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ANTIOXIDANT AND ANTI-CHOLESTEROL POTENTIAL OF *Cyperus rotundus* L. TUBER ESSENTIAL OIL AND ITS STABLE SELF-NANOEMULSIFYING DRUG DELIVERY SYSTEM FORMULATION

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ABSTRACT

This study investigated the antioxidant and cholesterol-lowering potential of *Cyperus rotundus* L. tuber essential oil using integrated in silico and in vitro approaches, followed by formulation into a self-nanoemulsifying drug delivery system (SNEDDS) to improve oral bioavailability. Gas chromatography–mass spectrometry identified 22 major phytochemicals, including β-pinene, D-limonene, and caryophyllene. PASS Online predicted moderate bioactivity, with probabilities of up to 0.565 for antioxidant and 0.435 for lipid-lowering effects. Drug-likeness, pre-ADMET, and toxicity analyses indicated favourable pharmacokinetic properties and oral safety. Molecular docking showed moderate binding affinities toward the Nrf2–Keap1 complex (−3.8 to −4.8 kcal/mol) and HMG-CoA reductase (−4.1 to −4.8 kcal/mol), supporting possible multi-target antioxidant and lipid-regulating mechanisms. In vitro assays demonstrated DPPH radical-scavenging activity (IC_{50}= 110.37 µg/mL) and dose-dependent cholesterol reduction (IC_{50} = 148.83 ppm). The optimised SNEDDS formulation, containing 1% essential oil, 20% Tween 80, and 15% propylene glycol, produced nanosized droplets (50–350 nm), neutral pH (7.13), low viscosity (~6 mPa·s), and excellent stability. Overall, these findings support the traditional use of C. rotundus and indicate its promise as a dual-action natural agent against oxidative stress and dyslipidaemia, while also demonstrating the utility of SNEDDS as a delivery platform to enhance its pharmaceutical applicability.

Keywords: *Cyperus rotundus,* Essential Oil, Antioxidant, Anti-Cholesterol, Molecular Docking, SNEDDS, Dyslipidaemia

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INTRODUCTION

Cardiovascular diseases remain the leading cause of death worldwide, and hypercholesterolemia is one of the most important modifiable risk factors contributing to this burden. In recent years, natural products have attracted significant attention as potential therapeutic agents for the management of chronic inflammatory and metabolic disorders, including hypercholesterolemia.[1] Essential oils, in particular, are rich in bioactive compounds that exhibit pharmacological properties with fewer side effects compared to synthetic drugs.[2] Among medicinal plants, *Cyperus rotundus* L. (purple nutsedge) has been widely used in

traditional medicine due to its antioxidant, anti-inflammatory, and lipid-modulating properties.[3] The tubers of *Cyperus rotundus* contain essential oils that contribute to its biological activities.[4] Hypercholesterolemia is a major risk factor for cardiovascular diseases, which remain the leading cause of global mortality. Conventional cholesterol-lowering drugs, such as statins, are effective but may cause adverse effects, including myopathy and hepatotoxicity.[5] This has encouraged research on plant-derived alternatives, including C. rotundus, which has demonstrated hypolipidemic effects in animal models. However, the molecular mechanisms underlying its anti-cholesterol activity remain unclear, warranting further investigation using in silico and in vitro approaches.[6] In particular, studies integrating phytochemical profiling, computational prediction, biological validation, and formulation development for C. rotundus tuber essential oil remain limited. This represents an important research gap, as an integrated approach is needed to substantiate its therapeutic potential and pharmaceutical applicability better. Oxidative stress also plays a central role in the pathogenesis of chronic diseases such as atherosclerosis, diabetes, and neurodegenerative disorders.[7] Although synthetic antioxidants are effective, their toxicity and low bioavailability limit their therapeutic use.[8] Therefore, a systematic evaluation of the antioxidant potential of C. rotundus essential oil through computational and experimental approaches is essential.[9] Beyond its scientific relevance, the exploration of plant-based bioactive agents may also contribute to sustainable health innovation, particularly in relation to SDG-3 (Good Health and Well-Being), by developing safer, more accessible therapeutic alternatives. In silico methods, including molecular docking and ADMET (absorption, distribution, metabolism, excretion, and toxicity) prediction, offer a cost-effective strategy to identify bioactive compounds and their potential molecular targets.[10] These approaches can predict interactions between C. rotundus constituents—such as β-pinene, D-limonene, and caryophyllene—with key enzymes, such as HMG-CoA reductase, and oxidative stress-related proteins (e.g., the Nrf2–Keap1 complex).[11] To validate computational predictions, in vitro assays such as DPPH radical scavenging and cholesterol inhibition tests are essential for confirming biological activity.[12] Furthermore, formulation into a self-nanoemulsifying drug delivery system (SNEDDS) can enhance the solubility, stability, and oral bioavailability of the essential oil.[13] Therefore, this study aims to evaluate the antioxidant and cholesterol-lowering potential of *Cyperus rotundus* L. tuber essential oil using in silico and in vitro methods, and to develop and characterise an SNEDDS formulation to support its use as a natural therapeutic agent. By addressing the limited integration of mechanistic prediction, experimental validation, and delivery-system optimisation, this study seeks to provide a more comprehensive basis for the development of C. rotundus essential oil as a promising natural candidate for managing oxidative stress and hypercholesterolemia.

EXPERIMENTAL

Materials and Methods

Research Material

The in-silico study was conducted using a 13th Gen Intel® Core™ i7-13650HX processor with Biovia Discovery Studio, AutoDock Vina, PASS Online, Pre-ADMET, Lipinski's Rule of Five, PyMOL, PubChem, Protein Data Bank (PDB), and ProTox-II. Major GC-MS–identified constituents of *Cyperus rotundus* L. tuber essential oil with similarity indices above 90%—including α-pinene, β-pinene, D-limonene, camphene, caryophyllene, copaene, and humulene—served as ligands, while sodium diclofenac and atorvastatin were used as reference drugs for the Nrf2–Keap1 (PDB ID: 6NF1) and HMG-CoA reductase (PDB ID: 1HWK) targets, respectively. In vitro assays and the SNEDDS formulation utilised standard laboratory glassware, a UV-Visible spectrophotometer, a particle size analyser, a viscometer, and a homogeniser. Reagents included H_2SO_4, acetic anhydride, pure cholesterol, ethanol, and chloroform, whereas Tween 80, propylene glycol, distilled water, and methylene blue were used in SNEDDS preparation to ensure accurate formulation and physicochemical characterisation.

Plant Material Collection, Extraction, and GC–MS Analysis

The tubers of *Cyperus rotundus* L. were collected from Deli Serdang, Medan, and taxonomically verified at the Herbarium Medanense (Specimen No. 2552/MEDA/2024). Cleaned and sorted tubers were extracted using microwave-assisted hydrodistillation (MAHD) with a modified Clevenger-type apparatus

at 600 W for 47.5 minutes, applying a liquid-to-solid ratio of 7 mL/g. The distillate was treated with 0.2 mL toluene for phase separation, and the organic layer was dried over anhydrous sodium sulfate, filtered, and stored in amber vials at 2–8 °C until analysis. Gas chromatography–mass spectrometry (GC–MS) was performed using an Agilent 7890B GC coupled with an MSD 5977A and an HP-5MS capillary column (30 m × 0.25 mm, 0.25 µm film). Helium was used as the carrier gas at 1 mL/min, with a 1 µL splitless injection at 250 °C. The oven temperature was programmed from 40 °C (1 min hold) to 300 °C at 10 °C/min and held for 4 minutes, while the MS operated in electron ionization mode (70 eV) with transfer line, ion source, and quadrupole temperatures of 270 °C, 230 °C, and 150 °C, respectively.

In-silico

Biological activity prediction was performed using PASS Online based on the SMILES of each *Cyperus rotundus* L. tuber essential oil compound. Lipinski's Rule of Five was evaluated from the 3D (.pdb) structures to assess drug-likeness, while pre-ADMET testing via pkCSM and Protox-II predicted pharmacokinetic and toxicity profiles. These analyses served as an initial screening to identify compounds with favourable biological and safety characteristics before docking simulation. Ligand and receptor preparation were carried out using Biovia Discovery Studio and AutoDock Vina. Ligands were converted from .sdf to .pdb, and receptor structures of HMG-CoA reductase (Fig.-1) and Nrf2–Keap1 complex (Fig.-2) were refined by removing water, native ligands, and adding Kollman charges. Validation was performed by redocking native ligands and calculating RMSD in PyMOL. Docking results were visualised in Biovia Discovery Studio to identify interacting amino acid residues.

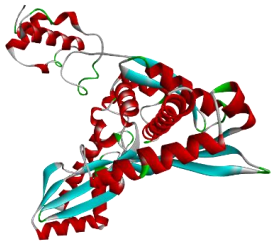

Fig.-1: HMG-COA Reductase Receptor

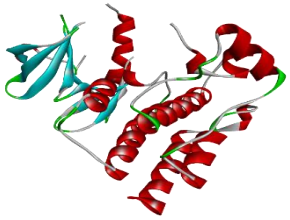

Fig.-2: Nrf2-Keap1 Complex Receptor

In-vitro

Antioxidant Activity

The antioxidant activity of *Cyperus rotundus* tuber essential oil was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. A 50 µg/mL DPPH solution was prepared in methanol, and quercetin (0.5–2.5 µg/mL) served as the standard. The essential oil stock solution (20,000 µg/mL) was prepared by dissolving 100 µL of oil in methanol and diluting to 40–200 µg/mL. For each test, 2 mL of sample or standard solution was mixed with 2 mL of DPPH reagent, vortexed, and incubated in the dark at room temperature for 30 minutes. The decrease in absorbance, indicating DPPH radical reduction, was measured at 517 nm using a UV–Vis spectrophotometer. Scavenging activity was calculated as the percentage inhibition relative to the control, and IC_{50} values were determined from the linear regression of concentration versus inhibition percentage.

Anti-cholesterol Activity

The antioxidant activity of *Cyperus rotundus* tuber essential oil was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. A 50 µg/mL DPPH solution was prepared in methanol, and quercetin (0.5–2.5 µg/mL) served as the standard. The essential oil stock solution (20,000 µg/mL) was prepared by dissolving 100 µL of oil in methanol and diluting to 40–200 µg/mL. For each test, 2 mL of sample or standard solution was mixed with 2 mL of DPPH reagent, vortexed, and incubated in the dark at room temperature for 30 minutes. The decrease in absorbance, indicating DPPH radical reduction, was measured at 517 nm using a UV–Vis spectrophotometer. Scavenging activity was calculated as the percentage inhibition relative to the control, and IC_{50} values were determined from the linear regression of concentration versus inhibition percentage.

Formulation and Evaluation

SNEDDS Formulation of *Cyperus rotundus* L. Tuber Essential Oil

The SNEDDS formulation was prepared using Tween 80, a nonionic surfactant that reduces interfacial tension between the oil and aqueous phases, thereby facilitating the formation of a stable nanoemulsion. Propylene glycol acts as a cosurfactant, enhancing the solubilising capacity of the system and improving the fluidity of the interfacial film, thereby supporting emulsification and increasing drug permeability. Distilled water (aquadest) serves as the continuous aqueous phase in which the oil droplets are dispersed, providing the medium for emulsion formation and maintaining the formulation's isotonicity and clarity. The optimal formulation was selected based on the ratio of SNEDDS components, with selection criteria including the minimal amount of surfactant required and the best homogeneity or emulsion stability as determined by visual observation.

Evaluation of SNEDDS Containing *Cyperus rotundus* L. Tuber Essential Oil

The SNEDDS formulation containing *Cyperus rotundus* L. tuber essential oil was evaluated for its physicochemical properties to ensure oral suitability. Assessments included organoleptic observation, pH measurement, centrifugation stability, emulsion type determination, viscosity, and particle size distribution. Organoleptic evaluation was conducted by observing the color, odor, clarity, and phase separation to detect potential instabilities. The pH was measured at room temperature using a calibrated digital pH meter to verify compatibility with physiological conditions. Physical stability was tested by centrifuging 2 mL of the formulation at 6000 rpm for 30 minutes and observing any color or phase changes. The emulsion type was determined by adding methylene blue; its uniform dispersion confirmed an oil-in-water (o/w) system. Viscosity was measured using a Brookfield viscometer to assess flow characteristics and ensure formulation stability and ease of administration. Particle size distribution was analysed using a Particle Size Analyzer (PSA) at the Nanomedicine Laboratory, Faculty of Pharmacy, Universitas Sumatera Utara, employing light scattering to determine droplet size within the 50–350 nm range, which characterises nanoemulsions with enhanced solubility and bioavailability.

RESULTS AND DISCUSSION

Essential Oil Yield and GC-MS Profile of *Cyperus rotundus* L. Tuber

The tubers of *Cyperus rotundus* L. collected from Deli Serdang, Medan, yielded a light-yellow essential oil with a distillation yield of 0.27% v/w. The results of the activity prediction for *Cyperus rotundus* L. tuber essential oil compounds were obtained using the Pa (probability for active molecule) parameter to identify potential antioxidant and anti-cholesterol compounds (Table-1).

Table-1: GC-MS Results and Biological Activity Prediction Results using PASSOnline

Compounds	Similarity index (%)	Biological activity	Pa value
Alfa-Pinene	96	Antioxidant	0.186
		Oxidizing agent	0.180
Camphene	97	Oxideructase inhibitor	0.261
Sabinene	96	Antioxidant	0.268
Beta-Pinene	95	Antioxidant	0.548
		LDL-lowering agent	0.328
		Free radical scavenger	0.511
O-Cymene	95	Antioxidant	0.180
		Oxidizing agent	0.234
D-Limonene	99	Antioxidant	0.511
		Free radical scavenger	0.565
Gamma-Terpinene	97	Antioxidant	0.311
		Oxidizing agent	0.309
Terpinolene	98	Antioxidant	0.116
(-)-Terpinen-4-ol	96	Antioxidant	0.564
		Oxidizing agent	0.552
Alpha-Terpineol	90	LDL-lowering agent	0.261
		Oxidizing agent	0.252

Geraniol	95	Antioxidant	0.548
		Oxygen scavenger	0.569
		Cholesterol synthesis inhibitor	0.201
Safrole	98	Antioxidant	0.480
		Oxideructase inhibitor	0.388
(-)-alpha-Cubebene	98	Oxidizing agent	0.261
		Oxygen scavenger	0.188
Copaene	99	Oxygen scavenger	0.109
		Oxideructase inhibitor	0.186
Caryophyllene	99	Oxygen scavenger	0.388
		LDL-lowering agent	0.435
Humulene	97	Oxidizing agent	0.516
6-Allyl-4-methoxy-1,3-benzodioxole	99	Oxideructase inhibitor	0.260
4-(p-Acetoxyphenyl)-2-butanone	98	Oxidizing agent	0.180
		Oxygen scavenger	0.118

GC–MS and PASS Online analyses revealed that the essential oil of *Cyperus rotundus* L. tubers contains several bioactive compounds with antioxidant and cholesterol-lowering potential. β-Pinene (Pa = 0.548 as antioxidant; 0.328 as LDL-lowering), D-limonene (Pa = 0.565 as free radical scavenger), and caryophyllene (Pa = 0.435 as LDL-lowering) exhibited promising activities, with Pa values > 0.3 indicating a high probability of involvement in lipid oxidation inhibition and cholesterol synthesis suppression.[13,14] Other constituents, such as geraniol (Pa = 0.569 as an oxygen scavenger; 0.201 as a cholesterol synthesis inhibitor) and (–)-terpinen-4-ol (Pa = 0.564 as an antioxidant), also contributed to hypocholesterolemic and oxidative stress–protective effects. These mechanisms are likely mediated by oxidoreductase inhibition, free radical scavenging, and regulation of LDL synthesis. The synergistic action among these compounds enhances the overall pharmacological potential of C. rotundus essential oil.[15]

Lipinski's Rule of Five Test

The physicochemical evaluation of *Cyperus rotundus* L. tuber essential oil compounds (Table-2) showed full compliance with Lipinski's Rule of Five (RO5), confirming favourable drug-like properties and oral suitability.[16] All compounds exhibited molecular weights between 134 and 206 Da, well below the 500 Da limit, ensuring efficient membrane permeability. Log P values (2.13–4.99) reflected balanced lipophilicity for optimal solubility and cellular uptake, while hydrogen bond donors (0–1) and acceptors (0–3) supported good oral bioavailability.[17] Molar refractivity values (43.75–68.90) were within the ideal range (40–130), indicating appropriate molecular polarizability and stability. The absence of RO5 violations across all analysed terpenoids and phenylpropanoids highlights their strong pharmaceutical relevance and consistency with previous findings on natural bioactive compounds possessing oral drug potential.[18] The consistent adherence to these rules across all analysed compounds strengthens the argument for further preclinical investigation into their therapeutic applications. The results are shown in Table-2.

Table-2: Lipinski's Rule of Five Test

No.	Parameter	Standard	Observed Range	Conclusion
1	Molecular Weight	< 500 Da	134–206 Da	Complies
2	Log P	< 5	2.13–4.99	Complies
3	HBD (H-bond donor)	< 5	0–1	Complies
4	HBA (H-bond acceptor)	< 10	0–3	Complies
5	Molar Refractivity	40–130	43.75–68.90	Complies

Pre-ADME Test

The pre-ADME evaluation of *Cyperus rotundus* L. tuber essential oil compounds (Table-3) demonstrated favourable pharmacokinetic characteristics, particularly in absorption and bioavailability. The bioavailability score of 0.55 meets the threshold for adequate systemic exposure, consistent with

Lipinski's Rule of Five compliance and supporting their potential as drug candidates.[19] Although six compounds showed TPSA values (18.46–94.77 Å²) outside the ideal range of 60–140 Å², indicating possible variation in membrane permeability and blood–brain barrier penetration, other parameters remained within acceptable limits. The Log Kp values (–6.37 to –3.89 cm/s) complied with the standard (–5.35 to –7.4 cm/s), suggesting good transdermal permeability, while most compounds exhibited optimal lipophilicity with Log Po/w values (2.22–4.31) close to the ideal range (2.25–4.75). Overall, these results indicate that C. rotundus essential oil constituents possess promising pharmacokinetic profiles for oral and transdermal delivery, although outliers in TPSA and Log Po/w merit further optimisation.[20]

Table-3: Pre-ADME Test

No	Parameter	Standard	Observed Range	Conclusion
1	TPSA	60-140 Å2	18.46-94.77 Å2	Six Compounds Do Not Comply
2	Bioavaibility	> 0.55	0.55	Complies
3	Log Kp	-5,35 -7,4 Cm/S	-6.37 To -3.89 Cm/S	Complies
4	Log $P_{o/W}$	2.25-4.75	2.22–4.31	One Compound Does Not Comply.

Toxicity Assessment

The toxicity evaluation of *Cyperus rotundus* L. tuber essential oil compounds (Table-4) indicated a favourable safety profile with no predicted AMES toxicity, hepatotoxicity, or skin sensitisation, suggesting low genotoxic and allergic potential.[21,22] These findings are consistent with the traditional use of C. rotundus and reinforce its safety as a natural therapeutic source. The acute oral toxicity (LD_{50}) analysis classified four compounds as Class IV (moderately toxic, $300 < LD_{50} \leq 2000$ mg/kg), twelve as Class V (slightly toxic, $2000 < LD_{50} \leq 5000$ mg/kg), and five as Class VI (practically non-toxic, $LD_{50} > 5000$ mg/kg). The predominance of Class V and VI constituents reflects a generally low toxicity level and supports their suitability for pharmacological applications. Nonetheless, the few Class IV compounds warrant further dosage optimisation and toxicological verification before clinical development.[23] The result is shown in Table-4.

Table-4: Toxicity Assessment

No	Parameter	Standard	Observed	Conclusion
1	AMES Toxicity	No	No	Complies
2	LD50 (mg/kg)	Class 1: ≤ LD50 Class 2: 5<LD50 ≤ 50 Class 3: 50<LD50 ≤ 300 Class 4: 300<LD50 ≤ 2000 Class 5: 2000<LD50 ≤ 5000 Class 6: LD50>5000	1016–5300 mg/kg	Four compounds in class IV Twelve compounds in class V Five compounds in class VI
3	Hepatotoxicity	No	No	Complies
4	Skin Sensitisation	No	No	Complies

Molecular Validation and Docking

The molecular docking analysis of *Cyperus rotundus* L. tuber essential oil compounds against the Nrf2–Keap1 complex revealed varied binding affinities, reflecting differential interaction strengths and possible modulatory effects on the Nrf2 signaling pathway. The native ligand exhibited the strongest binding energy (-6.000 kcal/mol), while sulforaphane, a known Nrf2 activator, showed a moderate affinity (-4.100 kcal/mol), serving as a benchmark for antioxidant potential. Among the essential oil constituents, caryophyllene (-3.800 kcal/mol) and humulene (-3.300 kcal/mol) displayed the most favourable binding, followed by (-)-α-cubebene and D-limonene (-3.500 kcal/mol). Although these values were slightly weaker than sulforaphane, the results suggest potential interactions within the Keap1 active site that could promote Nrf2 dissociation and activation. The findings indicate that sesquiterpenes and monoterpenes in the oil may function as mild modulators of the Nrf2-Keap1 complex, thereby enhancing antioxidant defence by indirectly activating Nrf2-mediated pathways. The structural diversity of these compounds also suggests the possibility of synergistic or additive antioxidant effects. Docking analysis against HMG-CoA reductase revealed comparable findings, where several essential oil constituents exhibited binding

affinities approaching those of the native ligand (-5.300 kcal/mol) and atorvastatin (-4.900 kcal/mol). Copaene (-4.800 kcal/mol), humulene (-4.200 kcal/mol), and caryophyllene (-4.100 kcal/mol) were the strongest binders, with copaene showing affinity nearly equivalent to atorvastatin, suggesting potential inhibitory activity on cholesterol biosynthesis. This correlates with PASS Online predictions that identified these compounds as likely LDL-lowering agents. Sesquiterpenes generally showed stronger interactions than monoterpenes, implying that larger molecular frameworks may fit more effectively into the enzyme's active site. However, certain monoterpenes such as α-terpineol (-4.200 kcal/mol) and (-)-terpinen-4-ol (-4.700 kcal/mol) demonstrated comparable affinities, likely due to hydroxyl groups facilitating hydrogen bonding. The observed variations among stereoisomers, such as α-pinene (-4.200 kcal/mol) versus β-pinene (-3.800 kcal/mol), highlight the influence of stereochemistry on enzyme interaction. Collectively, these findings suggest a potential synergistic mechanism among C. rotundus constituents, offering valuable insights for future optimisation of natural HMG-CoA reductase inhibitors. The results are shown in Table-5, and Fig.-3 shows the visualisation of the docking interaction between the receptor and the native ligand.

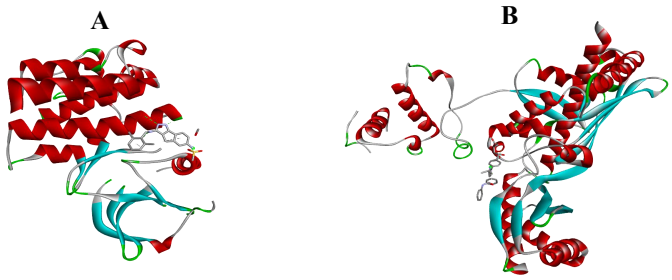

Fig.-3: Docking Visualisation of Interactions between (A) the Nrf2–Keap1 Receptor Complex and its Native Ligand, and (B) HMG-CoA Reductase and its Native Ligand

Table-5: Binding Affinities (kcal/mol) of *Cyperus rotundus* L. Tuber Essential Oil Compounds against Nrf2–Keap1 Complex and HMG-CoA Reductase

No.	Compounds	Nrf2–Keap1	HMG-CoA Reductase
1	Native Ligand ((2S)-3-[(3S)-1-(ethylsulfonyl)piperidin-3-yl]-2-{[3-(4-methylphenyl)imidazo[1,2-a]pyrazin-8-yl]amino}propan-1-ol)	–6.000	—
2	Sulforaphane	–4.100	—
3	Native Ligand (7-[2-(4 Fluoro-Phenyl)-5 Isopropyl-3-Phenyl-4 Phenylcarbamoyl Pyrrol-1-Yl]-3,5 Dihydroxy-Heptanoic Acid)	—	–5.300
4	Atorvastatin	—	–4.900
5	Caryophyllene	–3.800	–4.100
6	Humulene	–3.300	–4.200
7	(–)-α-Cubebene	–3.500	–3.800
8	6-Allyl-4-methoxy-1,3-benzodioxole	–2.800	–4.000
9	Copaene	–2.800	–4.800
10	4-(p-Acetoxyphenyl)-2-butanone	–2.900	–3.800
11	β-Pinene	–3.000	–3.800
12	Safrole	–3.000	–4.200
13	α-Terpinene	–2.700	–3.700
14	(–)-Terpinen-4-ol	–2.800	–4.700
15	α-Terpineol	–3.000	–4.200
16	3-Carene	–3.000	–3.400
17	Terpinolene	–2.800	–3.800
18	γ-Terpinene	–3.100	–3.600
19	α-Pinene	–3.200	–4.200
20	D-Limonene	–3.500	–3.900
21	Sabinene	–2.200	–3.500

22	Camphene	–2.100	–3.300
23	o-Cymene	–2.200	–4.000
24	Geraniol	–3.800	–3.800
25	β-Myrcene	–3.600	–3.300

Antioxidant Activity Analysis

Antioxidant activity was assessed using the DPPH radical scavenging assay, where DPPH, a stable nitrogen-containing free radical with strong UV absorption at 517 nm, changes colour from purple to yellow upon reduction by antioxidants. The IC_{50} values, indicating the concentration required to neutralise 50% of radicals, were measured for quercetin and the essential oil from the *Cyperus rotundus* tuber. Quercetin exhibited a low IC_{50} of 5.68 µg/mL, demonstrating very strong antioxidant activity, as values below 50 µg/mL are indicative of high potency. In contrast, the essential oil exhibited an IC_{50} of 110.37 µg/mL, indicating moderate antioxidant capacity. These findings suggest that while quercetin is a highly effective natural antioxidant, the essential oil also provides notable antioxidant effects, though to a lesser extent.[31]

Anti-Cholesterol Activity Analysis

The inhibition curve shows a strong positive correlation between sample concentration and percentage inhibition, with a regression equation of $y = 0.3889x – 7.8801$ and a high coefficient of determination ($R^2 = 0.9608$). This strong linearity indicates a clear dose-dependent inhibitory response, characteristic of biologically active compounds with potential therapeutic value.[32] Based on regression analysis, the IC_{50} value was calculated to be 148.83 ppm, representing the concentration required to inhibit 50% of the cholesterol reaction. According to standard classifications, compounds with $IC_{50} < 100$ ppm are highly active, 100–200 ppm moderately active, and >200 ppm weak inhibitors. Therefore, the essential oil of *Cyperus rotundus* L. tubers demonstrates moderate anti-cholesterol activity, supporting its potential as a natural lipid-lowering agent.[33]

SNEDDS Formulation Results of *Cyperus rotundus* L. Tuber Essential Oil

The SNEDDS (Self-Nanoemulsifying Drug Delivery System) formulation of *Cyperus rotundus* L. tuber essential oil was successfully developed using a combination of essential oil, Tween 80, propylene glycol, and distilled water. The optimised formulation consisted of 1% essential oil, 20% Tween 80 as the surfactant, 15% propylene glycol as the co-surfactant, and 64% distilled water, yielding a total volume of 50 mL. All components were homogenised at 3200 rpm for 1 minute to achieve a stable nanoemulsion system.[34]

Evaluation of SNEDDS Containing *Cyperus rotundus* L. Tuber Essential Oil

The SNEDDS formulation of *Cyperus rotundus* L. tuber essential oil was assessed for its physicochemical properties, including organoleptic characteristics, pH, centrifugation stability, emulsion type, viscosity, and particle size (Table-6). The nanoemulsion appeared clear, light yellow, and aromatic, with no phase separation or turbidity over 2 weeks, indicating good physical stability (Fig.-4).

Fig.-4: The Results of the Organoleptic Evaluation of the Formulation

The measured pH value of 7.13 falls within the physiological range for oral preparations (4.5–7.5), suggesting safety and mucosal compatibility. Centrifugation at 6000 rpm for 30 minutes revealed no separation or sedimentation, confirming mechanical stability. The methylene blue test verified an oil-in-water (o/w) emulsion type, ideal for enhancing lipophilic drug solubilization and absorption. Viscosity ranged from 6.08 to 6.82 mPa·s, suitable for oral nanoemulsions, ensuring ease of swallowing and

stability. Particle size analysis (50–350 nm) confirmed the nanoscale distribution, which is essential for improving dissolution, bioavailability, and uniform therapeutic performance. These findings collectively indicate that the SNEDDS formulation of C. rotundus essential oil meets the criteria for a stable and effective oral delivery system.

Table-6: Evaluation Results of SNEDDS

Evaluation Parameter	Result	Interpretation
Organoleptic properties	Clear, light-yellow, aromatic, no phase separation	Stable physical appearance over two weeks
pH value	7.13	Within the acceptable oral pH range (4.5-7.5)
Centrifugation test	No phase separation or precipitation observed	Stable under centrifugation stress
Emulsion type	Oil-in-water (o/w)	Suitable for an oral delivery system
Viscosity (mPa-s)	6.08, 6.21, 6.82	Acceptable viscosity for oral nanoemulsion
Particle size distribution (nm)	50-350	Meets the nanoscale requirement for nanoemulsion

CONCLUSION

Cyperus rotundus L. tuber essential oil contains key bioactive compounds, including β-pinene, D-limonene, and caryophyllene, and exhibits moderate antioxidant and cholesterol-lowering potential, as supported by in silico predictions and in vitro assays. Molecular docking revealed interactions with Nrf2–Keap1 and HMG-CoA reductase, and experimental results confirmed the corresponding biological activities. The optimized SNEDDS formulation produced nanosized droplets, demonstrated good stability, and exhibited favourable physicochemical properties, indicating improved oral bioavailability. Overall, these findings highlight the dual pharmacological potential and formulation feasibility of C. rotundus essential oil for future nutraceutical applications targeting oxidative stress and dyslipidemia. Further studies are recommended to confirm its efficacy and safety through in vivo evaluation and advanced formulation performance testing. In a broader context, these findings may also support SDG 3 (Good Health and Well-Being) by advancing the development of safer, potentially more accessible plant-based therapeutic alternatives for chronic metabolic disorders.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well-Being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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[RJC-9544/2026]