# Phytochemical, Antioxidant and Antimicrobial Properties of *Mirabilis jalapa*

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**DOI: https://doi.org/10.51584/IJRIAS.2024.907042**

**Received: 21 June 2024; Revised: 02 July 2024; Accepted: 23 July 2024; Published: 14 August 2024**

## ABSTRACT

**Background and Aim**: The phytochemical screening for the detection of plant secondary metabolites as well as the antimicrobial and antioxidant properties of the plant were evaluated in this study using standard chemical and instrumental methods. This study aims to determine the chemical constituents, antioxidant, and antimicrobial properties of *Mirabilis jalapa* plant.

**Experimental Procedure**: The phytochemical screening for the detection of plant secondary metabolites as well as the antimicrobial and antioxidant properties of the plant were evaluated using standard chemical and instrumental methods.

**Results**: A total of twenty-four (24) chemical compounds were identified in GC-MS analysis, among these the highest percentage composition was 9-octadecenamide (46.89%) followed by cholest-5-ene-3,16, 22, 26-tetrol (8.49%), octadecanoic acid (8.39%), hexadecanamide (7.14%), 8,11-octadecadienoic acid, methyl ester (6.41%) and hexadecanoic acid (6.40%). The antimicrobial activity of the various extracts on six selected microorganisms showed that the methanolic root extracts inhibited *E. coli* (18.5 mm) and *S. aureus* (18 mm) at higher inhibition zones which is more than the standard drugs. The total antioxidant capacity and strength of the plant extract revealed that the leaves is a powerful antioxidant.

**Conclusion:** The results of the study support folklore claims in treating infection and inflammation. It also revealed the immense potential of the plant for further research that aims at identifying the bioactive components responsible for the anti-oxidant and antimicrobial activity. The phytomedicinal attributes of the *M. jalapa* plant can be used for the formulation of antimicrobial and antioxidant drugs.

**Keywords:** Flavonoids, DPPH, $ABTS^{+}$, polyphenol, 9-octadecenamide, Hydroxyl radical.

## INTRODUCTION

The World Health Organization (W.H.O.) estimates that about 80% of people in emerging economies get their main healthcare from plants (1,2). Herbal medicine is the use of plants to treat disease and enhance

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume IX Issue VII July 2024

general health and well-being. Herbs can interact with other pharmaceutical medications and should be taken with care. Plant-based products used to treat diseases or maintain health are natural, botanical, or phytomedicines. A product made from plant sources and used only for internal use is called an herbal supplement (3,4). *Mirabilis jalapa* (MJ) is a member of *Nyctaginaceae* and is a widely utilized herb in various traditional remedies across the globe, commonly employed to remedy diverse illnesses (5,6). Interestingly, a protein extracted from *M. jalapa* root tubers was recently found to possess antiviral properties (7,8). *M. jalapa* (MJ) is used locally as a whole plant and as an individual plant to treat various human ailments. People from various nations use the entire plant component of MJ extensively to cure muscular aches, diarrhoea, and abdominal colic (5,9). This study was aimed at evaluating the secondary metabolites, and antimicrobial and antioxidant potential of *M. jalapa*.

## MATERIALS AND METHODS

### Sample Collection and Preparation

The stem, leaves, flowers, roots, and seeds of *Mirabilis jalapa* were collected from Ondo. The biology department at Adeyemi Federal University of Education in Ondo State verified the authenticity of the plant sample. ACE/BIOHER/23/001 was the generated specimen number. The biology laboratory kept a voucher specimen for future use as a guide. Every plant part received individual treatment. Each part of the plant portion was cleaned using distilled water, dried at room temperature in a ventilated space for about two weeks, and then ground into a fine powder, labelled, and kept in an airtight container at room temperature.

### Extraction of the plant sample

Extraction was performed using methanol and water. The powdered plant was extracted by soaking in methanol for 3 days, after which it was filtered and concentrated using a rotary evaporator at $\leq 45\ ^{o}C$ and stored in a refrigerator at $4\ ^{o}C$ for further analysis (10).

### Gas Chromatography and Mass Spectroscopy (GC-MS) analysis

The extract was analysed using Shimadzu GC-MS-QP2010 Plus (Japan). The separations were carried out using a Restek Rtx-5MS fused silica capillary column (5%-diphenyl-95%-dimethylpolysiloxane) of 30×0.25 mm internal diameter (di) and 0.25 mm in film thickness. The conditions for analysis were set as follows; column oven temperature was programmed from 60–280 °C (temperature at 60°C was held for 1.0 min, raised to 180 °C for 3 min and then finally to 280°C held for 2 min); injection mode, Split ratio 41.6; injection temperature, 250°C; flow control mode, linear velocity (36.2 cm/sec); purge flow 3.0 ml/min; pressure, 56.2 kPa; helium was the carrier gas with total flow rate 45.0 ml/min; column flow rate, 0.99 ml/min; ion source temperature, 200°C; interface temperature, 250°C; solvent cut time, 3.0 min; start time 3.5 min; end time, 24.0 min; start m/z, 50 and end m/z, 700. The detector was operated in an EI ionization mode of 70 eV. Components were identified by matching their mass spectra with those of the spectrometer data base using the NIST computer data bank, as well as by comparison of the fragmentation pattern with those reported in the literature (11).

### Preliminary Phytochemical Screening

**Test for Flavonoids**: 2g of the extract underwent a series of chemical tests. Initially, 20% sodium hydroxide was introduced dropwisely, resulting in the observation of a vivid and intense yellow colour formation. Subsequently, 70% dilute HCl was also introduced into the mixture, leading to the disappearance of the previously formed yellow colour. The formation and subsequent disappearance of the yellow colour served as a clear indicator of the presence of flavonoids in the extract (12).

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**Test for Alkaloids**: The following procedure was used to assess the presence of alkaloids in the sample extract: 1 g of each extract was combined with 1 ml of Marquis Reagent, along with 2 ml of concentrated sulfuric acid and a few drops of 40% formaldehyde. The emergence of a dark orange colour was a clear indicator of alkaloids within the samples (13).

**Test for Saponin:** The following procedure was used to determine whether the samples contained saponins: Two grams of each extract were mixed with six millilitres of distilled water and briskly shaken. Continuous foam production confirmed the presence of saponins (14).

**Test for Tannins**: The presence of tannins in the samples was detected using the following approach. 2 g of each extract was immersed in a 10% alcoholic ferric chloride solution. The appearance of a distinct brownish-green colour served as a reliable indicator of tannin content in the extracts (15).

**Anthraquinones:** In the analytical process, 500 mg of dried plant leaves were subjected to boiling in 10% HCl for 5 minutes. Afterwards, the resulting filtrate was left to cool. To this 2 mL filtrate, an equal volume of $CHCl_3$ was introduced, along with a few drops of 10% $NH_3$. The emergence of a distinct rose-pink colour in the mixture served as a conclusive indication of the presence of anthraquinones (16).

**Test for Steroids:** To investigate the presence of steroids within the plant extract, the following procedure was performed: 1 gram of plant extract was mixed with an equal amount of chloroform. A few drops of concentrated sulfuric acid were added to the mixture, resulting in the creation of a prominent brown ring. This brown ring indicated the presence of steroids in the extract (17).

**Test for Terpenoids:** A unique approach was used to examine terpenoids in the extract: 1 g of extract was mixed with 0.5 ml of chloroform, followed by a few drops of strong sulfuric acid. A reddish-brown precipitate was seen, indicating the presence of terpenoids in the extract (18).

**Test for Glycosides**: A specific approach was employed to detect the presence of cardiac glycosides in the sample extract: 1 g of each extraction was combined with 0.5 ml of glacial acetic acid and three drops of 1% aqueous ferric chloride solution. A large brown ring produced at the contact site indicated the presence of cardiac glycosides in the extract (19).

**Antimicrobial Screening**

The diffusion method was employed for the antimicrobial assay. The organisms used were obtained from the Microbiology Department at Bowen University, Iwo, Nigeria.

**The Diffusion method**

The antimicrobial test was performed on all samples and evaluated against bacterial strains. *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans*, and *Candida tropicalis* were the organisms tested. The culture was generated by immersing a loop full of bacteria cells in peptone water and incubating for 24 hours at 37 $^{o}$C. Following that, the optical density was measured at 625nm and normalized using the 0.5 McFarland standard. The Kirby-Bauer disc diffusion method was employed to conduct an antimicrobial susceptibility test, with Mullern Hinton agar (LABM) as the medium. The antibacterial potential of the sample extracts was evaluated by measuring the zone of growth inhibition against the test organisms. Augmentin (AUG) and gentamicin (GEN) were used as controls (20).

**Evaluation of Polyphenolic Content**

Using the Folin-Ciocalteu test as a standard reference, phenolic concentration was determined

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spectrophotometrically. Gallic acid was dissolved in 80% methanol to make a standard solution. 0.5 mL of the plant extract, 3 mL of distilled water, and 0.25 mL of Folin-Ciocalteu reagent were combined and mixed thoroughly to generate the reaction mixture. After allowing the solution to sit in the dark for five minutes, 1 mL of 7.5% $Na_2CO_3$ was added and allowed to remain at room temperature for 90 minutes. With distilled water, a blank for the reagent was also made. In comparison to the reagent blank, the sample's absorbance was taken at 760 nm. Milligrams of gallic acid equivalent (GAE) per 100 grams of sample was the unit used to calculate the extract's overall content of phenolic components. Every sample was examined twice. 1 mL of solutions containing 50, 100, 150, 200, 250, 300, 350, 400, and 450 µg/mL of Gallic acid were mixed with 5.0 mL of diluted Folin Ciocalteu reagent and 4.0 mL of sodium carbonate solution (75 g/L) to generate the calibration curve (Figure 7). At 760 nm, the absorbance was measured after 30 minutes. Using the same reagents that were used to create the calibration curve, 1 mL of the aqueous and methanolic extracts (1 g/100 mL) were blended separately (10).

**Determination of Flavonoid Content**

The flavonoid content was measured in mg/mL using an aluminium chloride assay. To put it briefly, a 10 mL test tube was filled with 0.5 mL of the extract and 2 mL of distilled water, and 0.15 mL of 5% $NaNO_2$ were included in each test tube. Following a five-minute incubation period, 0.15 mL of 10% $AlCl_3$ was added. Next, 1 mL of 1 M NaOH was added, and distilled water was used to raise the volume to 5 mL overall. After an incubation time of 10 minutes, the absorbance at 510 nm of the resultant solution was measured. All samples underwent double analysis. The calibration curve for the quercetin standard was calculated as $y = 0.0291\ x - 0.0397$, with an $R^2$ value of 0.9904 (21).

**Total Flavanols Determination**

Total flavanols were measured using the colourimetric method with aluminium chloride. Take a 10 mL volumetric flask and fill it with 4 mL of distilled water. Then add 1 mL of methanol extract or standard catechin solution (20, 40, 60, 80, and 100 µg/mL). Subsequently, the flask was filled with 0.3 mL of 5% sodium nitrite. 0.3 mL of 10% aluminium chloride was added following a 5-minute incubation period. The mixture was diluted with distilled water to a final volume of 10 mL after 6 minutes, and 2 mL of 1 M sodium hydroxide was added. A UV-visible spectrophotometer was used to detect the absorbance at 510 nm following a 15-minute incubation period at room temperature. The total flavonoids were expressed as milligrams of catechin equivalents (CAT) per gram of dry extract weight, which was estimated using a calibration curve with catechin (22).

**Determination of Reducing Power (FRAP)**

A 2.5 ml aliquot of the extract, 2.5 ml of 1% potassium ferricyanide, and 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) were mixed. 2.5 ml of 10% trichloroacetic acid was added to the mixture after it had been incubated at 50$^{o}$C for 20 minutes. A mixture of 5 millilitres of the supernatant, equal parts water, and 1 millilitre of 0.1% ferric chloride was added after centrifugation at 801 × g for ten minutes. The absorbance was measured at 700 nm, and ascorbic acid equivalents were used to calculate the ferric-reducing power (23).

**Determination of Total Antioxidant Capacity (ABTS)**

The total antioxidant activity was determined by 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic) acid ($ABTS^{\bullet+}$) radical. After reacting an aqueous solution of ABTS (7 mM) with $K_2S_2O_8$ (2.45 mM, final concentration) in the dark for 16 hours, ethanol was used to adjust the absorbance to 0.700 at 734 nm, resulting in $ABTS^{\bullet+}$. The absorbance at 734 nm was then measured after 15 minutes, when 2.0 ml of the $ABTS^{\bullet+}$ solution was mixed with 0.2 ml of an appropriate extract dilution. After that, Trolox was used as a

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standard to measure the Trolox equivalent antioxidant capacity (TEAC) (24).

**Determination of DPPH Free Radical Scavenging ability**

One millilitre of the extracts at the proper dilution was combined with one millilitre of a 0.4 mM DPPH radical solution in methanol. The mixture was then exposed to darkness for thirty minutes, at which point the absorbance at 516 nm was determined. A control was created by combining no-test samples with two millilitres of DPPH solution. Next, the percentage of the control was used to calculate the DPPH-free radical scavenging ability (25).

**Determination of $Fe^{2+}$ Chelating Ability**

218 µl of saline was added to 168 µl of 0.1 M Tris-HCl (pH 7.4), and the extracts (0-25 µl) to a reaction mixture, 150 µl of 500 µM $FeSO_4$ was added. 13 µl of 0.25% phenanthroline (w/v) should be added after 5 minutes of incubation. The $Fe^{2+}$ chelating capacity was then calculated at the absorbance of 510 nm (26).

**OH* Radical Scavenging Ability**

A mixture comprising 120 µl of 20 mM deoxyribose, 400 µl of 0.1 M phosphate buffer, 40 µl of 20 mM hydrogen peroxide, and 40 µl of 500 µM $FeSO_4$ was then mixed with dilutions of the extracts. 800 µl of distilled water was added to regulate the volume. Following that, the mixture was incubated at 37 degrees Celsius for 30 minutes. Add 0.5 mL of 2.8% trichloroacetic acid (TCA) and then 0.4 mL of 0.6% thiobarbituric acid (TBA) solution to halt the process. After 20 minutes of boiling water immersion, the tubes were taken out of the water, the absorbance at 532 nm was measured and the per cent scavenging capacity was calculated (27).

**Statistical Analysis:**

The data were presented as mean standard errors from three replicate trials. Tukey's post-hoc test using one-way ANOVA ($P < 0.005$).

## RESULTS

**GC-MS Analysis Result of Methanolic Extract of *M. jalapa***

A total of twenty-four (24) compounds were identified in GC-MS analysis, among these the highest percentage composition was 9-octadecenamide (46.89%) followed by cholest-5-ene-3,16, 22, 26-tetrol (8.49%), octadecanoic acid(8.39%), hexadecanamide(7.14%), 8,11-octadecadienoic acid, methyl ester (6.41%) and hexadecanoic acid (6.40%).

Table 1: Bioactive Compounds from Methanolic Leaf Extract of *Mirabilis jalapa*

| Compounds | Percentage Composition (%) | Retention Index |
|---|---|---|
| 2-ethyl-3-methoxypyrazine | 0.06 | 1070 |
| 5-caranol | 0.10 | 1125 |
| Carvacrol | 0.08 | 1262 |
| *tau*-cadinol | 0.19 | 1580 |
| *α*-cadinol | 0.17 | 1624 |
| diethyl phthalate | 0.13 | 1639 |
| pentadecanoic acid | 0.19 | 1869 |

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| | | |
|---|---|---|
| hexadecanoic acid, methyl ester | 0.18 | 1878 |
| Hexadecanamide | 7.14 | 2021 |
| 3,7,11,15-tetramethyl-hexadecen-1-ol | 0.27 | 2045 |
| 8,11-octadecadienoic acid, methyl ester | 6.41 | 2093 |
| Phytol | 0.60 | 2100 |
| Octadecanamide | 2.64 | 2220 |
| 9-octadecenamide | 49.06 | 2228 |
| 9-tricosene(Z) | 0.18 | 2315 |
| hexadecanoic acid | 6.98 | 2498 |
| octadecanoic acid | 8.70 | 2681 |
| ergosta-8,24(28)-dien-3-ol | 0.82 | 2765 |
| gorgost-5-en-3-ol | 1.77 | 2768 |
| *exo*-2-bornanol-5-one *p*-toluenesulfonylhydrazine | 0.23 | 2822 |
| Obtusifoliol | 1.87 | 2826 |
| 9,19-cyclolanostan-3-ol | 2.30 | 2834 |
| cholest-5-ene-3,16,22,26-tetrol | 8.49 | 3189 |
| 2,4-diamino-5-[p-[2-naphthylthio]phenyl]pyrine | 0.60 | 3467 |
| **Percentage Total** | **99.16** | |

**Phytochemical Components of the Extracts**

According to the table 2, the phytochemical contents include tannins, glycosides, anthraquinone, alkaloids, saponins, and flavonoids. Tannins and flavonoids were found in the combined plant extract, fresh leaves, fresh stem, fresh flower, fresh root, dried stem, dried seed, dried leaves and dried flower parts of the extract.

Steroids and essential oils were absent in all the plant parts. Glycosides was also present in combined plant parts, fresh leaves, fresh stem, fresh flower, dried stem, dried leaves and dried flower but absent in the dried seed and fresh root extract of the plant. Anthraquinone was also present in all plant parts but absent in the fresh root and dried seed. Alkaloids and terpenes were considerable in all the plant extracts. Saponin was sparingly present in the combined plant parts, fresh stem and dried stem but absent in other plant parts.

Table 2: The Phytochemical Components of the Extract

| Samples | Tannin | Steroids | Glycosides | Anthraquinone | Alkaloids | Terpenes | Saponins | Essential oils | Flavonoids |
|---|---|---|---|---|---|---|---|---|---|
| Combined (Stem/Root/leaves /flowers/seeds) | +++ | – | + | ++ | ++ | ++ | + | – | ++ |
| Fresh Leaves | + | – | +++ | ++ | ++ | ++ | – | – | ++ |
| Fresh Stem | ++ | – | + | + | + | ++ | + | – | + |
| Fresh Flower | + | – | +++ | + | ++ | +++ | _ | – | ++ |
| Dried Stem | ++ | – | + | + | ++ | + | + | – | + |
| Dried Leaves | + | – | +++ | ++ | ++ | ++ | – | – | ++ |
| Dried Flower | + | – | ++ | + | ++ | ++ | – | – | ++ |

KEY: + = Present – = Absent

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**Antimicrobial Sensitivity Results**

Table 3 presents the Zones of Inhibition (mm) of the anti-bacterial properties of the extract**.** The growth of the bacteria colonies was observed and the zone of inhibition was measured. The combined sample in Methanol showed the highest inhibitory activity with the highest effect on *E. coli*, followed by *Staphylococcus aureus* with a lesser effect at *Candida albican.* The methanolic combined extract inhibited *K. pneumonia* (6 mm), *S. aureus* (18mm), *P. aeruginosa* (7 mm), *E. coli* (18.5 mm) and *Candida albicans*. High inhibition was observed with *E. coli* (18.5mm) and *S. aureus* (18mm). The combined n-hexane extract showed low to moderate inhibition in *K. pneumonia* (10 mm), *S. aureus* (6 mm) and *C. albican* (6 mm). The methanolic fresh leaf extract showed no inhibition for all the microorganisms except for *C. albican* (9.5 mm). Methanolic fresh stem extract inhibited the growth of *P. aeruginosa* (10 mm) and *C. tropicalis* (2 mm). However, there was no inhibition with *K. pneumonia*, *S. aureus*, *E. coli* and *C. albican*.

Methanolic fresh flower extract also showed no inhibition with all the microorganisms except *E. coli* (9 mm). No inhibition was observed with *P. aeruginosa*, *C. albican* and *C. tropicalis*, and high inhibition was observed with *K. pneumoniae*. Methanolic dried stem extract showed low inhibition for *K. pneumonia* (9 mm) and *C. tropicalis* (8 mm), however *P. aeruginosa* (10 mm) and *E. coli* (10 mm) showed moderate inhibition. Methanolic dried seed extract showed moderate inhibition with *K. pneumonia* (10 mm) and *E. coli* (10 mm). Low inhibition was observed with *P. aeruginosa* (8 mm). *S. aureus* (18 mm) showed high inhibition with the seed extract. No inhibition was observed with *C. albican* and *C. tropicalis*. Methanolic dried leaf extract revealed low inhibition with *E. coli* (6 mm) and *C. tropicalis* (8 mm). *K. pneumonia* (12 mm) and *S. aureus* (11 mm) showed moderate inhibition however, *P. aeruginosa* showed no inhibition. The methanolic dried flower extract revealed low inhibition on *K. pneumonia* (4 mm), *S. aureus* (8 mm) and *C. tropicalis* (6 mm) but no inhibition for the other microorganisms.

The methanolic fresh root extract showed maximum inhibition of *E. coli* (22 mm) and *S. aureus* (20 mm) and even higher inhibition when compared with the control drug. This inhibition could be as a result of the presence of tannins, alkaloids and other secondary metabolites in the plant extract.

**Total Polyphenolic Contents, flavonoids and flavanol of the extract**

Table 4, 5 and 6 present the total polyphenolic contents, flavonoids and flavanol of the extract. Flavonoids have been acknowledged for their antioxidant activity, with significant implications for human nutrition and health. From Table 4, the total polyphenol content ranges from 6.65 – 38mg/g. The dried stem has the most polyphenolic content (29.45mg/g), while the fresh flower has the lowest polyphenol content. From table 5 shows that the combined extract has the highest flavonoid content (66.0 mg/g) while dried flowers contain the lowest flavonoid content (19.0 mg/g). Table 6 which showed the total flavanol content also revealed that the combined plant extract had the highest flavanol content (56 mg/g) while the dried leaves and flowers had the least flavanol content (22.0 mg/g).

Table 3: Zones of Inhibition (mm) of the Anti-bacterial Properties of the extract

| **S/N** | **SAMPLES** | | | | | | | | | | **CONTROL** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Organisms** | **CSM** | **CSN** | **FLM** | **FSM** | **FFM** | **FRM** | **DSM** | **DSDM** | **DLM** | **DFM** | **GEN** | **AUG** |
| *K. pnuemoniae* | 6 | 10 | – | – | – | 11 | 9 | 10 | 12 | 4 | 14 | 10 |
| S. aureus | 18 | 6 | – | – | – | 20 | – | 18 | 11 | 8 | – | 18 |
| P. *aeruginosa* | 7 | – | – | 10 | – | – | 10 | 8 | | – | 8 | 10 |
| E. coli | 18.5 | – | – | – | 9 | 22 | 10 | 10 | 6 | | 10 | 10 |

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| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *C. albican* | – | 6 | 9.5 | – | – | – | – | – | | – | – | – |
| *C. tropicalis* | 2 | – | – | 2 | – | – | 8 | – | 8 | 6 | 9 | 10 |

Keynote: – = No inhibition, 6 – 9mm = low inhibition, 10 – 15 mm = Moderate inhibition, and ≥15 mm = high inhibition, AUG = Augmentin, GEN = Gentamycin, CSM=Combined sample in methanol, CSN= Combined sample in n-Hexane, FLM=Fresh leaves in methanol, FSM= Fresh sample in methanol, FFM = Fresh flower in methanol, FRM= Fresh Root in methanol, DSM = Dried stem in methanol, DSDM= Dried seed in methanol, DLM = Dried leaf in methanol, DFM= Dried flower in methanol.

Table 4: The Polyphenolic content of the plant

| S/N | SAMPLES | Total Polyphenolic content: $C = C_1 \times V/m$ – mg/g, in GAE (Gallic acid equivalent) |
|---|---|---|
| 1 | Combined plant ext | 38.00 |
| 2. | Fresh Leaves | 8.5 5 |
| 3. | Fresh Stem | 10.45 |
| 4. | Fresh Flower | 6.65 |
| 5. | Dried Stem | 29.45 |
| 6. | Dried Leaves | 20.90 |
| 7. | Dried Flower | 13.30 |

Table 5: Total Flavonoids content of the plant

| **S/N** | **SAMPLES** | **Total Flavonoids Content (mgQE/gram)** |
|---|---|---|
| 1 | Combined plant ext | 66.00 |
| 2. | Fresh Leaves | 30.00 |
| 3. | Fresh Stem | 48.00 |
| 4. | Fresh Flower | 21.00 |
| 5. | Dried Stem | 48.00 |
| 6. | Dried Leaves | 41.00 |
| 7. | Dried Flower | 19.00 |

Table 6: The total Flavanol content of the plant

| S/N | SAMPLES | Total Flavanol Content (mgQE/gram) |
|---|---|---|
| 1 | Combined plant ext | 56.00 |
| 2. | Fresh Leaves | 22.00 |
| 3. | Fresh Stem | 29.00 |
| 4. | Fresh Flower | 32.00 |
| 5. | Dried Stem | 41.00 |
| 6. | Dried Leaves | 25.00 |
| 7. | Dried Flower | 22.00 |

**Antioxidant Capacity**

**DPPH (*α*, *α*-diphenyl-β-picrylhydrazyl)**

Figure 2 shows the overall antioxidant activity, determined using the DPPH method. The bars showed the

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mean +_SEM (n=3). The methanolic extract of the combined plant parts was statistically different from the control at P< 0.001. Aqueous extract of the combined plant parts (P<0.5), methanolic Mj (Mirabilis jalapa) leaf extract (P<0.001) and methanolic Mj flower extract (P< 0.01) were statistically different as compared with the methanolic Mj combined extract. Also, methanolic Mj leaf extract (P< 0.01) and methanolic MJ stem extract (P<0.5) were statistically different when compared with combined aqueous Mj extract. In addition, methanolic Mj stem extract (P<0.01) was statistically different when compared with methanolic Mj leaf extract. This clearly showed that the methanolic MJ combined extract exhibits scavenging ability but is lower than the control. The methanolic MJ leaf extract and methanolic MJ stem extract displayed higher scavenging activity compared with the methanolic combined MJ extract. The DPPH antioxidant assay results demonstrated that extracts from all plant sections may scavenge the radical to some extent. The presence of polyphenolic chemicals and flavonoids in the plant extract may contribute to its scavenging capacity.

**Ferric Reducing Antioxidant Power (FRAP)**

The FRAP values of the examined plant, presented in Figure 3, showed that the methanolic MJ leaf extract was statistically different when compared to the control (P< 0.01), and Mj leaf extract was statistically different from the combined aqueous MJ extracts at P<0.001. Methanolic MJ flower and stem extract however were statistically different from MJ leaf at P< 0.001. This result reveals that MJ leaf extract exhibited a high Ferric reducing property.

**Antioxidant Capacity**

**DPPH(*α*,*α*-diphenyl-β-picrylhydrazyl)**

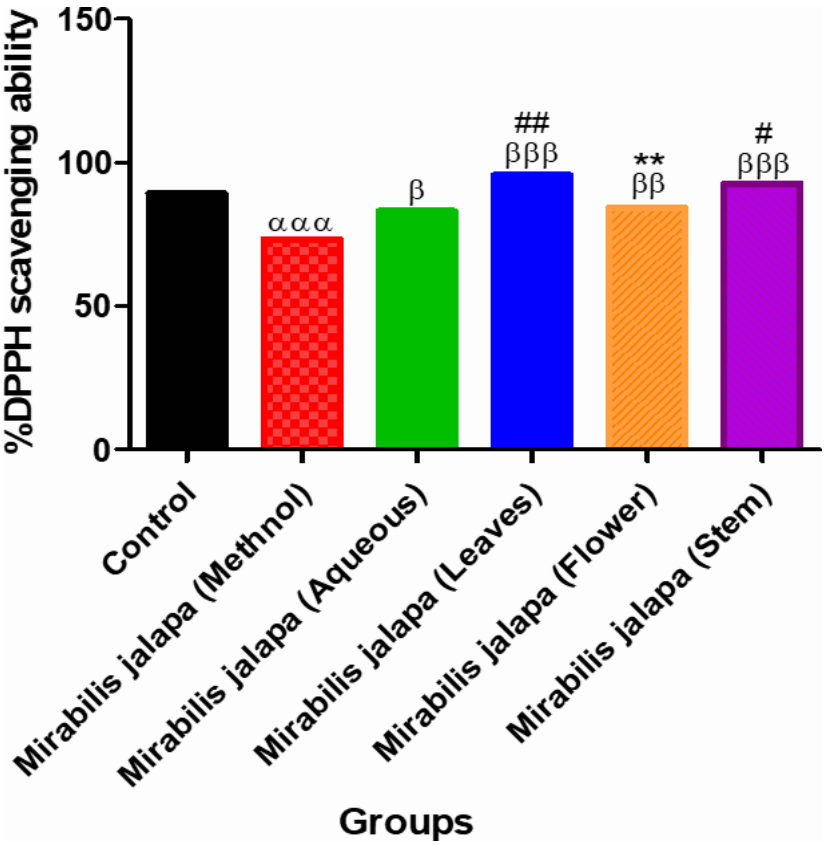


Fig. 2: The DPPH assay result. Bars represent mean ± SEM (n=3). Values are statistically different at $^{ααα}P < 0.001$, Vs Standard, $^{βββ}P < 0.01$, $^{ββ}P < 0.01$, $^{β}P < 0.5$ Vs *Mirabilis jalapa* (Methanol), $^{\#\#}P < 0.01$,$^{\#}P < 0.5$ Vs *Mirabilis jalapa* (Aqueous), **P < 0.01 Vs *Mirabilis jalapa* (Leaves).

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**Ferric reducing antioxidant power(FRAP)**

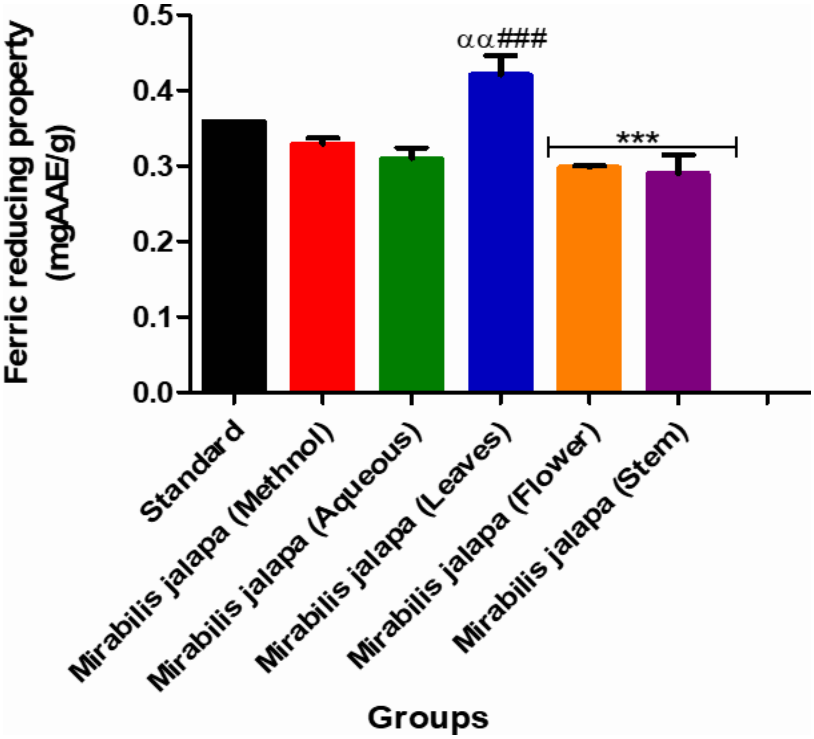


Fig. 3: The FRAP assay result. Bars represent mean ± SEM (n=3). Values are statistically different at $^{\alpha\alpha}P < 0.01$, Vs Standard, $^{\#\#\#}P < 0.001$, Vs *Mirabilis jalapa* (Aqueous), ***P < 0.001 Vs *Mirabilis jalapa* (Leaves).

**ABTS (2, 2'-azinobis-3-ethylbenzthiazoline sulphonate)**

Fig. 4 shows the ABTS activity of the extract.

To produce the stable ABTS radical cation, potassium persulfate was used. Once a stable absorbance was established, the antioxidant plant extract was added to the reaction medium, and the antioxidant efficacy was measured by observing the decolourization process. According to Fig. 4, the methanolic MJ floral extract and stem significantly differed from the control at P<0.001 and the combined methanolic extract at P<0.001. Methanolic MJ stems and flowers showed statistically significant differences from the Mj aqueous mixed extract (P<0.001) and the methanolic leaf extract (p<0.01). The figure reveals that MJ flowers and stem extracts have reduced ABTS+ scavenging ability, whereas MJ leaf extract has the highest scavenging activity.

**$Fe^{2+}$ Chelating Ability**

Fig. 5. Shows the $Fe^{2+}$ Chelating Ability of the extract. Ferrozine can form quantitative compounds with $Fe^{2+}$, but the presence of chelating substances disrupts this process, resulting in a decrease in the complex's red colour. The chelation of metal ions is the principal method for reducing the formation of reactive oxygen species (ROS) associated with redox-active metal catalysis. The MJ plant extract inhibited the formation of the ferrous and ferrozine complex, demonstrating chelation action in collecting ferrous ions before ferrozine. From Fig. 5, combined methanolic Mj extract, combined aqueous MJ extract and methanolic MJ leaf extract were statistically different when compared with the control (P<0.001). Methanolic MJ flower and stem were statistically different when compared with combined methanol extract (P<0.001), combined aqueous MJ (p<0.001) and MJ leaf (p<0.001). It is necessary to note that the Mj stem and flower exhibited $Fe^{2+}$ chelating activity when compared with the combined aqueous and methanolic extract and MJ leaf extract which has high activity.

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume IX Issue VII July 2024

**ABTS (2, 2'-azinobis-3-ethylbenzthiazoline sulphonate)**

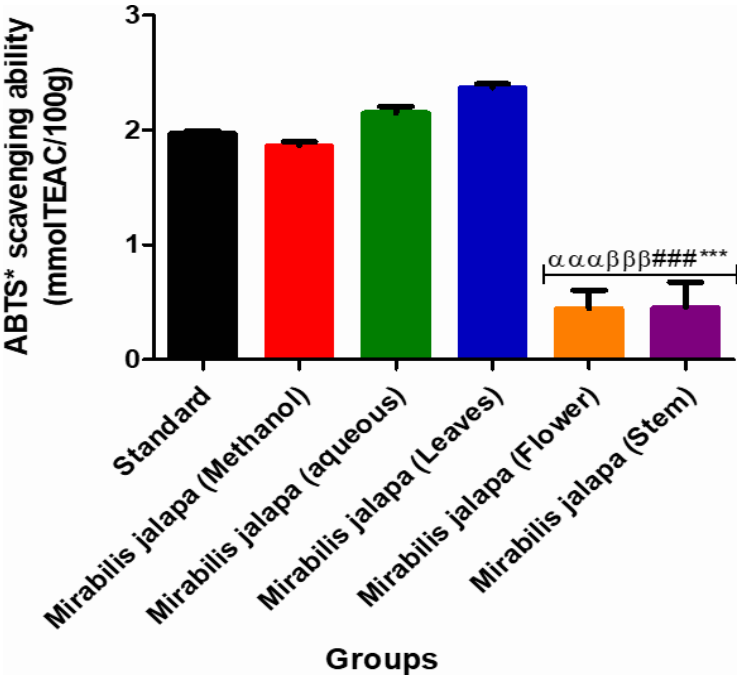


Fig. 4: ABTS scavenging activity of the extract. Bars represent mean ± SEM (n=3). Values are statistically different at $^{\alpha\alpha\alpha}P < 0.001$, Vs Standard, $^{\beta\beta\beta}P < 0.001$ Vs *Mirabilis jalapa* (Methanol), $^{\#\#\#}P < 0.001$, Vs *Mirabilis jalapa* (Aqueous), ***P < 0.001 Vs *Mirabilis jalapa* (Leaves).

**$Fe^{2+}$ Chelating Ability**

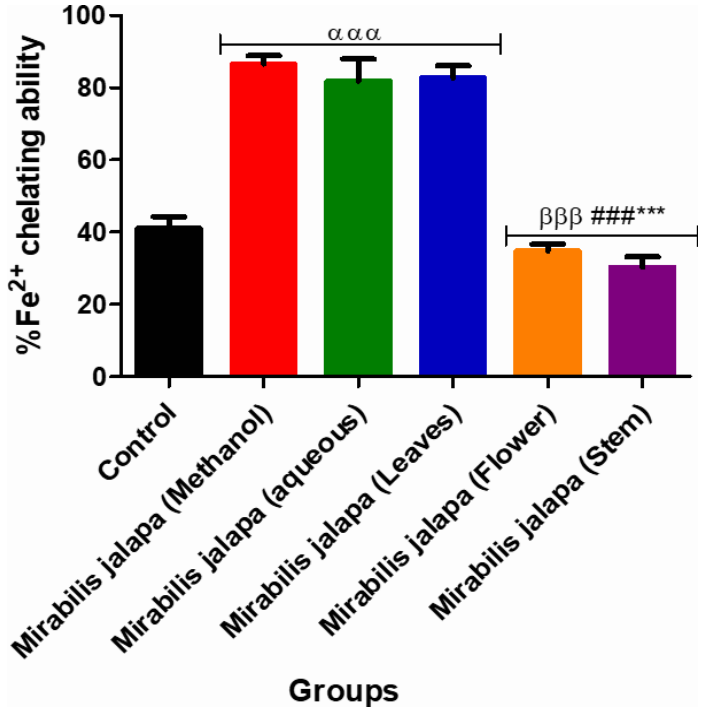


Fig. 5: The $Fe^{2+}$ Chelating ability of the extract. Bars represent mean ± SEM (n=3). Values are statistically different at $^{\alpha\alpha\alpha}P < 0.001$, Vs control, $^{\beta\beta\beta}P < 0.001$ Vs *Mirabilis jalapa* (Methanol), $^{\#\#\#}P < 0.001$, Vs *Mirabilis jalapa* (Aqueous), ***P < 0.001 Vs *Mirabilis jalapa* (Leaves).

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**Hydroxyl Scavenging Ability**

Fig. 6 shows the Hydroxyl scavenging ability of the extract**.** When compared to typical antioxidants, the plant extract demonstrated significant hydroxyl radical scavenging ability (Fig. 6), and it could be used to treat cancer by preventing the interaction of hydroxyl radicals with DNA. The extractives' capacity to quench hydroxyl radicals may have a direct impact on lipid peroxidation prevention. Figure 6 showew that methanolic MJ stem differs significantly from combined methanolic Mj extract at $p<0.05$, whereas MJ flower and stem vary from MJ leaf extract at $p<0.01$ and $p<0.001$, respectively. The results show that MJ leaf extract has higher scavenging activity than the conventional antioxidant.

**Hydroxyl Scavenging Ability**

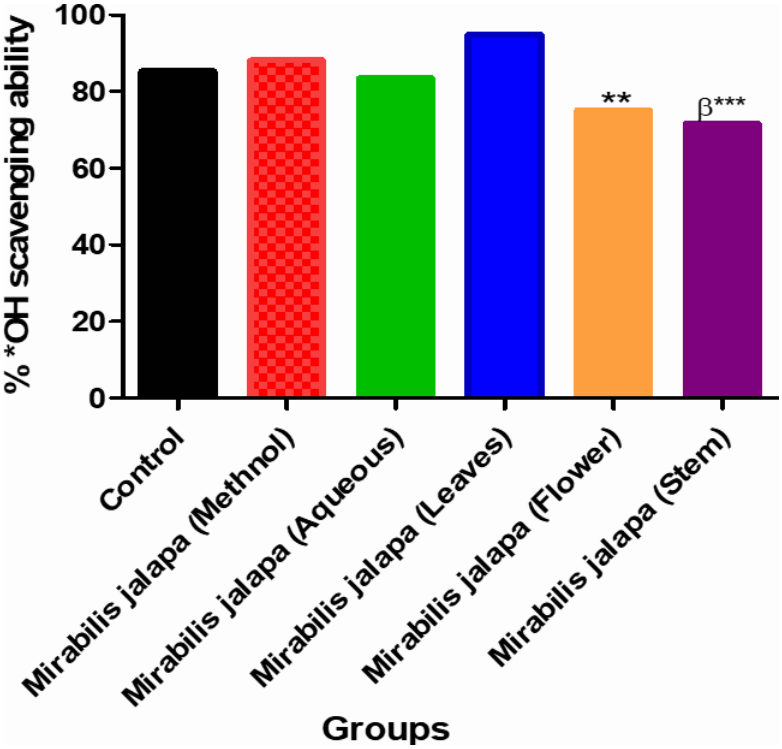


Fig. 6: The OH- Scavenging ability of the extract. Bars represent mean ± SEM (n=3). Values are statistically different at $^{\beta}P < 0.05$ Vs *Mirabilis jalapa* (Methanol), $^{**}P < 0.01$, $^{***}P < 0.001$, Vs *Mirabilis jalapa* (Leaves).

## DISCUSSION

Chemical compounds found in plant extracts are recognized to be pharmacologically active. *M. jalapa* plant parts were examined in this study to determine their proximate composition, antibacterial activity, and antioxidant activity. The GC-MS analysis of *Mirabilis jalapa* plant extract identified a diverse array of compounds, with 9-octadecenamide being the most abundant, comprising 46.89% of the total composition. This compound is known for its neurotransmitter regulation, anti-inflammatory properties, neuroprotective effects, pain modulation and lipid signalling potential. Cholest-5-ene-3,16,22,26-tetrol and octadecanoic acid were also prominent constituents, contributing 8.49% and 8.39% respectively. Hexadecanamide, 8,11-octadecadienoic acid methyl ester, and hexadecanoic acid constituted significant portions as well, each accounting for over 6% of the composition. Several authors have observed that the flavonoid and tannin groups are biologically active hence having antioxidant, anti-inflammatory, antibacterial, anti-angionic, anti-cancer, and anti-allergic effects. The study also evaluated the antimicrobial activity of different extracts

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume IX Issue VII July 2024

from Mirabilis jalapa against six different microorganisms: *Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Candida albicans,* and *Candida tropicalis*. The effectiveness of the extracts was compared to standard antibiotics, Gentamicin (GEN) and Augmentin (AUG). The results suggest that some extracts of *Mirabilis jalapa* possess antimicrobial properties, with varying effectiveness depending on the microorganism. The methanolic fresh root extract showed maximum inhibition of *E. coli* (22 mm) and *S. aureus* (20 mm) and even higher inhibition when compared with the control drug. The significant activity of combined samples in methanol against *S. aureus* (18 mm) and *E. coli* (18.5 mm) indicates potential for treating infections caused by these bacteria. The moderate activity of dried leaves in methanol against *K. pneumoniae* (12 mm) and *S. aureus* (11 mm) also highlights its potential use. The lack of activity of some extracts of *Mirabilis jalapa* against certain microorganisms in Table 3 suggests selective antimicrobial effects.

Synergistic actions of all phytochemicals in the extracts contributed tremendously to the antibacterial activities observed in this study. The development of resistance to conventional antimicrobials has been a serious issue in the invention of new drugs for both acute and chronic diseases. Scientists are now seeking natural antimicrobial drugs from plant origins with no or very low side effects due to bacteria resistance to common synthetic and conventional drugs, malicious use of antimicrobial drugs, and high rates of allergies coupled with side effects (28). The results obtained from this study showed that phenolic compounds such as flavonoids, flavanols, tannins etc. are part of the major phytochemicals that played major roles in the therapeutic activities of the leaf extract of the plant under investigation. Tungmunnithum et al. (2018) (29) and Tian et al. (2019) (30) stated that the therapeutic activities of polyphenol as anti-oxidative, anti-diabetic, anti-cancer, anti-atherosclerotic, anti-inflammatory, anti-mutagenic, ability to enhance immunity and its inhibition potential on free radicals and prevention of pathologies such as cardiovascular heart disease, skin diseases, cholesterol-lowering, neurodegenerative disorders, osteoporosis, organ protecting, anti-infection, bacteriostatic and obesity, as well as relief of menopausal symptoms cannot be overemphasized. Likewise, Ozcan et al. (2014) (31) and Williamson (2017) (32) reported that the consumption of the required amount of polyphenols in daily food nutrients and vegetables is essential to supply essentially high necessary concentrations in the blood because polyphenols are easily eliminated from plasma.

The extracts investigated in this study were observed to be a good natural radical scavenger and antioxidant agents even at very low concentrations. The results showed that the extracts were able to act effectively on strong free radical molecules. The DPPH assay (Fig.2) measures the ability of antioxidants to scavenge free radicals. The methanolic MJ combined extract has good scavenging ability but is less effective than the control. Methanolic MJ leaf and stem extracts exhibit higher scavenging activity compared to the methanolic combined extract. The presence of polyphenolic chemicals and flavonoids likely contributes to the scavenging capacity. The FRAP assay (Fig. 3) measures the reducing power of antioxidants. MJ leaf extract demonstrated a high ferric-reducing property, suggesting strong antioxidant activity. The ABTS assay (Fig. 4) measures the ability of antioxidants to quench the ABTS radical cation. MJ leaf extract exhibited the highest scavenging activity, while MJ flower and stem extracts showed lower scavenging abilities. The $Fe^{2+}$ chelating ability (Fig. 5) measures the capacity of antioxidants to bind metal ions, thereby preventing the formation of reactive oxygen species (ROS). MJ stem and flower exhibited notable $Fe^{2+}$ chelating activity, suggesting a strong ability to prevent ROS formation by binding metal ions. The hydroxyl scavenging ability measures the capacity of antioxidants to neutralize hydroxyl radicals, which are highly reactive and can damage biomolecules. MJ leaf extract demonstrated higher hydroxyl scavenging activity compared to the conventional antioxidant, indicating its potential in preventing lipid peroxidation and DNA damage. The antioxidant assays (DPPH, FRAP, ABTS, $Fe^{2+}$ chelating, and hydroxyl scavenging) collectively suggest that different parts of the *Mirabilis jalapa* plant have significant antioxidant properties.

A previous study carried out by Ololade et al. (2021) (25) showed that an increment in the intake of natural antioxidants from plants would ameliorate the damage caused by reactive oxygen species, through

scavenging the initiation or propagation of oxidative chain reaction, acting as free radical scavengers, quenchers of singlet oxygen and reducing agents. Moreover, natural antioxidants from vegetables exhibit broad therapeutic activities, such as anti-arthritic, anti-inflammatory, anti-malaria, antibacterial, antiviral, anti-ageing and anti-cancer (33,3).

## CONCLUSION

The study of *Mirabilis jalapa (M. jalapa)* plant extracts has revealed significant pharmacological potential through its diverse chemical composition, notable antimicrobial activity, and strong antioxidant properties. The GC-MS analysis identified 9-octadecenamide as the most abundant compound, known for its various therapeutic effects including neurotransmitter regulation and anti-inflammatory properties. The antibacterial assays demonstrated that *M. jalapa* extracts possess selective antimicrobial properties against a range of pathogenic microorganisms, with notable efficacy against *Staphylococcus aureus* and *Escherichia coli*. Antioxidant assays, including DPPH, FRAP, ABTS, $Fe^{2+}$ chelating, and hydroxyl scavenging, confirmed that *M. jalapa* extracts exhibit substantial radical scavenging and metal ion chelating abilities. Methanolic extracts, particularly from the leaves and stems, showed the highest antioxidant activities, likely due to the presence of polyphenolic compounds and flavonoids. These findings underscore the plant's potential as a natural source of antioxidants, capable of preventing oxidative stress-related damage and providing therapeutic benefits. The study suggests that *M. jalapa* could be developed into effective natural antimicrobial and antioxidant agents, addressing the growing concern of microbial resistance and the need for safer, plant-based therapeutic alternatives. Future research should focus on isolating and characterizing the individual bioactive compounds within *M. jalapa* to further elucidate their mechanisms of action and potential applications in medicine.

## CONFLICT OF INTEREST STATEMENT:

We declared that we have no conflict of interest.

## DATA AVAILABILITY STATEMENT:

The authors confirm that the data supporting the findings of this study are available within the article.

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