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Phytochemical Screening and Thin Layer Chromatography of Successive Solvent Extracts of the Medicinal Plant Maytenus emarginata

Research Article

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Abstract

Medicinal plants have long been recognized for their therapeutic potential, with phytochemical screening serving as a crucial step in understanding their bioactive constituents. *Maytenus emarginata*, a member of the Celastraceae family, has garnered attention for its diverse secondary metabolites. This study aimed to analyze the phytochemical composition of *M. emarginata* leaves through qualitative screening and thin-layer chromatography (TLC). Extraction was performed using chloroform, methanol, and aqueous solvents, followed by the identification of compounds such as flavonoids, alkaloids, tannins, and sterols. TLC profiling provided insights into compound separation, revealing varied retention factor (Rf) values across different extracts. The qualitative analysis of *M. emarginata* leaf extracts identified the presence of secondary metabolites. Alkaloids were detected in chloroform and aqueous extracts, while flavonoids and sterols were present across all extracts. Among the three solvents used, aqueous extraction yielded the highest extractive content (6.03%), followed by methanolic (3.95%) and chloroform (1.44%). TLC confirmed the presence of multiple phytoconstituents across extracts. The chloroform extract exhibited 6 spots under normal and short-wave UV and 7 under long-wave UV, indicating a broad range of compounds with Rf values from 0.62 to 1.0. The methanolic extract showed up to 4 spots under long-wave UV, while the aqueous extract displayed a single UV-active compound (Rf = 0.68). Qualitative phytochemical screening of *M. emarginata* suggests a complex chemical makeup, underscoring the need for quantitative HPLC analysis. Further research should emphasize the isolation and structural characterization (NMR, MS) of individual compounds.

Keywords: Celastraceae, *Maytenus emarginata,* Phytochemical screening, Secondary metabolites, Thin-layer chromatography.

Introduction

Historically, the plant kingdom has served as a substantial reservoir of bioactive molecules. A significant proportion of pharmaceuticals currently available and approved for clinical application are either naturally occurring compounds or their synthetic analogs. Despite the advent of novel synthetic drugs, phytomedicinals continue to be widely employed globally. Numerous secondary metabolites synthesized by medicinal flora function as precursor molecules in the development of novel therapeutic agents (1-3). Ethnobotanical practices, encompassing herbal medicine, represent an ancient and cross-cultural tradition. Medicinal plants have played a crucial role in pharmacognosy and remain integral to contemporary drug discovery pipelines. While traditionally foundational to therapeutics in developing nations, the utilization of herbal remedies has also witnessed increased adoption in developed countries, primarily attributed to their relative affordability, accessibility, and reliance on traditional empirical knowledge (1, 4).

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Recent scientific literature highlights the escalating research interest in the genus *Maytenus*, primarily driven by its extensive variety of bioactive constituents and its well-recognized role in traditional pharmacopoeia (1). *Maytenus emarginata* (Willd.) Ding Hou, a member of the plant family Celastraceae, is an evergreen arboreal species exhibiting tolerance to diverse abiotic stresses prevalent in arid environments. This taxon, vernacularly known as "Kankero" (Hindi) and "Thorny staff tree" (English), demonstrates a broad geographical distribution across various Indian states, including Madhya Pradesh, Uttar Pradesh, Punjab, Maharashtra, Gujarat, Delhi, Bihar, Tamil Nadu, and Rajasthan (5, 6).

The Celastraceae family, native to tropical and subtropical zones globally, encompasses approximately 88 genera and 1300 plant species. *M. emarginata* , known locally as Bharati, is an erect evergreen shrub within this family, characterized by obovate, glaucous green to reddish-brown leaves with an emarginate apex and serrate margin. Phytochemical analysis of M. emarginata has revealed the presence of sesquiterpene pyridine alkaloids (Emarginate A–E), triterpenes (β-amyrin), sterols (β-sitosterol), fatty acids (palmitic acid in leaves), maytansinoids (maytensine in leaves), and flavonoids (quercetin, myricetin, isorhamnetin in leaves). Traditional medicinal uses include the application of roots for dysentery and root juice for diabetes treatment (7, 8). The study showed that the

chemical profile of *M. emarginata* is characterized by a significant accumulation of triterpene quinone methides (pristimerin, tingenone, iguesterin) and the triterpenoid β-amyrin, as evidenced by their relatively higher extraction yields from different tissues. While sesquiterpene pyridine alkaloids (emarginatines A-G, maytansine, emarginatinine) and other isolated triterpenoids (including novel oxygenated lupanes) occur in trace amounts within stem extracts, flavonoids (myricetin, quercetin, isorhamnetin) and additional triterpenes (friedelan-3-one, 29-norcycloartanol) have been identified in leaf extracts (1, 9).

M. emarginata has been extensively investigated within the scientific community, with a particular focus on elucidating its therapeutic potential. Research findings indicate that the phytoconstituents present in *M. emarginata* demonstrate a diverse spectrum of pharmacological actions, encompassing antioxidant, anti-inflammatory, antimicrobial, and antineoplastic effects. These bioactivities are predominantly correlated with the presence of phenolic compounds, flavonoids, and other secondary metabolites identified within the plant matrix (1, 4, 5, 10).

The present study aims to investigate the phytochemical composition of *M. emarginata* through phytopharmacological screening and thin-layer chromatography to identify bioactive compounds that may contribute to the development of effective plant-based therapeutic agents.

Materials and methodology

Preparation of Plant Material

The leaves of *M. emarginata* (Figure 1) were collected from Nagpur in April. Following collection, the plant material underwent a cleaning process that included the removal of deteriorated components, washing with tap water followed by distilled water, and absorption of excess moisture with blotting paper. The cleaned leaves were then subjected to shade-assisted air drying for preservation.

Figure 1: Procurement of Leaves of *M. emarginata*

Preparation of Plant Extracts

The extraction of phytochemicals, essential for isolating bioactive secondary metabolites from plant matter, was performed on *M. emarginata* leaves. Specifically, 50 g of coarsely ground, shade-dried leaves were subjected to maceration with chloroform, methanol, and water, the maceration process consisted of 48 hours for the first maceration and 24 hours for the second maceration after filtration. (Figure 2) (11). Each solvent-containing filtrate was evaporated at 50°C to yield a concentrated extract. The percentage of extractable material for each solvent system was subsequently quantified based on the dry weight of the recovered extracts.

Figure 2: Extraction of plant leaves by the maceration process

Determination of percentage yield

The percentage yield serves as a quantitative metric for evaluating the overall efficiency of the extraction procedure. This value is determined through the following formula:

$$\textbf{Percentage Yield} = \frac{Weight\ of\ Extract}{Weight\ of\ Powder\ drug\ taken} x\ 100$$

Qualitative phytochemical screening

Qualitative phytochemical screening, employing established methodologies, was performed on the extract to identify diverse classes of natural compounds. This screening identified the presence of phenolics, flavonoids, tannins, saponins, alkaloids, and proteins based on characteristic color changes and precipitation reactions observed in freshly prepared extracts (12).

Detection of alkaloids

The presence of alkaloids in plant extracts was investigated through a series of analytical steps. Initially, extracts were dissolved in dilute hydrochloric acid and filtered to remove particulate matter. The resulting filtrates were then subjected to: (a) Hager's test, where the formation of a yellow precipitate upon addition of saturated picric acid confirmed the presence of alkaloids; and (b) Wagner's test, where a reddish brown precipitate observed after the addition of Wagner's reagent served as a positive indicator for alkaloids.

Detection of Glycoside

The different types of glycosides tests are as follows,

Borntrager's test

To 2 ml of filtered hydrolysate, 3 ml of choloroform is added and shaken, choloroform layer is separated and 10% ammomia solution is added to it. Pink colour indicates presence of glycosides.

Legal's test

50 mg of extract is dissolved in pyridine, sodium nitroprusside solution is added and made alkaline using 10% NaOH. Presence of glycoside is indicated by pink colour.

Keller-kilani test

Crude extract was mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of FeCl3. The mixture was then poured into another test tube containing 2ml of concentrated H2SO4. A brown ring at the interphase indicated the presence of cardiac glycosides.

Detection of flavonoids

The qualitative chemical characterization of the extract for the estimation of flavonoids involved two distinct color-based assays.

Shinoda test

Crude extract was mixed with a few fragments of magnesium ribbon, and concentrated HCl was added dropwise. Pink scarlet colour appeared after a few minutes, which indicated the presence of flavonoids.

Detection of diterpenes

Crude extract was dissolved in 2ml of chloroform and evaporated to dryness. To this, 2ml of concentrated H_2SO_4 was added and heated for about 2 minutes. A grayish colour indicated the presence of terpenoids.

Detection of phenols

The qualitative chemical characterization of the extract for phenolic content involved two distinct color-based assays. (a) The ferric chloride test, a rapid method for detecting phenols, relies on the formation of a colored complex (bluish-black) upon interaction with ferric chloride. (b) The Folin-Ciocalteu assay, a more sensitive but less specific test for phenolic antioxidants, was employed, with the appearance of a blue-green color indicating the potential presence of phenolic compounds.

Detection of proteins

The detection of proteins in extracts was performed using the xanthoproteic test, where a yellow color formation upon the addition of concentrated nitric acid served as a positive indicator.

Detection of saponins

The presence of saponins was confirmed by foam test. Following dilution of extracts to 20 mL with distilled water, the solution was mechanically shaken for 15 minutes in a graduated cylinder. The formation and persistence of a foam layer measuring ≥ 1 cm in height served as a positive indicator for the presence of saponins.

Detection of tannins

The gelatin Test serves as a qualitative assay for the presence of tannins. The procedure entails mixing a sample extract with a 1% gelatin solution containing sodium chloride. The formation of a visible white precipitate is interpreted as a positive qualitative indication of tannin presence.

Detection of Sterols

Libermann-Burchard's test

The extract (50 mg) is dissolved in 2 ml of acetic anhydride. To this, 1 or 2 drops of concentrated sulphuric acid are added slowly along the sides of the test tube. An array of colour changes shows the presence of phytosterols (13).

Separation and Identification

Separation and Identification of phytoconstituents in the extract of *M. emarginata* by thin layer chromatography (TLC). TLC profiling serves as a preliminary step toward the isolation and characterization of these phytoconstituents. Solvent extracts underwent one-dimensional ascending TLC on pre-coated silica gel 60F254 plates (7x6 cm, Merck), manually cut. Sample application (1 µL) was performed using glass capillaries, spotted 1 cm from the base in five tracks. Chromatographic development was conducted in a twin-trough chamber pre-saturated for 20 minutes with a toluene:ethyl acetate:formic acid (5:4:1 v/v/v) mobile phase for flavonoid analysis. Compound migration was quantified by Retention factor (Rf) values. Visualization of developed chromatograms was achieved under visible light, short-wave UV (254 nm), and long-wave UV (365 nm) using a TLC viewing cabinet (Electronic India). Once the chromatogram was developed the R_f Value of the spot was calculated using the formula (14):

$$\mathbf{Rf} = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

Results

% yield of leaves extract of *M. emarginata*

Table 1 presents the percentage yield (W/W) of different solvent extracts obtained from the leaves of *M. emarginata* . Among the three extracts, the aqueous extract exhibited the highest yield at 6.03%, followed by the methanolic extract with a yield of 3.95%. The chloroform extract showed the lowest yield, amounting to 1.44%.

Table 1: % Yield of leaves extract of *M. emarginata*

Sr. No	Extracts	% Yield (W/W)
1	Chloroform	1.44%
2	Methanolic	3.95%
3	Aqueous	6.03%

Result of phytochemical screening

Phytochemical screening of medicinal plants is crucial for assessing their potential therapeutic applications and identifying the bioactive compounds responsible for their established pharmacological effects. Table 2 presents the results of a qualitative phytochemical analysis conducted on chloroform, methanolic, and aqueous extracts of *M. emarginata* leaves to determine the presence of various secondary metabolites.

Phytochemical screening revealed that alkaloids were present in the chloroform and aqueous extracts, glycosides in methanolic and aqueous extracts, and flavonoids in all extracts. Phenols, proteins, carbohydrates, sterols, and saponins were present in both methanolic and aqueous extracts. Tannins were detected only in the aqueous extract. Diterpenes were absent in all, while saponins were consistently present across all extracts.

Separation and Identification by TLC

The phytochemical constituents present in the extracts of *M. emarginata* were separated and identified using TLC. The analysis was performed using a mobile phase consisting of Toluene: Ethyl acetate: Formic acid in the ratio of 5:4:1. The TLC plates were visualized under normal light, short-wave UV, and long-wave UV to identify the number and nature of phytoconstituents based on their Rf values. As shown in Table 3, the standard flavonoid quercetin exhibited a single spot with an Rf value of 0.64, consistently observed under normal light, short UV, and long UV conditions, which served as a reference for comparison.

Table 2: Result of phytochemical screening of the leaf extract of *M. emarginata*

S. No.	Constituents	Chloroform extract	Methanolic extract	Aqueous extract
1	**Alkaloids** Wagner's Test Hager's Test	 +ve +ve	 -ve -ve	 +ve -ve
2	**Glycosides** Borntrager's Test Legal's Test Keller-kilani Test	 -ve -ve -ve	 +ve +ve +ve	 +ve +ve +ve
3	**Flavonoids** Shinoda Test	 +ve	 +ve	 +ve
4	**Diterpenes** Conc. H_2SO_4 Test	 -ve	 -ve	 -ve
5	**Phenol** Ferric Chloride Test Folin Ciocalteu Test	 -ve -ve	 -ve +ve	 +ve +ve
6	**Proteins** Xanthoproteic Test	 -ve	 +ve	 +ve
7	**Saponins** Froth Test	 +ve	 +ve	 +ve
8	**Tannins** Gelatin test	 -ve	 -ve	 +ve
9	**Sterols** Libermann-Burchard's Test	 -ve	 +ve	 +ve

+Ve = Positive, -Ve= Negative

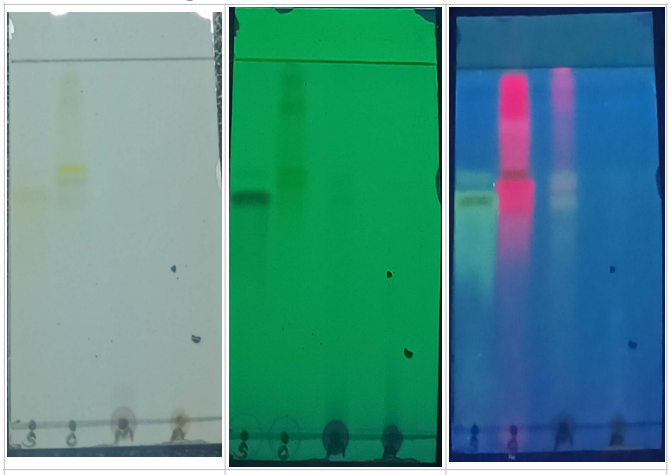
Table 3: TLC of the extract of *M. emarginata*

TLC of *M. emarginata* (Flavonoids)			
S. No.	**Mobile phase** Toluene: Ethyl acetate Formic acid (5:4:1)	**Distance of solute**	***Rf* value**
1	**(Quercetin)** Dis. travel by mobile phase= 5.0 cm No. of spot at normal light = 1 No. of spot at short UV = 1 No. of spot at long UV = 1	 Normal Light- 3.2 Short- 3.2 Long- 3.2	 Normal- 0.64 Short- 0.64 Long- 0.64
2	**(Chloroform extract)** No. of spot at normal light = 6 No. of spot at short UV = 6 No. of spot at long UV = 7	Normal Light- 3.1, 3.3, 3.5, 4.5, 4.8, 5 Short- 3.1, 3.3, 3.5, 4.4, 4.8, 5 Long- 3.1, 3.3, 3.5, 4, 4.4, 4.8	Normal- 0.62, 0.66, 0.7, 0.9, 0.96, 1 Short- 0.62, 0.66, 0.7, 0.88, 0.96, 1 Long- 0.62, 0.66, 0.7, 0.8, 0.88, 0.96
3	**(Methanolic extract)** No. of spot at normal light = 1 No. of spot at short UV = 2 No. of spot at long UV = 4	 Normal Light- 0.4 Short-3, 3.4 Long- 3, 3.5, 4.6, 5	 Normal- 0.08 Short- 0.6, 0.68 Long- 0.6, 0.7, 0.92, 1.0
4	**(Aqueous extract)** No. of spot at normal light = 0 No. of spot at short UV = 1 No. of spot at long UV = 1	 Normal Light- 0 Short- 3.4 Long- 3.4	 Normal- 0 Short- 0.68 Long- 0.68

The chloroform extract of *M. emarginata* showed a total of 6 spots under normal and short UV, and 7 under long UV, indicating the presence of multiple phytoconstituents (Figure 3). The Rf values ranged from 0.62 to 1.0, suggesting the presence of several distinct compounds. The methanolic extract exhibited 1 spot

under normal light, 2 spots under short UV, and 4 spots under long UV, with Rf values ranging from 0.08 to 1.0. This reflects a rich flavonoid and phenolic profile extractable in methanol. The aqueous extract showed no visible spots under normal light but revealed a single spot under both short and long UV light, with an Rf value of 0.68, indicating the presence of at least one UV-active compound.

Figure 3: TLC of Flavonoids



Normal light	Short UV	Long UV

1st spot= Standard Quercetin, 2nd spot= Chloroform extract, 3rd spot= Methanolic extract, 4th spot= Aqueous extract.

Discussion

An analytical study was undertaken to evaluate the phytochemical properties of *M. emarginata*. The plant's leaves were processed through dehydration and pulverization to enhance the efficiency of subsequent extraction. A series of solvents, specifically chloroform, methanol, and water, were utilized to facilitate the comprehensive extraction of the plant's phytochemical constituents. The purpose of the phytochemical analysis was to identify biologically active compounds, determine potential medicinal uses, assess overall quality, contribute to the advancement of pharmaceutical science, and optimize formulation design. Such analytical approaches are crucial for a comprehensive understanding of the therapeutic potential of medicinal flora, guaranteeing their safe and effective application and upholding rigorous quality control protocols. The phytochemical analysis of *M. emarginata* demonstrated the presence of alkaloids, flavonoids, phenolics, tannins, and saponins, compounds that have been extensively documented for their potential antidiabetic activity. TLC confirmed the diversity of these phytoconstituents, reinforcing the plant's pharmacological significance (8, 15).

Several previous investigations have highlighted the antidiabetic properties of medicinal plants rich in bioactive compounds. For instance, a study conducted by Chandak *et al*.(7) evaluated the antidiabetic effects of *M. emarginata* in alloxan-induced diabetic rats, revealing significant reductions in blood glucose levels. The phytoconstituents identified in the present study corroborate these findings, suggesting that the bioactive compounds found in *M. emarginata* may modulate metabolic pathways associated with glucose homeostasis.

Furthermore, prior ethnobotanical surveys by Kifle *et al*., (16) have documented over 1200 medicinal plants with hypoglycemic effects, emphasizing the therapeutic value of natural compounds in diabetes management. The current study aligns with these reports, reinforcing the necessity of further research into the pharmacological mechanisms of M. emarginata in glycemic regulation.

The presence of flavonoids and phenolics has been linked to antioxidant and insulin-sensitizing effects in diabetes, as documented by Alam *et al.,* (17). The study here does not provide mechanistic insights into how these compounds exert their influence. Future research should aim at isolating specific bioactive molecules and conducting detailed pharmacokinetic and clinical studies to determine their efficacy and safety.

Despite the promising findings of this study, several limitations must be acknowledged. The qualitative phytochemical analysis confirms the presence of key bioactive compounds, yet it does not quantify their concentrations, which is essential for determining their pharmacological potency. Without precise quantification, it remains challenging to assess the contribution of individual components to the observed antidiabetic effects. Future research should prioritize the isolation and characterization of bioactive compounds to identify precisely those responsible for the antidiabetic effects. Structural analysis using advanced spectroscopic techniques such as NMR and LC-MS would aid in determining the molecular identity and potential pharmacological targets of these compounds. Investigations into the mechanisms of action through in vitro enzyme inhibition assays and molecular docking studies could provide valuable insights into how *M. emarginata* influences glucose metabolism. Additionally, in vivo studies in diabetic animal models are necessary to validate its efficacy, alongside pharmacokinetic studies that assess absorption, distribution, metabolism, and excretion. Clinical trials must be undertaken to establish their safety, efficacy, and dosage parameters for human use. Furthermore, comparative studies evaluating *M. emarginata* alongside conventional treatments like Metformin could provide insight into its relative therapeutic potential. By addressing these gaps, future research can better elucidate the role of *M. emarginata* in diabetes management, paving the way for its potential integration into modern medicinal applications.

Conclusion

The present study highlights the rich phytochemical composition of *M. emarginata* and its potential as a natural antidiabetic agent. The detection of key bioactive constituents such as alkaloids, glycosides, and flavonoids, supported by TLC profiling, reinforces the therapeutic relevance of this plant in traditional medicine. These findings provide a scientific

basis for its use in diabetes management and warrant further investigation into the isolation, characterization, and pharmacological validation of its active compounds. Future studies involving in vivo models and clinical trials are essential to establish the efficacy, safety, and mechanism of action of *M. emarginata-derived* compounds in the treatment of diabetes mellitus.

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