

RESEARCH PAPER

Comparative Anti-oxidant Activity of Alkaloidal, Flavonoidal and Total Alcoholic Extracts of *Murraya paniculata* (L.) Jack

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ABSTRACT

Background: *Murraya paniculata* (L.) Jack (*Rutaceae*) is an ornamental and ethnomedicinal shrub and traditionally used as anti-inflammatory, antimicrobial and tonic medicinal uses etc., are attributed to its constituents, which includes alkaloids, flavonoids and other phytoconstituents.**Objective:** In this study the in vitro antioxidant activity of three phytochemical fractions of *M. paniculata* such as crude alkaloidal fraction (M1), crude flavonoidal fraction (M2) and total alcoholic fraction (M3) was measured and compared to the standard Ascorbic acid using DPPH radical-scavenging assay.**Methods:** Shade dried leaf and stem material was defatted with petroleum ether and acid-base partitioning was done for alkaloid rich extract (M1); acid hydrolysis followed by solvent partitioning was used for the flavonoid rich extract (M2) and 72 h cold maceration with ethanol was used for total alcoholic extract (M3). Then, the absorbance was measured at 517 nm after all the fractions (1-1000 µg/mL) were mixed with 0.1 mM DPPH and incubated for 30 min. The activity of radical scavenging was shown by all the fractions in a concentration dependent manner with good curve fitting ($R^2 \geq 0.986$).**Results:** The test fractions exhibited highest activity for M1 (IC 50 127.7 ± 0.02 µg/mL) followed by M2 (160.4 ± 0.03 µg/mL) and M3 (199 ± 0.04 µg/mL).**Conclusion:** The results of the present study demonstrate that the antioxidant potential of *M. paniculata* is moderate and fraction dependent with the highest potential in the alkaloidal fraction, which needs to be further bioassay guided isolated and investigated in vivo.**Keywords:** *Murraya paniculata*, Phytoconstituents, Ascorbic acid, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH), IC50**How to cite this article:** Singh UK, Shukla P, Shukla P, Bajpai V, Singh G, Mukherjee A. Comparative Anti-oxidant Activity of Alkaloidal, Flavonoidal and Total Alcoholic Extracts of *Murraya paniculata* (L.) Jack. *Int J Drug Deliv Technol.* 2026;16(81s): 1043-1049; DOI: 10.25258/ijddt.16.81s.133

INTRODUCTION

Superoxide anion, hydroxyl radical and hydrogen peroxide and other reactive oxygen species (ROS) are continually generated as byproducts of normal aerobically metabolising cells. Physiologically these species can be regulated by a complex and strategic network of enzymic (superoxide dismutase, catalase, glutathione peroxidase) and non-enzymic (ascorbic acid, tocopherols, glutathione, polyphenols from plants) antioxidant systems. In the presence of more ROS production than this defense capacity, oxidative stress occurs which damages lipids, proteins and nucleic acids and is now known to be a factor that contributes to aging and to the pathogenesis of atherosclerosis, diabetes mellitus, neurodegenerative diseases and carcinogenesis and other pathological conditions, as shown in figure 1. Therefore, the development of safe antioxidants of plant origin which can be used to boost body defense is still underway, in pharmacognostical and pharmacological studies [1, 2].

Murraya paniculata (L.) Jack (*Rutaceae*), also known as orange jasmine, kamini or mock orange, is a widely cultivated evergreen shrub used as an ornamental hedge

plant in South and Southeast Asian countries, and traditionally as a medicine [3]. Traditionally, various parts of these plants are used for their curative role in pain, diarrhoea, inflammatory and infective diseases and *Murraya* is documented to be rich sources of alkaloids, coumarins and flavonoids, some of which showed free-radical scavenging, anti-inflammatory and antimicrobial activity when used alone. Crude plant extracts are complex compound mixtures; the whole antioxidant activity of a plant could vary significantly depending on the class of phytoconstituents the plant extracts specifically [4].

Hence, the present study was conducted to prepare the three chemically different extracts of *M. paniculata* leaves and stem like crude alkaloidal (M1), crude flavonoidal (M2) and total alcoholic (M3) extract and compare their free radical scavenging activity in vitro with the help of well-established DPPH method with ascorbic acid as standard drug. A comparative approach was chosen because it enabled throwing up the main phytochemical class of the plant mainly responsible for its antioxidant activity with the subsequent bioassay guided isolation work [5].

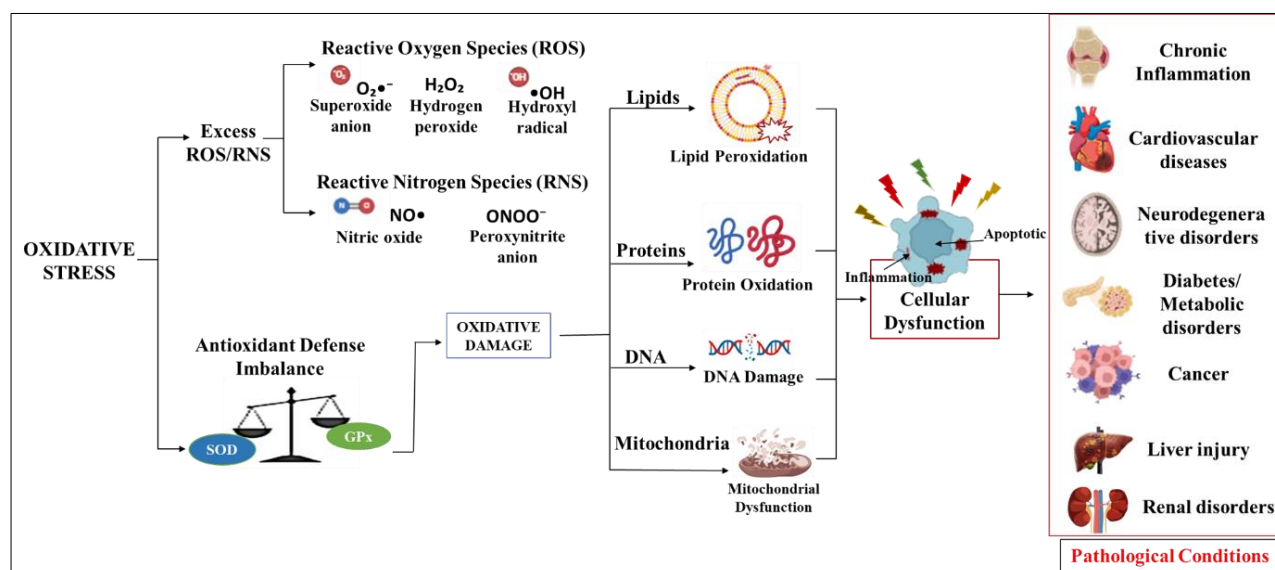


Figure 1: Oxidative stress: mechanisms and its effects

MATERIALS AND METHODS

Collection or authentication

The fresh healthy leaves and stems of *Murraya paniculata* (L.) were collected in the early morning hours (to avoid loss of thermolabile phytoconstituents) from the Herbal Garden of Paramedical Campus, UPUMS, Saifai, Etawah, Uttar Pradesh, India. The collected material was authenticated by Prof. (Dr.) Rajvir Singh, Head, Department of Botany, K.K.P.G College, Etawah, Uttar Pradesh, India. The authenticated leaves and stems were washed thoroughly with running tap water to wash off the adhering dust, soil. Leaves and stem were then shade dried at room temperature away from direct light and heat to prevent degradation of heat sensitive phytoconstituents. The dried leaves and stems were separately ground to coarse powder in mechanical grinding machine. The powder of the leaves and the stem was labelled and kept separately in cool and dry containers for further processing for extraction and phytochemical studies [6].

Chemicals and reagents

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from SRL Chemicals (SRL Chem, Cat. No. SR-29128). Ascorbic acid was bought from SRL, Cat. B.1501. No. 23006). A sample assay of methanol as solvent was made from the samples purchased from Cat. SD Fine-Chem. No. 109301C250). The extraction solvents, petroleum ether, chloroform, ethanol, ethyl acetate, n-amyl alcohol, dilute hydrochloric acid and dilute ammonia solution were analytical grade.

Extraction of crude alkaloid fraction (M1)

Coarsely powdered leaf and stem material was accurately weighed and defatted separately by continuous hot extraction using petroleum ether to remove the non-polar constituents such as fat, waxes and chlorophyll pigments. The defatted marc was air dried to remove residual solvent. The defatted material was basified to pH 8-9 with ammonia solution to convert naturally occurring alkaloidal salts to their free-base form, re-extracted by Soxhlet with

chloroform to selectively extract the free alkaloid bases. The chloroform extract was partitioned with acidified water (alkaloidal and polar components), This acid wash operation was done for three times so as to be sure of the complete transfer of the alkaloids. The acidic aqueous phase was combined and re-made basic with dilute ammonia (pH 9-10) and the free lipophilic alkaloid bases were re-extracted with chloroform. The pooled fractions of chloroform were dried and concentrated to yield the crude alkaloid fraction (M1) which was checked for the presence of alkaloids by Mayer's reagent, Hager's, Wagner's reagent, Dragendorff's reagent [6].

Crude flavonoidal fraction extraction (M2)

Coarsely powdered leaf and stem material was defatted with petroleum ether using continuous hot Soxhlet extraction, then the defatted marc was air dried. The defatted product was then gently and periodically (heated with stirring every 15 min) with distilled water and dilute hydrochloric acid then kept for 24 hours at room temperature to release the less polar aglycones of the flavonoids present in the plant. The cooled hydrolysate was then filtered. The filtrate was extracted thrice with ethyl acetate which was used for extracting the less polar flavonoid aglycones and the combined portions of ethyl acetate. The aqueous layer was re-extracted three times with n-amyl alcohol to extract the more polar flavonoid glycosides. Aqueous layer was discarded. Both fractions were dried and concentrated to yield the crude flavonoid fraction (M2). After that both fractions were subjected for identification test for flavonoids (Shinoda test, alkaline reagent test and the sulfuric acid test) [6, 7].

Total alcoholic extract (M3)

A portion of each part of the plant was taken and put in a sufficient amount of ethanol for cold maceration, in a closed container, for 72 h to ensure complete diffusion of soluble constituents from the plant material into the menstrum. The macerate was filtered through Whatman filter paper, marc was pressed and washed with fresh

ethanol. The washings were added to the main filtrate. The fraction of was dried and concentrated to yield the crude total alcoholic fraction (M3) [8].

In-vitro antioxidant activity

The free radical scavenging activity of alkaloidal (M1), flavonoidal (M2), total alcoholic (M3) extracts was determined through DPPH (2,2-diphenyl-1-picrylhydrazyl) assay with respect to ascorbic acid as reference compound. The dilution of the test sample or standard was prepared as a stock solution and 10 μ L of stock solution was transferred into 0.2 mL of 0.1 mM DPPH solution prepared in methanol in a 96 well microplate. Reactions were set up quadruplicate using 0.2 mL DPPH and 10 μ L of sample/standard dilution and duplicate blanks where no sample/standard was used. Wells without treatment and containing deionised water served as control, Wells without DPPH reagent served as blank. The plates were incubated in dark for 30 min and the absorbance was taken at 517 nm on an ELISA plate reader (ALTA ADX-150) [9, 10].

Activities of scavenging were expressed as percent inhibition over control and the %RSA (% Radical Scavenging Activity) is calculated for each test sample using the following equation:

$$\%RSA = (Abs_{Control} - Abs_{Sample}) / Abs_{Control} \times 100$$

Where, $Abs_{Control}$ is the absorbance for the control, Abs_{Sample} is the absorbance for the test sample/standard (both blank corrected)

The concentration of each sample that produced 50% inhibition in the reactions, expressed as the 50% inhibitory concentration or IC₅₀, was calculated by curve-fitting and plotting the dose-response curve of each sample (sample concentration on the X-axis, percent inhibition on the Y-axis) using non-linear regression (log(inhibitor)) versus normalized response variable slope) in GraphPad Prism 6. Since ascorbic acid was found to be more potent, it was tested over a lower concentration range (0–50 μ g/mL). The three extracts of plants with unknown potency were then tested over a wider range of concentrations (0–1000 μ g/mL) [11].

Statistical analysis

Tests were repeated four times and expressed as the mean and the standard error of the mean (SEM). IC₅₀ values with 95% confidence intervals were computed by non-linear regression, as above. One way ANOVA was used for analyzing the concentration dependence of the scavenging response of each sample, and a p value less than 0.05 was deemed statistically significant [12].

RESULTS

Preliminary Phytochemical Screening

Alkaloidal fraction (M1) was found positive to all alkaloid confirmatory tests performed (Mayer's, Wagner's, Hager's and Dragendorff's tests), confirming the presence of alkaloids. The flavonoids confirmatory tests carried out for flavonoid fractions (M2), included the Shinoda test, alkaline reagent test and sulfuric acid test was positive in all three tests suggesting presence of flavonoids, as shown in Table 1 and Figure 2.

Table 1 Phytochemical screening of the alkaloidal (M1) and the flavonoidal (M2) fractions

Fraction	Confirmatory test	Result
M1- (Alkaloidal Extract)	Mayer's test	Positive
	Wagner's test	Positive
	Hager's test	Positive
	Dragendorff's test	Positive
M2- (flavonoidal Extract)	Shinoda test	Positive
	Alkaline reagent test	Positive
	Sulfuric acid test	Positive

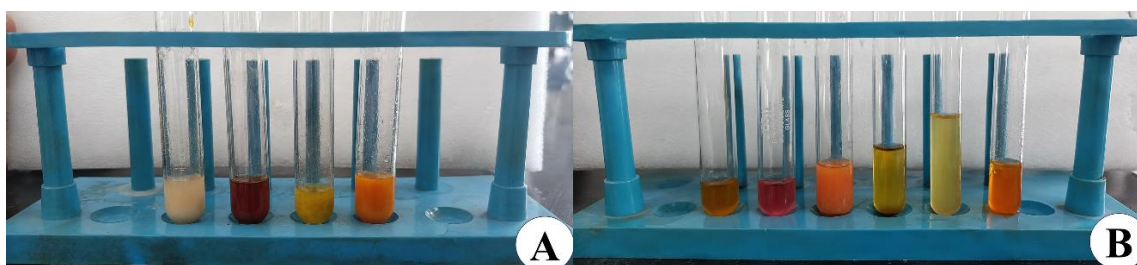


Figure 2: Qualitative Phytochemical Screening of Alkaloidal (A) and Flavonoidal Extract (B)

DPPH radical scavenging activities

In all the three extracts of *M. paniculata*, it was observed that there was a progressive fade of the characteristic purple colour of DPPH from the background into pale yellow in the presence of increasing concentration of the

sample throughout the microplate (Figure 3), indicating an increasing concentration dependent decolourisation of DPPH. The percentage inhibition (Mean $\pm$ SEM, n = 4) at each concentration tested is presented in Tables 1-4 and dose-response is presented in Figures 4-7.

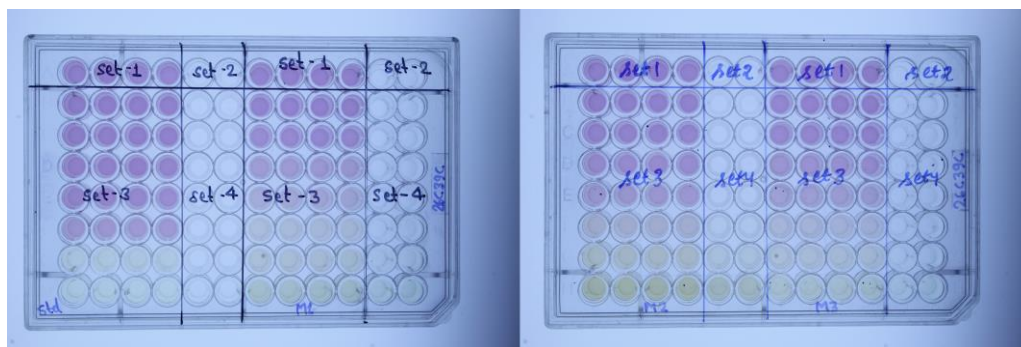


Figure 3: Decolourisation of DPPH assay in 96-well microplate at different concentrations for ascorbic acid standard and M1 and M2 and M3

Table 1. The Ascorbic Acid (Standard) exhibited the percentage inhibition of DPPH at various concentrations (Mean $\pm$ SEM, n = 4)

Concentration ($\mu\text{g/mL}$)	% Inhibition (Mean $\pm$ SEM)
0	0.00 $\pm$ 0.48
0.78	6.53 $\pm$ 0.43
1.56	12.06 $\pm$ 0.49
3.13	16.86 $\pm$ 1.25
6.25	27.38 $\pm$ 0.81
12.5	38.91 $\pm$ 0.63
25	58.96 $\pm$ 0.29
50	70.29 $\pm$ 0.19

Table 2. DPPH inhibition of M1 – Alkaloidal extract (mean $\pm$ SEM, n = 4, n = number of replicate values)

Concentration ($\mu\text{g/mL}$)	% Inhibition (Mean $\pm$ SEM)
0	0.00 $\pm$ 0.88
1	2.37 $\pm$ 0.44
10	11.33 $\pm$ 1.07
50	25.50 $\pm$ 0.58
100	45.71 $\pm$ 0.75
250	66.69 $\pm$ 1.14
500	79.33 $\pm$ 0.57
1000	84.38 $\pm$ 0.81

Table 3. M2 – Flavonoidal extract was used to determine percentage inhibition of DPPH at various concentrations (mean $\pm$ SEM, n = 4)

Concentration ($\mu\text{g/mL}$)	% Inhibition (Mean $\pm$ SEM)
0	0.00 $\pm$ 1.16
1	0.08 $\pm$ 1.10
10	5.58 $\pm$ 0.75
50	23.73 $\pm$ 1.09
100	32.68 $\pm$ 0.79
250	61.25 $\pm$ 1.09
500	80.13 $\pm$ 0.93
1000	95.48 $\pm$ 0.59

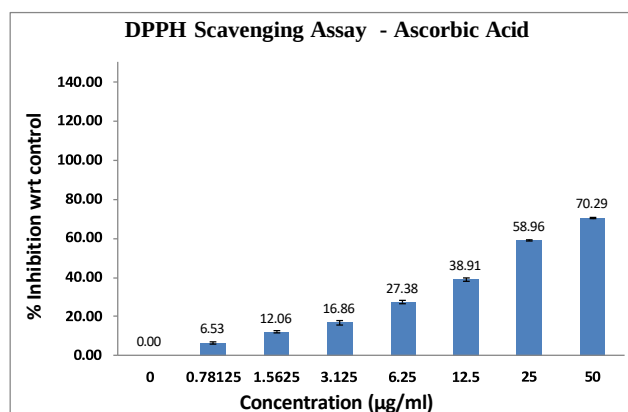


Figure 4: Dose–response curve for DPPH scavenging by ascorbic acid (standard)

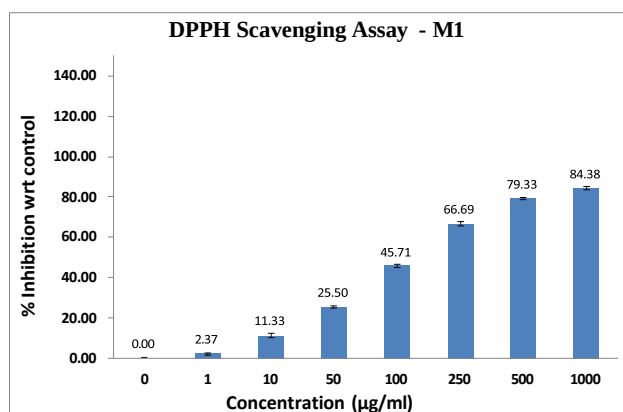


Figure 5: Dose–response curve for DPPH scavenging by the alkaloidal extract (M1)

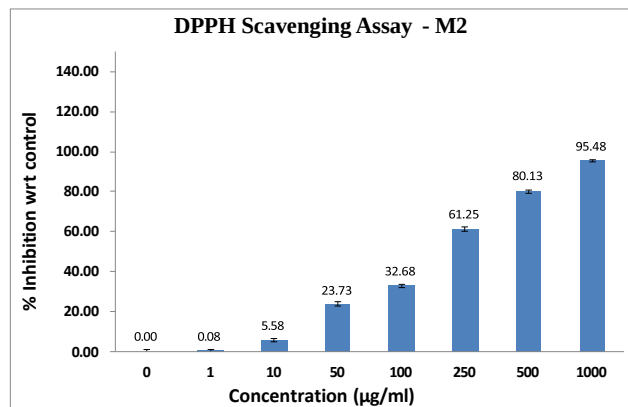


Figure 6: Dose–response curve for DPPH scavenging by the flavonoidal extract (M2)

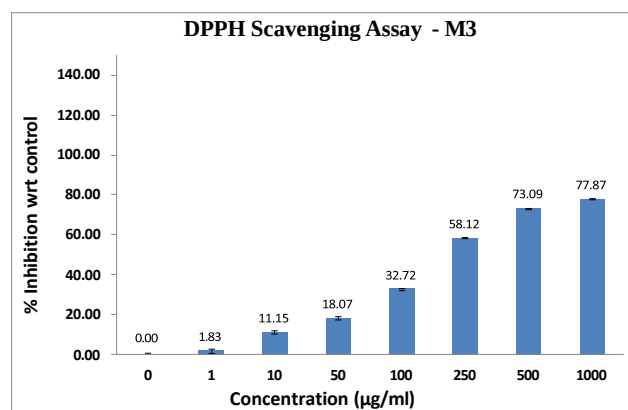


Figure 7: Dose–response curve for DPPH scavenging by the total alcoholic extract (M3)

Table 5. Summary of IC₅₀ values (µg/mL, mean ± SEM) and one-way ANOVA of the DPPH radical scavenging assay.

Sample	IC ₅₀ (µg/mL)	95% CI	R ²	ANOVA F (df 7,24)	p-value
Ascorbic Acid (Standard)	18.53 ± 0.01	16.51 – 20.78	0.9953	1520.93	< 0.0001
M1 – Alkaloidal Extract	127.7 ± 0.02	107.5 – 151.7	0.9941	1796.40	< 0.0001
M2 – Flavonoidal Extract	160.4 ± 0.03	133.0 – 193.5	0.9920	1527.54	< 0.0001
M3 – Total Alcoholic Extract	199 ± 0.04	156.9 – 252.3	0.9864	2035.12	< 0.0001

The alkaloidal fraction (M1) is the most active DPPH radical scavenger with the lowest IC₅₀ value (127.7 ± 0.02 µg/mL) as compared to the total alcoholic extract (M3) of IC₅₀ value (199 ± 0.04 µg/mL) and the flavonoidal extract (M2) of IC₅₀ value (160.4 ± 0.03 µg/mL). To achieve the same scavenging activity, 127.7, 160.4 and 199 µg of M1, M2 and M3 respectively were needed compared to merely 18.53 µg of ascorbic acid. This indicated that the extracted fractions had activity, although less potent than that of the purified standard ascorbic acid. The scavenging response was highly predictable by all curves that showed a very good linear fit (R² ≥ 0.986) and the responses to increasing concentration were significantly greater for all samples tested as shown by the one way-ANOVA (p < 0.0001 for all samples).

DISCUSSION

The DPPH assay is one of the most commonly used and repeatable in vitro assays used to quickly screen plant derived antioxidants. Based on the ability of a hydrogen or electron donating antioxidant to diminish the purple free radical DPPH, with a spectrophotometric measurement of the absorption loss proportional to the concentration of hydrogen or electron donating antioxidants. All three extracts showed significant dose dependent radical scavenging activity in the present investigation indicating that the plant has real free-radical scavenging activity and not an artefact of the test, as evidenced by the consistently high goodness of fit (R² ≥ 0.986) of the dose-response curve and the very high one-way ANOVA values (p < 0.0001) obtained for each one.

The alkaloidal fraction (M1; IC₅₀ 127.7 µg/mL) exhibited relatively higher potency as compared to the flavonoidal fraction (M2; IC₅₀ 160.4 µg/mL) and the total alcoholic extract (M3; IC₅₀ 199 µg/mL), suggesting that the antioxidant activity of *M. paniculata* under the extraction and assay conditions used here would largely be attributed to its alkaloidal constituents. This is an unexpected property as flavonoids are generally known for their potent DPPH scavenging activity probably because of possessing several phenolic hydroxyl groups. This discovery opens the possibility that some particular carbazole alkaloids characteristic of the genus *Murraya*, or synergy occurred among minor components in the same alkaloid fraction, may cause the greater activity noted. This is consistent with a simple dilution effect that the total alcoholic extract (M3) contains less amount of active alkaloidal and flavonoidal extract as compared to the two purified extracts, which are also present in lower concentration in the crude alcoholic extract (M3) but are inactive or less active.

As might be expected, ascorbic acid, which is a small purified compound and is very potent at a high level of activity - was significantly more active than any of the plant fractions (IC₅₀ 18.53 µg/mL vs 127.7, 160.4, 199 µg/mL for M1, M2, M3 respectively). This makes sense because a crude or semi-purified plant extract, has a lot of inactive material in it, which dampens down the activity or active constituent(s). This is similar to the reported IC₅₀ comparisons of purified standards with crude botanical extracts elsewhere. Overall, the present data support the fact that there is potential for the traditional use of *M. paniculata*, and the alkaloidal fraction appears to be the most interesting to pursue for bioassay-guided fractionation to identify a specific antioxidant principle that is responsible for this activity.

CONCLUSION

Murraya Paniculata (L.) Jack extracts were used in the study. Alkaloidal, flavonoidal, total alcoholic extracts of *Murraya paniculata* (L.) Jack were used in the study and exhibited concentration dependent and statistically significant DPPH radical scavenging activity; this demonstrates that the plant has in vitro antioxidant activity. The alkaloidal fraction (M1) appeared to be the most effective with IC₅₀ of 127.7 ± 0.02 µg/mL compared with the IC₅₀ of the ascorbic acid standard (18.53 ± 0.01 µg/mL), the flavonoidal fraction (M2) with IC₅₀ 160.4 ± 0.03 µg/mL, and the total alcoholic extract (M3) with IC₅₀ 199 ± 0.04 µg/mL. The obtained results are in accordance with its traditional usage and indicate that *M. paniculata* may serve as a good tool for future phytochemical and pharmacological studies involving the use of its alkaloidal content, which may serve as natural anti-oxidant agents.

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