



Phytoconstituents of *Datura metel* extract improved motor coordination in haloperidol-induced cataleptic mice: Dual-target molecular docking and behavioural studies

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ABSTRACT

Ethnopharmacological relevance: Parkinson's disease (PD) is a prominent health challenge characterized by complex aetiology and limited therapeutic breakthroughs. *Datura metel* (DM) is a medicinal plant containing active phytoconstituents with neuropharmacological potentials. In traditional medicine, it exerts anticholinergic, anti-inflammatory and antioxidant effects, and protection from organophosphate poisoning inclusively involved in the pharmacotherapy of PD. Its other PD-related medicinal potency includes treatment of motor sickness and bradycardia. However, the exact mechanisms of anti-PD effects of its phytoconstituents remain underexplored. **Materials and methods:** In this study, methanolic extract of DM was evaluated for anti-PD behavioural effects in *in vivo* haloperidol-induced cataleptic mice. The GC-MS-identified phytochemicals were studied for one-drug-multi-target inhibitory mechanisms against some key targets for PD treatment, alpha-synuclein (ASN) and dopa decarboxylase (DDC) using molecular docking.

Results: and discussion: Chronic administration of 50, 100 and 200 mg/kg of **DM extract** improved the 14-s latency time induced by haloperidol to 54, 54 and 57 s respectively, whereas **levodopa** (30 mg/kg) produced 47 s in rotarod tests. Similarly, the descending times for haloperidol-induced cataleptic mice were significantly reduced from 110 s to 17.7, 17.7 and 12.5 s by the respective chronic doses of **DM extract**, whereas levodopa-administered mice spent 17.5 s descending the same 30 cm pole. The interesting motor coordination enhancements are suggestively due to synergistic inhibition of ASN and DCC by the phytoconstituents of DM, especially, **atropine** and **scopolamine**. From the docking analysis, the two phytochemicals interacted more potently with the active therapeutic sites of the dual targets than **levodopa** and **carbidopa**.

Conclusion: Methanolic extract of DM contains active phytochemicals for multi-target-directed antiparkinsonian mechanisms amenable for further studies.

1. Introduction

Parkinson's disease (PD) is a progressive neurological disorder characterised by complex aetiology. Although the actual number of people suffering from PD is unknown, it is estimated that more than 10 million people (mostly above 60 years old) in the world are affected (Williams et al., 2018). The most implicated progressive neuropathology

of PD involves dopamine deficiency due to the neurodegeneration of dopaminergic neurons and dopamine-generating cells in the substantia nigra par compacta (Selvaraj et al., 2021; Thangavel et al., 2020). These induce the basal ganglia network, leading to cognitive and motor deficits in the central nervous system (CNS). In PD conditions, several motor coordination symptoms arise inclusively from the continuous loss of dopaminergic neurons in the substantia nigra followed by dopamine

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depletion in the striatum. These include bradykinesia/akinesia, postural instability, resting tremor, rigidity and extrapyramidal and cataleptic expressions as signals for impaired motor coordination. As such, the application of the induced-catalepsy model to study parkinsonian pathology and therapy is currently attracting scientific interest. Oftentimes, non-motor symptoms such as neurobehavioral problems including depression, autonomic dysfunction, gastrointestinal dysfunction and sensory processing disorder are also manifested (Yusuf Oloruntimehin Ayipo et al., 2021; Błaszczyk, 2016; Prasad and Hung, 2020; Waku et al., 2021).

To this moment, no effective therapeutic candidate has been identified as a curative agent for PD (Vittorio et al., 2020). Conventional drugs such as dopamine precursors (L-DOPA), dopamine agonists such as apomorphine and amantadine, monoamine oxidase (MAO) inhibitors including rasagiline and selegiline, and catechol-O-methyltransferase inhibitors (e.g. entacapone and tolcapone) only give symptomatic relief (Cacabelos, 2017). Some of them offer symptomatic amelioration of PD pathology by enhancing the dopamine level in the striatum. A well-established drug for restoring the dopamine level and improving motor symptoms is levodopa (a dopamine precursor). However, its use over a long period results in reduced efficiency and is associated with many adverse effects (Poewe et al., 2010). Thus, new therapeutic candidates with enhanced pharmacology and minimal side effects for the effective treatment of PD are required. The use of a suitable PD animal model to identify effective therapeutics for PD motor symptoms' treatment remains relevant. Progressively, haloperidol has become an interestingly relevant model for inducing catalepsy in mice to study PD-related motor coordination behaviour (Waku et al., 2021).

Moreover, the complexity surrounding the onset development and progression of the disease, implicating several pathways and biological targets often constitutes challenges in the treatment. For instance, the aggregation of the presynaptic α -synuclein proteins produce inclusions of toxic fibrils and oligomers in form of insoluble filamentous Lewy bodies. The unwanted accumulation of the protein induces cell damage and neuronal death, significantly implicated as the hallmark of PD and some other synucleinopathic diseases affecting man. Effective inhibition of the α -synuclein- (ASN)-inclined fibrillogenesis has been identified as a therapeutic pathway for the treatment of PD, making the protein an indispensable target. Several biomarkers have been identified as promising PD therapeutics acting through the inhibition of the ASN. These include natural products and some synthetic compounds (Fields et al., 2019; Sahihi et al., 2021; Selvaraj et al., 2021; Thangavel et al., 2020; Vittorio et al., 2020; Zhao et al., 2011). However, only a few have been progressed to clinical trial stages while none has been approved yet for PD treatment, making a further search for ideal candidates remains crucial.

The human dopa decarboxylase (DDC) is a pyridoxal 5'-phosphate-(PLP)-dependent enzyme abundantly present in the CNS and inclusively responsible for the biosynthesis of neurotransmitters, dopamine and serotonin which are strongly implicated in PD. The co-administration of levodopa with DDC inhibitors (DDCIs) such as carbidopa and benserazide reportedly enhances the inhibition of DDC in the peripheral circulation and has been identified as the most effective symptomatic therapy for PD. The DDCIs are commonly known for their inability to cross the blood-brain barrier (BBB), however, they protect the levodopa against the catalytic degradation, thereby enhancing its dosage concentration in the brain. Despite their intriguing efficacy, DDC inhibitors especially carbidopa and benserazide exhibit non-selective inhibition. They can bind irreversibly to either PLP itself or the PLP enzyme, making them prone to unpleasant side effects including involuntary movements (Boyd et al., 2017; Daidone et al., 2012; Krämer, 2019; Raboni et al., 2010). This also supports the need for a continuous search for improved candidates acting through one-drug-multiple target pharmacology.

Multitarget pharmacology is gaining an array of scientific explorations against neurological diseases with complex aetiology such as PD (Ramsay et al., 2016). Natural products have been demonstrated to

possess intriguing potentials for multi-target-directed mechanisms of action as potentials for treating PD. These include the anti-inflammatory effects to reduce oxidative stress, MAO inhibition for neuroprotection and anti-fibrillation against the synucleinopathies (Amro et al., 2018). Some recent studies have revealed multi-target-directed pharmacology of some natural compounds amenable for PD treatment using computational molecular docking analyses (Monteiro et al., 2018; Omoboyowa et al., 2021). Additionally, natural products are oftentimes preferred due to their efficiency, safety, vast availability and cheaper sources, and comparably fewer aftereffects than chemical drugs (Alok et al., 2013). Recently, extracts from *Boerhavia diffusa*, *Ophiorrhiza rugosa* and *Andrographis paniculata* have been reported with interesting anti-cancer/antioxidant, analgesic and antimicrobial effects respectively (Hossain et al., 2021; Sinan et al., 2021; Uddin Chy et al., 2021). Similarly, some natural metabolites from medicinal plants such as emodin, flavone folecetin, flavonoids and other metabolites have been experimented to possess strong pharmacological potentials against various cancers while cannabis oil has been documented with broad-spectrum effects against idiopathic disorders such as fibromyalgia (Ağagündüz et al., 2022; Akkol et al., 2021; Farooq et al., 2021; Fernández et al., 2021; Ferrarini et al., 2022), all supporting the wide therapeutic potentials of medicinal plants in pharmaceuticals.

Datura metel L. (WCSP in review) Species Plantarum (1753) (DM; Jimson weed in English and "Apikan" in Yoruba) is a medicinal plant enriched with essential phytochemicals and secondary metabolites such as alkaloids, polyphenols, flavonoids and terpenoids with diverse pharmacological potentials including anti-inflammatory (Nayyar et al., 2019; Tan et al., 2020), nephroprotective (Imo et al., 2019), antimicrobial (Hossain et al., 2014) and steroidal (Liu et al., 2021). In traditional context, the plant is used in Ayurvedic medicine as analgesic, antiepilepsy, anti-inflammation, antioxidant, antiulcer and protective agents from organophosphate (OP) pesticide effects (Namdeo and Tembhre, 2013; Sharma et al., 2021; Srivastava, 2020). Some of these ailments are strongly involved in the aetiology of PD, especially the OP poisoning (Chuang et al., 2017; Wang et al., 2014) implicating the inhibitory effects on acetylcholine (Namdeo and Tembhre, 2013). The motor symptoms commonly associated with PD have been experimentally shown to increase with the influence of metabolic interactions between the paraoxonase 1 and OP, resulting in neuronal cell death, a pathway to PD progression (Paul et al., 2017). In another context, anticholinergic agents are reportedly effective for the symptomatic management of PD. In recreational activities, DM is used for deliberate and unintentional hallucination, thereby exerting anticholinergic effects (Namdeo and Tembhre, 2013). A review of African medicinal plants have reported the strong anticholinergic effects of its phytochemicals, notably, the tropane alkaloids, although, including narcotic and stupefying effects (Kueete, 2014). The pharmacological mechanisms of the anticholinergic effects of the alkaloids involve the antagonism (blockade) of the muscarinic receptor, thereby inhibiting the central and peripheral muscarinic neurotransmission. As such, the alkaloidal contents implicate DM as muscarinic antagonists suggesting its interesting anti-PD pharmacology (Roy et al., 2018; Soni et al., 2012). However, as far as we are aware, no detailed experimental report has validated these claims, especially acting through selective mechanistic inhibition of ASN and DDC in multi-target pharmacology. Thus, this study was designed to evaluate the antiparkinsonian effects of the phytoconstituents of DM extracts using *in silico* and *in vivo* experiment in haloperidol-induced cataleptic mice model. The adopted objectives include the identification of major phytoconstituents of the methanolic extract using Gas chromatography-mass spectrometry (GC-MS), molecular docking to propose mechanisms of action on selected targets, predictions of absorption, distribution, metabolism, excretion and toxicology (ADMET) and PD-related neurobehavioural studies on haloperidol-induced catalepsy in mice through rotarod and pole tests. The summary of the workflow is illustrated in Fig. 1.

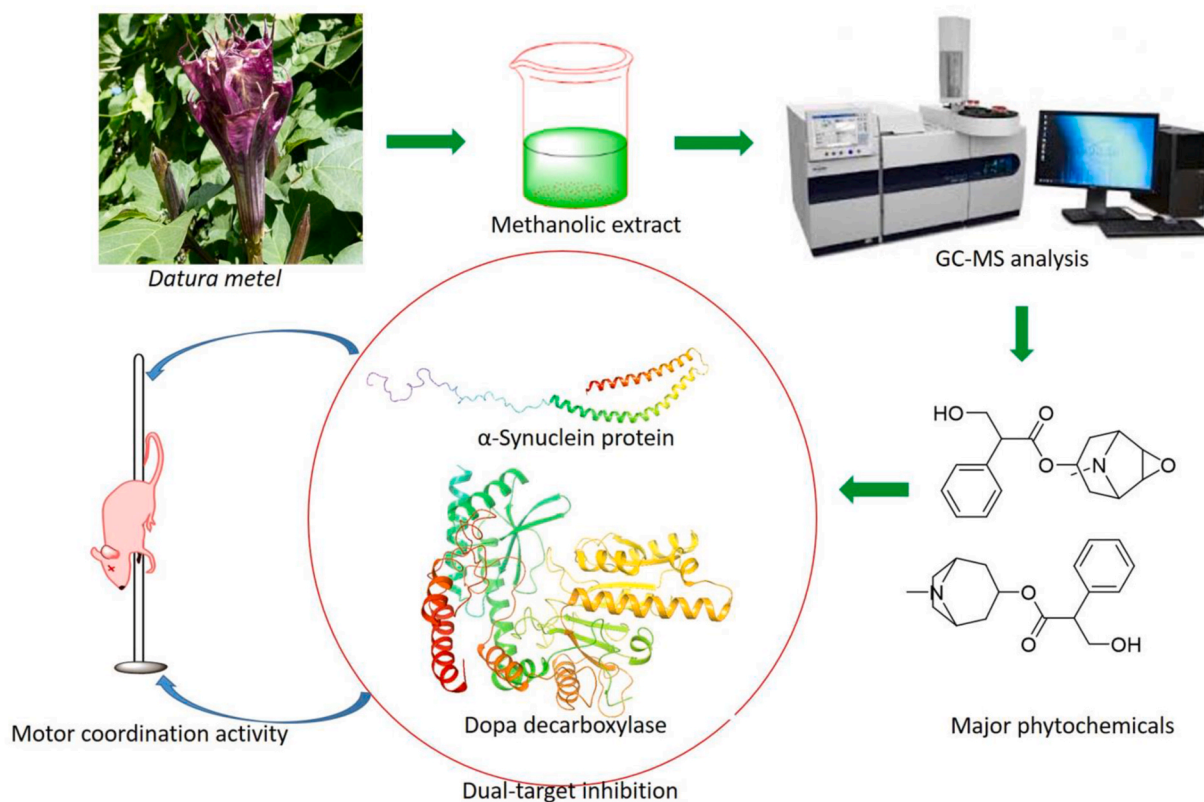


Fig. 1. Illustration of the study workflow.

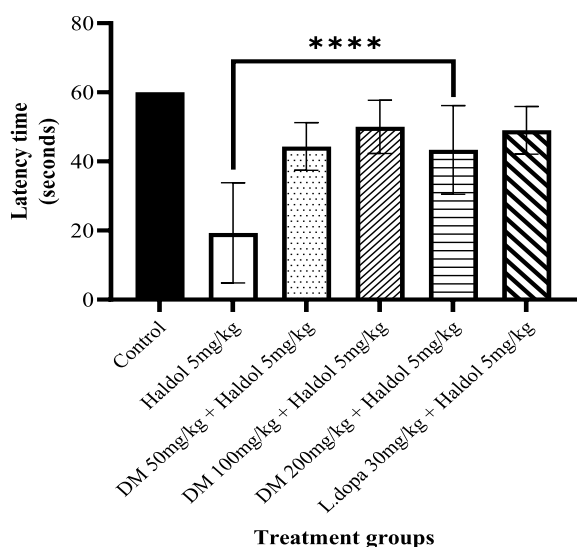


Fig. 2. Effect of acute administration of extract on the latency time of albino using the rotarod test. An acute study of the extract showed significant differences ($p < 0.05$) in the latency time of haloperidol-induced cataleptic rats when compared to the animals administered haloperidol only. The effect observed was similar to that of levodopa at 30 mg/kg.

2. Materials and method

2.1. Plant collection

Datura metel L. was collected at Jalala Estate, University of Ilorin, Ilorin, Kwara state, Nigeria in the month of January 2021. The plant sample was identified and authenticated by Mr. Bolu Ajayi, a taxonomist

in the Department of Plant Biology, Faculty of Life Science, University of Ilorin, Nigeria, and the herbarium specimen was deposited in the Department of Pharmacognosy and Drug Development Herbarium, Faculty of Pharmaceutical Sciences, University of Ilorin where voucher number FPS/PDDHI/025 was obtained.

Ethical approval

Ethical approval (Reference no. UERC/ASN/2022/2339) was obtained before the commencement of study in the Department of Pharmacology and Toxicology, University of Ilorin, Nigeria, and approved by the University Ethical Review Committee, University of Ilorin, Nigeria (2022).

2.2. Experimental animal preparation

Thirty (30) healthy Wistar albino mice of equally mixed sexes (each with a bodyweight of 22–25 g) were kept in clean housing cages each with a dimension of 33.0 × 20.5 × 19.0 cm, maintained in standard laboratory environmental conditions: temperature 25 ± 2 °C, 12/12 h of light/dark cycle and 50 ± 5% of relative humidity. The animals were allowed a free access to food and water *ad libitum*. They were acclimatized in the animal house of the Department of Pharmacology and Toxicology, University of Ilorin, Ilorin, Kwara State, Nigeria before the onset of testing.

Animals were cared for according to the United States National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH publication No 85–23) and allowed free access to animal feed and water (*ad-libitum*). Each group consists of five ($n = 5$) animals and the tests were performed in the light time (08: 00–16: 00 h).

2.3. Preparation of plant materials by extraction

Datura metel leaves were collected by handpicking and pulverized

using an electronic blender. Six hundred (600) g of the pulverized material was macerated with 4 L of methanol in a macerating jar for 72 h while shaking occasionally. The supernatant was transferred into a glass bottle, allowed to settle and filtered with Whatman filter paper No 1. The methanol extract was concentrated using a rotary evaporator and dried over a water bath at 45 °C after which the extract was stored in a desiccator.

2.4. Determination of extractive value

This was carried out according to the method of Khandelwal (2008). The dry pulverized plant material was extracted with water, chloroform, ethanol and petroleum ether using a maceration process. About 2 g of the roughly powdered plant material was weighed into a dry 250 mL conical flask. The flask was then filled separately with 30 mL of each solvent and kept with frequent shaking for 24 h at room temperature. The mixtures were filtered into a 50 mL measuring cylinder using Whatman No. 1 filter paper and then transferred into a previously weighed Petri dish. The extracts collected were concentrated and dried in vacuo for complete evaporation of the solvent.

The percentage extractive value was calculated using the formula:

$$\text{Extraction value (\%)} = \frac{\text{weight of dried extract}}{\text{weight of plant material}} \times 100$$

2.5. Determination of total ash value

This was carried out according to the method of Khandelwal (2008). About 20 g of the test sample was weighed into a previously ignited silica crucible with a known weight. The powdered sample was spread at the bottom of the crucible. The muffle furnace was incinerated with a gradual increase in temperature up to 450 °C, i.e. until the sample powder is free from carbon, then cooled in desiccators. The ash was weighed, and the percentage of ash was calculated for the air-dried drug sample.

$$\text{Ash value (\%)} = \frac{\text{weight of dried ash}}{\text{weight of plant material}} \times 100$$

2.6. Identification of major phytochemicals using GC-MS

A GCMS QP2010SE Shimadzu, Japan gas chromatography coupled with a mass spectrometer was used to analyse the extract for the identification of phytoconstituents. The phytoconstituents of the extract were separated on a silica capillary column (RTX-5MS) (5% phenyl methyl siloxane), 60 m × 0.2 mm and 0.25 µm film thickness. The temperature program was from 60 (kept 2 min) to 240 °C (kept 2 min), and finally to 290 °C (kept 2 min) at the rate of 12 °C/min. The injector temperature was 250 °C and the carrier gas was helium with a flow rate of 3.22 mL/min. The interface temperature was maintained at 250 °C. The GC-MS was run using splitless mode with the ion source temperature at 230 °C. The phytochemicals were eluted from the column into a detector for the production of electrical signals as chromatogram peaks when detected. The signal size is dependent on the phytochemical concentration while the period between the injection and elution was recorded as a Retention time (RT). The RT was represented by X-axis, while the signal strength was plotted on Y-axis to show the abundance (concentration) of a particular phytochemical in the injected sample. Individual chemicals were ionized when stroke with a stream of electrons upon elution from the gas chromatography column, causing them to break into fragments of charged ions with specific masses. The fingerprint of a molecule (mass spectrum graph) was used to calibrate the mass per charge (M/Z) ratio. The detected phytochemicals were signified by comparing their mass spectra fragmentations and retention indices with those in the computer library, NIST.LIB (Stein and Scott, 1994) and WILEY.LIB. (McLafferty, 2000).

2.7. Acute toxicity test using OECD guideline

The test was carried out according to the guideline of the Organization for Economic Co-operation and Development (OECD) Test No. 423 for the testing of chemicals (<https://mneguidelines.oecd.org/guidelines/>). Female nulliparous and non-pregnant mice between 8 and 12 weeks old, were used and housed under prevailing temperature and humidity conditions with a 12/12-h light/dark cycle. Conventional laboratory diets were used with drinking water ad libitum. Animals were randomly selected, and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions. Extracts were prepared shortly prior to administration and administered in a volume not exceeding 1 mL/100g of body weight over the range of doses to be tested. 2000 mg/kg body weight was used as the starting dose and administered in a single dose by oral gavage. A limit test was carried out at 5000 mg/kg body weight. Animals were fasted prior to dosing (food but not water was withheld over-night). Three (3) animals were used for each step and observed individually after dosing at least once during the first 30 min, periodically during the first 24 h, with special attention given during the first 4 h, and daily thereafter, for a total of 14 days. If no mortality was observed then the step was repeated with another three animals. All observations were recorded including expressions of toxicity such as changes in the eye, fur and skin, and mucous membrane, tremors, convulsion, salivation, diarrhoea, lethargy, sleep, and coma.

2.8. Experimental design

The test animals were randomly selected into six groups of five mice each. The first group received vehicle (distilled water + tween 80) and served as the control, the animals in the second group were administered 5 mg of haloperidol per kg of body weight, while the third, fourth and fifth groups received DM extract in doses of 50, 100 and 200 mg/kg respectively. Then the sixth group received 30 mg/kg of levodopa.

Test samples of DM extract (50 mg/kg, 100 mg/kg, 200 mg/kg) was dissolved in 5% tween 80 and administered orally while 30 mg/kg of the control drug, levodopa was administered intraperitoneally, and 30 min preceding infusion of haloperidol (5 mg/kg) for one week. The selected dose of haloperidol was drawn from the pilot study, where it produced a moderate degree of catalepsy, enough to allow the attenuation of the phenomenon to be adequately detected (Waku et al., 2021).

Acute study: The degree of catalepsy was measured on the first day of administration at 30 min after haloperidol administration by using the rotarod and the pole test protocols as described in the relevant literature (Carter et al., 2001; Matsuura et al., 1997; Waku et al., 2021).

Chronic study: DM extract, levodopa and haloperidol were administered similarly to the acute study, however, for additional consecutive 6 days. Catalepsy was again measured on day 7 at 30 min post-haloperidol administration using the rotarod and pole test protocols accordingly (Carter et al., 2001; Matsuura et al., 1997; Waku et al., 2021).

2.9. Behavioural assessment models

(i) Rotarod test

The rotarod test is a test of motor coordination and motor learning. The protocol allows an adequate assessment of the ability of the animal to maintain balance on a 1-inch-diameter rod revolving at a constant rate of 20 revolutions/min. The motor coordination demonstrated was measured at 30 min post haloperidol (5 mg/kg, i.p.) administration. The latency to fall off the rotarod was also observed before and after drug administration (Carter et al., 2001).

(ii) Pole Test

The mouse is placed head-upward on the top of a rough-surfaced

vertical pole of ≈ 30 cm height and the time spent by the animal descending to the floor is recorded to study the mouse's locomotor activity. The motor function was then evaluated by measuring the time in seconds required for the body and head of the animal to turn completely downward (T-turn) as well as the total time taken to descend to the ground (T-total) (Matsuura et al., 1997).

(iii) Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) with a GraphPad Prism 8 (version 8.4.2) software. P values < 0.05 were considered significant at 95% confidence interval.

2.10. Molecular docking

The mechanisms of interactions of the major GCMS-identified phytochemicals in the methanolic extract of DM leaves were studied against the selected therapeutic targets for PD, ASN protein and DDC using the ligand-receptor extra precision (XP) docking module of Maestro 12.2 Schrodinger Release 2019–4 (Maestro Schrodinger, LCC, New York, NY, 2019). Firstly, the chemical structures of the 25 major phytochemicals were drawn in 2D and retrieved in SMILE format using ChemDraw 20.1.1. The retrieved ligand SMILE files including those of the reference drugs, carbidopa, levodopa and phenylbutyrate were imported into the workspace of the Maestro 12.12 for preparation using the LigPrep module available in Maestro 12.2 (LigPrep, Schrodinger, LCC, New York, NY, 2019). The protocols for the preparation including the assignment of correct geometry, protonation states, energy minimization and partial atomic charge calculation using the optimized potentials for liquid simulations 3e (OPLS-3e) force field were adopted from relevant previous studies (Ayipo et al., 2022; Harder et al., 2016). The minimized and protonated phytoconstituent conformers for docking were finally saved in LigPrep.out file.

Similarly, the crystal structures of human ASN (1–19) fused to maltose-binding protein with a co-crystallized phenylbutyrate (PDB 3Q25) and that of DDC in complex with carbidopa (PDB 1JS3) were retrieved in.pdb formats from the RCSB protein data bank (PDB) (<https://www.rcsb.org>) (Burley et al., 2021). The representative structures were carefully selected based on relevant previous studies and their respective high resolutions of 1.90 and 2.25 Å (Burkhard et al., 2001; Daidone et al., 2012; Facchiano et al., 2019; Krämer, 2019; Omoboyowa et al., 2021; Zhao et al., 2011). The structures were imported into the workspace of the Maestro 12.12 for preparation using the Protein preparation wizard of Maestro 12.2 (Protein preparation, Schrodinger, LCC, New York, NY, 2019). They were separately pre-processed by assigning the correct bond orders, and protonation states and filling the missing loops (Madhavi Sastry et al., 2013). Other preparation protocols including refining, optimization and energy minimization steps involving the conversion of heavy atoms to a relative mean standard deviation with an RMSD of 0.3 Å using the OPLS-3e force field were adopted from relevant literature (Ayipo et al., 2021; Harder et al., 2016). The enclosing grid boxes for ligand docking were generated with automatically assigned x, y and z coordinates by selecting the centroids of the workspace ligands. The flexible amino acid residues at the active sites were identified and receptor grid boxes for ligand docking were saved in gridbox.zip files.

Molecular docking analysis was conducted to evaluate the inhibitory interactions of the prepared phytochemical conformers against the selected targets through the XP module of Maestro 12.2 following some previous protocols (Ayipo et al., 2022; Y.O. Ayipo et al., 2021). The docked ligands were sorted in ascending order of the XP scores, representing the inhibitory binding affinities of the selected phytochemicals against the receptors. The bonding and nonbonding interactions between the ligands and active pocket amino acid residues were analyzed using the 2D diagrams of binding poses generated from the ligand-receptor docking. The precision and reproducibility of the

ligand-receptor docking protocols were validated by redocking the co-crystallized ligand of the DDC, carbidopa within the selected active pocket of the receptor and computing RMSD in comparison with the retrieved crystal structure. The redocked ligand produced a similar pose to the original ligand in the crystal structure and an RMSD value of 0.629 Å, thereby validating the protocols as ideally precise and reproducible (Castro-Alvarez et al., 2017; Ramírez and Caballero, 2018).

2.11. Physicochemical, pharmacokinetics, druggability and toxicity predictions

The pkCSM-pharmacokinetics server-based tools (<http://biosig.unimelb.edu.au/pkcsml/>) were used to predict the physicochemical, pharmacodynamics, pharmacokinetics and toxicological properties of the major phytochemicals. These define the profile of the phytochemicals for absorption, distribution, metabolism, excretion and toxicity (ADME), essentially for assessing the drug-likeness and safety delivery of the DM extract to targets for pharmacological expression with insignificant toxicity. The tools utilise a distance-based graph structural signature through accurate and precise estimations of atomic pharmacophore frequency counts, general molecular properties and toxicophore fingerprints for probing druggability with the input of SMILE strings of the compounds (Pires et al., 2015). The predicted properties include molecular weight (MW), number of rotatable bonds, hydrogen bond acceptor/donor (HBA and HBD), lipophilicity (log P), solubility (Log S), topological polar surface area (TPSA), quantifying the physicochemical and pharmacokinetic properties of the selected phytochemicals. The druggability of the identified phytochemicals was also predicted, expressing the ability for crossing the blood-brain barrier (BBB) and human gastrointestinal absorption. Also determined were their inter-active potentials with the p-glycoprotein (pg) and metabolic cytochrome P450 isoenzymes for toxicological expressions. The result parameters were referenced to the BDDC rules of 5 (RO5) and druggability, and Veber's standard for the molecular property influence on oral bioavailability (Benet et al., 2016; Veber et al., 2002).

3. Results and discussion

3.1. Evaluation of extractive values of *Datura metel* leaves

Extractive values play a great role as indicators of the nature of phytoconstituents present in a crude drug (Mireku-Gyimah et al., 2017). From the results of the extractive values (water, petroleum ether, ethanol, and methanol), moisture content and total ash value (Table 1), the highest percentage was obtained with methanol (20%), followed by ethanol and water (13.3% respectively) and then the lowest value with petroleum ether (6.67%). This indicates the presence of polar constituents in the plant and based on this result methanol was used for the total extraction of the crude drug. The moisture content of a crude drug is a determinant of the drug's tendency to be subject to microbial contamination and stability (Alam and Us Saqib, 2015). The moisture content of the leaves was found to be 1.3%, and this value is acceptable according to standards (Nair et al., 2008), indicating that the plant sample can be stored for a long period with a low risk of contamination by microbes. The ash content of a medicinal plant shows the amount of inorganic

Table 1
Physicochemical analysis of *Datura metel* leaves for extraction and total ash values.

S/N	Parameters	Values (%)
1	Water soluble extractive value	13.3
2	Petroleum ether extractive value	6.67
3	Ethanol extractive value	13.3
4	Methanol extractive value	20.0
5	Moisture content	1.3 ± 0.19
6	Total Ash value	15.93 ± 0.07

matter present in the plant and also indicates the purity of a plant, proving whether it has been adulterated or not. The total ash value was found to be $15.93 \pm 0.07\%$, showing the percentage of inorganic matter present in the DM leaves, showing the presence of some inorganic matter.

3.2. Acute toxicity testing

The acute toxicity testing of DM leaves extract on albino mice was conducted according to OECD Guidelines. From the results, there were no observable changes in respiration, skin and fur, eyes and mucous membranes. There were also no observed tremors, convulsions, salivation, diarrhoea, lethargy or sleep and coma. No mortality at any of the dose levels (50–2000 mg/kg) employed. A limit test was conducted at 5000 mg/kg where one mortality was observed (Table 2). Thus, our extract can be categorized as category 5: $2000 \text{ mg/kg} < \text{LD}_{50} < 5000 \text{ mg/kg}$. This suggests that our extract is of relatively low acute toxicity hazard but may, under certain circumstances, present a danger to vulnerable populations. Based on the nature of this study (including a chronic administration study), the three doses of extract selected for administration was tapered down to 50 mg/kg, 100 mg/kg and 200 mg/kg.

3.3. Behavioural models

The haloperidol-induced catalepsy model was used to investigate the anti-parkinsonism effect of the DM extract using rotarod and pole tests. The pole descending test accesses the motor coordination of the mouse while the rotarod test evaluates the neurological deficits in the motor coordination of rodents whereby a mouse is challenged to stay upright on a rotarod which gradually accelerates along its axis (Giraldo et al., 2018). Haloperidol-induced catalepsy reportedly occurs from the blockade of the D2 dopaminergic receptors in the nigrostriatal pathway and has been used extensively as a rodent model for the study of motor impairment episodes of PD and the screening of potential anti-PD candidates (Bhangale and Acharya, 2016) (see Fig. 2).

(i) **Rotarod test:** The results of the rotarod test model show that compared to the haloperidol group, DM methanolic extract at 50, 100 and 200 mg/kg had significant effects on the latency time (seconds) of the mice on the rotarod; $p < 0.05$ for the acute and chronic studies. Dose-dependent effects were observed only in chronic cases. The most significant difference when compared with the haloperidol group, in the chronic study was seen with the 200 mg/kg extract group and the 30 mg/kg Levodopa; $p < 0.05$ Fig. 2, Fig. 3 and Supplementary Figure S1). The increased effect on the improved motor coordination of the mice and, thus, longer latencies to fall off the rotarod is suggestively due to an increased level of dopamine at those doses. In comparison with other studies, the lack or loss of dopaminergic neurons hypothetically leads to increased levels of extracellular dopamine leads to increased activities that can be manifested in the increase in repetitive behaviours (Giraldo et al., 2018). Interestingly, the inhibitory effects of the major phyto-constituents identified in DM extract on ASN and DDC as shown in the molecular docking part of this study support the pathways for the regulation of dopamine neurotransmitters for anti-PD expression. These could constitute the behavioural motor coordination factor underlying the longer latencies to fall off the rod and might also be connected to their reduced anxiety as compared to the control (Riout-Pedotti et al.,

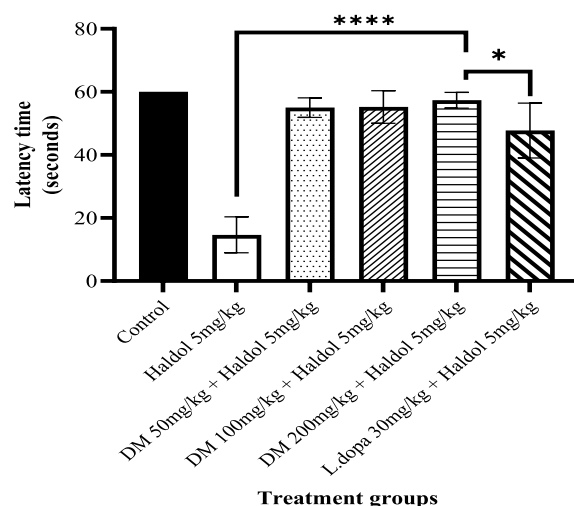


Fig. 3. Effect of Chronic administration of extract on the Latency time of albino mice on a rotarod at 20 revolutions/min using the rotarod test. The extract showed significant differences ($p < 0.05$) in the latency time of haloperidol-induced cataleptic rats when compared to the animals administered haloperidol only. The extract at $\geq 200 \text{ mg/kg}$ improved the latency time of haloperidol-induced cataleptic rats more significantly than levodopa at 30 mg/kg ($P < 0.01$).

2015; van Rumund et al., 2021; Venda et al., 2010).

(ii) **Pole test:** The Pole test results show also that as compared with the Haloperidol treated group, DM methanol extract at 50, 100 and 200 mg/kg has a significant effect on the completion time (T-turn; time taken by mouse to orient itself downward + T total; time taken by mouse to descend to the ground; $p < 0.05$ for both acute and chronic studies) when compared with the negative control. (Fig. 4, Fig. 5 and Supplementary Figure S2). The time to align head-down and descend the vertical pole was significantly longer as compared to their control. The faster time to descend the vertical pole and longer latency to fall off the rotarod suggest improved motor performance.

Several phytochemicals belonging to various natural product classes were identified upon analyses of the methanolic extract of the DM leaves as shown in the GC-MS spectra illustration (Fig. 6). Among the identified

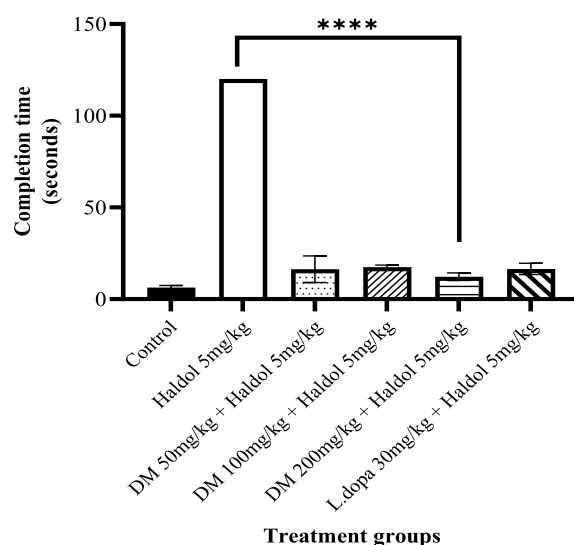


Fig. 4. Effect of Acute administration of extract on the time spent by albino mice to descend/travel down a 30 cm pole using pole test. There was a significant reduction in the time spent to descend by the pole haloperidol-induced cataleptic rats following administration of the extract which was as effective as levodopa positive control at 30 mg/kg ($P < 0.05$).

Table 2

Acute Toxicity Test of the extract on Albino Mice.

Dose (mg/kg)	Number of animals	24 h mortality	14 day mortality
50	6	0	0
300	6	0	0
2000	6	0	0
5000	3	1	–

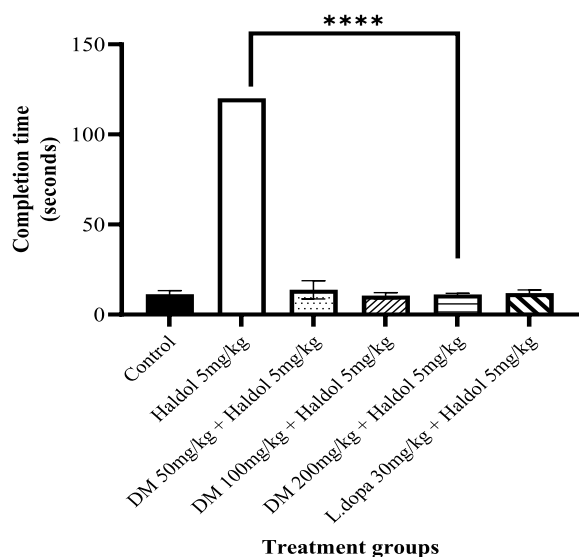


Fig. 5. Effect of Chronic administration of extract on the time spent by albino mice to descend/travel down a 30 cm pole using pole test. Chronic study of the extract at 50–200 mg/kg reduced the time spent to descend by haloperidol-induced cataleptic rats. There was no significant difference in completion time between the extract and the positive control ($P < 0.05$). Identified phytochemicals in *D. metel* using GC-MS.

compounds, those with %Area of peak ranging from ≈ 1 and above were selectively categorized as major phytochemicals (Table 3) while the rest are classified as minor. Most of the identified compounds including scopolamine, atropine, hyoscyamine and litorine have been reported as prominent components of *Datura* species including DM (CÉSPEDES-MÉNDEZ et al., 2021; Jakabová et al., 2012; Nayyar et al., 2019; Sharma et al., 2021), thereby supporting the results obtained herein. The chemical structures of all detected phytochemicals are provided in Supplementary Figure S3. The phytochemical studies indicated the presence of alkaloids, flavonoids, tannins, saponins and

glycosides and the absence of steroids in the extract. This correlates with phytochemical test results as previously described by Cinelli and Jones (2021). The presence of the bioactive constituents of interest i.e alkaloids (especially tropane alkaloids) accounts for the dopaminergic property of the plant. The synergistic effects of the phytochemicals are essential for the overall pharmacological anti-PD activities of the extract as could be observed in the *in silico* and experimental parts of the study.

3.4. Molecular docking

3.4.1. Inhibition of α -synuclein

From the molecular docking results (Table 4), the selected phytochemicals especially 15 and 17 showed higher binding affinities of -12.352 and -11.560 kcal/mol respectively to the fibrillation site of the ASN than the reference inhibitor, **phenylbutyrate**, a recently identified inhibitor of ASN in clinical trials for PD (<https://clinicaltrials.gov/ct2/show/NCT02046434>) and **levodopa**, a renowned anti-PD drug both of which scored -8.692 and -11.150 kcal/mol respectively. The amino acid constituents of the site as revealed by the ligand interaction diagram using Maestro 12.2 include Asn 13, Asp 15, Lys 16, Trp 63, Ala 64, Asp 66, Arg 67, Glu 112, Glu 154 and Pro 155. According to the supporting literature on the receptor (PDB ID 3Q25), they constitute the fibre-forming segments of the protein (Zhao et al., 2011). Moreover, the residues cut across the amphipathic region, non-amyloid- β component (NAC) and acidic tail are inclusively responsible for protein-small molecule interactions, ASN aggregation and misfolding in the pathogenesis of PD (Yusuf Oloruntoyin Ayipo et al., 2021). As such, interactions of compounds with the residues could express inhibitory effects on the protein activities and channel a therapeutic pathway for mitigating PD initiation and progression (Fields et al., 2019).

From the 2D ligand-receptor interaction diagram (Fig. 7), the references and selected phytoconstituents demonstrated interesting pharmacological hydrophilic and hydrophobic interactions against the residues. The referenced **phenylbutyrate** showed H-bond interactions to Trp 63 and Arg 67 through OH and CO groups. It also formed a salt bridge with Arg 67 and π - π interaction with Tyr 156. **Levodopa**, a reference anti-PD drug showed H-bonding interactions with Trp 63, Arg 67 and Glu 112, π -cation to Asp 66 and Arg 67, and a π - π interaction with

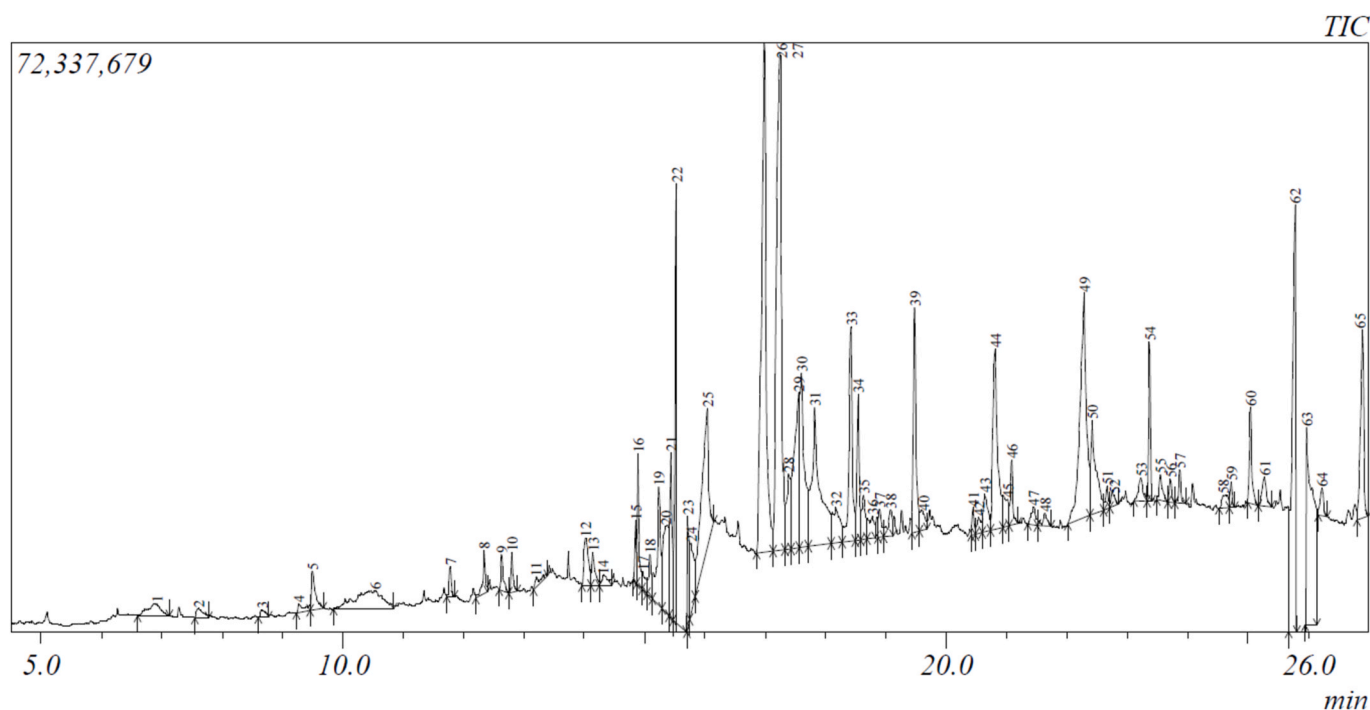


Fig. 6. GC-MS spectra of major phytochemicals identified in ethanolic extract of *Datura metel*.

Table 3

Major phytochemicals, retention time and percentage area of peak.

Comp No	Peak No	Rt	% Area	Compound identified
01	6	10.533	2.14	2-Propanone, 1-[4-(1-methyl-2-pyrrolidinyl)-1-methyl-2-pyrrolidinyl]-
02	19	15.235	1.79	9,12,15-Octadecatrienoic acid, methyl ester
03	20	15.350	2.14	8-Azabicyclo [3.2.1]octan-3-ol
04	21	15.436	1.55	Methyl hexadec-9-enoate
05	22	15.525	2.68	Hexadecanoic acid, methyl ester
06	24	15.767	1.08	Isophytol
07	25	16.042	4.15	l-(+)-Ascorbic acid 2,6-dihexadecanoate
08	26	16.986	9.60	Linolenic acid, methyl ester
09	27	17.242	10.17	Phytol
10	28	17.383	1.32	2-Butenoic acid, 2-methyl-,8-methyl-6-(1-oxopropoxy)-8-azabicyclo [3.2.1]oct-3-yl ester, [1R-[1.alpha.,3.beta.(E),5.alpha.,6.alpha.]]-
11	29	17.550	3.43	Citronellyl isobutyrate
12	30	17.600	3.16	Linolenic acid
13	31	17.817	4.64	Scopolamine
14	32	18.175	1.05	Phytol acetate
15	33	18.417	3.14	Atropine
16	34	18.5391	1.43	8-Azabicyclo [3.2.1]octane-3,6-diol, diacetate (ester)
17	39	19.473	2.46	Scopolamine
18	44	20.808	3.51	Glycerol 1-palmitate
19	49	22.283	5.88	Butyl 9,12,15-octadecatrienoate
20	50	22.417	2.01	Bacteriochlorophyll-c-stearyl
21	54	23.369	1.06	Squalene
22	60	25.039	0.96	Gamma Tocopherol
23	62	21.783	5.64	Vitamin E
24	63	25.967	5.46	3-(Methoxymethoxy)cholest-4-ene
25	65	26.900	2.60	Campesterol

Table 4Molecular docking scores of major phytochemicals of *Datura metel* against α -synuclein and dopa decarboxylase.

ASN (PDB ID 3Q25)		DCC (PDB ID 1J53)	
Compound	Docking score (kcal/mol)	Compound	Docking score (kcal/mol)
15	-12.352	18	-6.226
17	-11.56	17	-5.844
Levodopa	-11.15	15	-5.764
10	-9.917	19	-5.65
Carbidopa	-9.417	14	-5.63
Phenylbutyrate	-8.692	10	-5.226
Adrenaline	-8.521	16	-4.79
16	-8.387	Carbidopa	-4.611
24	-8.265	20	-3.925
12	-8.219	01	-3.881
18	-7.709	Levodopa	-3.745
19	-7.051	09	-3.404
22	-6.967	24	-3.29
25	-6.683	02	-3.172
03	-6.392	03	-3.135
14	-6.12	06	-3.076
09	-6.059	Phenylbutyrate	-2.949
02	-5.772	04	-2.849
08	-5.772	25	-2.848
20	-5.681	12	-2.737

Tyr 156. The selected phytoconstituents of DM, especially **15** and **17** interacted similarly but more potently with the active amino acid residues than **phenylbutyrate**, **levodopa** or both. For instance, compound **15** displayed salt bridge interactions with Trp 63, Arg 67, Tyr 156 and Trp 231 in addition to π -cation and H-bonding interactions with Asp 15 and Asp 66 respectively. Similarly, compound **17** showed bonding hydrophilic interactions through salt bridges to Trp 63, Tyr 156 and Trp 231, H-bonding to Trp 63 and Asp 66, π -cation to Asp 15 and Ala 64. The stronger molecular interactions consistently with the binding affinities

support higher inhibitory potentials of the phytoconstituents **15** and **17** against the fibrillogenesis of the ASN than the referenced known inhibitors, **phenylbutyrate** and **levodopa**.

3.5. Inhibition of DOPA-decarboxylase

The molecular docking of the major phytochemicals within the active site of the human DDC generated the XP docking scores, representing the binding affinities ranked in ascending order (Table 4). From the results, it could be observed that compounds **15** and **17** showed higher binding affinities than the referenced anti-PD drugs, **carbidopa** and **levodopa**, consistently with the results obtained with the ASN (PDB ID 3Q25). The two compounds interacted with pharmacologically relevant amino acid residues in similar patterns to the references. From the 2D binding poses (Fig. 8), **carbidopa** showed H-bonding interactions with Pro 81, Ala 148 and His 302 through its CO and OH groups. It also formed a salt bridge and π electrostatic linkage Lys 303 through its aromatic and O^- moieties. The anti-PD **levodopa** interacted with Ala 148 and Asp 271 through H-bonding, Lys 303 through π -cation and His 192 through π - π . Similarly, the selected phytochemicals, **15** and **17** both displayed H-bonding with His 302, salt-bridge formation with Lys 303 and π - π interaction with His 192. These amino residues and others such as Ser 147, Ser 149, Ser 193, Thr 246 and Asn 300 shown by the binding poses (Fig. 8) are reportedly essential for hydrophilic and hydrophobic bonding and nonbonding interactions of small molecules to express inhibitory effects on the human DCC (Daidone et al., 2012; Krämer, 2019). The consistency in the bonding and non-bonding interactions between the selected phytochemicals and standard drugs with amino acid residues at the active pocket of the human DCC indicates potential similarities for anti-PD pharmacology. However, higher binding affinities of the selected phytochemicals potentiate stronger inhibitory effects.

3.6. Physicochemical, pharmacokinetics, pharmacodynamic and druggability predictions

The predicted physicochemical properties are presented in Table 5. The parameters were assessed for all the identified major compounds to evaluate the overall pharmacokinetics, druggability and safety of the crude methanolic extract of DM. All the identified major phytochemicals have MW of <500 g/mol except compound **20**. They all have a fraction of carbon atoms with sp³ (Fraction Csp³) scoring above 0.42, ideal for drug candidates (Wei et al., 2020). The Log P values were mostly within -0.7 - +5.0, especially for compounds **15** and **17**, indicating good lipophilicity with ideal numbers of rotatable bonds for flexibility. They mostly possess <10 HBA, $\approx$ 5 HBD and TPSA between 20 and 130 Å², especially the main focused compounds **15** and **17**. Interestingly, the MW of <500 g/mol and rotatable bonds of $\approx$ 7 are reported to potentially influence >65% fractions of the compounds for $\geq$ 20% oral bioavailability in a whole rat system (Veber et al., 2002). Selectively, compounds **15** and **17** have an ideal weight, flexibility, solubility, saturation and polarity for pharmacokinetics within the expected ranges for lead-like candidates (Fig. 9) (Daina et al., 2017; Pires et al., 2015).

Consistently, most of the phytochemicals display high intestinal absorption, especially compounds **15** and **17** (Table 6), however, only a few including compound **15** were predicted with the ability to cross the BBB, a common challenge to natural products (Bertrand et al., 2019). The inability of the major phytochemicals especially **17** to cross the BBB advantageously supports their promising candidacy as DDC inhibitors. In spite of its wide clinical application as effective symptomatic medication for PD, levodopa extensively undergoes decarboxylation to dopamine in peripheral tissues, cumulating its drawbacks. This means that only diminutive concentrations of the drug reach the brain, its targeted site for anti-PD actions. To mitigate this, it is administered in large quantity which of course could engender adverse events including drug-drug interactions translating to side effects including postural



Levodopa

violations (Table 6) and distinct their potential as pro-drug candidates (Benet et al., 2016; Daina et al., 2017; NaAllah et al., 2021; Pires et al., 2015; Veber et al., 2002).

Medicinal plants remain relevant globally for their unique nutraceutical, pharmaceutical and other valuable influences for maintaining human wellness. Research has also shown that these plants have a special status in the health and well-being of communities. They continuously constitute models for the discovery of effective drugs for combating the current and emerging diseases (Rabiei et al., 2019). Relevantly to PD, the currently available interventions only provide symptomatic relief in patients and minimal efficacy for reversing the underlying neuropathological mechanisms of PD as curative agents. Therefore, scientific efforts toward identifying effective therapeutic

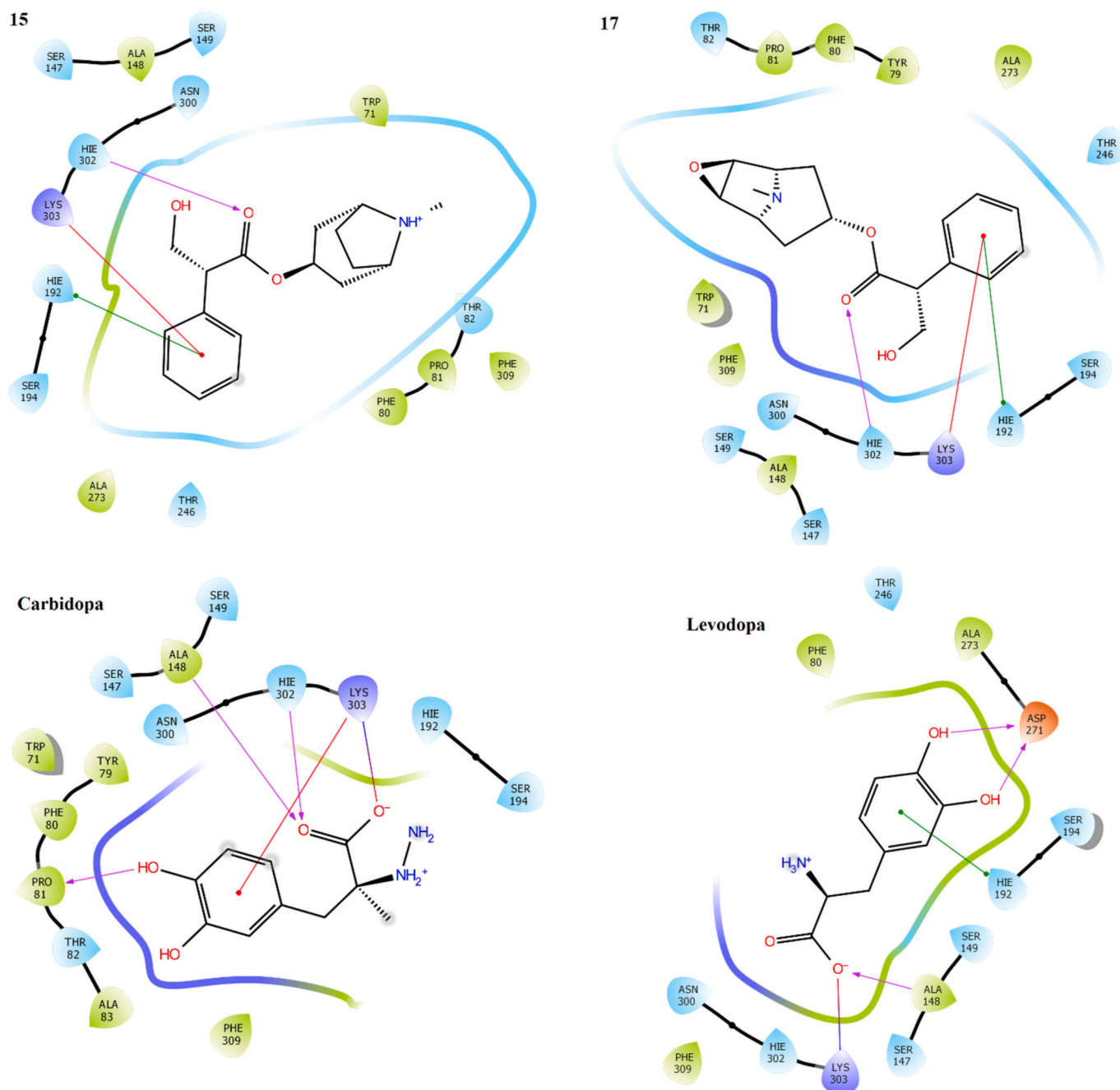


Fig. 8. 2D Binding pose illustrations of interactions (bonding and non-bonding) of selected compounds and references with amino acid residues at the active site of dopa decarboxylase (1JS3). Interactions are displayed as H-bonding (magenta arrow), π -cation (blue line), salt bridge (red-blue line) and π - π stacking (green line). The selected compounds, **15** and **17** showed similar bonding electrostatic interactions with active site amino acid residues with the referenced drugs, levodopa and carbidopa, a propensity for similar/improved pharmacological effects. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

agents for PD pathophysiology remain essential. One of such paradigms is to explore the propensity of safer and vast natural products to pharmacologically interfere with PD pathology.

In this study, the pharmacognostic properties and neurobehavioral effect of methanolic extract of DM were evaluated, and the neuro-behavioural (specifically motor coordination) activity was studied based on positive outcomes from computational studies on the efficacy of DM in the management of Parkinson's disease. The phytochemical analyses showed the presence of alkaloids, flavonoids, tannins, saponins, and glycosides and the absence of steroids and terpenoids in the extracts. This correlates with phytochemical test results as described by Cinelli and Jones, 2021) (Cinelli and Jones, 2021). The presence of the

bioactive constituents of interest especially tropane alkaloids, atropine and scopolamine account for the dopaminergic effect of the plant extract on motor coordination in haloperidol-induced cataleptic mice. The clinical significance of this includes the putative mechanistic inhibition of ASN (with currently no FDA-approved candidate) and DDC, notably, by atropine and scopolamine. The compounds displayed interesting pharmacological potentials, making them worthy of further development as standalone or adjuvant chemotherapeutics for PD. Moreover, their synergistic effects confer the overall neuroprotective and neuropharmacological prospects on DM, amenable for further exploration as medicinal supplements for PD and other dopamine-inclined neurological disorders.

Table 5
Predicted physicochemical and pharmacokinetic parameters of the major phytochemicals.

Compd.	MW	Fraction Csp3	#Rot. bonds	#HBA	#HBD	TPSA	Log P	Log S	Syn. Access.
01	224.34	0.92	3	3	0	23.55	1.45	-1.64	3.02
02	292.46	0.63	14	2	0	26.3	5.55	-4.69	3.1
03	127.18	1	0	2	2	32.26	0.62	-0.81	2.95
04	268.43	0.82	14	2	0	26.3	5.26	-4.59	2.93
05	270.45	0.94	15	2	0	26.3	5.54	-5.18	2.53
06	296.53	0.9	13	1	1	20.23	6.18	-5.75	3.89
07	652.94	0.87	34	8	2	119.36	9.69	-10.5	7.27
08	292.46	0.63	14	2	0	26.3	9.69	-10.5	7.27
09	296.53	0.9	13	1	1	20.23	6.22	-5.98	4.3
10	295.37	0.75	6	5	0	55.84	2.2	-2.91	4.79
11	226.36	0.79	8	2	0	26.3	4	-3.75	2.95
12	278.43	0.61	13	2	1	37.3	4	-3.75	2.95
13	303.35	0.59	5	5	1	62.3	1.38	-2.21	4.03
14	338.57	0.86	15	2	0	26.3	6.67	-6.47	4.53
15	289.37	0.59	5	4	1	49.77	2.06	-2.67	4.33
16	227.26	0.82	4	5	1	64.63	0.64	-1.27	4.07
17	303.35	0.59	5	5	1	62.3	1.38	-2.21	4.03
18	330.5	0.95	18	4	2	66.76	4.64	-4.69	3.88
19	334.54	0.68	17	2	0	26.3	6.5	-5.49	3.48
20	841.46	0.62	23	8	1	89.38	-8.61	-10.49	10
21	410.72	0.6	15	0	0	0	-8.61	-10.49	10
22	416.68	0.79	12	2	1	29.46	7.83	-8.29	5
23	430.71	0.79	12	2	1	29.46	8.29	-8.6	5.17
24	430.71	0.93	8	2	0	18.46	7.13	-7.7	6.55
25	400.68	0.93	5	1	1	20.23	6.89	-7.54	6.17

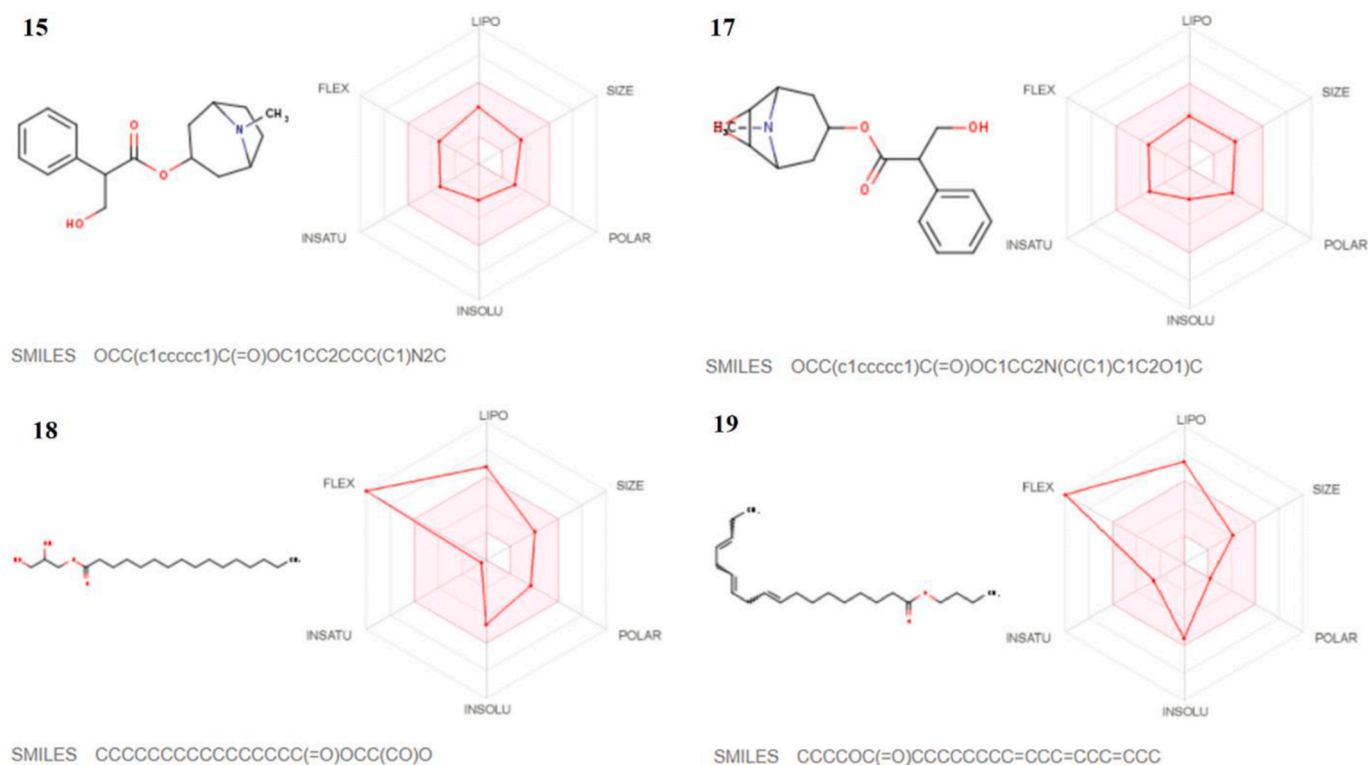


Fig. 9. Illustration of physicochemical parameters expected for drug-like molecules. Phytochemicals 15 and 17 of *Datura metel* extract have properties that fall within the expected range while 18 and 19 failed in flexibility and lipophilicity predictions.

4. Conclusion

Methanolic extract of *Datura metel* was found to contain major phytoconstituents including the tropane alkaloids, **atropine** and **scopolamine**. The phytochemicals displayed potent inhibitory interactions with ASN and DCC, some prominent markers implicated in PD pathogenesis and improved motor coordination in haloperidol-induced cataleptic mice, hallmarks of PD treatment.

Although, isolation of individual phytochemicals and target-based *in vitro* analysis could provide more succinct validation on the mechanisms of actions of each phytochemical individually. Moreover, robust pre-clinical *in vivo* studies are required to quantitatively assess the real-time pharmacokinetics, toxicological profiles and side effects of the extract as well as its phytoconstituents. However, the study provides a scientific basis for such laborious and resource-demanding evaluations of *Datura metel* as a potential natural source for medicinal antiparkinson

Table 6

Predicted pharmacodynamic, druggability and toxicological profiles of the major phytochemicals.

Comp.	GI abs.	BBB	Pgp sub.	CYP1A2 inhib.	CYP2C19 inhib.	CYP2C9 inhib.	CYP2D6 inhib.	CYP3A4 inhib.	log Kp (cm/s)	Lipinski #viol.
01	High	No	No	No	No	No	No	No	−6.98	0
02	High	Yes	No	Yes	No	Yes	No	No	−3.62	1
03	High	No	No	No	No	No	No	No	−6.87	0
04	High	Yes	No	Yes	No	No	No	No	−3.41	1
05	High	Yes	No	Yes	No	No	No	No	−2.71	1
06	Low	No	Yes	No	No	Yes	No	No	−2.55	1
07	Low	No	Yes	No	No	No	No	No	−0.3	2
08	Low	No	Yes	No	No	No	No	No	−0.3	2
09	Low	No	Yes	No	No	Yes	No	No	−2.29	1
10	High	Yes	No	No	No	No	No	No	−6.26	0
11	High	Yes	No	No	No	Yes	No	No	−4.27	0
12	High	Yes	No	No	No	Yes	No	No	−4.27	0
13	High	No	No	No	No	No	Yes	No	−7.45	0
14	Low	No	Yes	No	No	Yes	No	No	−2.15	1
15	High	Yes	No	No	No	No	Yes	No	−6.77	0
16	High	No	No	No	No	No	No	No	−7.36	0
17	High	No	No	No	No	No	Yes	No	−7.45	0
18	High	Yes	Yes	No	No	No	Yes	No	−3.82	0
19	Low	No	No	Yes	No	Yes	No	No	−3.05	1
20	Low	No	Yes	No	No	No	No	No	−3.74	2
21	Low	No	Yes	No	No	No	No	No	−3.74	2
22	Low	No	Yes	No	No	No	No	No	−1.51	1
23	Low	No	Yes	No	No	No	No	No	−1.33	1
24	Low	No	No	No	No	No	No	No	−2.48	1
25	Low	No	No	No	No	No	No	No	−2.5	1

phytochemicals for further studies.

Author contribution

BAL and YOA: Project conceptualization, data collection and manuscript draft preparation; AOA, UMB and WAA: Data collection and literature survey; MOA and MNM: Project supervision and manuscript revision.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jep.2022.115753>.

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