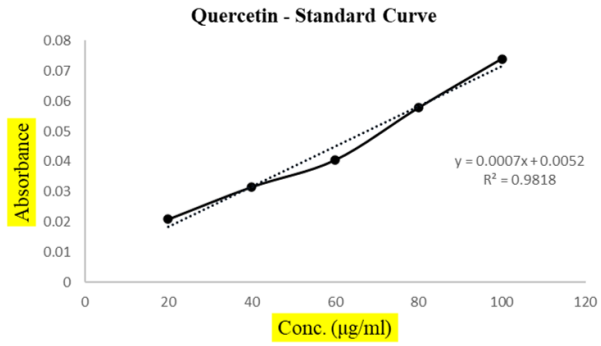

Figure 1: Standard Curve of Quercetin

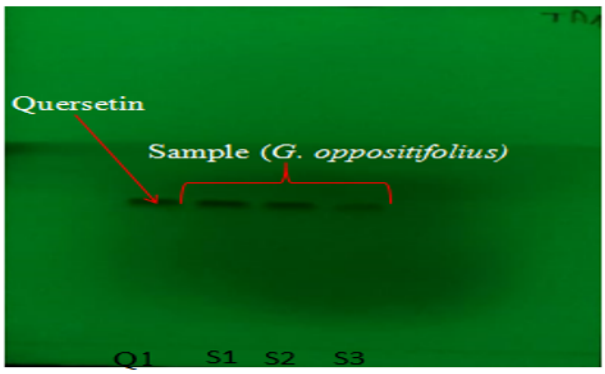

Figure 2: HPTLC Plate Showing Bands of Standard Quercetin (Q1)and Sample (S1-S3) at 254nm

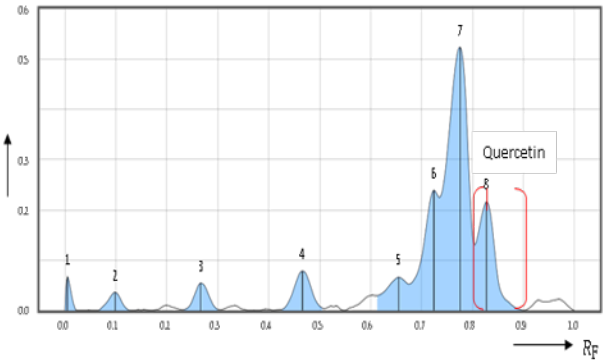

Figure 3: HPTLC Chromatogram of *Glinus oppositifolius* (L.)

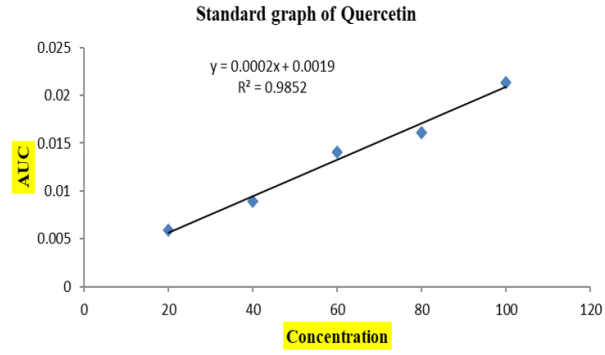

Figure 4: Regression Curve of Standard Quercetin

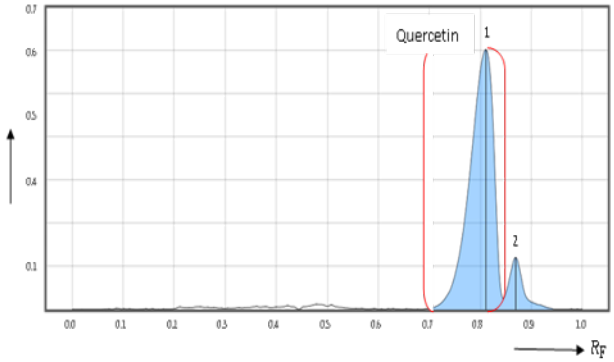

Figure 5: HPTLC Chromatogram of Standard Quercetin

curve - y = 0.0007x + 0.0052; R^2 = 0.9818 (Figure 1) used for the determination of total flavonoid content in the sample extracts, as shown in the below.

Total flavonoid content represented as Quercetin equivalent (QE) per gram of sample was tested in the methanol extract. The absorbance for each extract was measured in triplicate and results were calculated as their mean. Total flavonoid content in the methanol extract of *Glinus oppositifolius* (L.) 102.95 ± 3.85 mg QE/ gm.

Solvent Selection

A clear, compact, and well-resolved band for quercetin was obtained via ascending development using ethyl acetate-toluene-formic acid (5:4:0.2v/v/v). This band's R_f value was 0.81±0.06. Figure 2 shows band of standard quercetin (Q1) and different volume of sample solution (S1-S3) at 254nm.

HPTLC Fingerprinting

The results of the HPTLC fingerprinting of methanol extract of *Glinus oppositifolius* (L.) at 254 and 366 nm are given in Figure 3. The methanol extract showed a maximum of 8 well resolved peaks, at 2μL sample volume, with R_f values of 0.005, 0.098, 0.266, 0.466, 0.655, 0.724, 0.776 and 0.827.

Among these peaks, the peak at R_f value 0.827 showed the highest peak area of 16.22%. The peak corresponding to R_f value 0.827 showed a sharp peak and significant area of 16.22%, was identified as quercetin.

Method Validation

According to the calibration plot in Figure 4, the response is a linear function of quercetin concentrations between 20-100μg/ml.

The slope, intercept, and correlation coefficient were 0.00019, 0.001859 and 0.9852 respectively. Table 2 displays the linear regression information for the quercetin calibration curve.

Recovery Study

Results from recovery studies, which are listed in Table 3, showed good accuracy and fell within acceptable ranges (91.29 to 103.06%).

Precision

Table 4 displays the findings from the determination of repeatability and intermediate precision, expressed as SD (%). For repeatability, RSD fell between 0.98 and 1.33, and for intermediate precision, it fell between 0.88 and 1.01. These small values demonstrated the method's accuracy.

Robustness

Results of robustness are shown in Table 5. Low values of %RSD (0.09- 1.30) were obtained after introducing small deliberate change into the densitometric TLC procedure proved the robustness of the propose HPTLC method.

Limit of Detection and Limit of Quantification

The suggested approach's LOD and LOQ for quercetin were determined to be 34.62 and 104.91 μg/spot, respectively, demonstrating that the method may be utilised successfully for quercetin detection and quantification in a range of conditions.

Method Development

The mobile phase composition was optimised with the goal of creating a reliable and accurate densitometric HPTLC technique for the measurement of quercetin. The mobile phase Ethyl acetate-Toluene-Formic acid 5:4:0.2 (%v/v/v) exhibited a crisp, symmetrical, and well-resolved peak at R_f value of (0.81 ± 0.06) (Figure 5).

Quantification of Quercetin in the Methanolic Extract of *Glinus oppositifolius* (L.)

By contrasting the single spot at R_f = 0.81 ± 0.06 (Figure 5) of the quercetin peaks from the methanolic extracts of the leafy vegetable *Glinus oppositifolius* (L.) with those obtained by chromatography of the standard under the same circumstances. Using the standard concentration and AUC, the quercetin content in methanolic extracts of *Glinus oppositifolius* (L.) is $0.258\pm$ 0.0047 mg of quercetin /100g sample.

DISCUSSION

Plant medicines have been used to treat illnesses for as long a time in the history of mankind. Many contemporary medications have their origins in higher plants. Ayurveda, the traditional medical system of India, has made a variety of therapeutic claims regarding these plant-based medicines. However, it is crucial to support the varied medical benefits of the herbs with scientific evidence.

For a variety of reasons, the stage of ignorance for plant medicine is fast changing. First, issues with side effects of contemporary medications have rekindled interest in plants as a significant source of novel drug candidates. Second, since the majority of the already known lead structures have already been used, pharmaceutical scientists are in search for newer lead compounds from plants [20]. Traditional wisdom of the indigenous people can provide new sources for discovering leads. Third, herbal remedies have achieved remarkable success in the recent years.

The present study was undertaken to prepare the HPTLC Fingerprint profile of the plant *Glinus oppositifolius* (L.) (also known as Gima shaak) for the identification of compounds present in its extracts, as well as to quantify the identified flavonoid from it. Literature survey was conducted to find out the ethnomedicinal uses of the plant in several tribes of India, as well as in other countries like Taiwan, Mali and Bangladesh.

The methanol crude extract of *G. oppositifolius* (L.) was used for the identification of the plant secondary metabolites or phytochemicals present by the standard phytochemical screening tests. The methanol extract tested positive for most of the phytochemical tests, indicating presence of alkaloids, glycosides, flavonoids, terpenoids, tannins and saponins (Table 1).

The total flavonoid content of the plant extracts was estimated using the Aluminium chloride method, using quercetin as the standard. The principle behind this assay procedure is that aluminium chloride forms acid-stable complexes with the C-4 keto groups and either the C-3 or C-5 hydroxyl groups of flavones and flavonols present in the sample. Additionally, it combines with the ortho-dihydroxyl groups on the A or B ring of flavonoids to produce complexes that are acid labile [21]. The concentration of flavonoids present in the reaction media linearly affects the intensity of the light absorbed, thus useful in estimating the flavonoid content in the sample. In this study, higher amount of TFC was obtained in the ethanolic extract (102.95 ± 3.85 mg QE/ gm sample).

Following development, 10% H_2SO_4 solution spraying reagents are applied to the TLC plates. After being sprayed with a 10% H_2SO_4 solution, quercetin turned up as a red colour spot in both the sample and the reference. Under chromatographic conditions, the R_f value of quercetin extracted from *Glinus oppositifolius* (L.) was almost identical to that of

the reference standard. One of the solvent systems was discovered to be suitable for running the sample after all 2 were analyzed. Therefore the Ethyl acetate: Toluene: Formic acid (5: 4: 0.2 v/v/v) can be considered as good solvent system (Figure 3).

High Performance Thin Layer Chromatography (HPTLC) is a very useful technique for the analytical validation of novel natural product forms. HPTLC is currently becoming one of the best methods for ensuring product quality, purity, stability, as well as for identification, or, validation of an herbal product's complex composition. In this study, HPTLC was used to develop the chromatographic fingerprint profile of the methanolic extract of *Glinus oppositifolius* (L.) in the optimized mobile phase Ethyl acetate: Toluene: Formic acid (5: 4: 0.2 v/v/v). The pattern of the fingerprint profile obtained from this study can be used for the quality control of the plant sample. The identified compound from the chromatogram of this plant is Quercetin, having R_f value 0.82. Quantification of quercetin was also carried out to estimate the amount of quercetin content per gram of plant sample. Quercetin content was found to be 0.258 ± 0.0047 mg of quercetin /100gm sample.

CONCLUSION

The presence of flavonoids in the methanolic extract of the plant could be responsible for its anti-inflammatory, anti-viral and immunomodulatory activity. HPTLC Fingerprint profile of the methanolic extract was obtained, which could be used for authentication and quality control of the herbal plant or other herbal formulations containing this plant. The flavonoid identified from the plant is quercetin, at R_f value 0.82, which is being reported for the first time from this plant *Glinus oppositifolius* (L.). The flavonoid was quantified by comparing the area under the curve with standard quercetin and the quantity of quercetin is 0.258 ± 0.0047 mg of quercetin /100g sample.

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Conflict of Interest

The authors declare that there is no conflict of interest.

REFERENCES

[1] N Hari and V P Nair. Preliminary Phytochemical Evaluation and HPTLC Fingerprint Profile of Jasminum azoricum L. *Int J Sci Res Sci Eng Technol*, 4:791–796, 2018.

[2] C Y Ragasa, D L Espineli, E H Mandia, M J Don, and C C Shen. A new triterpene from Glinus oppositifolius. *Chinese Journal of Natural Medicines*, 10(4):284–286, 2012.

[3] S T Ramaseshan, P Pitchaiah, V Bharti, K K Ramakrishna, V Gaddam, D Tewari, and D K Singh. Pharmacognostical, Phytochemical and Nutritional Evaluation of Glinus oppositifolius (L.) Aug. DC. *Pharmacognosy Journal*, 8(1), 2016.

[4] R K Dewangan, S Dubey, and A K Dixit. Pharmacognostical and Qualitative Phytochemical Study of Glinus oppositifolius (Linn.) Aug. DC-An Important Ethnobotanical Plant. *GIS Sci J*, 8:2105–2119, 2021.

[5] T Thirunarayanan. Ethnobotanical survey on Folk Medicine in the management of animal bite poisons in the forest tract of Salem region of Tamil Nadu, India. *International Journal of Pharmacology and Clinical Sciences*, 2(2), 2013.

[6] N Dongarwar, Uma Thakur, and S Dongarwar. Ethnomedicines Among Some Tribes Of Nandurbar District Of Maharashtra (India). *Life Sci Leafl*, 4:48–53, 2012.

[7] G Siromoney, L D Giles, and C Livingstone. Herbal medicines of the Narikoravas. *Folklore*, 14(10):363–369, 1973.

[8] C P Khare. Indian medicinal plants: an illustrated dictionary. 2008. Springer Science and Business Media.

[9] M Chowdhury and A P Das. Inventory of some ethno-medicinal plants in wetlands areas in Maldah district of West Bengal. *Pleione*, 3(1):83–88, 2009.

[10] K T Inngjerdingen, S C Debes, M Inngjerdingen, S Hokputsa, S E Harding, B Rolstad, and B S Paulsen. Bioactive pectic polysaccharides from Glinus oppositifolius (L.) Aug. DC., a Malian medicinal plant, isolation and partial characterization. *Journal of ethnopharmacology*, 101(1-3):204–214, 2005.

[11] P Sahakitpichan, W Disadee, S Ruchirawat, and T Kanchanapoom. L-(−)-(N-trans-Cinnamoyl)-arginine, an Acylamino Acid from Glinus oppositifolius (L.) Aug. DC. *Molecules*, 15(9):6186–6192, 2010.

[12] N Hoque, M Z Imam, S Akter, M E H Mazumder, S R Hasan, J Ahmed, and M S Rana. Antioxidant and antihyperglycemic activities of methanolic extract of Glinus oppositifolius leaves. *Journal of Applied Pharmaceutical Science*, pages 50–53, 2011.

[13] R Bhowmik, M R Saha, M A Rahman, and M A U Islam. Ethnomedicinal survey of plants in the Southern District Noakhali. *Bangladesh Pharmaceutical Journal*, 17(2):205–214, 2014.

[14] C Y Ragasa, E C Cabrera, O B Torres, A I Buluran, D L Espineli, D D Raga, and C C Shen. Chemical constituents and bioactivities of Glinus oppositifolius. *Pharmacognosy Research*, 7(2):138, 2015.

[15] O O Odebiyi and E A Sofowora. Phytochemical screening of Nigerian medicinal plants II. *Lloydia*, 41(3):234–246, 1978.

[16] G E Trease and W C Evans. Phenols and phenolic glycosides. In *Textbook of Pharmacognosy*, volume 12, pages 343–383, 1989. Tindall and Co Publishers, London, UK.

[17] B N Shah and A K Seth. Textbook of Pharmacognosy and Phytochemistry. 2010.

[18] T Adhikari and P Saha. Quantitative Estimation of Immunomodulatory Flavonoid Quercetin by HPTLC in Different Leafy Vegetables Available in West Bengal. *Pharmacognosy Research*, 14(4), 2022.

[19] R C Guy. International Conference on Harmonisation. *Encycl Toxicol Third Ed*, 2:1070–1072, 2014.

[20] B Patwardhan. Ayurveda: The designer medicine. *Indian drugs*, 37(5):213–227, 2000.

[21] G C Bag, P G Devi, and T H Bhaigyabati. Assessment of total flavonoid content and antioxidant activity of methanolic rhizome extract of three Hedychium species of Manipur valley. *International Journal of Pharmaceutical Sciences Review and Research*, 30(1):154–159, 2015.