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ORIGINAL ARTICLE

UPLC-ESI-QTOF-MS phenolic compounds identification and quantification from ethanolic extract of *Myrtus communis* 'Variegatha': *In vitro* antioxidant and antidiabetic potentials

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Abstract Global public health is seriously threatened by diabetes and its complications. Although several synthetic drugs are currently employed for managing diabetes, however, the adverse effects associated with their use cannot be underestimated. Thus, the quest for a safe and cost-effective alternative is highly imperative. In the present study, the phenolic contents, antioxidant, antidiabetic, and cytotoxic potentials of 70% ethanolic crude extract of *Myrtus communis* 'Variegatha' were investigated using *in vitro* biochemical protocols. The total polyphenols content was 116.44 mg GAE/g, flavonols (6.74 mg QE/g), flavanols (2.46 mg CE/g) and the ferric reducing antioxidant power (FRAP) value was 1267.28 µmol AAE/g, 2,2-diphenyl-1-picrylhydrazyl (DPPH) (1165.37 µmol TE/g), and Trolox equivalent antioxidant capacity (TEAC) (775.52 µmol TE/g). High-resolution ultra-performance liquid chromatography coupled with electrospray ionisation/quadrupole-time-of-flight-mass spectrometry (UPLC-ESI-QTOF-MS) was explored to identify the phenolic compounds, most of which were flavonoids. The extract demonstrated a strong

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α-glucosidase inhibition potential in a concentration-dependent manner with IC_{50} (3.159 μg/mL), which was higher than epigallocatechin gallate (EGCG) (6.208 μg/mL), a positive control antidiabetic drug. A slight increase in glucose utilization was observed after 24 h of treatment in C3A hepatocytes at 25 μg/mL whereas an increase in glucose uptake was recorded at 25 and 50 μg/mL. The extract exhibited a cytotoxic effect (IC_{50} 76.85 μg/mL) against C3A hepatocytes at 100 μg/mL, which correlates to the glucose utilization and uptake recorded. The findings from the study show the prospect of *M. communis* 'Variegatha' as a promising source of bioactive compounds that could be used in the development of new anti-diabetic agents, thus, further research into the plant is recommended.

1. Introduction

Diabetes mellitus is a chronic metabolic condition defined clinically by elevated blood glucose levels. It is one of the world's most serious public health concerns in the 21st century, affecting approximately 347 million people globally (Moodley et al., 2015; Sulaimon et al., 2020). It has been predicted that its prevalence may double by 2030 if measures were not taken (Eleazu et al., 2013). The destruction of β-cells in type 1 diabetes results in insulin insufficiency. Type 2 diabetes, on the other hand, is the most prevalent (Unnikrishnan et al., 2016); it is prompted by a gradual insulin secretory defect in the context of insulin resistance; other types include gestational diabetes, monogenic diabetes syndromes, exocrine pancreas diseases, and drug or chemical-induced diabetes (Sulaimon et al., 2020). The release of insulin hormone from the pancreas is triggered by a rise in blood glucose levels shortly after a meal (Komatsu et al., 2013). Remarkably, insulin stimulates the liver to metabolize glucose, as well as fat and muscle cells to eliminate glucose from the bloodstream, thereby normalising blood sugar levels. The blood sugar level in a diabetic patient remains high because the pancreas produces insufficient insulin (Dean and McEntyre, 2004).

Modern medical developments have led to the manufacturing of several pharmaceuticals, including insulin, which is the most common antidiabetic drug. Despite the hypoglycaemic potential of these drugs, several adverse effects are usually associated to their use (Jacob and Narendhirakannan, 2019). Due to the severity of diabetes and its complications, any choice of therapeutic invention to address this metabolic disorder must be devoid of adverse effects. As a result, natural sources of anti-diabetic agents have continued to gain significant attention over the years due to their safety and cost-effectiveness compared to the existing synthetic drugs (Sulaimon et al., 2020). Diabetes has been managed with many plants and plant-derived compounds, thus, making medicinal plants a possible source of hypoglycaemic remedies (Choudhury et al., 2018). Plants have abundant phytochemical compounds such as flavonoids, alkaloids, phenolics, and tannins that possessed anti-diabetic activity by boosting the secretion of insulin or reducing glucose absorption from the intestine to increase the effectiveness of pancreatic tissues (Kooti et al., 2016).

Myrtus communis is a Mediterranean shrub of the Myrtaceae family that grows in South Europe, West Asia, and North Africa. It may grow up to 2 m tall and prefers acidic soil that is protected from winds. Among these Myrtle species, *M. communis* L is the most common one. Many insects, notably honeybees, rely on the flowers as a major supply of pollen (Petretto et al., 2015). Moreover, it produces berry fruits rich in polyphenolic compounds (Hayder et al., 2008). *M. communis* L has been utilized as an antibacterial (Mir et al., 2020), antioxidant (Serreli et al., 2017), antidegenerative (Cevikelli-Yakut et al., 2020), antiproliferative (Yangui et al., 2021), antidiabetic (Tas et al., 2018), and anti-inflammatory activity (Touaibia 2020). The antioxidant activity of the essential oil of *M. communis* L has been investigated in the literature (Petretto et al., 2016). *M. communis* L has also been reported to reduce oxidative stress *in vitro* and *ex vivo* (Tas et al., 2018). For example, Wu et al. (2020) reported on the antibacterial activity of phloroglucinol derivatives from *M. communis* L. However, the plant, *Myrtus communis* 'Variegata' often referred to as Variegated Myrtle, is an evergreen shrub with coloured leaves that is mostly planted in the Mediterranean region. This plant has a long and storied history of usage as a traditional antiseptic and disinfectant (Scora, 1973). Terpenoids, flavonoids, and derivatives of phloroglucinol are the primary components of Myrtus plants, according to phytochemical studies documented in the literature (Alipour et al., 2014; Ghnaya et al., 2013;).

Considering the research on *M. communis* L, to the state of the art, the only study on *M. communis* 'Variegatha' was on the antibacterial activity of its phloroglucinol derivatives; as a result, there has never been any reference of its antidiabetic potential has been documented. Therefore, the present study aimed at investigating the phytochemical compounds, antioxidant activity as well as *in vitro* antidiabetic and cytotoxic potentials of 70% ethanolic leaf extract of *M. communis* 'Variegatha' obtained from a botanical garden in Cape Town, South Africa.

2. Materials and methods

2.1. Plant collection and identification

The fresh leaves of the plant were collected on the 6 September 2021 at the Botanical Garden at Cape Peninsula University of Technology, Bellville campus, Cape Town, South Africa. The plant was authenticated by Mr. Gordon Dreyer (a botanist at the Department of Horticultural Sciences) and a specimen with a voucher number 3342 was deposited in the herbarium.

2.2. Plant extraction

The leaves were washed thoroughly with tap water followed by distilled water to remove all impurities, air-dried for 10 days at room temperature under shade and pulverized with a grinding machine. One hundred grams (100 g) of leaves powder were dissolved in 1500 mL of 70% ethanol contained in a 2000 mL flask and kept on a magnetic stirrer for 48 h (Zhao et al., 2022). Thereafter, the mixture was filtered using Whatman no. 1 filter paper and the supernatant was transferred to a rotary evaporator, where the solvent was evaporated at 48 °C under reduced pressure to collect the crude extract, and thereafter, the remaining crude extract was freeze-dried (Fig. S1), and the dried powder was kept at 4 °C until further use.

2.3. Determination of total polyphenols

To determine the total polyphenol content of the extract, the Folin-Ciocalteu technique investigated by Okafor et al.

(2021) was used. The extract (1.0 mg/mL) was treated with 5 mL of 10% Folin-Ciocalteu reagent and 4 mL of 7.5 % w/v sodium carbonate. The mixture was vortexed for 15 s before being kept at 40 °C for 30 min to develop a characteristic blue color. Using a spectrophotometer, the extract's absorbance at 765 nm was determined (ANALYTIK JENA 200–2004 spectrophotometer, Germany). The total phenolic content of the extract was calculated using a curve made with gallic acid concentrations ranging from 0 to 250 mg/L; the result is represented as mg of sample gallic acid equivalent (GAE)/g.

2.4. Determination of total flavanols and flavonols

The total flavonol content was determined using the method outlined by Yermakov et al. (1987). Specifically, 2.0 mL of the extract (1 mg/mL) was combined with 2.0 mL of a 50 g/L sodium acetate solution and 3.0 mL of a 20 g/L aluminum trichloride solution. A spectrophotometer was used to test the mixture's absorbance at 440 nm after 2.5 h of incubation at 20 °C. A standard curve with quercetin concentrations ranging from 20 to 80 mg/L was used to determine the total flavonol. To express the results, milligram of quercetin equivalent per gram sample (QE/g) was employed. A colorimetric approach was used to assess the amount of total flavanol in the extract. Using 4-(Dimethylamino)-cinnamaldehyde (DMACA), the Delcour and Varebkke (1985) method was employed and the effect was determined colorimetrically at 640 nm. The result was given as milligram catechin equivalents (CE) for each gram of sample (g).

2.5. Trolox equivalent antioxidant capacity (TEAC)

The radical cation decolourization test, which employs the 2, 2′-azinobis (3-ethylbenzothiazolie-6-sulfonate) diammonium salt (ABTS) method in line with the method reported by Re et al. (1999) with minor modification was used to measure the TEAC of the extract. 8 mM ABTS (in water) and 3 mM potassium persulfate were combined to create $ABTS^{\bullet+}$, which was then allowed to stand for 16 h. The $ABTS^{\bullet+}$ solution was then diluted with ethanol (approximately 1:90, v/v) to obtain an absorbance of 0.7 ± 0.02 at 734 nm and subsequently, 100 µL diluted extract or Trolox standard solution (0–500 µM) was added to 2.4 mL $ABTS^{\bullet+}$ solution, and absorbance was measured at exactly 6 min at 734 nm by using a Multiskan Spectrum plate reader (Thermo Fisher Scientific, USA). Results were presented as micromole Trolox Equivalent per gram sample (TE)/g, with Trolox serving as the analytical reference. The standard curve was linear between 0 and 1000 µM Trolox.

2.6. Ferric reducing antioxidant property (FRAP)

The process outlined by Benzie and Strain (1996) was used for FRAP analysis. To prepare the FRAP reagent, tripyridyl triazine (TPTZ) (10 mM in 40 mM HCl), 20 mM of $FeCl_3{\cdot}6H_2O$, and acetate buffer (300 mM, pH 3.6) were well mixed. Subsequently, in a 96-well plate, 10 µL of the diluted extract was added to 300 µL of the FRAP reagent, and the mixture was allowed to sit for 30 min at room temperature. The plate was then read at 593 nm (wavelength) using a Multiskan Spectrum plate reader (Thermo Fisher Scientific, USA). Ascorbic acid served as the standard antioxidant drug.

2.7. DPPH assay

Following the description of Jimoh et al. (2019), the ability of the plant extract to scavenge DPPH radicals was evaluated. Briefly, a solution of 0.135 mM DPPH was prepared in a dark bottle served as the source of the DPPH radical. The plant extract and Trolox standard were serially diluted to equivalent concentrations of 0.08, 0.04, 0.02, 0.01, and 0.005 mg/mL, respectively, then mixed in a ratio of 1:1. The mixture followed vertexing and a 30 min incubation at room temperature. Thereafter, a volume of 300 µL dispensed in a 96-welled microplate was read at an absorbance of 517 nM. The results were presented as micromole/Trolox equivalent per g dry weight (µmol TE/g).

2.8. UPLC-ESI-QTOF-MS of phenolic analysis

The LC-MS analysis was conducted using a QA Waters Synapt G2 quadrupole time-of-flight mass spectrometer. It was fitted with a Waters ultra-pressure liquid chromatography (UPLC-MS) using Waters ms^E technology and photodiode array detection. The phenolic method and specification of the instrument was reported by and adopted from Stander et al. (2017), in the negative ion mode with minor modifications. The solvents A and B in the positive ion mode each contained 0.1% formic acid, while the mobile phase in this mode was made up of water and acetonitrile. After 0.5 min of 100 % solvent A, the gradient switched to 100% B for over 0.5 min to 12.5 min. Thereafter, 13 min into the runtime, it then changed to 100% A for the following 2 min in a total run time of 15 min. The flow rate was 0.4 mL/min, the seal wash was 5 min, and the column temperature was maintained at 55 °C. Ionizing electrospray 275 °C desolvation temperature, 15 V cone voltage, and ESI Pos. Leucine encephalin was injected as a lock mass in the background and sodium formate was employed for calibration to get precise mass measurements. The MassLynx software platform supplied with Waters Mass spectrometers was used for manually processing each chromatogram.

2.9. Identification of compounds using UPLC-ESI-QTOF-MS

The identified metabolites were given preliminary names based on accurate mass matches that were automatically searched in databases such as Metlin, massBank, NIST, and other libraries like PubChem, mass fragmentation patterns of compounds searched in the databases, and number of carbon atoms for isotope relative abundance. All compounds were identified as unidentified using accurate mass match if their accurate mass error (AME) was more than 5 ppm (Zubarev and Makarov, 2013). To identify a particular compound based on the retention time, mass fragmentation, and ionization modes, a few standards of phenolic compounds were spiked under identical LC/MS conditions (positive and negative ion modes). Considering that it was possible to get all standards and many compounds could be detected using UPLC-MS, the MS and MS^2 fragment ions of other compounds that were like those being annotated were employed. Compound structures were

elucidated using MS-MS analysis of the sample's compounds that were fragmented to match to product ion mass spectra. If isotope abundances were available, the number of carbon atoms in the peak was computed as a final step. False annotations were minimized by using the predicted number of carbon atoms in the putatively recognized molecule.

2.9.1. Reagents

All reagents and chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless stated otherwise. Minimal Essential Medium (MEM) and PBS with and without Ca^{2+} and Mg^{2+} were purchased from Cytiva (Marlborough, MA, USA). Foetal Bovine Serum (FBS), non-essential amino acids and penicillin/streptomycin were purchased from Biowest (Nuaillè, France). Bis-benzamide H 33,342 trihydrochloride (Hoechst) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Human hepatoma derived C3A hepatocytes were purchased from American Type Culture Collection (ATCC, Manassas, Virginia, USA). MEM/EBSS and Park Memorial Institute (RPMI) 1640 Medium, as well as PBS with and without Ca^{2+} and Mg^{2+} were purchased from Cytiva (Marlborough, MA, USA).

2.9.2. Cell culture maintenance

All cell cultures were incubated at 37 °C in a humid atmosphere with % CO_2. The L6 myoblast cells were grown in an antibiotic-free medium comprising RPMI 1640 and 10% foetal calf serum, while the HepG2 cells received a growth medium consisting of RPMI 1640 medium every 2 to 3 days. After 90 percent confluence, the cell lines were divided into subcultures.

2.9.3. MTT cytotoxicity assay

A final concentration of 100 mg/mL of the plant extract was obtained by reconstituting it in dimethyl sulfoxide (DMSO), which was then sonicated and kept at 4 °C until needed. The cells (C3A hepatocytes) were kept in 10 cm culture dishes with full media (MEM with 1 % non-essential amino acids, 10% FBS, penicillin/streptomycin) and incubated at 37 °C in a humid environment with 5% CO_2. Afterward, the cells were seeded in 96-well plates at a density of 4000 cells per well (100 µL aliquots) and allowed to attach for an additional day. Thereafter, 100 µL of three different concentrations of the plant extract (200, 100, and 50 µg/mL) were then treated with the cells in the 96-well plate for 48 h at 37 °C, 5% CO2, and melphalan (100 mM stock) was used as a positive control at 30 µM. After aspirating the medium and treatment, 100 µL of the staining solution—Hoechst 33,342 nuclear dye (5 µg/mL)—was added to each well, and the plates were then let to sit for 30 min. The plates were immediately photographed using the DAPI and Texas Red filters after the incubation time, when 10 µL of propidium iodide (PI) solution (100 µg/mL) was added to each well.

Using a 10 Plan Fluor objective, DAPI and Texas Red filter cubes, and an ImageXpress Micro XLS Widefield Microscope (Molecular Devices), it was possible to quantify both living and dead cells. Per well, nine image sites were captured, which represents approximately 75% of the well's surface area. The MetaXpress program and the Multi-Wavelength Cell Scoring Application Module were used to analyze the acquired images. Data processing and analysis were conducted on acquired data once it was uploaded to an EXCEL spreadsheet.

2.9.4. Alpha-glucosidase determination

For this test, all chemicals and reagents were freshly prepared and purchased from Sigma-Aldrich (St. Louis, MO, USA). The α-glucosidase inhibitory test was carried out using a modified version of the Sekhon-Loodu and Rupasinghe (2019) method. Briefly, the plant extract was reconstituted in dimethyl sulfoxide (DMSO) to a final concentration of 100 mg/mL and diluted in assay buffer (potassium monobasic anhydrous phosphate (67 mM, pH 6.8)) to concentrations range of 20 – 1.25 µg/mL. Ten microlitres (10 µL) of the extract and 70 µL of the α-glucosidase from Saccharomyces cerevisiae (50 g/mL) were put to a 96-well clear plate, and the mixture was incubated at 37 °C for 10 min. Thereafter, 20 µL of p-nitrophenyl-D-glucopyranoside (10 mM) was added and the reaction was terminated by adding 25 µL Na_2CO_3 (100 mM) and the absorbance was measured at 410 nm using a BioTek® PowerWave XS spectrophotometer (Winooski, VT, USA). The positive control, epigallocatechin gallate (EGCG), was mixed with the enzyme and substrate without inhibitors. Sample controls and blanks, on the other hand, were mixes of sample and control, but with α-glucosidase in place of buffer, respectively. The following formula was used to determine the percentage of α-glucosidase inhibition:

$$\%\,\alpha - \textit{glucosidase}\,\text{inhibition} = \frac{(\text{A410nm of control} - \text{A410nm of test sample})}{\text{A410nm of control}}\ \text{x}\ 100$$

IC_{50} values were determined using GraphPad Prism 4.0.

2.9.5. Hepatocyte glucose uptake of the plant extract

The effect of the plant extract on glucose uptake and utilisation in C3A hepatocytes was determined in accordance with the method of van de Venter et al. (2008), with modifications. The plant extract was ultrasonically reconstituted in DMSO at a stock concentration of 100 mg/mL, then kept at 4 °C until needed. The cells were kept in 10 cm culture dishes loaded with a complete medium (MEM with 1% NEAA, 10% FBS, and 1% Pen-Strep) and incubated at 37 °C in a humid environment with 5 % CO_2. The cells were seeded in 96 well plates (2×10^4 cells/well, 100 µL aliquots) and left overnight to attach. Incubation of the cells with the plant extract at various concentrations (25, 50, and 100 µg/mL) lasted for 24 h. Following the aspiration of the medium, 25 µL of incubation buffer (RPMI-1640 mixed with PBS containing 0.1% BSA to a final glucose concentration of 8 mM) was added to the cells before being rinsed with 100 mL of PBS and the media was removed. A positive control was employed, which was insulin (1 µg/mL). After the cells had been incubated for 4 h, 200 µL of freshly prepared glucose oxidase test reagent (0.5 M PBS, pH 7.0, 2.5 U/mL horseradish peroxidase, and 1 mU/mL glucose oxidase from *Aspergillus niger*) was added to the plates. A BioTek® PowerWave XS spectrophotometer was then used to measure the absorbance at 510 nm after the reaction had been incubated at room temperature for 15 min (Winooski, VT, USA). To track changes in the concentration of glucose in the used culture medium as a function of the red-colored quinoamine dye complex produced, which was measured spectrophotometrically at 510 nm, a colourimetric glucose oxidase/peroxidase assay, based on the procedure described by Trinder (1969), was used. The glucose standards were the cell-free wells with incubation buffer and full culture media.

As a function of the amount of glucose (mM) left, glucose uptake and consumption were calculated and expressed as the difference between the means of the standard and test samples.

To confirm that any detected variations in glucose uptake or utilization were not caused by variations in cell viability, the MTT (3–4,5 dimethylthiazol-2,5-diphenyltetrazolium bromide) experiment was carried out. The reduction of a yellow, water-soluble tetrazolium salt to a purple, insoluble formazan product is the base of the MTT test. The absorbance was determined at 540 nm and the purple-colored formazan crystals were dissolved in dimethyl sulfoxide (DMSO). The quantity of healthy, live cells is then correlated with the formazan concentration (Twentyman and Luscombe, 1987).

2.10. Data analysis

All the data were triplicated and expressed as mean ± standard deviation. The data were analyzed using a one-way Analysis of variance (ANOVA). For the cytotoxicity test, the IC_{50} values were calculated using the graph pad prism. A p-value < 0.05 was considered significant.

3. Results and discussion

3.1. Phenolic contents

It has been well established that prevention appears as a potent strategy for reducing the risk of detrimental chronic conditions such as diabetes as therapy options do not always ensure a long-term recovery (Averilla et al., 2019). Interestingly, plants have been employed as a reference source for isolating lead compounds for the development of drugs that could be used in the management of chronic diseases in humans (Naz et al., 2019). Presently, the extraction of phytochemicals, such as phenolic compounds, has gained popularity since they are one of the most significant natural antioxidants or free radical terminators with a variety of therapeutic advantages and coupled with the fact that their use is associated with fewer side effects (Banothu et al., 2017; Hassan et al., 2021). It is worth noting that the solvents used in soaking and shaking for maceration extraction also play a vital role (Azwanida et al., 2015). Remarkably, previous studies have demonstrated that the solvent mixture (alcohol and water), as opposed to ethanol and other solvents, is better for the extraction of phenolic compounds (Obied et al., 2005; Moo-Huchin et al., 2019; Shahinuzzaman et al., 2019; Shahinuzzaman et al., 2020). It is important to note that the presence of water in an aqueous alcohol mixture during extraction enables the plant components to absorb water and increase in size, allowing the alcohol to easily penetrate the solid matrix and leading to higher extraction and bioactive components of the plant (Galanakis et al., 2013; Okafor et al., 2021).

In the present study, 70% (v/v) ethanol was employed to extract the phenolic compounds of *M. communis* 'Variegatha'. A crude extract yield of 20.22 g was obtained from 100 g of dried leaf powder in 1.5 L of 70 % (v/v) ethanol for 48 h. The polyphenols content was 116.44 ± 3.26 mg GAE/g, flavonols (6.74 ± 0.50 mg QE/g), flavanols (2.46 ± 0.03 mg CE/g). Flavonoids are the largest and predominant group of plant polyphenols with nutraceutical importance (Lokesh and Venkatarangana, 2015). Flavonols, the most predominate group of flavonoids, are present in most plants and consequently their TEAC (Singleton et al., 1999). In addition to scavenging free radicals and inhibiting enzymes that generate ROS, flavonoids also regulate the body's antioxidant defenses (Mishra et al., 2013). Also, flavonoids protect the biomembrane lipids from lipid peroxidation-caused damage. Thus, flavonoids act as antioxidants and help to prevent a variety of chronic caused by oxidative stress (Ramchoun et al., 2009).

Likewise, the antioxidant activity of plant extracts was assessed by using some well-established assays such as DPPH, TEAC and FRAP (Rajurkar and Hande 2011). The FRAP was estimated to be 1267.28 ± 38.23 µmol AAE/g, DPPH (1165.37 ± 45.21 µmol TE/g), and TEAC (775.52 ± 20.96 µ mol TE/g). The results from the present study corroborate with the findings of Aryal et al. (2019) from *Alternanthera sessilis* and Tirado-Kulieva et al. (2021) from *Mangifera indica* about the strong antioxidant capacity demonstrated by the crude extract, which may be attributed to its rich phenolic compounds. Free radicals have been identified as one of the main players in endogenous enzyme inactivation and other vital cellular components caused by oxidative stress (Halliwell and Gutterdige, 2015). To restore redox equilibrium and either inhibit or reverse detrimental free radical impacts, the investigated plant extracts may modulate and neutralize these free radicals *in vivo* (Moriasi et al.., 2020). The 70% ethanolic leaf extract of *M. communis* 'Variegatha' also had a high ferric reducing power, which implies that it may reduce ferric ions to ferrous ions to assess its antioxidant potential. Intriguingly, it aids in predicting the extract's capacity to mimic the body's natural antioxidants, such as bilirubin and uric acid, in reducing oxidative stress (Singh et al., 2016).

3.2. Total antioxidant capacity

Total antioxidant capacity assays have the benefit of being able to assess the antioxidant contents of a sample in a global way (Rubio et al., 2016). Diabetes and its complications have been linked to oxidative stress, which is caused by an imbalance favouring free radicals over cellular antioxidants (Ojo et al., 2019). In the present study, ABTS, DPPH, FRAP, and TEAC assays were widely used to assess the antioxidant capacity of the extract and it was observed that the extract demonstrated strong antioxidant activity (1165.37 ± 45.21 µ mol TE/g) against DPPH radical, TEAC (775.52 ± 20.96 µmol TE/g) and the significant antioxidant capacity demonstrated by the extract could be due to the presence of the phenolic compounds identified in Tables 1 and 2 (Oleinik et al., 2022). The potential of the extract to reduce ferric to ferrous was also investigated (Bartosz 2010). The FRAP was observed to be 1267.28 ± 38.23 µmol AAE/g. An important mechanism of the phenolic antioxidant effect is electron donating activity, which is frequently measured by Fe (III) reduction (Vadivukkarasi et al., 2014). The FRAP reducing power capacity of any compounds is often dependent on the existence of reductones, which serve as antioxidants by donating one atom of hydrogen to break the chain of free radicals (Meir et al., 1995; Duh et al., 1999).

The extract had a considerable reducing power potential, which served as a significant indication of its antioxidant activity by causing the Fe^{3+}/ferricyanide complex to be reduced to

Table 1 Phytochemicals screened from 70% ethanol extract of *M. communis* 'Variegatha'.

No	t_R (min)	UV λ^{max} (nm)	*m/z* $[M-H]^-$	MS/MS	Tentative name	Identification
1	1.10		215.0326	207, 209, 195, 179, 165	Xanthotoxin	New
2	1.17		191.0561	191, 89	Quinic acid	Taamalli, et al., 2014
3	1.96	231	191.0201	131, 127, 161	Quinic acid	Taamalli, et al., 2014
4	4.45	274	343.0672	191, 147, 127	Galloyl quinic acid isomer	Taamalli, et al., 2014
5	6.90	264	782.0615	316, 633, 162	Quercetagetin-7-O-galloylprocatechuoylhexoside	New
6	8.03	274	495.0761	169, 343, 191, 125	Digalloyl quinic acid	Taamalli, et al., 2014
7	8.34	265, 274	783.0643	301, 495, 708	Cornusiin C isomer 1	Taamalli, et al., 2014
8	11.47	237	431.1889	385, 421, 179	Neorehmannioside	Taamalli, et al., 2014
9	11.84	264, 349	631.0911	489, 316, 271	Quercetagetin-7-O-galloylglucoside	New
10	12.52	262, 307, 357	479.0818	316, 287, 271	6-hydroxyquercetin7-O-β-glucopyranoside	New
11	13.40	259, 348	615.1011	381, 447, 477	Quercetagetin-O-procatechuoylhexoside	New
12	14.13	259, 349	463.0865	316, 300, 271	Ellagic acid hexoside	Taamalli, et al., 2014
13	16.31	256, 345	447.0943	301	Quercetin-3-O- rhamnoside	Taamalli, et al., 2014
14	17.25	244, 287, 325	359.0757	161, 197, 191	Syringic acid hexoside	New
15	17.50	244	417.2121	407, 187, 161, 179	1,3-O-Caffeoyl-dihydrocaffeoylglycerol	New
16	19.84	273	571.2371	305, 533, 247	Epigallocatechin derivative	New
17	21.95	272	567.2070	169, 447, 125, 271, 324, 461	Gallomyrtucommulone C	Taamalli, et al., 2014
18	24.03	238, 271	209.0804 503.3405	117	unknown	
19	24.44	288	445.2232	431, 265, 225, 165, 139, 123	Apigenin-6-C-glucoside derivative	New
20	24.66	281, 404	431.2065	209, 311	Apigenin-6-C-glucoside	New
21	24.79	281, 404	431.2066	195, 209	Apigenin-8-C-glucoside	New

the ferrous form (Sulaimon et al., 2020). Antioxidants are also reported to react with certain precursors of peroxide, thus preventing peroxide formation (Kumar et al., 2021; Sharifi-Rad et al., 2020). It has been established in the literature that non-enzymatic antioxidants such as phenolic compounds can act through one of the different antioxidant mechanisms to scavenge free radicals (Kumar et al., 2012). By either boosting the body's built-in antioxidant defense system or taking proven numerous health benefits, the plant's antioxidant capacity suggests that it may be utilized to lower the risks of chronic illnesses and prevent disease development. Therefore, the search for natural antioxidants is, therefore, a top priority (Ali et al., 2008). The antioxidant activity of medicinal plants is revealed by their involvement in reducing free radical-caused tissue injury (Kasote et al., 2015; Sylvie et al., 2014).

3.3. UPLC-ESI-QTOF-MS analysis

3.3.1. Characterisation of phenolic compounds

A total of 42 metabolites could be identified. Figure 1 and 2 show the base peak chromatogram for 70% ethanol extract of M. communis. A number of phytochemicals were identified; hydroxybenzoic acids including ellagic acid derivatives, hydroxycinnamic acids, flavonols, flavanols, flavones and coumarins and furanocoumarins

Hydroxybenzoic acids: Peaks 4, $[M-H]^-$; *m/z* 343, peak 6 and 36, and 17 were considered as galloyl derivatives, which exhibited major ions in MS^2 fragmentation, *m/z* 169 corresponding to that of gallic acid (Fig. 1) (Khallouki et al., 2015), owing to UV absorption of about 276 nm and *m/z* 125 [gallic acid $-H_2O-CO_2]^-$. The compounds were also previously identified (Clifford et al., 2007, Taamalli et al., 2014).

Hydroxycinnamic acids: two peaks, 14 and 15 peaks have been characterised and hydroxycinnamic acid derivatives. Peak 14 was tentatively identified as syringic acid hexoside; [M -H]$^-$, *m/z* 359, showing a typical major fragment; 197 [syringic acid -H]$^-$, 329 [M –H- $CH_3]^-$, 161[M –H- syringic acid]$^-$. Peak 15 was a phenylpropane glycerides identified as 1,3-O-caf feoyl-dihydrocaffeoylglycerol in the literature with a similar MS^2 fragmentation pattern (Ma et al., 2007; Kang et al., 2016).

Flavonols: Eleven compounds belonging to flavonol peaks: (5, 9, 10, 11, 12, 13, 24, 25, 26, 27 and 29) were characterised from 70% ethanol extract of *M. communis*. Most flavonols were detected at UV absorption at 258 and 348 nm. Peaks 27 and 13 were identified to be those of quercetin and quercetin-3-O-rhaminoside, respectively as earlier identified

Table 2 Phytochemicals screened from 70% ethanol extract of *M. communis* 'Variegatha'.

No	t_R (min)	UV λ^{max} (nm)	*M/z* $[M + H]^+$	MS/MS	Tentative nomenclature	Identification
22	0.68	239, 259	203.0535	192	Xanthotoxol	New
23	1.35	268	163.0614	145	Hydroxy coumarin	New
24	2.75	240, 260	479.0839	153, 125, 309	Isorhamnetin-7-O-galactoside	New
25	3.56	259, 286	319.0445 481.0957	153, 319	6-hydroxyquercetin7-O-β-glucopyranoside	New
26	3.83	258, 287	319.0466	319, 147	6-hydroxyquercetin	New
27	4.20	267, 353	303.0517	155, 227	Quercetin	
28	4.42	254, 370	211.1699^{**}	193, 175, 135	Scopoletin	New
29	5.17	275	315.0713	153, 165, 221, 255	6-Hydroxykaempferol methyl ester	New
30	5.92	239, 282	221.1545^{*H2O}	193	Xanthotoxol	New
31	6.30	248	475.1626^{Na}	313, 375, 298, 105	Epicatechin-3-O glucoside	New
32	7.00	275	279.1611^{**}	219, 149	Leptophyllin	New
33	7.21	273	237.1489	237	unknown	
34	8.06	278, 234	313.1094^{Na}	237	Epicatechin	New, identified using a standard
35	8.50		200.2017		unknown	
36	9.39	293	496.3383	184, 125, 104, 478	Digalloyl quinic acid	Taamalli, et al., 2014
37	9.72	239, 293	433.2227	197, 179, 415, 151	Apigenin-8-C-glucoside	New
38	10.08	292	447.2373	429, 211	Apigenin-8-C-glucoside derivative	New
39	10.60	436	373.2750 653.3666	373, 593, 293, 413	Epicatechin or catechin derivative	New
40	11.52	408	593.2747	533, 461, 433, 505, 277, 415	Apigenin-di-C-hexoside	New
41	11.83	264	373.2741	237	Epicatechin or catechin derivative	New
42	12.49	410	621.3067	unfragmented	Cratenacin (4^{111}-acetylvitexin-2^{11}-O-rhamnoside)	New

$^{*H20} = [M + H\text{-}H_2O]^+$, $^{Na} = [M + Na]^+$, and $^{**} = [M + NH_4]^+$ in the positive ion mode. ^{New} means it has been reported from *M. communis* 'Variegatha' for the first time, although it has been previously identified from other plant sources.

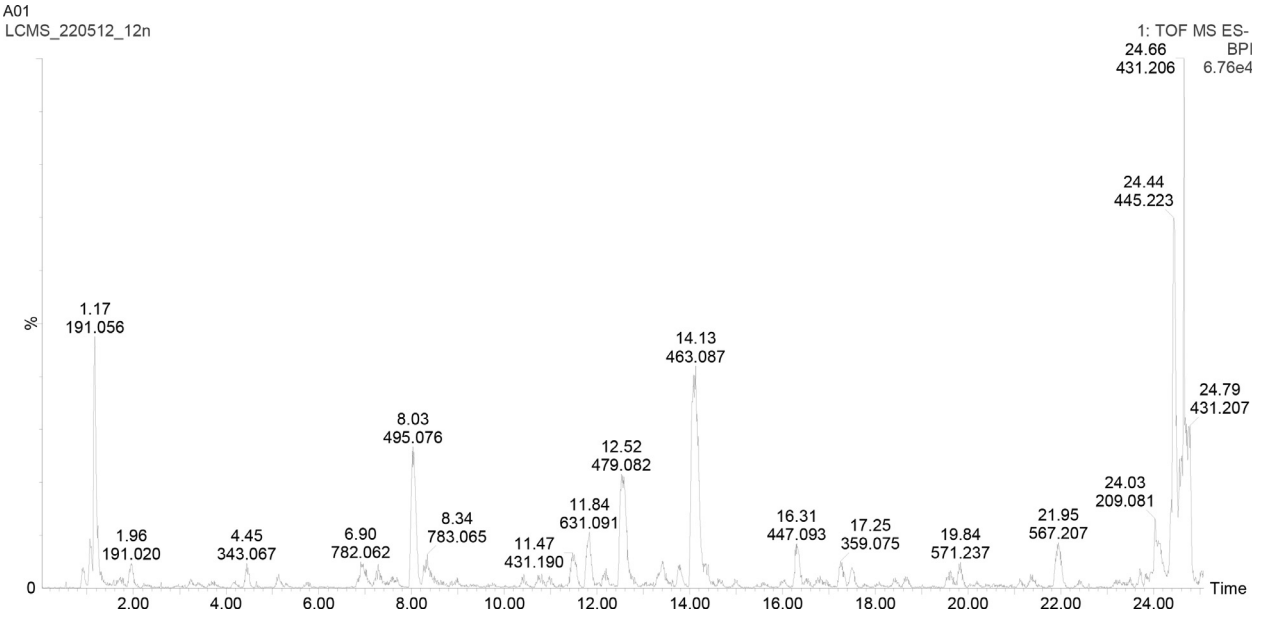

Fig. 1 UHPLC-ESI-MS base peak chromatogram for 70% ethanol extract of *M. communis* 'Variegatha' analysed in the negative ion mode.

(Taamalli et al., 2014; Wang et al, 2001). Quercetin; $[\text{quercetin} + H]^+$; *m/z* 303 with common MS^2 fragment 155 $[^{1,3}A^-]$, formed through retrocyclization cleavages of the C-ring of the aglycone involving 1 and 3 bonds (bonds 1 and 3 refer to the O—C—2 and C-3—C-4 bonds of the C-ring) (Tsimogiannis et al., 2007). Quercetin aglycone underwent sugar conjugation by the addition of a rhaminoside (146 Da) (peak 13). Peak 26, *m/z* 319 ion, ($[M-H]^-$ ion) was previously

identified as 6-hydroxyquercetin (Parejo et al., 2004, Shahzadi and Shah, 2015). Peaks 10 and 25 were a sugar conjugates of 6-hydroxyquercetin (Parejo et al., 2004, Shahzadi and Shah, 2015). They displayed major MS *m/z* 481. Peaks 9 and 11 were identified as quercetagetin-7-O-galloylglucoside and quercetagetin-O-procatechuoylhexoside, respectively as previously identified (Parejo et al., 2004). The CID spectrum of the quercetagetin *m/z* 317 ions, ([M−H]$^-$ ion) showed MS^2 main fragment, 149 [^{1,3}B$^-$], in negative ionisation mode ([^{1,3}B$^-$], bonds 1 and 3, here refers to the O—C—1 and C-3—C-4 bonds of the C-ring bond cleavage) (Tsimogiannis et al., 2007). Exhibiting the characteristic ion fragments due to the *retro*-Diels-Alder cleavage at *m/z* 149. Quercetagetin aglycone was conjugated with O-linked galloyl glucoside and procatechuoylhexoside (peaks 9 and 11) Table 1. Further esterification of procatechuoylhexoside with galloyl could give compound 5. Peaks 29 were identified as those of 6-hydroxykaempferol methyl ester, owing to UV max absorption, 339, 340 nm and prior report of this compound and as methyl ester adding 14 u on the aglycone (Parejo et al., 2005, Shahzadi and Shah, 2015). Peak 24 was identified as isorhamnetin-7-O-galactoside after glucoside conjugation to isorhamnetin aglycone (315 Da) with product ions *m/z* 153, 125, 309 (Parejo et al., 2004).

Ellagic acid derivatives: Peaks 7 and 12 were previously characterised as cornusiin C isomer 1 and ellagic acid hexoside (Taamalli et al., 2014).

Flavanols: Five flavanols were characterised (peaks 16, 31, 34, 39 and 41). Compound 34 was identified as epicatechin because its fragmentation pattern was consistent with that of the standard. Peak 31 was identified as epicatechin-3-O glucoside in which 162 Da was added. Peaks 16, 39 and 41 had MS^2 fragments, similar to that of the aglycone largely (Escobar-Avello et al., 2019) and therefore identified as derivatives of epicatechin.

Flavones: Seven flavones from peaks 19, 20, 21, 37, 38, 40 and 42. Many of these are being reported for the first time from this plant. However, MS^2 fragmentation behaviour for compounds 20, 21, 37, 40, and 42 is similar to earlier report (Stander et al., 2017). Apigenin-6-C-glucoside derivative was identified from peak 38 due to similar fragmentation pattern and UV absorption with that of peak 20 and 21.

3.4. Coumarins and furanocoumarins

Coumarins and furanocoumarins were detected at peaks 1, 22, 23, 28, 30 and 32. Most of which exhibited UV λ^{max} at 274 nm, characteristic of coumarin. Peak 1 was identified in negative ion mode as xanthotoxin consistent with previous reports (Kerebba et al., 2022; Yang et al., 2010). Peak 23 was identified as umbelliferone (hydroxyl coumarin) peak, consistent with the literature (Yang et al., 2010). Peak 28 was tentatively identified as scopoletin via its ammonium adduct in the positive ion mode with MS^2 of characteristic ion, *m/z* 193 [M + H]$^+$, 175 [M + H- OH]$^+$ and 135 [M + H-OCH$_3$ –OH-O]$^+$. From Fig. 2, peaks 22 and 30 were identified to be furanocoumarin; xanthotoxol (8-hydroxypsorale), as identified in the previous report (Kerebba et al., 2022; Yang et al., 2010), although they have been reported from this plant for the first time. Peak 32 was identified as leptophyllin in accordance with the literature (Yang et al., 2010). It displayed with [M + NH$_4$]$^+$; *m/z* 279 and MS^2 fragments *m/z* 219 [M + H- CO$_2$]$^+$ Table 2.

3.4.1. UPLC-QTOF-MS quantitation of phenolic compounds

The UV/vis absorptions, capable of distinguishing phenolic subclasses, were considered a starting point for compound quantification. Based on the qualitative analysis, four chemical markers, namely phenolic acids and derivative (neochlorogenic acid (peak 1), caffeic acid (peak 3), ferulic acid (peak 6), and coumaric acid (peak 5), flavonol (peak 7), dihydrochalcone

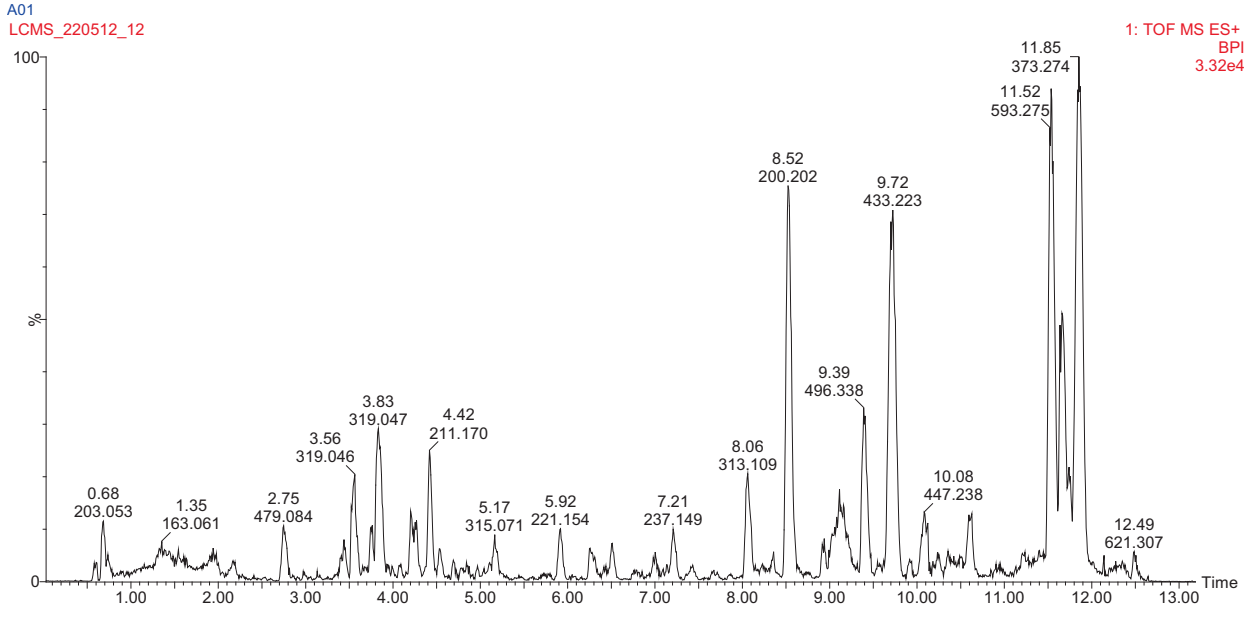

Fig. 2 UHPLC-ESI-MS base peak chromatogram for 70% ethanol extract of *M. communis* 'Variegatha' analysed in the positive ion mode.

(peak 8), and flavano-3-ols (peak 2/4) were selected for simultaneous quantitative determination (Fig. 3). They were the most abundant in many reported plant ethanolic extracts. They have been well represented in this study. Based on the UV spectrum of the markers, UV detection wavelengths were chosen. The phenolic acids and derivatives had the strongest UV absorption at 300, 308, and 325 nm, whereas flavonols at 254, 255, and 354 nm, dihydrocalcones at 284 nm, and finally flavan-3-ol at 277 nm.

Different concentrations of the standard mixture (3.9, 7.8, 15.6, 31.3, 62.5, 125.0, and 250.0 mg/L), were injected for quantification. The linearity of the calibration curve was checked by plotting the peak areas against the series of standard solution concentrations (mg/L) and determining the correlation coefficient using a linear regression model. In all cases, the system was linear when r greater than 0.99. Limits of quantification and detection LOQ and LOD respectively, presented in Table 3 were calculated by the parameters of the analytical curves (standard deviation of the response and slope). The standard deviation of the y-intercepts of regression lines was used as the standard deviation of the blank. The LODs and LOQs were estimated as 3.3 and 10 times the standard deviation of the blank/slope ratio of the calibration curve, respectively. According to LODs and LOQs, the phenolic compounds were highly detectable and quantifiable using the methods specified in the 70% ethanol extract of *M. communis* 'Variegatha' (Fig. 4). (Table 3). This approach may be used to profile phenolic compounds from the samples examined, according to the observed limits of detection and quantification. The quantification of phenolic compounds has been expressed as neochlorogenic acid, caffeic acid, ferulic acid, coumaric acid, rutin, catechin, epicatechin, phloridzin equivalents.

3.5. Alpha-glucosidase inhibition

In the present study, Fig. 5 depicts the inhibition of alpha-glucosidase activity by an ethanolic extract of *Myrtus communis* 'Variegatha'. Remarkably, a concentration-dependent (1.25–20 μg/ml) manner was observed and the highest activity (98.95%) was attained at the higher concentration of 20 μg/mL, which is higher than the control drug, EGCG (45.87 μg/mL ≈100 μM). From the above result, the plant extract exhibited a strong alpha-glucosidase inhibition, with an IC_{50} of 3.159 ± 1.03 μg/mL comparable to that of the positive control EGCG (6.208 ± 1.02 μg/mL ≈ 13.54 μM). The alpha-glucosidase inhibitory activity recorded in the present study is highest than those reported in the literature. For instance, the ethanol extract of *Myrica gale* demonstrated the highest inhibition of alpha-glucosidase with IC_{50} values (27 mg/L) (Sekhon-Loodu and Rupasinghe, 2019). Extract from *Cornus capitata* Wall. showed inhibition effect, with 98.37% inhibition (IC_{50} 12.5 μg/mL) (Bhatia et al., 2019). With IC_{50} values of 12.5 μg/mL and 24.8 μg/mL, respectively, both 50% ethanol and *Tamarix nilotica* crude in aqueous inhibited alpha-glucosidase in a concentration-dependent manner (Daou et al., 2022).

The quest for novel drugs from natural resources, particularly plants, has become a promising strategy for treating postprandial hyperglycemia to validate the traditional claims of some medicinal plants (Kifle et al., 2020; Pringle et al., 2021). Alpha glucosidase catalyses the final stage of starch digestion by hydrolysing terminal glucose molecules from the non-reducing ends of oligosaccharides. The breakdown of dietary starch and conversion of the oligosaccharides to glucose by the carbohydrate hydrolytic enzymes alpha-amylase and alpha-glucosidase results in an increase in postprandial glucose (Ojo et al., 2019; Sekhon-Loodu and Rupasinghe, 2019). Maltase-glucoamylase (MGAM) and sucrose-isomaltase (SI), two membrane-bound enzymes found in the brush edge of epithelial cells in the small intestine, are alpha-glucosidase (Tundis et al., 2010; Rose et al., 2018). Alpha-glucosidase inhibitors prevent postprandial hyperglycaemia by slowing down the digestion of carbohydrates and consequently the rate at which glucose can be absorbed and enter the general circulation. Alpha-glucosidase inhibitors such as acarbose act via a

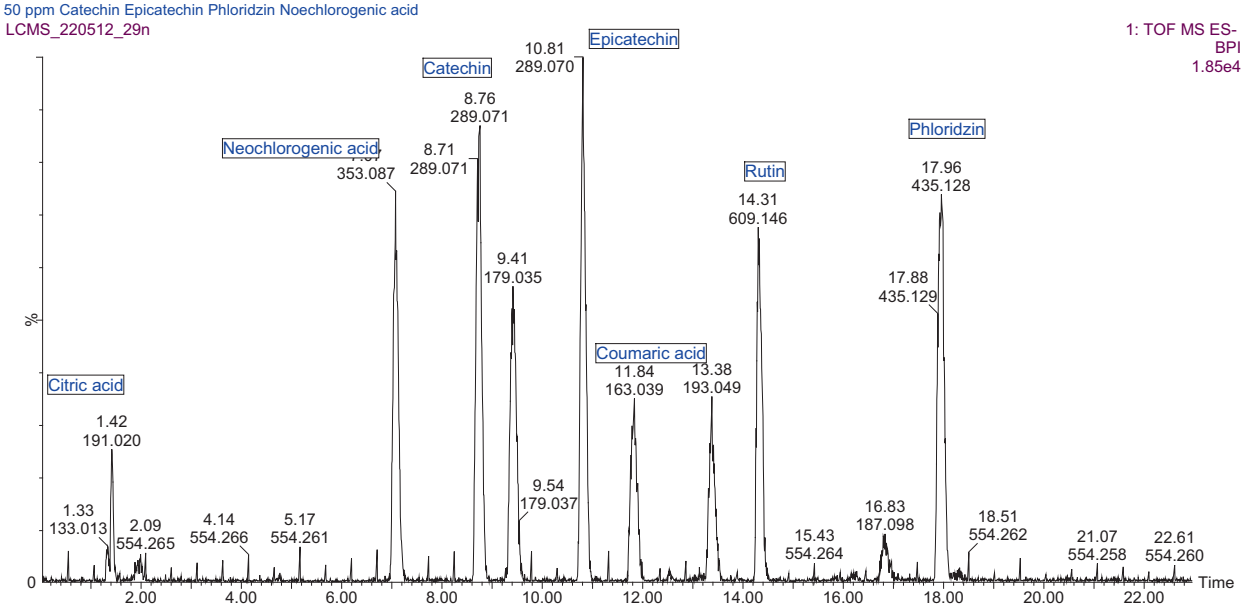

Fig. 3 UHPLC-ESI-MS base peak chromatogram for the standard mix in negative ion mode.

Table 3 Linearity, LOD and LOQ of chemical markers used as reference.

No	Name	t_R (min)	UV_{λ}^{max} (nm)	Regression equation	Linear range mg/L	R^2	LOD mg/L	LOQ mg/L
1	Neochlorogenic acid	7.1	300, 325	y = 3.0x + 7.9	3.9–31.3	0.994	10.0	30.4
2	Catechin	8.7	278	y = 1.6x + 94.2	3.9–31.3	0.999	5.3	16.0
3	Caffeic acid	9.4	300, 325	y = 1.9x-1497.4	3.9–15.6	0.993	6.2	18.9
4	Epicatechin	10.8	277	y = 1.2x + 1859.3	3.9–31.3	0.996	4.0	12.2
5	p-Coumaric acid	11.8	300, 308	y = 3.9x + 2855.3	7.8–62.5	0.993	12.9	39.1
6	Ferulic acid	13.4	300, 323	y = 4.2x + 1098.7	7.8–31.3	1.000	13.8	41.7
7	Rutin	14.5	254, 255, 354	y = 1.9x + 449.7.3	7.8–31.3	0.994	6.1	18.6
8	Phloridzin	17.9	284	y = 1.4x + 1603.3	3.9–15.6	1.000	4.7	14.2

NB: Limit of detection (LOD), Limit of quantification (LOQ).

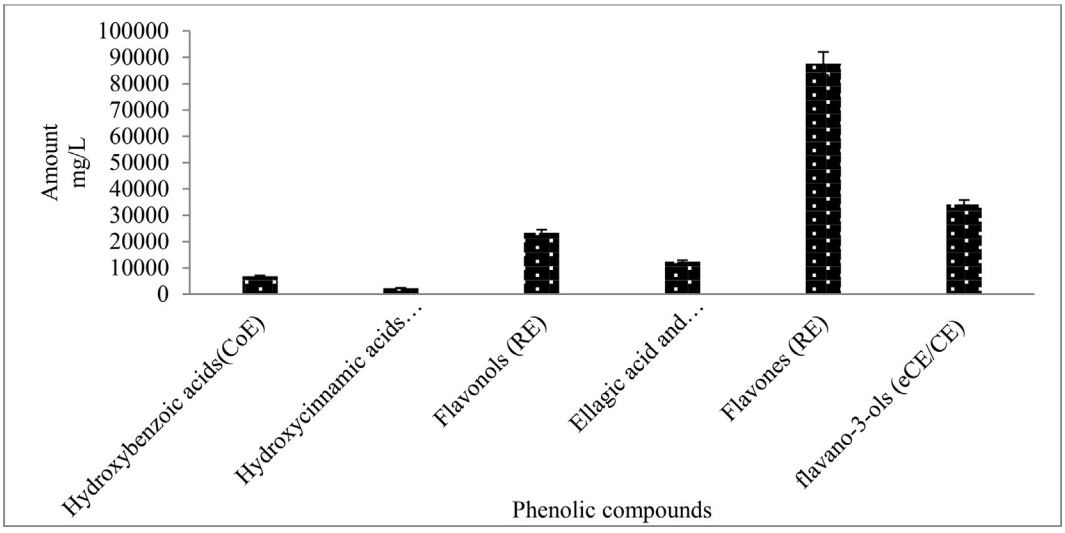

Fig. 4 The amount of phenolic compounds quantitated from 70 % ethanol extracts of *M. communis* 'Variegatha'. CoE = coumaric acid equivalents, CE = Catechin equivalents, CAE = caffeic acid equivalent, RE = rutin equivalent, eCE = epicatechin equivalent.

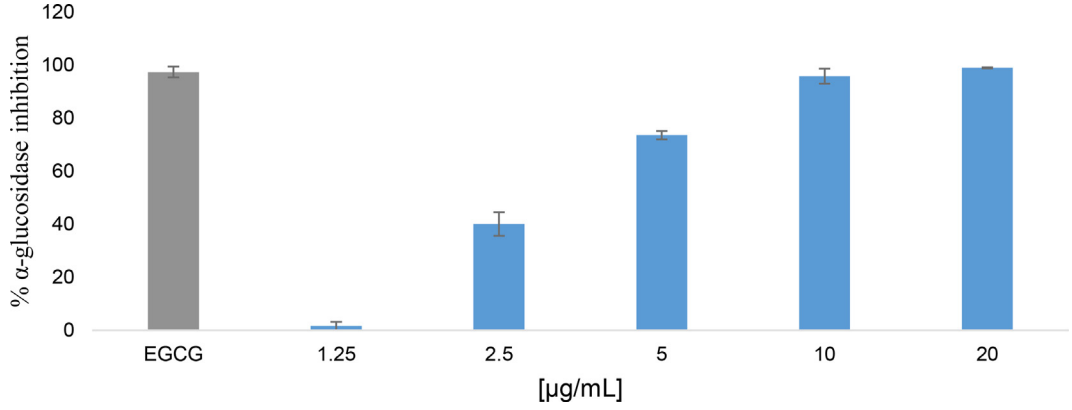

Fig. 5 Alpha-glucosidase inhibition effect of ethanolic extract of *Myrtus communis* 'Variegatha'. ECGC (100 µM) was used as a positive control. Error bars indicate the standard deviation of the mean.

competitive inhibition mechanism and should ideally bind to all four catalytic domains of the enzyme for it to effectively inhibit the hydrolysis of oligosaccharides (Roskar et al., 2015). Thus, alpha-glucosidase inhibitors are frequently used as oral antidiabetic drugs in the early stages of T2D to combat postprandial hyperglycaemia and obesity (Ojo et al., 2019). As a result, the quest for alpha-glucosidase inhibitors from natural sources such as plants has gained attention.

In this study α-glucosidase inhibitory compounds such as, ellagic acid hexoside, quercetin, and their conjugates, eriodictyol, catechin and epicatechin, coumarins such as leptophyllin and Xanthotoxol. For example, quercetin, and their

conjugates are strong α-glucosidase inhibitors (You et al., 2012). Catechin has suppressed the activation of NF-κB system through the inhibition of pro-inflammatory cytkines productions (Mostafa et al., 2014), eriodictyol also suppresses the activation of NF-κB system and reduces TNF-α, intercellular adhesion molecule 1 (ICAM-1), vascular endothelial growth factor (VEGF), and endothelial NOS (eNOS) (Zhang et al., 2012). Epicatechin has previously exhibited α-glucosidase inhibitory activity of IC_{50} = 29.85 ± 2.20 compared with quercetin (IC_{50} = 5.30 ± 0.11 mol/L) (Shibano et al., 2008). Quercetin showed IC_{50} = 0.53 mmol/L, vs 1.7 mmol/L for acarbose, a positive control (Escandón-Rivera et al., 2012). Vitexin, one of the compounds identified in this study has previously demonstrated inhibitory activity against α-glucosidase obtained from rat intestine with IC_{50} of 0.51 mmol/L (Shibano et al., 2008). Many polyphenols and coumarins with similar skeleton to ones identified here have shown promising α-glucosidase inhibitory activity. For example, coumarins compounds with isolated from the root extract of *Rosa rugosa* showed potent sucrase inhibitory activity (61.88 ± 3.19% to 84.70 ± 3.07%) at a concentration of 1.0 mmol/L (IC_{50} ranging between 0.25 ± 0.04 to 0.48 ± 0. 12 mmol/L), and the activity was comparable with that of acarbose (50.96 ± 2.97 % inhibition, IC_{50} < 0.5 mmol/L) (Thaoet al., 2014). Gallic acid, an important constituent of many plants' species (Zhao et al., 2009), showed strong inhibitory activity against glucosidase both *in vitro* and *in vivo*; its IC_{50} value (24.3 mol/L) was lower than that of acarbose (59.5 mol/L). Methyl gallate obtained from the dried stem and bark extracts of *Terminalia superb* (IC_{50} = 11.5 mol/L) (Wansi et al., 2007) and propyl gallate isolated from green tea extracts (IC_{50} = 11.5 mol/L, Ki = 43.12 mol/L) (Gamberucci et al., 2006) showed strong α-glucosidase inhibitory activity.

3.6. Glucose utilization

Fig. 6 depicts the glucose utilization of 70% ethanolic extract of *Myrtus communis* 'Variegatha' in C3A hepatocytes at different concentrations (25, 50, and 100 µg/mL). The highest glucose utilization was attained at 25 µg/mL while at higher concentrations a slight decrease in glucose utilization was recorded. The glucose utilization potential of the extract may be due to the presence of phytochemicals compounds identified in Tables 1 and 2. The presence of these phytochemical compounds may reduce the increase in postprandial glucose level and different mechanisms of action may be involved in which they achieved this action (Das and Devi 2015). The phytochemical substances can cause the fluids of the small intestine to thicken, which also makes it more challenging for glucose to permeate into the bloodstream from the lumen. Additionally, glucose has the capacity to form bonds with phytochemicals, which lowers the concentration of those compounds in the small intestine lumen. Furthermore, the phytochemicals may inhibit carbohydrate-digestive enzymes, which break down starch, limiting the digestion of carbohydrates and reducing postprandial hyperglycemia. The findings from the present study corroborate with the reports of other research reports documented in the literature (Aladejana et al., 2020; Hassan et al., 2017; Odeyemi and Dewar 2019).

3.7. Glucose uptake

A family of glucose transporters (GLUTs), which serve as vehicles to carry sugar across the cell surface, distribute glucose (Sayem et al., 2018). This rapid glucose-transport mechanism is also essential for the body's ability to store insulin-dependent glucose in muscle and adipose tissues after meals, helping the body continuously to maintain appropriate blood glucose levels (Bouche et al., 2004). Fig. 7 depicts the effects of ethanolic extract of *M. communis* 'Variegatha' on glucose uptake in C3A hepatocytes at different concentrations. It was observed that the glucose uptake effect decreases with an increase in the extract concentration. At 25 µg/mL, the highest glucose absorption effect (70.21%) was recorded, and the effects at 25 µg/mL (70.21%) and 50 g/mL (63.50%) were both higher than the standard antidiabetic drug, insulin (62.67%). At 100 µg/mL, the insulin had a higher glucose uptake effect than the extract (56.23%). It has been established that glucose uptake by cells is influenced by the type of cells

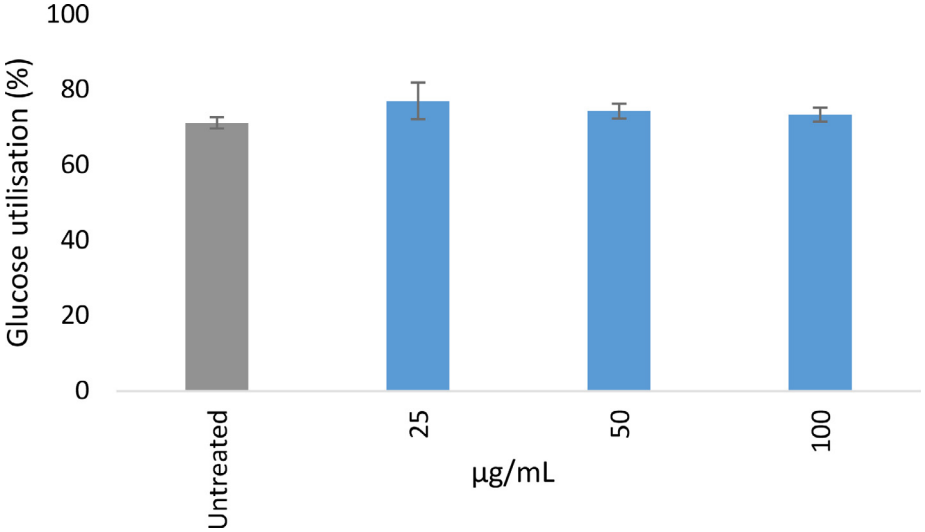

Fig. 6 Glucose utilisation (%) after 24 h of treatment in C3A hepatocytes. Results were normalised to cell viability as determined using the MTT assay. Error bars indicate the standard deviation of the mean of at least 3 replicates from a single experiment.

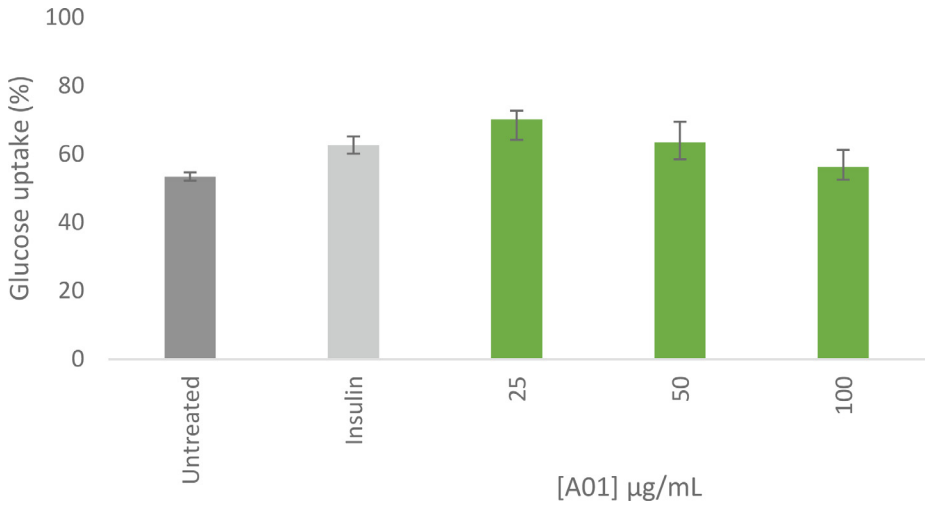

Fig. 7 Glucose uptake (%) after 4 h in C3A hepatocytes, following 24 h pre-treatment. Results were normalised to cell viability as determined using the MTT assay. Error bars indicate the standard deviation of the mean of at least 3 replicates from a single experiment.

used in the assay. Glucose uptake in human body cells, for example, differs significantly from those of other eukaryotic cells such as yeast cells. It is interesting to note that the internal concentration of glucose in cells, as well as the rate at which glucose is metabolized in the cells, have a great influence on glucose uptake (Rehman et al., 2018).

Once the internal sugar is converted into other metabolites, the internal glucose concentration drops, thereby favouring high glucose uptake into the cell. The glucose uptake by C3A hepatocytes mediated by the extract could be attributable to both facilitated diffusion and enhanced glucose metabolism, according to the findings of this study. According to past research, insulin binds to the tautomeric insulin receptor subunit on the cell membrane of myocytes, triggering a series of processes that drive the translocation of the GLUT 4 receptor into the membrane and enable glucose absorption into the cytosol (Sayem et al., 2018). Polyphenols' hypoglycemic effects are primarily explained by their capacity to inhibit the absorption of carbohydrates in the intestine, alter glucose uptake by altering enzyme activities, enhance β-cell function and insulin action, start the release of insulin, and possess antioxidant and anti-inflammatory properties (Iwai et al., 2006).

3.8. Cytotoxicity assay

The cytotoxic test was carried to determine the most practicable and best concentration for use in anti-diabetic drug administration (Buttner et al., 2021). The MTT assay is a sensitive and quantitative colourimetric assay used to measure the viability of cells (Mosmann, 1983). 3-(4,5-dimethylthiazol-2yl)-2, 5-diphenyl tetrazolium bromide (MTT) is water soluble and yields a yellow colour when prepared in medium or buffers. The test is based on the theory that the water-soluble yellow tetrazolium can be reduced by cellular dehydrogenase enzymes of living cells to generate the purple, insoluble formazan result. Due to the impermeability of the cell membrane, this purple formazan product is unable to leave live cells and instead accumulates inside the cell (van Meerloo et al., 2011). Therefore, the amount of formazan product formed is directly proportional to the number of living cells. After solubilization of the formazan product using DMSO the amount produced can be measured and quantified spectrophotometrically at 540 nm (Lin et al., 2019; Kamiloglu et al., 2020). In this study, the cytotoxic effect of *M. communis* 'Variegatha' was assessed against C3A cells and the results is depicted in Fig. 8. After 48 h of treatment, processed live cells are shown in Fig. 8. At the lowest concentration of 50 µg/mL, the extract demonstrated approximately 50% cell death, and the number of viable cells decreased with increasing concentrations. To accurately estimate an IC_{50}, the plant extract was tested at various concentrations. The concentration of an extract that will make 50 % of cells non-viable is known as an IC_{50}. In the present study, the IC_{50} value is 76.85 µg/mL ($P < 0.05$).

Based on UPLC-ESI-QTOF-MS identification and quantification, flavonols and flavones flavanols were the most identified subgroups of flavonoids in the ethanol extract. Flavonoids and phenols are important components of diets and medications due to their inherent health-promoting properties, which include antioxidative, anti-inflammatory, antimutagenic, anti-carcinogenic, antimicrobial, antidiabatic and larvicidal properties (Panche et al., 2016). The antioxidant activity is both the individual and joint effect of the metabolites in the plant sample. Our study reveals that the antioxidant activities of the ethanol extracts correlate with the number of peaks or phytochemicals. In the present study, the cytotoxic effect of the studied extract could be due to the identified phytochemicals in the extract, particularly flavonoids. Phenolic acids, for both solvents of extraction, is the highest annotated class of compound. According to the report by Saibabu et al. (2015), phenolic compounds help in disease prevention and therapy through cell growth promotion using their antioxidants and anti-inflammatory properties (Saibabu et al., 2015), which is contrary to the activity of flavonoids, particularly against cancerous cells. This implies that flavonoids do not promote cell proliferation, unlike phenolic acids. Other classes of compounds with health benefits such as coumarins and related compounds were also annotated in the ethanolic extract of *M. communis* 'Variegatha'. These compounds have become relevant in recent years due to their various biological activities. Coumarins and related compounds in their natural

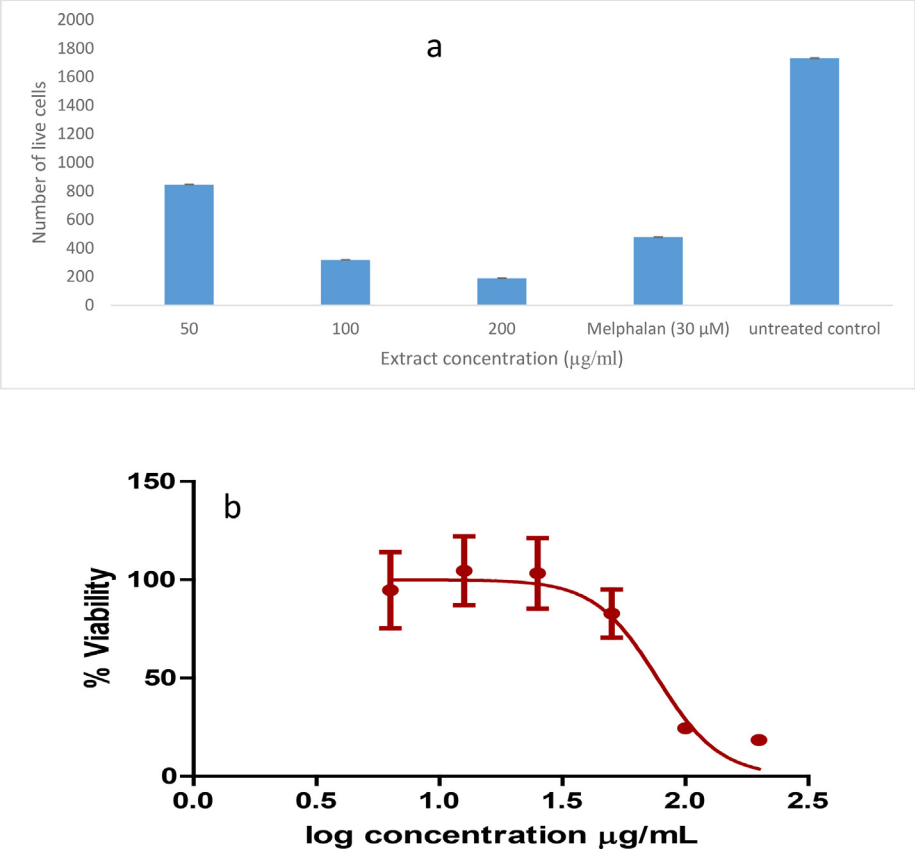

Fig. 8 Cytotoxicity of *M. communis* 'Variegatha' tested against C3A cells. Cells were treated for 48 h. Error bars indicate the standard deviation of quadruplicate values done as a single experiment. Cytotoxicity assay of ethanolic extract of *M. communis* 'Variegatha' by MTT assay. The IC_{50} is generated from GraphPad Prism.

or synthetic forms have been reported to be used as antibiotics (Musicki et al., 2000), anti-inflammatory and antinociceptive (Tosun et al., 2009), photochemotherapy and anti-HIV therapy (Bartnik and Facey, 2017), cancer and antioxidant (Akkol et al., 2020).

4. Conclusion

The present study was the first to investigate the *in vitro* antidiabetic potential of a 70 % ethanolic extract of *M. communis* "Variegatha". Identification of the phytochemical compounds in the plant extract revealed that it comprises of phenolic acids, flavanols, and flavonols and the crude extract demonstrated a strong inhibitory effect on α-glucosidase, which was more effective as compared to epigallocatechin gallate (EGCG), a positive control antidiabetic drug and this indicates that the plant could be used to ameliorate postprandial hyperglycaemia. Furthermore, the highest glucose utilization was attained at 25 µg/mL while at higher concentrations a slight decrease in glucose utilization was recorded. Furthermore, it was found that the glucose uptake effect decreases with an increase in the extract concentration. The biological effects exhibited by the plant extract is due to the identified phytochemical compounds as previously document, which indicates it as a promising source for the isolation of lead compounds that could be used for the development of new antidiabetic agents. Taking this into consideration, further studies such as *in silico* molecular docking of the identified compounds with carbohydrates digestive enzymes as well as *in vivo* studies are encouraged. Additionally, targeted isolation of bioactive compounds responsible for these biological activities is of much significance in studying the biological activities of this plant. Thus, the present findings would be useful for future research directions on the application of traditional medicinal plants in the development of nutraceuticals and pharmaceuticals.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.arabjc.2022.104447.

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