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GC-MS PROFILING OF PHYTOCOMPONENTS IN *MORMORDICA CHARANTIA* L., *IN-SILLICO* ADMET PREDICTION AND *IN-VIVO* ACUTE TOXICITY EVALUATION USING ADULT ZEBRAFISH

Sanjib Kumar Mohanty[1], Sai Aparna[2], Achyutananda Samal[3], Yashaswi Nayak[1]* and Manorama Patri[4]*

[1]Department of Zoology, School of Applied Sciences, Centurion University of Technology and Management, Bhubaneswar Campus, Jatni, Ramchandrapur, Odisha, India.

[2]Department of Zoology, School of Life Sciences, Central University of Odisha, Koraput, Odisha, India

[3]Stress Physiology and Neurotoxicology Laboratory, Ravenshaw University, Cuttack, Odisha, India

[4]Centre for Computational Biology and Bioinformatics, School of Life Sciences, Central University Himachal Pradesh (CUHP), Himachal Pradesh, India.

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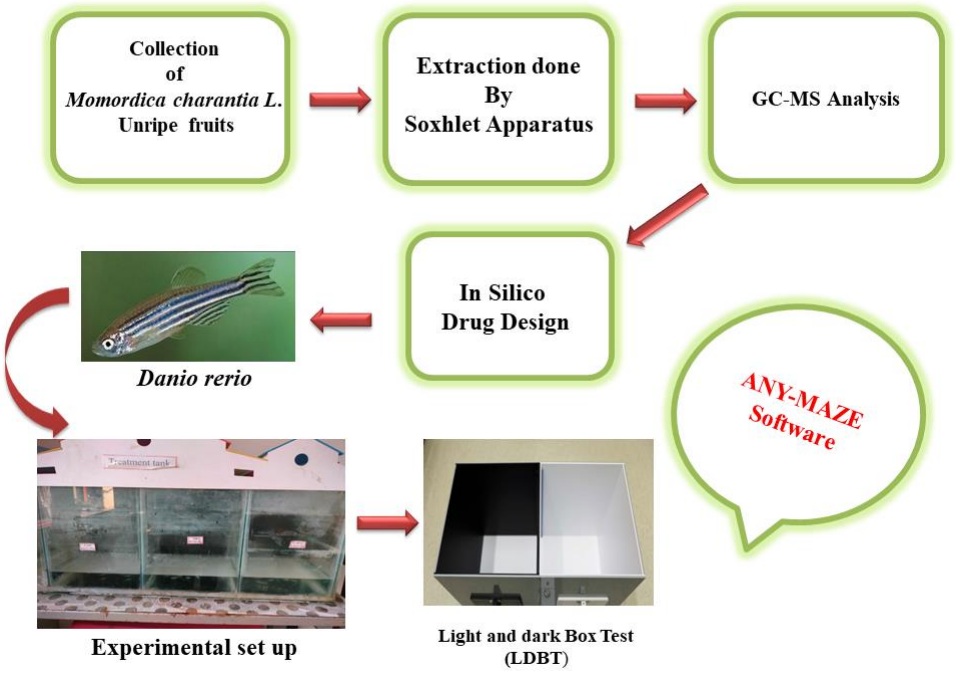
***Corresponding Author**

Yashaswi Nayak

Department of Zoology, School of Applied Sciences, Centurion University of Technology and Management, Bhubaneswar Campus, Jatni, Ramchandrapur, Odisha, India.
mohantysanjib698@gmail.com, saiaprna@gmail.com, amansamal521@gmail.com, yashaswi.nayak@cutm.ac.in, patri_manoroma@hpcu.ac.in,

Graphical Abstract



Methanolic extraction and GC-MS analysis of Phytocomponents of Momordica charantia L. and acute toxicity evaluation through assessing behavioural alterations in adult Zebrafish.

ABSTRACT

Bitter melon *(Momordica charantia),* both plant and its derivatives commonly known for its significant antiproliferative properties and medicinal use modulating the key signalling pathways. Despite its therapeutic potential, the toxicological effects of its components are not well understood. This study presents a comprehensive evaluation of the phytocomponents of *Momordica charantia* L., with an aim for assessment of cytotoxicity through Mass Spectrometry (GC-MS), *in-silico* prediction as well as *in-vivo* studies using zebrafish model. The phytochemical profiling was conducted using Gas Chromatography for assessment of the drug-likeness of its components; an ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profiling was performed *in silico*, providing insights into their pharmacokinetic properties and safety profiles. Among the bioactive compounds, quinine was highlighted for further investigation due to its significant pharmacological impacts. Acute toxicity of quinine was assessed in adult zebrafish (*Danio rerio*) by following OECD guidelines. In this study, the adult zebrafish were exposed to different concentrations of quinine (0.125 mg/L, 0.250 mg/L, 0.50 mg/L, 2.5 mg/L, 5 mg/L and 10 mg/L) for 21 days. The behavioral phenotype was evaluated by light and dark preference test (LDPT). The findings showed an altered behavioral pattern in response to higher concentration of quinine as compared to control groups. Current study identified a high-risk dose response (0.50 mg/L) proposing a safer alternative dose of 0.250 mg/L for further experiments. These findings suggested that *M. charantia* phytocomponents, particularly quinine, exhibit promising therapeutic potential with an optimized safety margin to bridge the gap between experimental discoveries and clinical possibilities in drug development.

KEYWORDS: *Momordica charantia* L.; GC-MS profiling; Quinine; Acute Toxicity; LDPT; Adult zebrafish.

INTRODUCTION

The quest for novel therapeutic agents from natural sources has significantly intensified in recent years, driven by the need to discover safer and more effective treatments for various diseases. Among the huge range of medicinal plants, *Momordica charantia L.*, commonly known as bitter melon or bitter gourd, stands out due to its diverse pharmacological properties. This plant, traditionally used in various cultures for its medicinal benefits, has been extensively studied for its anti-diabetic, anticancer, anti-inflammatory, and anti-viral activities (Chidozie et al., 2014). Previous studies have highlighted the therapeutic potential

of *M.charantia* in various disease models. For instance, its anti-diabetic activity has been attributed to the presence of compounds such as charantin, vicine, and polypeptide-p, which exhibit hypoglycaemic effects (Hussin et al., 2002). Additionally, cucurbitane-type triterpenoids found in bitter melon have demonstrated anti-inflammatory and antioxidant properties by inducing apoptosis and inhibiting neuronal disorder (Anilakumar et al., 2015). Despite these promising findings, the detailed chemical composition and comprehensive bioactivity profiles of M.charantia extract remain underexplored.

Behavioural studies are becoming more crucial in basic and applied research as they provide a new insight to scientists how the organisms are giving responds to different external and internal stimuli. The studies in animal models are critical for understanding human neurological and psychiatric problems, assessing therapeutic efficacy and safety, and investigating the impact of genetic and environmental variables on behaviour (Crawley, 2007). Zebrafish are widely used in behavioural studies due to their unique combination of biological, genetic, and experimental advantages as they share about 70-80% (approx.) of their genes with humans and have a comparable neurotransmitter system, making them very useful to study neurological disorder (Chia et al., 2022).

Zebrafish exhibit a range of measurable behaviours such as shoaling, aggression, anxiety, learning, and memory, which can be tested using well-established paradigms like the elevate plus maze test, novel tank test or light/dark preference test. The model organisms exhibit anxiety-like behaviours such as avoidance of open or brightly lit areas, increased freezing or grooming, and reduced exploration. Such behavioural patterns are used to evaluate the anxiolytic or anxiogenic effects of any drugs, making them vital tools in neuroscience and pharmacological research (Cryan, J. F., & Holmes, A. 2005). In our present study the experimental and behavioural analysis were conducted in adult zebrafish to assess the neuroactive efficacy of the phytocomponents present in Momordica charantia L., with particular attention to dose-dependency. By regulating the behavioural parameters, phytochemical constituents of *M. charantia* identified through GC-MS and the favourable safety and absorption profiling predicted via *in-silico* ADMET analysis, supporting the potential therapeutic relevance of the extract in modulating stress-related behaviours.

GC-MS is a powerful analytical technique that combines the features of gas chromatography and mass spectrometry to identify different substances within a test sample. This method is particularly effective for the separation, identification, and quantification of volatile and

semi-volatile compounds in complex mixtures (Xu et al., 2021). By employing GC-MS profiling, the chemical constituents of *Momordica charantia* extract can be accurately determined, providing a comprehensive overview of its phytochemical composition. These phytochemicals are often responsible for the observed therapeutic effects and can serve as lead compounds for drug development (Saini et al., 2017).

In silico ADMET analysis is a computational approach used to predict the pharmacokinetic and toxicological properties of compounds. This method aids in the early-stage drug discovery process by evaluating the potential efficacy and safety of bioactive molecules before in -vivo testing (Dearden et al., 2007). By integrating in -silico ADMET prediction, this study aims to assess the drug-likeness of the identified compounds from *Momordica charantia* extract, thereby identifying candidates with favourable pharmacokinetic profiles and minimal toxicity risks (Adelusi et al., 2021). Zebrafish models are valuable in pharmacological and toxicological research due to their genetic similarity, rapid development, and transparency. Adult zebrafish are ideal for studying acute toxicity and behavioural effects of bioactive compounds, providing insights into their potential impact on higher vertebrates (Bhattacharya et al., 2023). This study aims to bridge this gap by employing GC-MS profiling to elucidate the chemical constituents of *Momordica charantia* extract and utilizing *in silico* ADMET analysis to predict their pharmacokinetic and toxicological properties. Furthermore, acute toxicity and behavioural assessments in adult zebrafish to elucidate critical insights into the safety and potential adverse effects of the extract. By integrating these approaches, the research seeks to identify novel bioactive compounds with therapeutic potential and establish a foundation for future pharmacological studies and drug development efforts.

2. MATERIALS AND METHODS

2.1 Collection and Authentication of Plant Materials

About 10 kg unripe fruits of bitter melon were identified and then purchased from the Soul Souk Organic Farm (www.soulsoak.com) in the month of October 2023 with verification of the taxonomic characteristics and authentication by Department of Botany, Centurion University of Technology and Management Bhubaneswar, Khordha, District Odisha, India.

The recognized fruit material was deposited as a voucher specimen under Herbarium number M # 120 in the Centurion University herbarium for future reference.

2.2 Chemicals and Reagents

The chemicals and reagents required for this experiment were purchased from Sigma-Aldrich.

2.3 Experimental Design

Zebrafish were collected from the Central Institute of Freshwater Aquaculture in Odisha, India, and maintained in a 50-L aquarium at 22-24 °C (OECD 2019). The pH of water was maintained between 6.8 –7.5, with sodium bicarbonate used when needed. The fish were acclimatized for 5 days, and fed twice per day. Waterborne exposure of quinine towards zebrafish was persuaded by taking test solution in DMSO (CAS NO-67-68-5). The experimental setups were divided into seven groups (n=7) were exposed to different concentrations of Quinine, Group 1 was the control group and received only DMSO with no addition of quinine phytocomponents . Starting from Groups 2 to Group 7 the zebrafish were exposed with increasing quinine concentrations, for instance, 0.125 mg/L, 0.250 mg/L, 0.50 mg/L, 2.5 mg/L, 5 mg/L and 10 mg/L, respectively. Accordingly, each group was given quinine with above mentioned concentration by dissolving in DMSO (<0.01%) for 21 days.

2.4. Preparation of Plant Extract

The purchased amount (10Kg) of bitter melon was cleaned and cut into little pieces. Later it was oven-dried for one day at 50^{o}C. Dry material was grinded in to a fine powder using electronic blender (SR no. LAMK1WD184195) and kept in 4^{o}C until needed. After good mixing, 850 g of powder was added to 1L of 90% methanol which was then constantly extracted in a Soxhlet extractor for three days until the extraction procedure was complete. The extract was filtered through Wattman No. 1 filter paper and further evaporated using a rotary evaporator (Yamato, Rotary Evaporator, model-RE 801, Japan) at 190-220 rpm and 40-50°C for 24 hours under decreased pressure to get an amorphous solid mass about 15mg.

2.5 GC-MS Analysis Method

Aqueous and methanolic extract of *Momordica charantia* was evaluated qualitatively for the confirmation of phytochemicals using a SHIMADZU QP-2010 Gas Chromatography-Mass spectroscopy equipped with a fused silica column packed with 5% Diphenyl 95% Dimethyl polysiloxane, 30 mm × 0.25 mm × 0.25 µm). The sample extract of 2 µL is injected into the instrument. It was detected by the turbo gold mass detector with the aid of the Turbo mass 5.2 software. The oven was maintained at a temperature of 110°C with 2 min holding. The temperature of the injector was 250°C The mass spectra were collected within the range of 45

to 450 Da with a scan time of 0.5 S and at 70 eV. Detection of MS was done within less than 36 minutes. Interpretation of GC-MS mass spectrum data was made using NIST's database containing over 62,000 patterns. The NIST library contains the spectrum of unknown components. The names, molecular weights, and structures of the test materials were used to identify their constituent parts.

2.6 *In silico* ADMET Analysis

2.6.1 Ligand Preparation and Filtration

Bioactive phytocompounds of *Momordica charantia* were obtained through the GC-MS study by methanolic extracts. The three-dimensional structures of these compounds were obtained from the PubChem database. Clean ligands were prepared by computing their 3D coordinates with the help of "prepare ligand" process in Discovery Studio 4.0. Then, these molecules were selected based on Lipinski's "rule of five," which decides their bioavailability through the molecular properties of a compound. It must have a log P of no higher than five, molecular mass less than 500 Daltons, and no more than five hydrogen bond donors and ten hydrogen bond acceptors (Jonker et al., 2011).

2.6.2 Drug-Likeness and medicinal Property

Drug-likeness refers to a compound’s potential to become an orally active drug based on its physicochemical properties and adherence to certain drug design rules. These rules help predict whether the compound can be absorbed, distributed, metabolized, and excreted effectively. Lipinski’s Rule of Five rule assesses the likelihood of good oral bioavailability. A compound that violates two or more of these rules is less likely to be an effective oral drug. In in silico ADMET, adherence to Lipinski’s rule can predict absorption and oral bioavailability (Chen et al., 2020). Ghose, Veber, Egan, Muegge Filters are filters extend beyond Lipinski’s rule by considering properties like molecular weight, LogP (partition coefficient), hydrogen bond count, and rotatable bonds. Adhering to these rules helps ensure that the compound can be effectively distributed and absorbed, while avoiding toxicity due to poor permeability or excessive molecular complexity (Reddy et al., 2021). Drug-likeness, defined by rules like Lipinski's Rule of Five, predicts whether a compound will have favourable absorption, distribution, metabolism, and excretion (ADMET) properties, which are crucial for its effectiveness as an oral drug. Medicinal chemistry properties, such as bioavailability score (BAS) and PAINS alerts, further refine this by assessing the compound's suitability for therapeutic use, focusing on bioavailability, safety**,** and synthetic accessibility

(Jon et al.,2017)**.** Together, these properties ensure the compound is not only biologically active but also feasible for drug development, linking its potential as a therapeutic agent with its ability to perform well in pharmacokinetic and safety evaluations.

2.7 Preparation and Quantification of Experimental Dose

To prepare a waterborne exposure, quinine test solutions, the compound was first solubilized in DMSO (CAS NO-67-68-5). An appropriate amount of quinine powder was dissolved in DMSO to obtain a concentration was adjusted 0.01% (V/V) (Zhao et al., 2018) In vivo waterborne dose calculation, particularly for zebrafish acute toxicity studies, it requires consideration of factors such as the body weight of the zebrafish, the volume of water in the test tank, and the pharmacokinetic properties of the phytochemicals (Hsieh et al.,2019). All the animals were weighed, coded and then grouped on day zero. Low, medium, and high concentrations (0.125 mg/L and 0.250 mg/L, 0.50 mg/L and 2.5 mg/L, and 5 mg/L and 10 mg/L) doses were applied during a seven-day period. On a daily basis, every animal's, food consumption, appearance, abnormal symptoms, and mortality were also recorded and also maintained waterborne exposure.

2.9 Behavioural Assay

2.9.1 Light & Dark Preference Test (LDPT)

Crawley and colleagues were the first to develop and implement the light and dark preference test (Crawley and Goodwin, 1980). The light and dark preference test is one of the most widely used tests in behavioural neuroscience to measure anxiety-like behaviour in animal model. This test is based on natural avoidance of zebrafish to bright light areas, as well as their spontaneous exploratory behavioural patterns in changing environments. The light and dark box is composed of glass and has a partition dividing it into two equal chambers. One side of the box is dark, while the other is transparent and exposed to bright light. The base of the box has an entrance for the exploration behaviour. The LDPT apparatus is a rectangular acrylic tank (15 height × 30 length × 16 cm width) that rests on a level surface that is divided into two equal vertical portions demarcated by black-and-white coloration. After completion of exposure period, zebrafish were individually placed in a rectangular tank and their behaviour was monitored for a 5-minute period using the Any-maze video tracking software, which recorded the time spent in each zone, the number of transitions between zones, and the latency to enter the light zone (Magno et al., 2015). These parameters served as indicators of anxiety levels, with increased time spent in the light area , increase number of transitions and

more time spent in the light zone suggesting an anxiogenic effect. Data were collected and analysed to determine dose-dependent effects of quinine on Zebrafish anxiety-like behaviour (Komleva et al., 2021).

3. Statistical Analysis

To evaluate the acute toxicity and standardize doses of *Momordica charantia* phytocomponents, a comprehensive statistical analysis was conducted as Mean ± SEM. Dose-response graphs were generated to visualize the relationship between concentration, Toxicity assessment was triplicated to minimize the possible handling errors and for the standardization of safe and effective doses for subsequent studies using Graph Pad Prism 8.0.2). The significant difference was considered at $P < 0.05$.

4. RESULT AND DISCUSSION

4.1 GC-MS Analysis Methods

Different phytochemicals/bioactive components of *M.charantia* fruit extracts were analysed by GC-MS. The chromatograms of the plant extract (Methanol) were shown in **Fig 1** and summarised in **Table 1.** GC-MS chromatogram of *M.charantia* fruit extract showed 34 peaks, which indicated the presence of 34 different bioactive/phytochemicals compounds and major compounds are described in **Table 1**. The main compounds identified based on the relative contents were 2-Cyclopenten-1-one (alicyclic ketone), 2-hydroxy-, Dodecane, Tetradecane, Stigmasterol, Retinol, Ergosta-5,22-dien-3-ol, (3.beta.,22E) and Quinine(alkaloid). Quinine exhibits anti-inflammatory, antioxidant and cardio protective activity, while Tetradecane has been reported to exhibit antimicrobial activity. stigma sterol is a phytosterol with potential cholesterol-lowering properties that benefit in the prevention against cardiovascular diseases. The other small peaks of phenolic compounds detected include Retinol and 2-Cyclopenten-1-one. The phenolic compounds have already been identified as antioxidants, thus adding to the general antioxidant ability of the plant. Moreover, the presence of phytocomponents in *M. charantia* may have the neutraceutical activity for the traditional management of oxidative stress-related disorders.

4.2 In-silico Swiss ADMET Analysis

4.2.1 Physicochemical Properties

Development of a potential drug candidate with caution on ethical and physicochemical considerations may be inevitable since these aspects would rather critically determine the ADMET, which in turn affects other phases of drug development. Assessments as to potential

interactions of drugs with the living thing and determining which molecular properties can be desirable.

Table 2 lists the molecular weights for all compounds that are found to be below 500 g/mol. As shown in Table 2, all the compounds had a maximum number of 10 rotatable bonds and a molar refractivity of 40 to 130. Another significant property of drug bioavailability is Topological Polar Surface Area or TPSA; compounds having TPSA below 75 Å² are generally very orally accessible and passively absorbed (Reddy et al., 2021). All the selected compounds shown in Table 2 are polar. Among all the selected compounds, this compound, cineole has the lowest TPSA value. The TPSA values indicated a good potential for oral bioavailability ranging from 29.54 Å² to 71.69 Å². Along With Log S/Log P values ranging from 2.03 to 2.78, all of the six drugs in Table 2 exhibited a good to moderate water solubility and hence ensured efficient oral absorption. Results from this study shows that Quinine, mostly, the Retinol and Ergosta-5 were shown to be moderately soluble whereas only 2-Cyclopenten-1-one,2-hydroxy compound is soluble. Apart of these phytocompounds, all rest phytocomponents were proved to be poorly soluble.

4.2.2 Pharmacokinetics & Pharmacophore Properties

It deals with the rates at which drugs are disposed of in the body, and therefore, factors such as clearance, volume of distribution, elimination half-life, and bioavailability are involved. The bioavailability for a given medication refers to the percentage it enters the blood stream when administered. In addition, pharmacokinetics gives knowledge on when and how long drugs should be administered. Most drugs are metabolized by a member of the CYP superfamily, excluding drugs that activate transporters. Another important parameter is drug permeability across cell membranes, expressed through a partition coefficient, Kp. The greater the value for Kp (cm/s) shows lower membrane permeability.

In Table 3, the result revealed that 2-Cyclopenten-1-one, 2-hydroxy shows high absorption in the gastrointestinal tract (GIA), Crosses the blood-brain barrier (BBB), Inhibits CYP2C19 and CYP2D6 enzymes with Log Kp value was -6.76 cm/s, indicating low skin permeability. Dodecane phytocompounds shows Low gastrointestinal absorption(GIA), does not cross the blood-brain barrier(BBB), Inhibits CYP1A2 only with carry Log Kp value was -3.01 cm/s. Tetradecane Similar properties to dodecane, with low absorption and no BBB penetration. Inhibits CYP1A2 and has a slightly better skin permeability (-2.40 cm/s). Octadecane shows Low absorption (GIA), no BBB penetration, Inhibits CYP1A2 only with Log Kp value was -

1.20 cm/s, indicating better skin permeability compared to shorter alkanes. Quinine shows High absorption (GIA), Can cross the blood-brain barrier (BBB), does not inhibit any of the listed CYP enzymes with carries Log Kp: -6.23 cm/s, indicating low skin permeability. Retinol shows high absorption (GIA), Crosses the blood-brain barrier (BBB), Inhibits CYP1A2 and CYP2C9 carried Log Kp value was -4.01 cm/s. Ergosta-5 Low absorption(GIA), does not cross the blood-brain barrier (BBB), Inhibits CYP2C9 and Log Kp value was -3.45 cm/s.

4.2.3 Drug likeness and Medicinal Chemistry

Drug-likeness, defined by Lipinski's Rule of Five, predicts a compound's absorption, distribution, metabolism, and excretion properties, crucial for oral drug effectiveness. Medicinal chemistry properties, like bioavailability score and PAINS alerts, assess a compound's suitability for therapeutic use. All compounds passed ADMET screening tests, suggesting a lead-like approach for lead optimization in virtual screening (jon et al., 2017), which was described in Table 4.

In Table 4, the result described that 2-Cyclopenten-1-one, 2-hydroxy passed the Drug-likeness property like Lipinski, Veber, Egen, but fails to Ghose and Muegge filters. But in Medicinal Chemistry, it was shown “No” PAINS alerts, so here was not shown any lead like property and with a Moderate synthetic accessibility score is 2.42. Dodecane passed the Drug-likeness property likes Lipinski, Ghose, Veber, Egen, but fails Muegge. In medicinal Chemistry it shows “No” PAINS alerts, not lead-like. Easier to synthesize with a score of 1.83.Tetradecane passed Drug-likeness property likes Lipinski and Egen, but fails Ghose, Veber, and Muegge. But in medicinal Chemistry it was shown “No” PAINS alerts, not lead-like. Synthetic accessibility score is 2.04. Octadecane: passed only Drug-likeness property likes Lipinski and Egen, fails the rest. Whereas medicinal Chemistry showed “No” PAINS alerts, not lead-like and having Synthetic accessibility is 2.49. Quinine passed all filters like (Lipinski, Ghose, Veber, Egen, and Muegge) of Drug-likeness property ,whereas Medicinal Chemistry showed “No” PAINS alerts and considered lead-like, but has a higher synthetic accessibility score of 4.34, indicating more difficulty in synthesis. Retinol showed Drug-likeness property like Lipinski, Ghose, Veber, and Egen, but fails Muegge., In medicinal Chemistry shows “No” PAINS alerts, not lead-like. Synthesis is moderately difficult (4.28). Ergosta-5 passes Drug-likeness property like Lipinski and Veber, but fails Ghose, Egen, and Muegge. In medicinal Chemistry showed “No” PAINS alerts, not lead-like, and is the most

difficult to synthesize with a score of 6.21. Table 4, shown that Quinine stands out as the most drug-like compound, passing all filters, while Dodecane and 2-Cyclopenten-1-one, 2-hydroxy show moderate drug-likeness but fail certain rules. Synthetic accessibility varies, with ergosta-5 being the hardest to synthesize, while dodecane is the easiest. Lead-likeness is only positive for quinine, suggesting it may be a better candidate for further development.

4.2.4 Bioavailability Radar

In *in silico* ADMET analysis**,** the bioavailability radar is a visual tool used to assess a compound's drug-likeness and its potential for oral bioavailability. The radar typically evaluates multiple key parameters, including lipophilicity (Log $_P$)**,** size (molecular weight)**,** polarity (TPSA)**,** solubility**,** flexibility (number of rotatable bonds) and saturation (fraction of sp3 carbons). By plotting these properties on a radar chart, researchers can quickly determine how well a compound aligns with ideal drug-likeness criteria (Chong et al., 2024). From the bioavailability radar, it is clear that the Swiss ADMET prediction performance for Six substances have competent chemotherapeutic potential since they show an ideal range for each of the six features described in Figure 1.

4.2.4 Light & Dark Preference Test (LDPT)

The Light-Dark Preference Test (LDPT) is a widely used behavioral assay for zebrafish experiments, offering significant insights into anxiety-related responses and exploratory behavior. This test capitalizes on the innate preference of zebrafish for darker environments while simultaneously allowing the quantification of their exploratory drive in Lighter spaces. The test results indicated that lower doses exposure with 0.125 mg/L and 0.250 mg/L groups spent time is slightly increased (112.5 ± 3.491,110.5 ± 6.104) as compared to control group 98.19±1.55), indicating that, there is no significant difference ($P < 0.05$). Moderate doses of 0.50 mg/L and 2.5 mg/L groups resulted in significantly increased time spent(149.8 ± 4.355 ,154.6 ± 4.344 respectively) in the light zone as compared to the control group described in graph 3[a]. Conversely, Group with higher doses of 5 mg/L and 10 mg/L led to reduced time in the light zone (139.3 ± 5.006, 133.3 ± 4.408) suggesting potential adverse effects. The latency towards the light zone decreased (23.29 ± 1.539) in the control as compared with 0.50 mg/L and 2.5 mg/L exposure group, (17.76 ± 1.068,15.00 ± 0.568 respectively) indicating an increased willingness to explore the light zone described in graph 3[b], latency towards the light zone, which decreased significantly($P < 0.05$) at moderate doses of 0.50 mg/L and at 2.5 mg/L exposure groups (17.76 ± 1.068, 15.00 ± 0.559) as compared to the control group.

Zebrafish treated with 0.125 mg/L and 0.250 mg/L groups showed a slightly reduced latency (21.81 ± 1.02, 22.46 ± 1.142), suggesting quicker movement toward the light zone and not having any significant difference. In contrast, higher doses maintained low latency period (12.19 ± 0.578, 12.84 ± 0.486) possibly due to sedation. Additional analysis revealed that the number of transitions to the light zone peaked (22.86 ± 1.10, 20.43±2.136) in group exposure with 2.5 mg/L and 0.50mg/L as compared to controls(10.43 ± 0.895) showed a significant difference ($P < 0.05$), described in graph 3[c], but declined at higher doses (17.86 ± 1.895, 16.43 ± 1.343) as compared with moderate dose. Whereas, groups treated with 0.125 mg/L and 0.250 mg/L showed slightly increased no. of transitions (11.86 ± 0.633, 11.14 ± 1.056), remaining above the control group. These results suggest that the 0.250 mg/L dose may effectively enhance exploratory behaviour while being the safest option compared to higher doses, which exhibited reduced activity may enhance exploratory behaviour more effectively and is likely the safest option compared to the higher dose.

Table 1: Shows Highest Amount Chemical composition of Methanol extract through GC-MS Analysis.

Sl no	R.Time	Area	Area %	Name	Formula
1	5.342	776507	14.86	2-Cyclopenten-1-one,2-hydroxy	$C_6H_8O_2$
2	14.318	375245	7.18	Dodecane	$C_{12}H_{26}$
3	20.333	281462	5.39	Tetradecane	$C_{14}H_{30}$
5	30.247	56020	1.07	Octadeca	$C_{18}H_{38}$
6	31.028	100526	1.92	Neophytadine	C20H38
7	32.911	314334	6.02	Quinine	$C_{20}H_{24}N_2O_2$
8	33.002	300561	5.75	Benzene propanic acid,methyle	$C_{10}H_{13}NO$
9	36.391	231531	4.43	9,12,15-Octadecanic acid,methyle	$C_{37}H_{74}N_2O_2$
10	55.343	243533	4.66	Stigma sterol	$C_{29}H_{48}O$
11	55.452	214284	4.10	Retinol,acetate	$C_{22}H_{32}O_2$
12	56.310	220480	4.22	Ergosta-5,22-dian-3ol	$C_{28}H_{50}O_2$

Table 1 List of Phytocomponents of *M. charantia*

The table describes there are 12 phytocomponents from *M.charantia* showing the highest peak in methanolic extract of *M.charantia* Fruit. Out of these components, Compound 1 (2-Cyclopenten-1-one, 2-hydroxy) with a retention time of 5.342 minutes has the highest area percentage at 14.86%, suggesting it's one of the most abundant compounds in the sample. Quinine appears at a retention time of 32.911 minutes with an area percentage of 6.02%, highlighting its significant presence in the sample, which is relevant considering your research interest in this compound**.**

Table 2: Represented the Prediction of the physiochemistry of a few chemicals found in *M. charantia* Linn.

Sl No	Compound	Formula	Mol. weight	Fraction Csp3	RB	HBA	HBD	MR	TPSA	ESOL	Lipophilicity Consec o Log p
1.	2-Cyclopenten-1-one,2-hydroxy	C6H8O2	112.13 g/mol	0.50	0	2	1	30.14	37.30 Å²	Soluble	1.38
2.	Dodecane	C12H26	170.33 g/mol	1.00	9	0	0	59.80	0.00 Å²	P. soluble	3.82
3.	Tetradecane	C14H30	198.39 g/mol	1.00	11	0	0	69.41	0.00 Å²	P. soluble	4.23
4.	Octadecane	C18H38	254.49 g/mol	1.00	15	0	0	88.64	0.00 Å²	P. soluble	5.23
5.	Quinine	C20H24N2O2	324.42 g/mol	0.45	4	4	1	99.73	45.59 Å²	M. soluble	3.36
6.	Retinol	C20H30O	286.45 g/mol	0.50	5	1	1	94.67	20.23 Å²	M. Soluble	3.89
7.	Ergosta-5	C28H44O	396.65 g/mol	0.71	5	1	1	129.37	20.23 Å²	M. soluble	5.00

Table 2 shows Fraction Csp3 ≤ 0.25, permissible range: & lt; 500 g/mol Acceptable ranges for HBA and HBD are ≤10 and ≤5, respectively, for hydrogen bond acceptors and donors. ESOL stands for water solubility, Log p for high lipophilicity (recommended range: ≤5), Molar refractivity (MR): 40 to 130 is the acceptable range. Topological polar surface area or TPSA.

Table 3: Prediction of the pharmacokinetics of pharmacophores obtained from *M. charantia.*

SL. No.	Compound	GIA	BBBP	P-gpS	CYP1A2 Inhibitor	CYP2C19 Inhibitor	CYP2C9 Inhibitor	CYP2D6 Inhibitor	CYP3A4 Inhibitor	Log Kp
1.	2-Cyclopenten-1-one,2-hydroxy	High	Yes	No	No	Yes	No	Yes	No	-6.76 cm/s
2.	Dodecane	Low	No	No	Yes	No	No	No	No	-3.01 cm/s
3.	Tetradecane	Low	No	No	Yes	No	No	No	No	-2.40 cm/s
4.	Octadecane	Low	No	No	Yes	No	No	No	No	-1.20 cm/s
5.	Quinine	High	Yes	No	No	No	No	No	No	-6.23 cm/s
6.	Retinol	High	Yes	No	Yes	No	Yes	No	No	-4.01 cm/s
7.	Ergosta-5	Low	No	No	No	No	Yes	No	No	-3.45 cm/s

Table 4: Prediction of the drug-likeness and medicinal chemistry of a few chemicals found in *M. charantia.*

Drug Likeness										
Sl no.	Compound	Lipinski	Ghose	Veber	Egen	Muegge	BAS	PAINS	Lead Likeness	Synthetic accessibility
1.	2-Cyclopenten-1-one,2-hydroxy	Yes	No	Yes	Yes	No	0.55	0 alert	No	2.42
2.	Dodecane	Yes	Yes	Yes	Yes	No	0.55	0 alert	No	1.83
3.	Tetradecane	Yes	No	No	Yes	No	0.55	0 alert	No	2.04
4.	Octadecane	Yes	No	No	No	No	0.55	0 alert	No	2.49
5.	Quinine	Yes	Yes	Yes	Yes	Yes	0.55	0 alert	Yes	4.34
6.	Retinol	Yes	Yes	Yes	Yes	No	0.55	0 alert	No	4.28
7.	Ergosta-5	Yes	No	Yes	No	No	0.55	0 alert	No	6.21

Figure 1: GC-MS chromatogram of *Momodica charantia* fruit extract Methanol Extract.

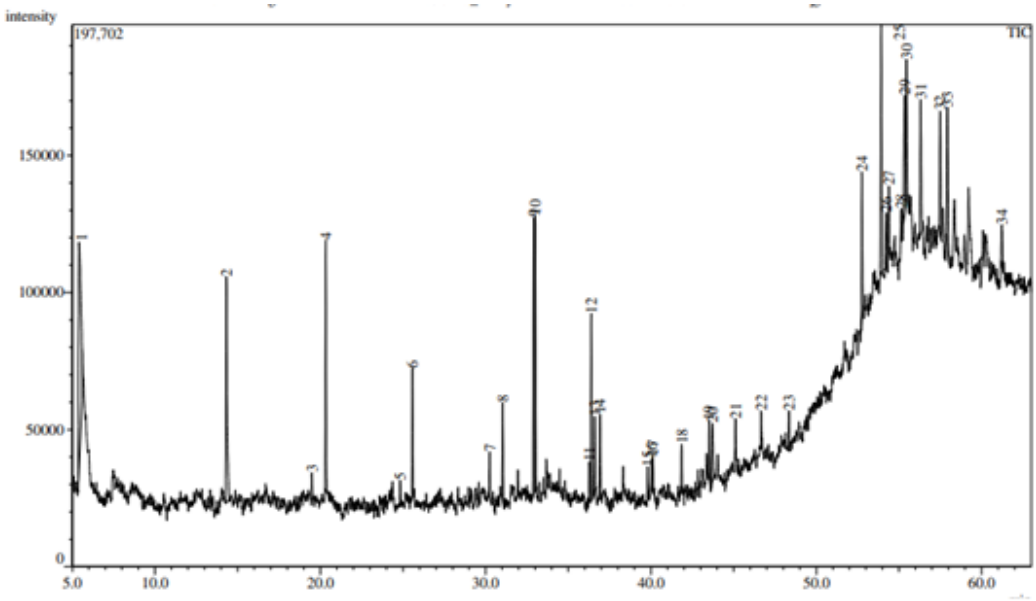
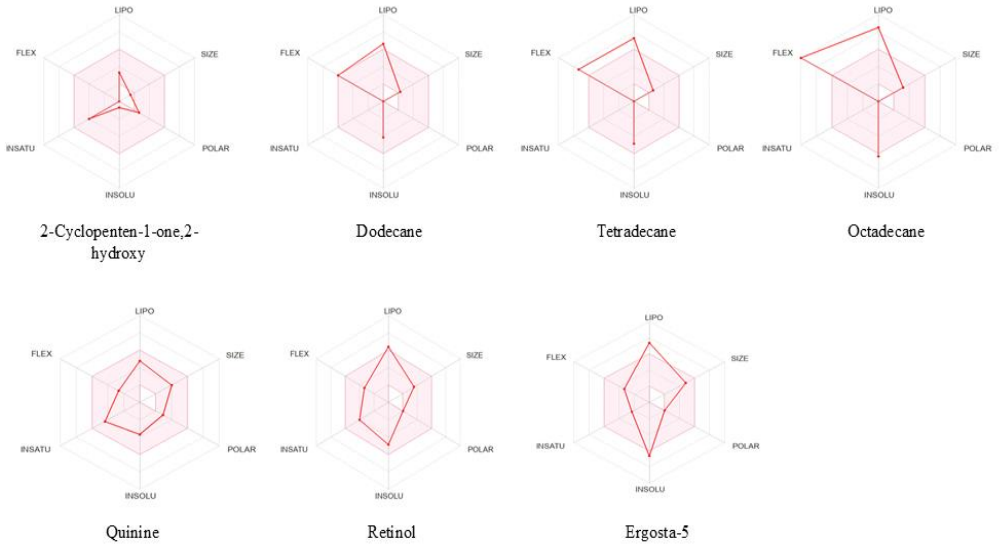


Fig 1 showed Compound 1 (2-Cyclopenten-1-one, 2-hydroxy) with a retention time of 5.342 minutes has the highest area percentage at 14.86%, suggesting it's one of the most abundant compounds in the sample. Quinine appears at a retention time of 32.911 minutes with an area percentage of 6.02%, highlighting its significant presence in the sample, which is relevant considering your research interest in this compound.

Figure 2: Illustrated the compounds under investigation- A.2-Cyclopenten-1-one.2-hydroxy, B. Dodecane, C. Tetradecane, D. Octadecane, E.Quinine, F. Retinol, G. Ergosta-5



The bioavailability radar (pink area) shows the ideal range for each particular property: LIPO (lipophilicity as XLOGP3), SIZE (Size as molecular weight), POLAR (polarity as TPSA (topological polar surface area), INSOLU (insolubility in water by log S scale), INSATU (instauration as per fraction of carbons in the sp3 hybridization), and FLEX (flexibility as per rotatable bonds.

Figure 3: showed the Light & Dark Preference Test (LDPT) Of Quinine phytocomponents induced behavioural alteration in Adult Zebrafish (*Danio rerio*) by Any-MAZE software.

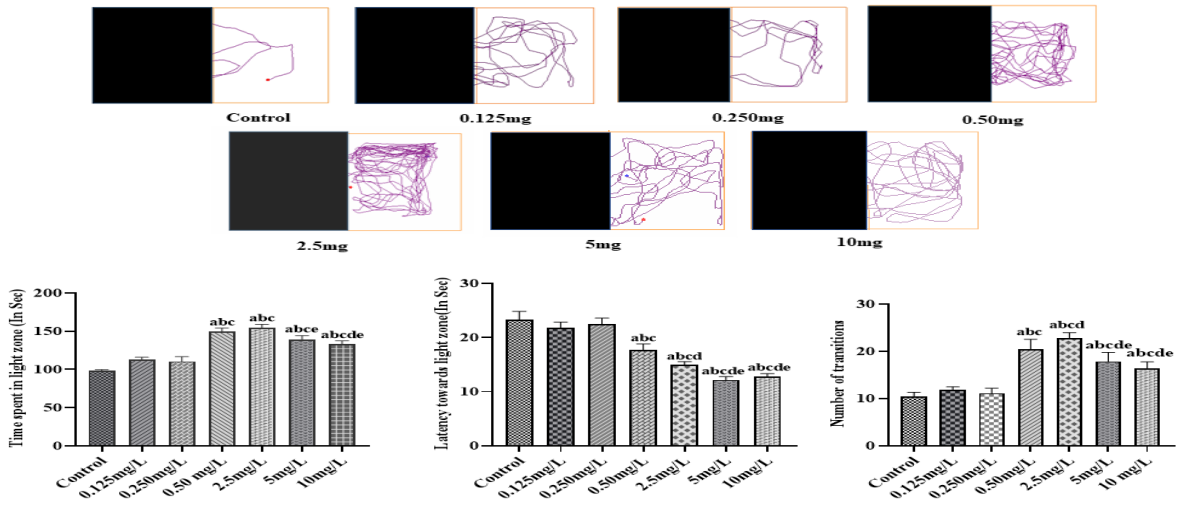


Fig. 3. Behavioral analysis: Typical outcomes from the Light-dark preference test that was adapted for adult Zebrafish. Figure showing: 3[a] *Time spent in the light zone,* 3[b] *Latency towards the light zone and* 3[c] *Number of transitions* in LDPT following exposure to different doses of Quinine. Denotation of "a" represents when compared with control (DMSO); "b" represents when compared with 0.125mg/L; "c" represents when compared with 0.250mg/L; "d" represents when compared with 0.50mg/L; "e" represents when compared with 2.5mg/L; "f" represents when compared with 5mg/L. Data are represented as mean ± SEM and a significant difference were considered at $p < 0.05$ (n=7).

5. DISCUSSION

The identification of quinine in *Momordica charantia* is not insignificant, as documented studies on this compound already determine its antimalarial, anti-inflammatory, antimicrobial, and neuroprotective activity **(**Tisnerat et al., 2022**)**. As per data shows in Table 3 , 2-Cyclopenten-1-one, 2-hydroxy, quinine, and retinol show high absorption and blood-brain barrier penetration, making them potential candidates for central nervous system (CNS)

drug applications. Dodecane, tetradecane, and octadecane have low absorption and no BBB penetration, making them less effective for systemic applications. The CYP inhibition data help identify potential drug-drug interactions, especially for compounds that inhibit multiple enzymes, such as retinol and 2-Cyclopenten-1-one, 2-hydroxy.

In-Silico ADMET profiling of quinine was conducted to predict its pharmacokinetics and safety in a biological system. Quinine was found to have favourable absorption properties with good gastrointestinal absorption potential, suggesting that it may be effectively taken up by the body when administered orally. Distribution predictions revealed that quinine has the capacity to cross the blood-brain barrier, which is crucial for its neuropharmacological effects, but this also raises concerns about potential neurotoxicity at higher concentrations.

The ADMET analysis of quinine phytocomponents revealed a significant absence of inhibitory effects on various cytochrome P450 (CYP) enzymes, specifically CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4. These findings suggest that quinine may have a favourable metabolic profile, as the lack of inhibition on these key enzymes indicates a reduced potential for drug-drug interactions described in table 4. Inhibitory effects on CYP enzymes can lead to altered drug metabolism, which may result in increased toxicity or reduced efficacy of co-administered medications. The results of our analysis indicate that quinine phytocomponents do not interfere with the metabolism of commonly prescribed drugs that are substrates of these CYP isoenzymes. This finding highlights the potential for drug-drug interactions if quinine is used concurrently with other medications metabolized by the same enzyme. Quinine also exhibited moderate excretory properties, primarily via renal elimination, with a low potential for bioaccumulation. However, toxicity profiling flagged potential risks at higher doses, particularly in relation to hepatotoxicity and neurotoxicity, warranting caution in dose selection.

The GC-MS analysis confirmed the presence of quinine in the *Momordica charantia* extract, along with other phytocomponents. Quinine was identified as one of the major alkaloids, known for its antimalarial, antipyretic, and analgesic properties. Given its prominence and therapeutic potential, quinine was selected for further investigation, particularly in understanding its effects on behaviour and toxicity in an animal model. This approach aimed to determine the safe and effective dosage for quinine use, while minimizing its adverse effects. The Zebrafish model was utilized to assess the acute toxicity of quinine and evaluate its impact on behaviour, leveraging Any-maze software for detailed behavioural tracking.

Zebrafish are a well-established model for toxicity testing due to their physiological similarities with humans and their transparency, which allows for real-time observation of internal changes. The study tested six doses of quinine phytocomponents on Zebrafish using Any-Maze software to track their behaviour. Results indicated that waterborne exposure to quinine at lower concentrations does not any morbidity or behavioural alterations in adult zebrafish. Contrarily, a surging behavioural alteration was found on exposure to further higher concentrations.

At the increasing concentration of quinine dose of 0.50 mg/L, significant adverse effects were observed within 24 hours post-exposure. Zebrafish displayed abnormal behaviour, including decreased locomotion, erratic swimming patterns, and increased freezing episodes. These behavioural alterations suggest neurotoxicity at this concentration, likely due to quinine crossing the blood-brain barrier and exerting effects on the central nervous system. The Any-maze software provided quantitative data on these behavioural changes, confirming the dose-dependent impact of quinine on Zebrafish activity levels. Anxiety-like behaviour or overall neural function was measured as part of behavioural assessment using the light and dark box test by Any-Maze software. To identify a safer dose for future studies, the quinine concentration was reduced in lower dose respectively . At this lower dose, Zebrafish exhibited normal behaviour with no significant deviations from baseline activity. Swimming patterns, response to stimuli, and overall locomotion were consistent with untreated control groups. This finding is crucial, as it establishes mg as a safer dosage for future experimentation, allowing researchers to harness quinine's therapeutic potential while minimizing its toxicological risks. These findings demonstrate a clear dose-dependent effect, with moderate doses exhibiting the most significant anxiolytic and exploratory behaviour. High doses, show diminished efficacy, potentially due to toxicity or sedative effects. Statistical analysis underscores the significance of these observations, with superscript annotations denoting differences from the control and among the dose groups.

The present findings stated that the lower concentration of quinine proved to be relatively safe, whereas the higher concentrations showed increasing toxicity causing behavioural alteration in zebrafish. Additionaly, it clearly focuses on a better understanding of the safe profiling of quinine for further investigation concerning quinine neurotoxicity mechanisms and potential safe therapeutic applications of quinine in future studies.

6. CONCLUSION

In conclusion, the comprehensive evaluation of the phytocomponents of *Momordica charantia* revealed quinine as a key phytocomponents with significant therapeutic potential for its future neutraceutical approach. *In silico* ADMET analysis not only highlighted the quinine's favourable pharmacokinetic properties but also raised concerns regarding its neurotoxicity at higher concentration. Zebrafish behavioural analysis using the Any-maze software definitely confirmed the findings that while 0.50 mg/L of quinine induced significant behavioural alterations, the safer concentration of Quinine (0.250 mg/L) showed no significant behavioral changes. Hence, the pressent study identified 0.250 mg/L as the optimal concentration for future experiments, offering a balance between efficacy and safety, and underscores the importance of precise dose management in therapeutic applications.

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