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Phytochemical profiling and antibacterial assessment of *Mappia nimmoniana* (J. Graham) Byng & Stull

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ABSTRACT

Mappia nimmoniana (J.Graham) Byng & Stull, a medium-sized tree, is distributed in the states viz. Odisha, Maharashtra, Goa, Kerala, Assam, Tamil Nadu and Jammu & Kashmir. It is known for its therapeutic potentials including anti-cancer activity. The present study explores the phyto-chemical profiling, antimicrobial activity and DPPH radical scavenging assay of *M. nimmoniana* leaves. The study detected various phyto-chemical compounds such as tannin, saponin, terpenoid, alkaloid, steroid and reducing sugar. Further quantification of the phyto-chemical compounds revealed abundant tannin content (22.15 mg/100 g), phenol content (2.17 mg/100 g), comparatively low flavonoid content (0.458 mg/100 g) and saponin of 5.994 %. Although no significant antibacterial activity was observed against *E. coli*, the aqueous extract showed high radical scavenging activity of 54.04% at the lowest concentration of 0.125 mg ml^{-1}, which correlates with the presence of bioactive compounds in the polar solvents. As the extraction shifts towards non-polar solvents, the radical scavenging activity is reduced to half in the ethanolic extract and is negligible as it reaches the lowest polarity of n-hexane. The present study showed that *M. nimmoniana* could be a source of bioactive compounds for future drug development and also highlighted the need for sustainable extraction of plant parts for drug development works.

Key words: Phytochemical, plant collection, radical scavenging**,** secondary metabolites

INTRODUCTION

Medicinal plants play an important role in India due to their deep-rooted connection with traditional healthcare systems, cultural and religious activities. India is one of the world's richest countries in terms of plant diversity and most people inhabiting in the country still rely on plant-based remedies for primary healthcare. Ayurveda, Unani and Siddha are ancient therapeutic means for treating a wide range of ailments, from common colds to chronic diseases. India has a rich bio-resource of medicinal plants with innumerable bioactive components that are the source of many pharmacological drugs (Jedage et al., 2018). Medicinal plants in India still provide affordable and accessible healthcare, especially in rural and tribal areas, where people have good knowledge of the utilization of medicinal plants. It also contributes significantly to India's pharmaceutical and herbal industries. India is a major exporter of herbal products, raw plant materials and traditional medicines, supporting livelihoods for farmers, collectors and small-scale industries. The growing demand for natural and herbal products has further increased their commercial value. *Mappia nimmoniana* (J. Graham) Byng & Stull is a medium-sized tree with therapeutic importance and distributed in countries like Sri Lanka, China, Taiwan, South East Asia, the Philippines and India (Sharma et al., 2010). In India, it is distributed in Odisha, Maharashtra, Goa, Kerala, Assam, Tamil Nadu, Sikkim, West Bengal and Jammu & Kashmir (Das et al., 2020; Shamal et al., 2025). Reports have shown that *M. nimmoniana* have a

potential alkaloid such as camptothecin that has an anti-cancer potential (Khan et al., 2013; Shwetha et al., 2020). Further medicinal properties for the treatment of malaria, HIV and fungal and bacterial infection have been recorded (Rather et al., 2018). Due to its therapeutic potential, the plant parts such as leaves, stems, roots and seeds are exported from India to countries like the USA, Japan and Spain (Das et al., 2020). Considering its therapeutic importance, a study has been conducted to find the qualitative and quantitative phyto-chemical analysis of *M. nimmoniana* and antimicrobial activity has been carried out to validate the therapeutic claims from different sources.

MATERIALS AND METHODS

Collection of plant sample

During the field survey in Deomali Hills of Koraput, Odisha, in November 2025, leaves of *M. nimmoniana* were collected (Fig.1) and photographs were taken for taxonomic identification using available published literature (Das et al., 2020) and e-herbarium specimens (IPNI). Plant parts were also collected and herbarium specimen was prepared with the necessary field information. Standard fungicide (2% $HgCl_2$) was applied to protect the herbarium specimen from fungal attack (Jain and Rao, 1976).

Fig. 1. *Mappia nimmoniana* plant parts: a) Apical stem bearing flowers, b) fruits, c) leaves d) collection of leaves in the field

Qualitative phytochemical analysis

Preparation of extracts

5 g of the coarsely ground leaves of *M. nimmoniana* was placed in a stoppered container with different solvents (50 ml each) and allowed to stand for 24 hours. The mixture was then strained, pressed and filtered. The process was repeated for the different selected solvents (distilled water, ethanol and n-hexane). The filtrate was then used for the qualitative analysis of the bioactive components (Abubakar and Haque, 2020; Jena et al., 2024; Marndi et al., 2024).

Secondary metabolites

Test for tannins

One ml of the leaf extract was taken and three to five drops of 10 % lead acetate solution were added. The white gelatinous precipitate formation confirms the presence of tannins.

Test for saponin

One ml of the leaf extract was taken and 1 ml of distilled water was added and shaken well. The formation of persistent froth confirms the presence of saponin.

Test for flavonoids

One ml of the leaf extract was taken and two ml of 2% NaOH solution and 3 to 4 drops of diluted HCl were added. The colour initially turned to an intense yellow colour with NaOH solution and later became colourless. This change in colour confirms the presence of flavonoids.

Test for terpenoids

One ml of the leaf extract was taken and 5-6 drops of chloroform were added. It was then placed in the water bath for few minutes. Later, 5-6 drops of concentrated sulphuric acid were added. The appearance of a reddish-brown interface confirms the presence of terpenoids.

Test for phenolic groups

One ml of the leaf extract was taken and a few drops of 5% ferric chloride solution were added. The dark bluish-black appearance confirms the presence of phenolic compounds.

Test for reducing sugars

About 1 ml of the leaf extract was taken and 2 drops of Fehling's solution A followed by Fehling's solution B were added. It was then kept in the water bath for some time. The presence of red-orange precipitate confirms the presence of reducing sugar.

Test for steroids

One ml of the leaf extract was taken and 1 ml of chloroform and 1 ml of concentrated sulphuric acid were added to it. The presence of upper red and lower yellow colours with green fluorescence confirms the presence of steroids.

Test for alkaloids

One ml of the leaf extract was taken and 3 to 4 drops of Dragendorff's reagent were then added. The formation of a reddish-brown precipitate confirms the presence of alkaloids.

Quantitative phyto-chemical analysis

The quantitative analysis of the plant extracts was conducted using standard methods to assess their medicinal value.

Estimation of total tannin content

Extraction

0.5 mg of tannic acid was mixed with 1 ml of distilled water and from this solution, 5, 15, 25, 35 and 50 µl were taken in different test tubes. The volume was made to 1 ml. The 0.5 ml of Folin reagent and 2.5 ml of 20% sodium carbonate were added to each test tube. The mixed solutions were then shaken for 5 minutes in the dark conditions. The solution of each test tube was left for 40 minutes. Absorbance was then taken at 720 nm wavelength and a standard graph was plotted (Sadasivam and Manickam, 2009).

Estimation

5 mg of leaves was crushed and transferred into a 250 ml conical flask to which 75 ml of distilled water was added and boiled for 30 minutes. The whole solution was centrifuged at 2000 rpm for 20 minutes. Supernatant was taken in a 100 ml volumetric flask and made up to the volume. 1 ml of sample was taken from a 100 ml volumetric flask and 75 ml of distilled water, 5 ml of Folin reagent and 10 ml of 20% sodium carbonate were added. The volume was made up to 100 ml. After shaking for 5 minutes, the absorbance was taken at 720 nm wavelength. The amount of tannin was calculated from the tannic acid standard graph (Fig. 2).

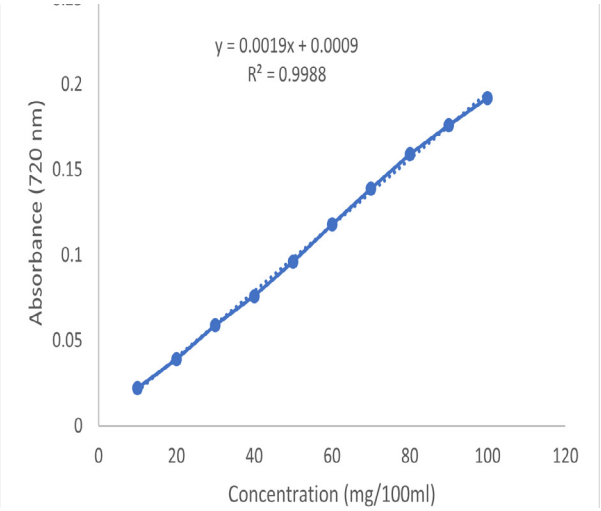

Fig. 2. Standard graph for tannic acid for the estimation of tannin

Estimation of total phenol content

Extraction

Three replicas of 0.5 g of leaves were taken and crushed with 60% methanol in a mortar and pestle. Samples were centrifuged 5 times at 5000 rpm for 20 minutes.

Estimation (in dark condition)

Seven test tubes were taken, including a blank and two test tubes for each replica. 0.1 and 0.2 ml of the sample were taken in each test tube except the blank. 60% methanol was added to each test tube to bring the volume up to 1 ml. 1 ml of 0.1 N HCl was added and allowed to stand for a few minutes. 1 ml of sodium nitrite molybdate mixture was added, shaken well and allowed to stand for a few minutes, then diluted with 5 ml of distilled water. After dilution, 2 ml of 1 N NaOH was added and allowed to stand for 20 minutes. Absorbance was taken at 515 nm wavelength. The amount of phenol present in the sample was calculated from the gallic acid standard graph (Swain and Hills, 1959; Fig. 3).

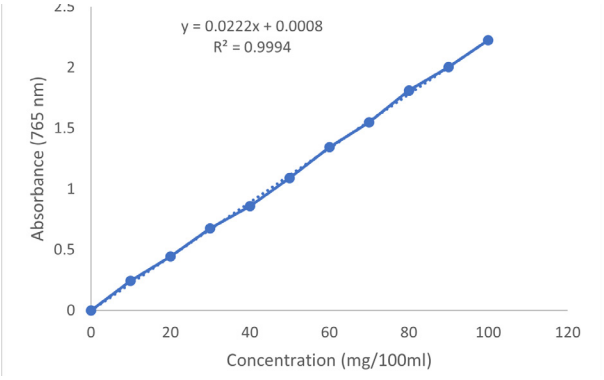

Fig. 3. Standard graph for gallic acid for the estimation of phenols

Estimation of flavonoids

Extraction

100 µl of the leaf aqueous extract was mixed with 100 µl of 5% sodium nitrite and allowed to stand for 5 minutes. Next, 150 µl of aluminium chloride was added and incubated for 6 minutes, followed by the addition of 200µl of 1 M NaOH. The total volume of the mixture was made up to 3 ml with distilled water and incubated for 15 minutes at room temperature in the dark for taking absorbance at 510 nm wavelength. The amount of flavonoid present in the sample was then calculated from the standard graph. The standard graph was prepared for quercetin (Chang et al., 2002; Shirazi et al., 2014; Raina and Misra, 2023; Fig. 4).

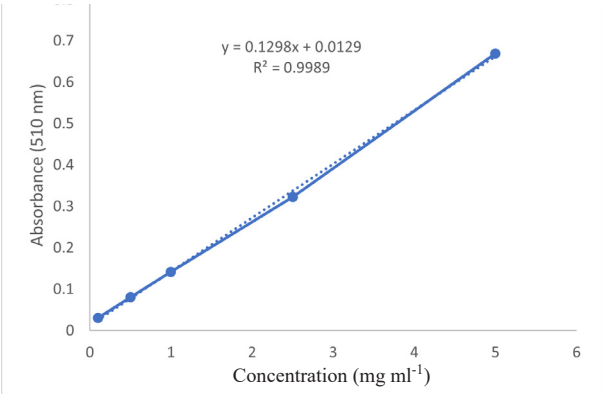

Fig. 4. Standard graph for quercetin for estimation of flavonoids

Estimation of saponin

The determination of total saponin was done by the method of Obadoni and Ochuko, (2001) with slight modifications. 5 g of crushed leaves was added to 100 ml of 20% aqueous ethanol and stirred for 30 minutes in a flask. It was heated for 4 h at 45 °C. The mixture was filtered using Whatman filter paper No. 1 and the residue was then extracted with an additional 100 ml of 25% aqueous ethanol. The combined extracts were concentrated and then transferred into a separator funnel and later extracted twice with 20 ml of diethyl ether. The ether layer was discarded, while the aqueous layer was kept and re-extracted with 30 ml n-butanol. The n-butanol extract was washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was evaporated. After evaporation, the sample was dried in the oven at 40 °C to a constant weight. The saponin content was then calculated.

Percentage (%) of saponin = (final weight of sample/initial weight of extract) x 100

Analysis for antibacterial activity

Soxhlet extraction

The Soxhlet extraction technique was used to assess antimicrobial activity. The method relies on repeated circulation of a solvent through reflux and siphoning to extract compounds from a solid sample. Dried leaf material of *M. nimmoniana* was placed in a thimble within the Soxhlet apparatus and a pure solvent was heated in a flask. The solvent vapour condensed, percolated through the sample and dissolved the phytochemicals. Once the solvent reached the siphon level, it returned to the flask and the cycle was repeated for 6-7 hours. The extract was then concentrated using a rotary evaporator to obtain the final plant extract (Suwari et al., 2017).

Disc diffusion assay

Disc Diffusion assay of the leaf extracts of *M. nimmoniana* was carried out according to the National Committee for Clinical Laboratory Standards (NCCLS). Inoculum was spread on the sterile nutrient agar plates with the sterile swabs of *E. coli* suspension. Different concentrations of the leaf extracts (6.25 mg ml^{-1}, 25mg ml^{-1}, 50mg ml^{-1} and 100mg ml^{-1}) were prepared using the different solvents. Whatman filter paper No. 1 was cut into 6mm disc pieces and allowed to suspend in the different concentrations of the

different leaf extracts for about 5 hours. Filter paper discs suspended in different concentrations of the solvent extracts were then placed on the nutrient agar plate with *E. coli* using a sterile forceps on the petri-plates with approximately equal distance. It was then placed for incubation at 37ºC for 24 hours. Ampicillin disc suspended in a 5mg ml^{-1} ampicillin solution was also placed as a positive control. Three replicates were carried out with each test organism. (Owusu et al., 2021).

Broth dilution assay (minimum inhibitory concentration)

Serial dilutions were made from the selected solvents of the leaf extract or stock solution (100 mg ml^{-1}). Different concentrations of the leaf extract (100 mg ml^{-1}, 50 mg ml^{-1}, 25 mg ml^{-1}, 12.5 mg ml^{-1} and 6.25 mg ml^{-1}) were prepared from the stock solution of the different solvents of leaf extracts, respectively. 100 µl of the bacterial inoculum and 500 µl of the leaf extract were added to the sterile nutrient broth. This incubation was carried out for different concentrations of the leaf extracts for the selected solvents. For positive control, inoculum was taken in sterile nutrient broth and as a negative control, sterile nutrient broth was taken and all were incubated at 37ºC for 24 hours. After 24 hours, turbidity was checked, compared and recorded (Owusu et al., 2021).

Analysis for antioxidant activity

Maceration method for plant extraction

1g of dried leaves was ground and placed in stoppered containers with 10 ml of solvent and was allowed to stand for 48 hours with occasional stirring. The reduced mixture was then strained and pressed. The process was followed with the two different selected solvents (distilled water and ethanol). It was then filtered. The filtrate was then dried in a hot-air oven to obtain crude extract and was diluted in 1% Dimethyl Sulfoxide to obtain the working extract. The technique (Abubakar and Haque, 2020) was followed with slight modifications.

Preparation of DPPH solution (0.1 mM)

2,2-Diphenyl-1-picrylhydrazyl (DPPH) stock solution was prepared by dissolving 3.94 mg of DPPH in 100 ml of methanol. A 0.1 mM working solution was then prepared by diluting 3 ml of the stock solution to 50 ml with methanol in a volumetric flask. The solution was wrapped in aluminium foil to protect it from light (Baliyan et al., 2022).

DPPH radical scavenging assay

Different concentrations of the leaf extracts were prepared in their respective solvents. DPPH solution was mixed with the extracts in a 1:1 ratio. Sample blanks containing extract and solvent (without DPPH) were used for background absorbance correction. 0.1 mM DPPH solution was used as a control, taking methanol as a blank. All the reaction mixtures were incubated in the dark at room temperature for 10 minutes and the absorbance was taken at 517 nm using a UV-Visible Spectrophotometer. The percentage of radical scavenging activity was calculated using the formula mentioned below. A percentage inhibition versus concentration graph was plotted using the resultant data for comparison and quercetin was taken as a standard (Majhi et al., 2026).

$$\% \text{ Inhibition} = \frac{\text{Absorbance}_{(control)} - \text{Absorbance}_{(sample)}}{\text{Absorbance}_{(control)}} \times 100$$

RESULTS AND DISCUSSION

Qualitative phytochemical analysis

The qualitative phytochemical screening of the leaf extract of *M. nimmoniana* revealed notable variations in the presence of bioactive compounds (Table 1). The aqueous extract showed the presence of tannins, saponins, terpenoids and alkaloids. The presence of saponins and terpenoids suggests that some moderately polar constituents are also soluble in aqueous medium. Ethanol extract exhibited saponins, terpenoids, reducing sugars and steroids. The presence of steroids, along with reducing sugars, indicates that ethanol is particularly efficient in extracting a diverse spectrum of bioactive constituents. The n-hexane extract, however,

showed only the presence of reducing sugars. Most bioactive compounds present in the leaf extract of *M. nimmoniana* are polar to moderately polar in nature. Camptothecin (CPT) is a well-known alkaloid obtained from *M. nimmoniana* plant parts that has been used as an anticancer agent (Ankad et al., 2015; Shamal et al., 2025). The compound is known to be present in leaves, stems, bark, roots and seeds. Other phytochemical compounds such as 9-methoxy camptothecin, mappicine, 9-amino camptothecin, topotecan, irinotecin and SN 38 are also reported from this plant (Nazeerullah et al., 2012; Khan et al., 2013; Godbole et al., 2023).

Table 1. Qualitative phytochemical analysis of *M. nimmoniana* leaves

Solvent used	Phytochemicals present
Aqueous extract	Tannin, saponin, terpenoid and alkaloids
Ethanol extract	Saponin, terpenoid, reducing sugar and steroid
n-hexane extract	Reducing sugar

Quantitative phytochemical analysis

The quantitative phytochemical analysis revealed the variation in the concentration of different bioactive compounds present in the leaf extract of *M. nimmoniana*. The tannin content was found to be the most abundant (22.15 mg/100 g) concentration. This high level of tannin correlates with a potential for antioxidant and antimicrobial activities, as tannins are known for their ability to precipitate proteins and inhibit microbial growth.

Table 2. Quantitative phytochemical analysis of *M. nimmoniana* leaves

Phytochemical compounds	Concentration
Tannin	22.15 mg/100 g
Phenol	2.171 mg/100 g
Flavonoid	0.458 mg/100 g
Saponin	5.994 %

The content of phenolic compounds was 2.171 mg/100 g, as recorded. Phenols also play a crucial role in antioxidant activity by scavenging free radicals and preventing oxidative stress. Flavonoids were present in significantly lower concentration (0.458 mg/100 g). Saponin content was detected with 5.994%, which demonstrates a considerable proportion in relation to other phyto-chemicals (Table 2). Saponins are also known for their surfactant properties and exhibit a wide range of biological activities, including antimicrobial, anti-inflammatory and immune-modulatory effects.

Antibacterial activity

The ethanolic leaf extract of *M. nimmoniana* demonstrated good antibacterial activity as compared to the aqueous and n-hexane extract against *E. coli.* The ethanolic extract of 100 mg ml^{-1} concentration recorded the highest zone of inhibition ZI (20 ± 0.0), followed by the concentration of 50 mg ml^{-1} with ZI (19 ± 0.2). The least or negligible zone of inhibition was recorded by the n-hexane extract. The Minimum Inhibitory Concentration (MIC) was observed at 100 mg ml^{-1} (Table 3). Earlier studies have revealed the antibacterial activity against *Xanthomonas campestris* and *Aeromonas hydrophila,* which indirectly correlated to the therapeutic potential of diarrhoea and dysentery (Britto et al., 2014). Further, reports of antibacterial activity have been reported against *E. coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and antifungal activity against *Candida albicans* (Shrivastava et al., 2023). Shrivastava and his team

in 2023 also reported that the methanol leaves extract of *M. Nimmoniana* shows good antibacterial activity against *Escherichia coli* (21 ± 0.0) mm and *Klebsiella pneumoniae* (23 ± 0.0) mm.

Table 3. Zone of inhibition produced by different solvents extracts of *M. nimmoniana* against *E. coli*

Test Samples	Zone of inhibition (mm)		
	n-hexane	Ethanol	Aqueous
100 mg ml^{-1}	7 ± 0.0	20 ± 0.0	15 ± 0.2
50 mg ml^{-1}	7 ± 0.1	19 ± 0.2	9 ± 0.0
25 mg ml^{-1}	7 ± 0.0	16 ± 0.1	8 ± 0.0
6.25 mg ml^{-1}	7 ± 0.0	15 ± 0.0	8 ± 0.0
Standard (Kanamycin) 5 mg ml^{-1}	30 ± 0.0	30 ± 0.0	30 ± 0.0

Antioxidant activity

The antioxidant activity of *M. nimmoniana* leaf extracts showed visible differences between the two tested solvents that were selected on the basis of the phytochemical screening results. The aqueous extract showed higher radical scavenging activity as compared to the ethanolic extract. At the lowest concentration of 0.125 mg ml^{-1}, the aqueous extract showed 54.04% inhibition, which is more than double that of the ethanolic extract (25.59%). This trend was observed with increasing concentration, reaching 58.26% for the aqueous extract and 39.42% for the ethanolic extract at 1 mg ml^{-1} (Fig. 5). The stronger activity of the aqueous extract can be linked to the presence of polar bioactive compounds such as the trigonelline (alkaloid) and pumiloside (monoterpene glycoside), both of which are water soluble and possess active radical scavenging potential (Khan et al., 2013). Presence of other compounds, many of which have significant cytotoxic activity but moderate antioxidant potency, like gypsogenin and enoxolone of the terpenoid class, camptoth ec in derivatives (alkaloid) and steroids like stigmast-5-en-3-β, 7-α-diol and sitosteryl-β-D-glucoside, may contribute to the radical scavenging activity observed in the ethanolic extract (Godhaniya and Ganatra, 2021). On the other hand, the standard quercetin showed very high radical scavenging activity even at much lower concentrations. At 8.33 µg ml^{-1}, it already showed 96.85% inhibition, increasing only slightly to 97.5% at 66.67 µg ml^{-1}. This indicates the potent antioxidant capacity of quercetin as a pure bioactive compound (Qi et al., 2022), far exceeding that of the crude plant extracts. The minimal increase across concentrations also shows a near-saturation effect, where quercetin achieves maximal radical scavenging efficiency even at the lowest dose.

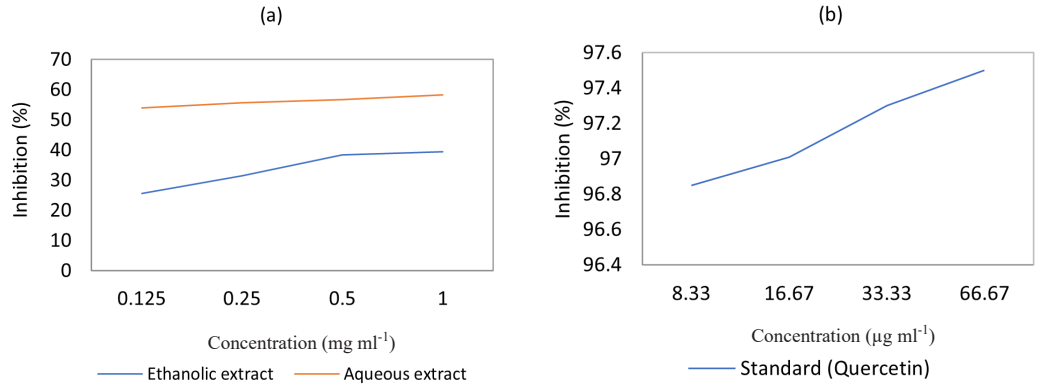

Fig. 5 (a, b). Percentage inhibition against concentrations showing DPPH free radical scavenging activity of (a) aqueous and ethanolic leaf extracts of *Mappia nimmoniana* and (b) quercetin standard

CONCLUSION

The present study demonstrates that the bioactive compounds soluble in the polar solvents show various bioactivities, including antibacterial and antioxidant activity. The study is supported by the presence of secondary metabolites such as tannin, saponin, terpenoids and alkaloids. The abundant presence of alkaloids is also linked to the antioxidant activity and may possess anti-cancer potential. Present study may help to identify bioactive compounds and for future drug development, keeping in view the sustainable extraction of plant parts and conservation of *M. nimmoniana.*

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