DOI: https://doi.org/10.52756/ijerr.2024.v40spl.021 ***Int. J. Exp. Res. Rev.*, Special Vol. 40: 245-255 (2024)**

Original Article | *Peer Reviewed* | *Open Access*

International Journal of Experimental Research and Review (IJERR)

ISSN: 2455-4855 (Online)
www.iaph.in
ISSN 2455-4855

Secondary Metabolite Fingerprinting and Antioxidant Potency of *Eleusine indica*: An Integrated Chromatography and *In silico* Investigation

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Article History:

Received: 09th Feb., 2024
Accepted: 26th Jun., 2024
Published: 30th June., 2024

Keywords:

Eleusine indica; chromatography and fingerprint; antioxidants metabolites; molecular docking and toxicity prediction.

How to cite this Article:

Tapan Kumar Sahu, Nityananda Sahoo, Gurudutta Pattnaik, Himansu Bhusan Samal, Amulyaratna Behera and Biswakanth Kar (2024). Secondary Metabolite Fingerprinting and Antioxidant Potency of *Eleusine indica*: An Integrated Chromatography and *In silico* Investigation. *International Journal of Experimental Research and Review*, *40(spl.)*, 245-255.

DOI:
https://doi.org/10.52756/ijerr.2024.v40spl.021

Abstract: Natural regimens and constituents serve as the primary sources for drug conservation, and each herbal regimen possesses unique ethnomedicinal and biological properties. Therefore, researchers are currently more interested in exploring the biological activity of existing plant species with the most systematic approach to be used in the specific therapeutics proposed, either crude extracts or bioactive metabolites. The present study also explores the antioxidant potency of ethanolic extracts of *Eleusine indica* (a weed in an agricultural field) through chemical fingerprinting analyses through high-performance thin-layer chromatography (HPTLC), followed by gas chromatography-mass spectrometry (GCMS) and *in silico* investigation in a systematic way. Briefly, this investigation aims to observe the presence of secondary metabolites associated with antioxidant activity. After extraction, a fingerprint study was carried out with the mobile phase consisting of ethyl acetate, acetic acid, formic acid, and water in the ratio 10:1.1:1.1:2.3 by volume, where more than eight fluorescent and non-fluorescent bands were found, with the highest density peak area at Rf 0.87 and the lowest at Rf 0.18. As per the GCMS report ethanolic extracts of *E. indica* are composed of several metabolites. Further, based on the higher Rf value, we have selected seven (**EI_1** to **EI_7**) that constitute molecular docking against three target enzymes: cyclooxygenase-2 (PDB ID: 5F1A), peroxiredoxin-5 (PDB ID: 1HD2), and haemoxygenase 1 (PDB ID: 3CZY) to assess the antioxidant potency. Overall, the results revealed that **EI_7** (2,7-dioxatricyclo [4.4. 0.0(3,8)] decane-4,5-diol) showed similar potency with ideal drug-ability profiles similar to ascorbic acid and was considered a lead for further therapeutic use as a complementary agent.

Introduction

Eleusine indica (L.) Gaerth, commonly known as Indian goosegrass or crowfoot grass, exhibits remarkable diversity and is widely distributed across tropical and subtropical regions worldwide (Saidi et al., 2016; Li et al., 2022). This resilient species thrives in a variety of environments, from agricultural fields and pastures to roadsides and urban areas. Its adaptability to different soil types and climatic conditions allows it to colonize disturbed sites effectively, often becoming a dominant weed in these habitats (Yemets et al., 2003; Elmore et al., 2022). The plant's morphological diversity, which includes variations in leaf shape, size, and growth habits, further enhances its ability to survive and proliferate in diverse

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ecosystems. This widespread distribution and adaptability not only make *E. indica* a significant agricultural weed, but also highlight its ecological versatility and potential as a study subject in plant biology and environmental science. On the other hand, *E. indica* serves as a remedy for several ailments in many parts of Asia and Africa (Kumar et al., 2016; Abioye et al., 2022; Sahu et al., 2024). Traditional healers prepare decoctions and infusions from the leaves and stems to treat conditions such as fever, headaches, and gastrointestinal disorders (Kumar et al., 2016; Iqbal and Gnanaraj, 2012; Gonzaga et al., 2023). People also use the plant for its antidiabetic, antipyretic, and anti-inflammatory properties (Al-Zubairi et al., 2011; Ong et al., 2017). In some cultures, *E. indica* is applied topically in the form of poultices to alleviate skin conditions, wounds, and insect bites. People sometimes chew the roots to relieve toothaches, and they use the entire plant as a diuretic. Modern research has begun to validate these ethnomedicinal uses, uncovering a rich array of bioactive compounds in *E. indica* that contribute to its therapeutic potential (Magadelin et al., 2023; Sukor et al., 2023). This integration of traditional knowledge and contemporary science emphasizes its value as a resource in ethnomedicine. However, its biological activities were limited as a weed or unwanted grass in the rice field, and now, according to ethnomedicinal records, it is receiving special attention from scientists investigating its medicinal potency.

Chemical fingerprinting of plant extracts is a pivotal technique for exploring their biological activity, providing an in-depth understanding of the myriad of compounds present within the extracts and their interactions (Zhang et al., 2008; Li et al., 2018; Bārzdiņa et al., 2022). This analytical method involves creating a detailed chemical profile, or 'fingerprint', which represents the unique composition of the plant extract. Generally, the identification and quantification of bioactive compounds present in crude extracts really help researchers correlate them with specific biological activities. This correlation is fundamental for elucidating the mechanisms of action and optimizing the extraction and formulation processes to maximize efficacy (Zhang et al., 2008; Li et al., 2018; Bārzdiņa et al., 2022). Moreover, chemical fingerprinting allows for the identification of synergistic effects, where multiple compounds work together to produce a greater therapeutic effect than any single compound alone. This understanding of synergistic interactions is crucial for the development of more effective and holistic plant-based therapies. Furthermore, chemical fingerprinting ensures the quality and consistency of plant extracts, which is essential for reliable biological activity. Researchers and manufacturers primarily use the chemical profile as a benchmark for quality control, ensuring that each batch of extract maintains its intended bioactivity. This consistency is vital for the reproducibility of scientific studies and the production of standardized herbal products (Zhang et al., 2008; Li et al., 2018; Bārzdiņa et al., 2022; Bhatta et al., 2023; Hore, and Bhaben, 2023). Additionally, chemical fingerprinting guides bioassay-guided fractionation, a process that involves separating the extract into smaller fractions and testing each for biological activity. This targeted approach helps isolate and identify the most active compounds, facilitating the discovery of new therapeutic agents.

In this regard, advanced thin layer chromatography (TLC), namely, high-performance thin layer chromatography (HPTLC), is a potent analytical tool in the quantitative analysis of phytocompounds (Attimarad et al., 2011; Kowalska and Sajewicz, 2022; Nakkala and Laddha, 2022). In general, both TLC and HPTLC are widely used chromatographic methods that are efficient, faster, reliable, and reproducible. TLC serves as the primary and essential step in identifying the phytoconstituents in a sample, while HPTLC is an upgraded method that provides an electronic image of the chromatograms with fingerprint and densitometry studies for the detection of phytomarker compounds. This method not only saves time but also reduces the cost per analysis. Technically, the HPTLC method offers much better separation, resolution, and results, making it possible to separate secondary metabolites with better accuracy (Attimarad et al., 2011; Kowalska and Sajewicz, 2022; Nakkala and Laddha, 2022). Thus, HPTLC finds widespread use in both academia and pharmaceutical research to identify secondary metabolites. In the present study, we used the advantages of HPTLC and a computer-aided drug discovery (CADD) approach to systematically identify potential antioxidant lead candidate(s) from *E. indica* crude extract.

Materials and Methods

Plant material collection and processing

For crude extraction, we obtained synthetic-grade reagents, solvents, plasticware, and glassware from SRL, Tarsons Pvt. Ltd., Mumbai, India, through local vendors. The entire computational work was analyzed with several cheminformatics software packages (Swain et al., 2021; Sahoo et al., 2022a). In August 2023, we collected the *E. indica* (L.) Gareth (whole plant) from the local flora near Berhampur town in Ganjam district, Odisha, and verified its authenticity with plant taxonomist Dr. A. Leelaveni (Assistant Professor, PG Department of Botany,

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Berhampur University, Odisha). Further, the collected plants were cleaned properly and shade-dried at room temperature. Next, we cursed the dried plants and transformed them into powered forms for extraction. We defatted the powdered plant material with n-hexane, followed by chloroform, and then extracted it with ethanol using a Soxhlet apparatus for 24 hours. We finally evaporated the solvent and collected dry crude extracts for further analysis.

Chemical profiling and fingerprint analysis

In this study, we dissolved 100 mg of ethanol extract in one mL of HPTLC-grade methanol, rinsed it properly, and centrifuged it at 3000 rpm for 5 minutes (Li et al., 2018; Koli et al., 2024). We separately loaded 200 µL of test solution on pre-coated HPTLC silica gel (Merck) 60 F 254 aluminum sheets (100×100 mm) using a Hamilton syringe of 100 µL capacity with the help of a Linomat 5 applicator and a Camag ATS 4 attached to a Camag HPTLC system with filling speed and dosage speed of 15 µL/s and 150 µL/s, respectively, which was programmed through the software LABSERVER, version 3.1.21109.3 (Li et al., 2018; Koli et al., 2024). After applying the spots, we dried them with spray gas. Then, the chromatogram was developed in a twin-trough glass chamber (20×10 cm), which was previously saturated with the selected mobile phase for 20 minutes. Further, the plates were air dried at room temperature for 5 minutes and kept in a photo documentation chamber (Camag Reprostar 3), and images were developed by Camag TLC visualizer 2 and captured at visible white light, UV 254 nm and 366 nm. The chromatograms were developed by using a derivatizing reagent (10% methanolic sulphuric acid) and dried at 100 °C for 3 minutes (Li et al., 2018; Koli et al., 2024). Herein, the fingerprint analysis was carried out with the mobile phase consisting of ethyl acetate, acetic acid, formic acid, and water in the ratio 10:1.1:1.1:2.3 by volume for this extract. All images were scanned with a TLC Scanner 4 with a scanning speed of 20 mm/s, and for UV scanning, a deuterium lamp was used. The peak number, its height, area, and retardation factor (Rf) values of fingerprint data were recorded by Vision CATS software.

Antioxidant metabolites study

Using 2,2-diphenyl-1-picryl hydrazyl (DPPH), we conducted an antioxidant study. Test solutions were loaded separately on pre-coated Merck HPTLC silica gel 60F254 aluminum sheets (100×100 mm) with the help of a Linomat 5 applicator and a Camag ATS 4 attached to a Camag HPTLC system with filling speed and dosage speed of 15 µL/s and 150 µL/s, respectively, which were programmed through the software Server LABSERVER, version 3.2.22308.1 (Pandey et al., 2018; Zahiruddin et al., 2021; Yadav et al., 2024). After the application of spots, these were dried with spraying gas air, and then the chromatogram was developed in a twin-trough chamber (20×10 cm), which was previously saturated with the mobile phase for 20 minutes. For five minutes, the plates were air-dried at room temperature. The developed chromatograms were dipped in DPPH reagent and dried at 100°C for 3 minutes. Dipping reagent was exposed for 0.87 s, and images were scanned with a TLC Scanner 4 with a scanning speed of 20 mm/s. Fluorescence was the mode of investigation and was detected by a mercury lamp. All the HPTLC experimental studies were made with a system suitability test (Pandey et al., 2018; Zahiruddin et al., 2021; Yadav et al., 2024).

GC-MS analysis, molecular docking and toxicity prediction

We will conduct further GC-MS studies and analyse the compounds using the NIST library for additional computational study (Konappa et al., 2020; Sahu et al., 2024). We performed the analysis on a Perkin Elmer Turbo Mass Spectrophotometer (USA) model Claurus 590 Gas Chromatography/Claurus SQ 8S Mass Spectrometer (equipped with a liquid auto sampler). The total run time was 35 minutes. We analysed GC-MS using electron impact ionization at 70 eV and analysed the data using a total ion chromatogram. Further, to demonstrate the antioxidant potency, we have conducted molecular docking studies with selective phytoconstituents similar to previously GC-MS studies (Sahu et al., 2024).

Based on high-intensity GC-MS spectra, we have selected seven constituents (**EI_1** to **EI_7**) along with their chemical information as ligands for the docking study (Table 1). All phytoconstituent structures were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) by recording their unique ids (Table 1). Further, cyclooxygenase-2 or COX-2 (PDB ID: 5F1A), peroxiredoxin 5 or PRDX5 (PDB ID: 1HD2), and haem oxygenase 1 or HO-1 (PDB ID: 3CZY) were retrieved from the protein data bank as three target enzymes for use in the docking study (Sahoo et al., 2021a and 2021b). The molecular docking study was performed using AutoDock 4.2 software, taking two antioxidant standard drugs, ascorbic acid and celecoxib, for comparison of potency, and the protein-ligand interaction study was analysed using the software BIOVIA-DSV-2019 (Sahoo et al., 2021a and Swain et al., 2022).

Currently, computational tools are used to predict drug-likeness-relevant profiles, leading to the selection of lead candidates. Physicochemical profiles, known as the Lipinski rule of five (RO5) profiles of **EI_1** to **EI_7**, along with standard profiles, were predicted using the

DOI: https://doi.org/10.52756/ijerr.2024.v40spl.021

SwissADME tool (http://www.swissadme.ch/index.php) with reference to the PubChem database (Sahoo et al., 2022b). Similarly, the possible toxicity profiles, including hepatotoxicity (HT), carcinogenicity (CG), immunotoxicity (IT), mutagenicity (MG), cytotoxicity (CT), and toxicity class (TC) of all candidates, including standards, were predicted using the ProTox tool (http://tox.charite.de/protox_II/) (Sahoo et al., 2021; Swain et al., 2022).

Results

Fingerprint analysis and antioxidant metabolite observation

We conducted the current study to screen *E. indica*'s ethanol extract for secondary metabolites and develop chemical fingerprints using TLC and HPTLC techniques. The analyses revealed the presence of clear, well-defined, and dose-dependent resoluted bands, including some fluorescent and non-fluorescent ones, when we saturated the TLC chamber with the respective mobile phase for 15 to 20 minutes at room temperature without humidity. We observed more than eight bands or spots, both fluorescent and non-fluorescent, without any overlap (Figure 1A). We subjected the major bands or spots to densitometry scanning at 366 nm in both reflection and absorbance modes. The densitometry study indicates that the highest peak area percentage was 23.19% and the lowest peak area percentage was 1.799%, with Rf values of 0.877 and 0.187, respectively (Figure 1B).

Simultaneously, yellow colors spots were seen from Figure 1C which indicates presence of antioxidants. Bands at R_F-0.13,0.18,0.31,0.42,0.5 and 0.56 were showing yellow color after application of DPPH reagent. Intensity of color bands were less in light and without derivatizing reagent but after application of 10 % methanolic sulphuric acid as derivatizing reagent all the spots or bands were more prominently visualized at 366 nm. This indicates that some active compounds are fluorescent in nature on interaction with 10 % methanolic sulphuric acid as derivatizing reagent. This derivatizing reagent is used mostly for esterification, derivatization of fats and also to detect certain types of glycoside. From the fingerprint study few bands at more R_f value were affected by the polarity of mobile phase and needs some changes in mobile phase. Concentrated active compounds can be obtained by isolation of this specific band. Bands 9 at R_F-0.13, 0.18, 0.31, 0.42, 0.5 and 0.56 shows yellow color and intensity of the yellow color, signifies the potency of antioxidants (Figure 1B). All the active components of the ethanolic extract were distributed into nine bands which are seen from the densitometric study of bands at 366 nm scanning. Major distribution percentage concentration of bands, represented in form of peaks were shown in the densitometric study. This densitometry study reveals the band observed at R_f 0.87 has a higher concentration than other bands (Figure 1).

Molecular docking, physicochemical, and toxicity study

The quantitative phytochemical study via GC-MS analyses on *E. indica* ethanolic extracts revealed the presence of a variety of phytoconstituents at a molecular weight within 126 to 278 g/mol. Among all, we have selected seven (EI_1 to EI_7) constituents according to higher GCMS spectral peaks and R_f values. Further, selected compounds, **EI_1** (1,2,4-benzenetriol), **EI_2** (1,3,5-benzenetriol), **EI_3** (4-mercaptophenol), **EI_4** (1,10-hexadecanediol), **EI_5** (D-mannotetradecane-1,2,3,4,5-pentaol), **EI_6** (1,2,3,4,5-cyclopentanepentol), and **EI_7** (2,7-Dioxatricyclo [4.4. 0.0(3,8)] decane-4,5-diol) were verified and retrieved from PubChem with individual IDs, molecular formula, and physicochemical properties of compounds (Table 1).

The molecular docking score of individual docking scores of selected seven constituents (**EI_1** to **EI_7**) along with two standard drugs (ascorbic acid and celecoxib) against three target enzymes (COX-2, PRDX5, and HO-1) was recorded (Table 2). According to Auto Dock software, a lower docking score (kcal/mol.) represented higher binding efficacy. According to the recorded docking score, phytoconstituents showed their potency within -4 to -7 kcal/mol., similar to ascorbic acid, where **EI_7** (2,7-Dioxatricyclo [4.4. 0.0(3,8)] decane-4,5-diol) had the most potential (Table 2). On the other hand, the standard celecoxib is within -6 to -9 kcal/mol., and the indicated individual phytoconstituents are less active than celecoxib. To better understand the potency of **EI_7**, we have evaluated their interactions with three target enzymes (Figure 2) and revealed that interacts with a number of H-bonds, van der Waals bonds, and pi-pi bonds. However, because of these bioactive phytoconstituents, the ethanolic extracts of *E. indica* exhibited antioxidant activity in their crude form. Furthermore, the percent of bioactivity compounds in crude need to be observed to be used in a specific

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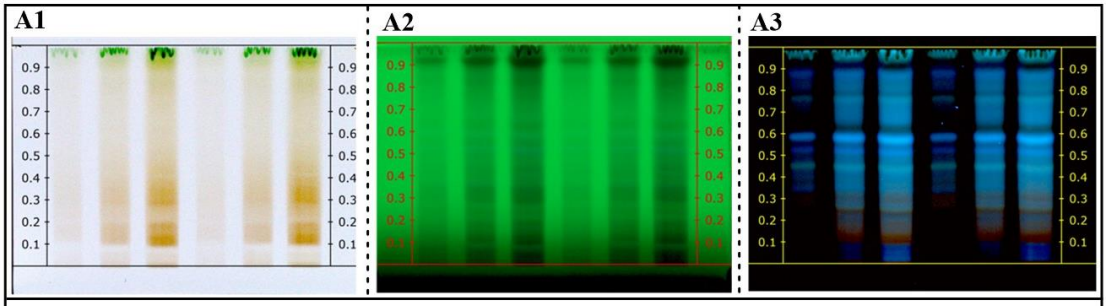

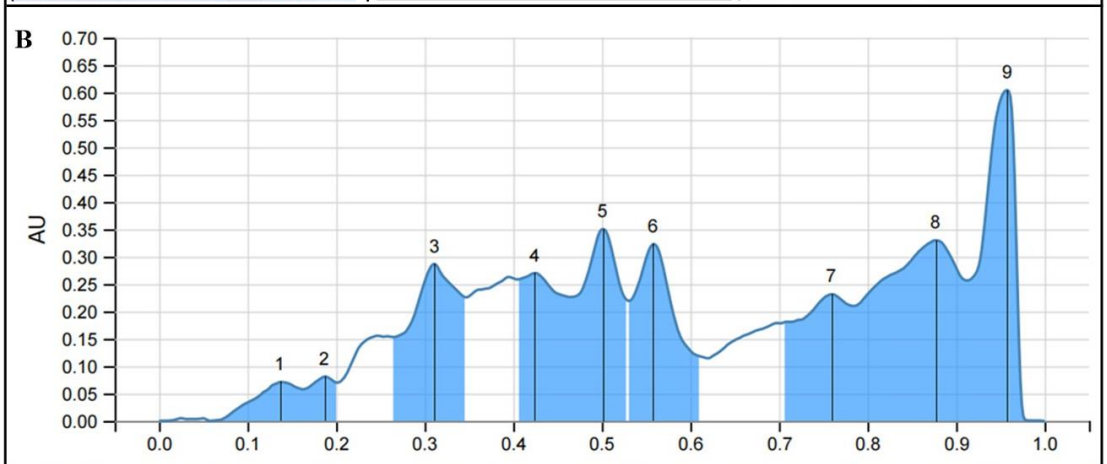

Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.058	0.0000	0.137	0.0710	2.79	0.163	0.0578	0.00418	2.71
2	0.163	0.0578	0.187	0.0808	3.17	0.202	0.0699	0.00276	1.79
3	0.265	0.1531	0.311	0.2865	11.25	0.347	0.2257	0.01857	12.03
4	0.405	0.2584	0.424	0.2699	10.60	0.463	0.2262	0.01458	9.44
5	0.463	0.2262	0.502	0.3503	13.76	0.529	0.2200	0.01835	11.88
6	0.531	0.2191	0.558	0.3228	12.68	0.618	0.1149	0.01862	12.05
7	0.705	0.1793	0.760	0.2314	9.09	0.784	0.2099	0.01625	10.52
8	0.784	0.2099	0.877	0.3298	12.95	0.913	0.2565	0.03581	23.19
9	0.913	0.2565	0.958	0.6040	23.72	0.984	0.0006	0.02532	16.39

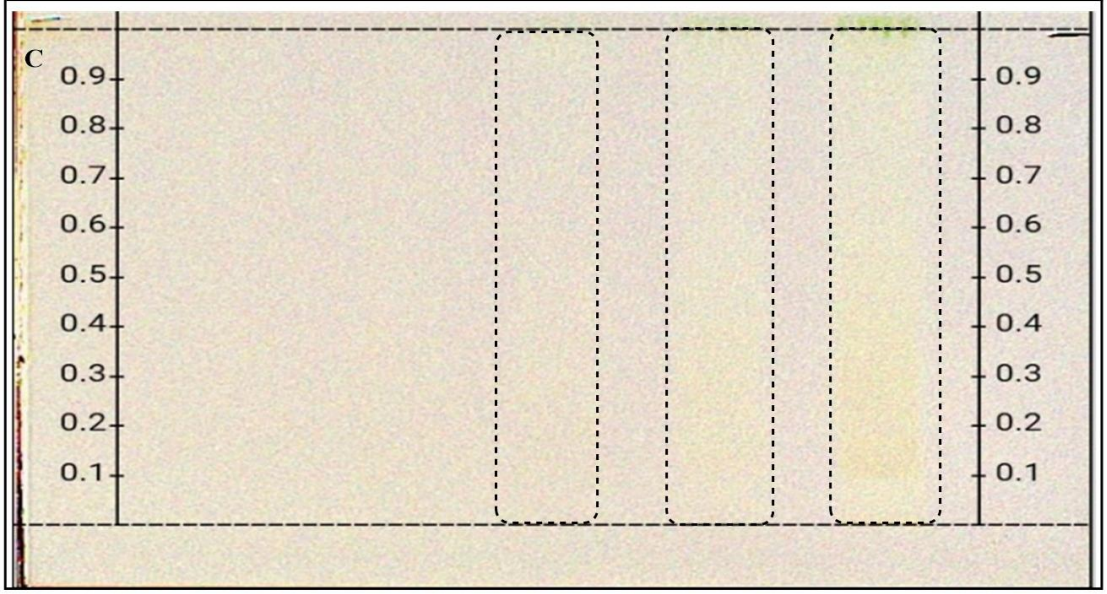

Figure 1. Overall presentation of fingerprint analysis and antioxidant metabolite observation of the ethanolic extract of E. indica. (A1), Image at white light after derivatization with 10% methanolic sulphuric acid; (A2), Image at 254 light after derivatization with 10% methanolic sulphuric acid; (A3), Image at 366 light after derivatization with 10% methanolic sulphuric acid; (B), Densitometric study of bands at 366 nm scanning with data in tabular form (AU-area under, RF-retardation factor); and (C), Antioxidant activity (yellow color indicates presence of antioxidant compounds).

DOI: https://doi.org/10.52756/ijerr.2024.v40spl.021

Table 1. Chemical formula, physicochemical profiles (molecular weight, XlogP, h-bod donor, h-bond acceptor, topological polar surface area), along with the PubChem ID of selected seven phytochemicals and two standard drug candidates.

Sl. No	Selected Phytoconstituents	MF	MW	XLogP (≤ 5)	HBD (≤ 5)	HBA (≤ 10)	tPSA (≤ 142)	PubChem CID
1	1,2,4-Benzenetriol	$C_6H_6O_3$	126.11	1.5	3	3	60.7	10787
2	1,3,5-Benzenetriol	$C_6H_6O_3$	126.11	0.2	3	3	60.7	359
3	4-Mercaptophenol	C_6H_6OS	126.18	1.1	2	2	21.2	240147
4	1,10-Hexadecanediol	$C_{16}H_{34}O_2$	258.44	5.4	2	2	40.5	543836
5	D-Mannotetradecane-1,2,3,4,5-pentaol	$C_{14}H_{30}O_5$	278.38	1.6	5	5	101	552347
6	1,2,3,4,5-Cyclopentanepentol	$C_5H_{10}O_5$	150.13	-3.1	5	5	101	552295
7	2,7-Dioxatricyclo [4.4. 0.0(3,8)]decane-4,5-diol	$C_8H_{12}O_4$	172.18	-1.0	2	4	58.9	558032
8	Ascorbic acid	$C_6H_8O_6$	176.12	-1.6	4	6	107	54670067
9	Celecoxib	$C_{17}H_{14}F_3N_3O_2S$	381.4	3.4	1	7	86.4	2662

Serial numbers 1–7 represent phytochemicals, while 8 and 9 represent standard drugs. MF, molecular formula; MW, molecular weight (g/mol.); H-BD, h-bond donor; H-BA, h-bond acceptor; tPSA, topological polar surface area.

Table 2. Molecular docking score (kcal/mol.) of selected seven phytoconstituents against three oxidative-related target enzymes, predicted toxicity profiles, along with two standard drugs, ascorbic acid and celecoxib, for comparison study.

Sl. No.	Selected Phytoconstituents	Molecular docking score			Predicted toxicity profiles					
		COX-2	PRDX5	HO-1	HT	CG	IT	MG	CT	TC
1	1,2,4-Benzenetriol	-5.0	-5.4	-5.0	IA(0.77)	A(0.59)	IA(0.97)	A(0.66)	IA(0.91)	III
2	1,3,5-Benzenetriol	-4.7	-5.3	-4.7	IA(0.78)	IA(0.73)	IA(0.99)	IA(0.97)	IA(0.95)	III
3	4-Mercaptophenol	-4.4	-4.1	-4.3	IA(0.73)	IA(0.62)	IA(0.99)	IA(0.98)	IA(0.90)	IV
4	1,10-Hexadecane..	-5.4	-4.3	-5.2	IA(0.87)	IA(0.60)	IA(0.92)	IA(0.97)	IA(0.84)	IV
5	D-Mannotetra…..	-5.7	-4.9	-5.9	IA(0.92)	IA(0.67)	IA(0.98)	IA(0.89)	IA(0.84)	VI
6	1,2,3,4,5-Cyclo….	-4.9	-5.2	-4.9	IA(0.88)	IA(0.53)	IA(0.99)	IA(0.68)	IA(0.69)	VI
7	2,7-Dioxatricyclo..	-5.7	-5.8	-5.7	IA(0.82)	IA(0.60)	IA(0.94)	IA(0.63)	IA(0.74)	VI
8	Ascorbic acid	-5.1	-5.4	-5.1	IA(0.86)	IA(0.92)	IA(0.99)	IA(0.87)	IA(0.65)	V
9	Celecoxib	-8.7	-6.1	-8.7	IA(0.60)	IA(0.56)	IA(0.99)	IA(0.75)	IA(0.91)	IV

formulation, and the individual constituents may be used as complementary agents with celecoxib like mainstream drugs to enhance their potency.

In addition, recorded physicochemical and toxicity profiles indicated that all candidates followed RO5 as potential bioactive candidates with a non-toxic nature. The lower molecular weight with an XlogP value > 5 is a good indication, and interactions similar to those of ascorbic acid and a higher toxicity class suggest that EI_7 could be considered an ideal candidate for further study. Furthermore, the docking score confirmed the presence of the aforementioned antioxidant metabolites in crude extracts.

Cyclooxygenase-2 or COX-2 (PDB ID: 5F1A); peroxiredoxin 5 or PRDX5 (PDB ID: 1HD2) and Haem oxygenase 1 or HO-1 (PDB ID: 3CZY); HT, hepatotoxicity; CG, carcinogenicity; IT, immunotoxicity; MG, mutagenicity; CT, cytotoxicity; TC, toxicity class. Four different colors represented the toxicity profiles; green color represented for safe/ non-toxic, light cyan-color moderates safe, while red color high-toxic/risk and light-pink for moderate toxicity profile.

DOI: https://doi.org/10.52756/ijerr.2024.v40spl.021

and cost-effective source of bioactive compounds but also

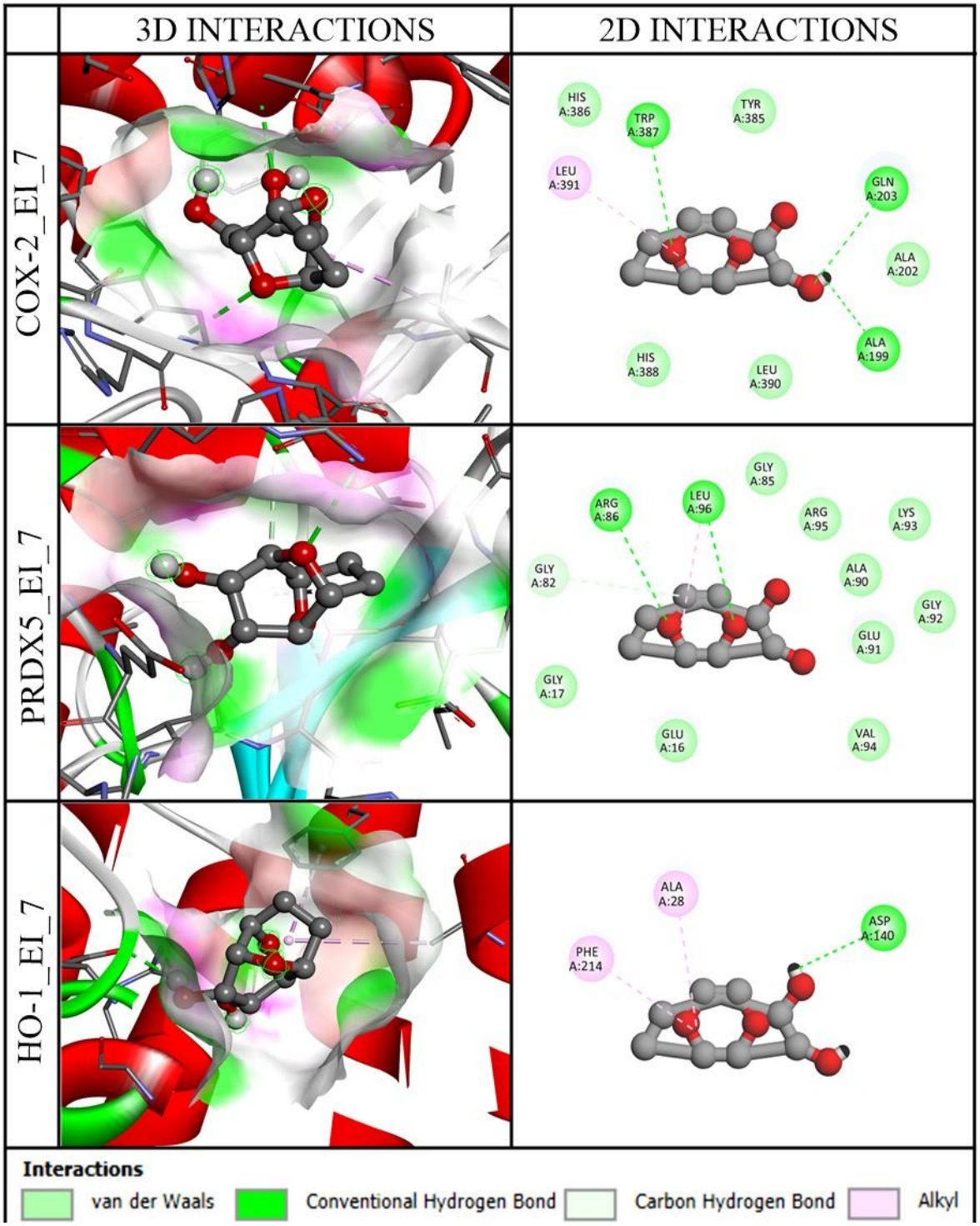

Figure 2. Graphical presentation of protein-ligand interactions between EI_7 and three target enzymes. The BIOVIA-Discovery Studio Visualizer software visualized the interaction study, while ChemDraw 20.0 software presented it.

Discussion

Exploring the biological activity of *E. indica*, a common weed, opens up the potential for discovering new therapeutic agents and the trends is going on (Al-Zubairi et al., 2011; Ong et al., 2017; Magadelin et al., 2023; Sukor et al., 2023). This approach not only provides a sustainable promotes the proper utilization of underappreciated natural resources. *E. indica*, known for its resilience and widespread distribution, contains a rich array of phytochemicals with significant medicinal properties, such as antioxidants, anti-inflammatories, and antimicrobials. By systematically studying these compounds, researchers can uncover novel treatments for various health conditions, transforming this pervasive

DOI: https://doi.org/10.52756/ijerr.2024.v40spl.021

weed into a valuable asset in our daily lives (Al-Zubairi et al., 2011; Ong et al., 2017; Magadelin et al., 2023; Sukor et al., 2023). This process underscores the importance of re-evaluating and harnessing the hidden potential of commonly overlooked plants, contributing to the development of new, evidence-based herbal medicines and promoting biodiversity conservation.

The chemical fingerprinting method also backs up ethnopharmacological claims by connecting certain compounds to their traditional uses and checking biological activities, such as the antioxidant activity seen in this study (Li et al., 2018; Zahiruddin et al., 2021; Rami et al., 2023; Sarkar et al., 2024; Yadav et al., 2024; Koli et al., 2024). In drug development, detailed chemical profiling and the subsequent understanding of biological activity lay the foundation for the creation of new pharmaceuticals. By identifying active compounds and comprehending their mechanisms, researchers can design and synthesize novel therapeutic agents with enhanced efficacy and safety profiles. Additionally, researchers can use chemical fingerprinting to examine how environmental conditions and genetic variations influence the production of bioactive compounds (Banerjee et al., 2014; Li et al., 2018; Hijam et al., 2024; Zahiruddin et al., 2021; Yadav et al., 2024; Verma et al., 2024; Koli et al., 2024; Hijam et al., 2024; Jakkana and Yamala, 2024). This information is valuable for optimizing cultivation practices to enhance the yield of desired compounds. Therefore, chemical fingerprinting is an indispensable tool in exploring the biological activity of plant extracts, followed by a comprehensive GC-MS study to identify bioactive compounds, understand synergistic interactions, ensure quality and consistency, guide fractionation processes, validate traditional uses, and facilitate drug development.

Additionally, using advanced bioinformatics and chemoinformatics tools to create drug-able analyses such as target-specific binding efficacy, physicochemical profiles, and toxicity profiles helped choose the most drug-able candidates early on and pushed for more studies that had a better chance of being done (Sahoo et al., 2021; Swain et al., 2022). Mainly, experimental validation of each compound through hit-and-trial methods is expensive and time-consuming, whereas CADD is a more ideal platform to predict the drug's ability and further proceed with the most ideal candidates, helping to reduce the cost and time involved in the lead candidate's selection (Sahoo et al., 2021; Swain et al., 2022). Therefore, in academic and pharmaceutical research, bioinformatics tools are widely used to locate most lead candidates from lists or libraries with higher clinical success. However, each approach has its own advantages and limitations, and in any research field, proper hypotheses and background knowledge are always essential. In conclusion, this method for identifying potential antioxidant candidate **EI_7** (2,7-dioxatricyclo [4.4. 0.0(3,8)]decane-4,5-diol) is more methodical and has the potential to serve as complementary therapeutic agents following pharmacological validation.

Conclusion

Chemical fingerprinting and antioxidant potency analysis of *E. indica* ethanol extract using integrated chromatography and *in silico* methods have taught us a lot about the plant's phytochemical profile and its potential as a medicine. The detailed chemical fingerprinting revealed a diverse array of bioactive compounds, which are known for their antioxidant properties. The *in silico* analysis further supported the identification of key compounds and predicted their biological activities, reinforcing *E. indica*'s traditional use. This comprehensive approach not only validates the medicinal potential of this widespread weed but also highlights the importance of combining advanced analytical techniques with computational methods to explore and harness the therapeutic benefits of natural products. The findings suggest that *E. indica* could be a valuable source of natural antioxidants, paving the way for its inclusion in the development of new herbal health supplements. In conclusion, this method for identifying potential and non-toxic antioxidant candidate **EI_7** (2,7-dioxatricyclo [4.4. 0.0(3,8)] decane-4,5-diol) through systematic fingerprinting in combination with *in silico* investigations, and could be used as a complementary therapeutic agent after some pharmacological validation.

Acknowledgements

The authors graciously acknowledge Dr. Saurov Dey and his team at CAIF, Guwahati Biotech Park Technology Incubation Centre, for the GCMS study. The authors are also thankful to Dr. A. Leelaveni, Assistant Professor, PG Department of Botany, Berhampur University, Odisha, for the authentication or identification of the plant. The authors also want to thank Salixiras Research Pvt. Ltd., Bhubaneswar, Odisha for providing computational facilities and assisting in paper writing and editing.

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How to cite this Article:

Tapan Kumar Sahu, Nityananda Sahoo, Gurudutta Pattnaik, Himansu Bhusan Samal, Amulyaratna Behera and Biswakanth Kar (2024). Secondary Metabolite Fingerprinting and Antioxidant Potency of *Eleusine indica*: An Integrated Chromatography and *In silico* Investigation. *International Journal of Experimental Research and Review*, *40*(spl.), 245-255.

DOI: https://doi.org/10.52756/ijerr.2024.v40spl.021