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In silico, In vitro studies of Anti-Oxidant and Anthelminthic Abilities of Phytoconstituents from *Rhynchosia cana* (Wild.) DC.

Praveena Yempada[*,1], Arya Lakshmi Marrisetti[*], Ganga Rao Battu , Girija Sastry Vedula[*]

[*]Pharmacognosy and Phytochemistry Research Division, University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, Andhra Pradesh, India

Abstract

Helminthiasis is a significant economic burden on grazing cattle. Increased resistance to currently available synthetic anthelmintics used to treat helminthiasis, and anthelmintic residues in meat and dairy products pose a significant worldwide health threat. These obstacles require the development of new anthelmintics capable of combating drug resistance while also exhibiting improved safety profiles. *Rhynchosia cana* (Fabaceae) is a herb that has historically been used as a worm expeller. To evaluate the phytochemical profile and explore the anti-oxidant and anthelmintic effects of different extracts of *Rhynchosia cana* (*R. cana*) by *In silico* and *In vitro* methods. Using standardised chemical tests as defined in the literature, phytochemical research was carried out. Using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Hydrogen peroxide (H_2O_2) radicals scavenging assay, *In vitro* free radical scavenging behaviour of different extracts was quantitatively estimated, whereas *In-vitro* anthelmintic activity was measured against *Pheretima posthuma* (*P. posthuma*) (Annelida). The molecular docking analysis was then carried out to establish compounds with good efficiency for anti-oxidant activity against the catalase, superoxide dismutase, glutathione-S-transferase, glutathione reductase, glutathione peroxidase and tubulin-colchicine enzyme for anthelmintic activity. Furthermore, ADME/T profiles have been tested by ADMET SAR. The various extracts of *R cana* potentially inhibited the reactive oxygen species (ROS) and possessed anti-oxidant activity. In anti-oxidant assays, the IC_{50} values ranged from 62.08 to 440.08 µg/mL for PERC, EARC, and MERC. All the extracts demonstrated anthelmintic behaviour on *P. posthuma* that was dose-dependent and statistically relevant. On the other side, molecular docking analysis reveals that Gallocatechin has the best fitness score of -7.1 kcal/mol with tubulin-colchicine enzyme; Rhynchosin, Luteolin-3',4'-dimethyl ether, Isoorientin and Orientin has the best fitness scores with different targets related to the oxidation process. In addition, all compounds were in the array of expected properties to fulfil the Lipinski law of five to be accepted as drug-like potential. The observation indicates that the *R. cana* possesses anti-oxidant and anthelmintic activity *In vitro* and *In silico* assays. However, further research was needed to elucidate their primary molecular mechanism of action, safety, toxicity, and bioavailability.

Keywords: Anthelmintic, Antioxidant, *Rhynchosia cana* (Wild.) DC., *In silico, In vitro studies*

Introduction

Throughout the globe, the usage of medicinal plants predates the advent of antibiotics and other modern medicines. It has been demonstrated that higher plant origin products are efficient sources of chemotherapeutic agents without underscoring side effects. [1, 2]. In treating chronic illnesses, including Alzheimer's disease, many culinary herbs and spices have been assessed for their biological activities [3-5]. Reactive oxygen species (ROS)/free radicals have been implicated in developing over 100 diseases [6-10]. Evidence has been given in laboratory, clinical, and epidemiological trials to support ROS's cancer aetiology function [11]. There are anti-oxidant protection mechanisms for all aerobic organisms, including humans, which protect against oxidative destruction. Nevertheless, the natural anti-oxidant protection mechanisms may be ineffective, and thus dietary consumption of anti-oxidant components is necessary and suggested. Interest in seeking natural anti-oxidants for use in foods or pharmaceutical goods to supplement synthetic anti-oxidants, which are limited due to their undesirable reactions, such as carcinogenicity, has recently risen dramatically. Therefore, plants represent the principal reservoir of natural anti-oxidant molecules capable of removing or neutralising the deleterious ROS. Helminth infections were prevalent in marginalised, low-income, and resource-constrained regions of the world, with over 1 billion people in developing areas of sub-Saharan Africa, Asia, and the Americas infected with one or more helminth species [12]. Humans utilised plants to cure a variety of illnesses, but the practice was eventually abandoned. Researchers have grown increasingly interested in alternative medicines derived from plants due to their structural variety, low toxicity, ease of accessibility, and varied mode of action. [13-15].

[1]Corresponding author E-mail: navya.praveena.26@gmail.com
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The use of medicinal plants can significantly decrease medical expenditures in developing nations, which is sadly plagued by hunger and poverty. In developing nations, these diseases have a significant effect, provided that there is no regular supply of a new drug or that the parasitic strain has established a tolerance to the available medicine.

Besides, multidrug-resistant and extremely drug-tolerance helminths are observed in patients in developed countries. On the other side, pain is a massive global phenomenon that decreases living standards and significantly affects well-being and the economy [16, 17]. It will also minimise all the risks involved with the drugs that are currently accessible by utilising a herbal plant with significant biological activity against pathogens and acute or chronic pain.

Rhynchosia cana (*R. cana*) belongs to the family Fabaceae, which has high medicinal value. There are approximately 300 *Rhynchosia* species distributed all over the world. This plant finds use in traditional medicine viz., the bark decoction for dysentery [18], leaves used for wounds, cuts, boils, and rheumatic pains. The phytochemicals of *R. cana* explored were vitexin, Vicenin-2, Orientin, Isoorientin [19]. The flowers of *R. cana* demonstrate anti-inflammatory and antipyretic activity [20]. Recent studies also confirmed the genus' pharmacological and biological activities, such as anti-oxidant, antimicrobial, anti-inflammatory, anti-angiogenic, and antityrosinase antiproliferative and allelopathic activities [21].

Ethical concerns about the use of animals in science have increased significantly in recent years, necessitating exploring alternative techniques like *In silico* approach that do not include experimental animals, thus reducing animal use. Methods were developed in silico to identify and model toxic effects in humans and the environment. [22, 23]

In silico methods help integrate more intelligently diverse up-to-date computational and experimental techniques than a set of exploratory laboratory analyses [24]. In medicinal chemistry, methods such as virtual screening are used in silico to evaluate plant compound pharmacological behaviour and receptor associations, and these strategies are cost-effective and straightforward [25]. Various online programming systems have been developed for in-silico research by different laboratories. Detailed pharmacological profiles of phytoconstituents and novel biological activities of these phytoconstituents are expected using these programmes. PASS is based on a comprehensive analysis of structure operation relationships and allows more accurate predictions for phytoconstituents belonging to a new chemical class [26, 27].

The purpose of this analysis was to examine computationally the possible biological effects of the primary compounds found in the extract of *Rhynchosia cana*, with an emphasis on anti-oxidants, a property implicated in several pathologies, and anthelmintic properties, also to quantify potential risks of toxicity.

Materials and Methods

Collection of Plant Material

Rhynchosia cana whole plant (leaves, root and stem) was collected from Tirumala hills, district Chittoor, Andhra Pradesh, India, in November 2016. It was authenticated by Dr Madhava Chetty, Department of Botany, Sri Venkateswara University, Tirupati. The plant parts gathered were washed with water and dried at room temperature in the shade. The dry components were ground coarsely. For the duration of the analysis, the powdered plant was stored in an airtight, light-resistant package.

Extraction procedure

Powdered plant material (500 g) was taken in separate beakers and immersed in 1000 ml of petroleum ether, ethyl acetate, and methanol; along with constant shaking, the beaker with the contents was kept for 14 days.

Preliminary phytochemical analysis

All the extracts were qualitatively tested using different chemical methods for different classes of phytoconstituents. Carbohydrates were detected using the Molisch's test; proteins were detected using either Biuret test or Millon's test and amino acids using Ninhydrin test. Steroids were detected by Salkowski, Libermann-Burchards and Libermann's tests, alkaloids were identified with freshly prepared Dragendroff's, Mayer's, Hager's and Wagner's reagents by the presence of turbidity or precipitation. The flavonoids were detected using four tests, Shinoda, sulphuric acid, aluminium chloride, lead acetate and sodium hydroxide. Tannins were detected by gelatin, lead acetate, potassium dichromate, and ferric chloride. The froth, emulsion, and lead acetate tests were applied for the detection of saponins. Steroids were detected using a combination of acetic anhydride with sulphuric acid or acetic chloride with sulphuric acid. Sample extracted with chloroform was treated with sulphuric acid to test for the presence of terpenoids. Ammonia solution and ferric chloride solutions were used to detect the presence of anthraquinones. [28-30].

In-vitro anti-oxidant activity

DPPH radical scavenging activity

Using 2,2-diphenyl-1-picrylhydrazyl (DPPH), radical scavenging assay was performed according to Blois [31] and Desmarchelier et al. [32], using ascorbic acid as a reference standard?)

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging behaviour was calculated by the Halliwell et al. technique [33].

Anthelmintic activity

Collection and authentication of worm

The Indian earthworms *Pheretima Posthuma* (Annelida) employed in the present study were collected from moist and muddy soil of the Paderu region, Visakhapatnam, Andhra Pradesh, India, the average size earthworm being 6-8 cm. Worms were washed with normal saline to remove all the faecal matter. It was authenticated by the Department of Zoology, Andhra University. Visakhapatnam.

In-vitro Anthelmintic activity

The anthelmintic activity was performed according to Mehta et al. on the adult Indian earthworm *Pheritima posthuma*. A total amount of 51 earthworms had collected and were divided up into groups, each made up of three worms. Various concentrations (5 mg/ ml, 10 mg/ml, 15 mg/ml, and 20 mg/ml) of the extracts, i.e., *R. cana* petroleum ether extract (PERC), *R. cana* ethyl acetate extract (EARC), and *R. cana* methanol extract (MERC), as well as Standard (Mebendazole), had been prepared in distilled water of 10 ml. The Control group was treated with only distilled water. The earthworms have been formerly washed in regular water before these were launched into 10 ml of the corresponding Petri dish. Promptly after releasing the earthworms in the concerned Petri dishes, the time of launching was stated, and also consequently, the motility of the earthworms was observed. Time of paralysis was seen if the earthworms revealed no activities apart from when worms have trembled very and also the time of death was seen after finding that earthworms neither relocated as soon as shiver extremely neither when showered with hot water (40–50°C) [34-36].

GC-MS analysis

Using Agilent Technology GC systems with the GC-7890A/MS-5975C model, the GC-MS study of bioactive compounds of methanolic extracts of *R. cana* was carried out.

Drug-likeliness

The phytochemical components of the compounds were determined by using DruLiTo software [37, 38].

In silico docking studies

The docking studies of the compounds vitexin, isovitexin, Vicenin-2, Orientin, and Isoorientin were carried out using the interaction between ligand and target protein tools from PyRx and Discovery Studio Biovia 2020. For the present study, five enzymes of the cellular anti-oxidant mechanism, viz. catalase (PDB ID: 1DGH), superoxide dismutase (PDB ID: 5YTU), glutathione-S-transferase (PDB ID: 5H5Q), glutathione reductase (PDB ID: 1XAN), and glutathione peroxidase (PDB ID: 13GS), were selected and to determine the anthelmintic activity, β-Tubulin (PDB ID: 1OJ0) was used and to estimate the inhibitory potential of the constituents and metabolites of *R. cana* on these (Figure 1).

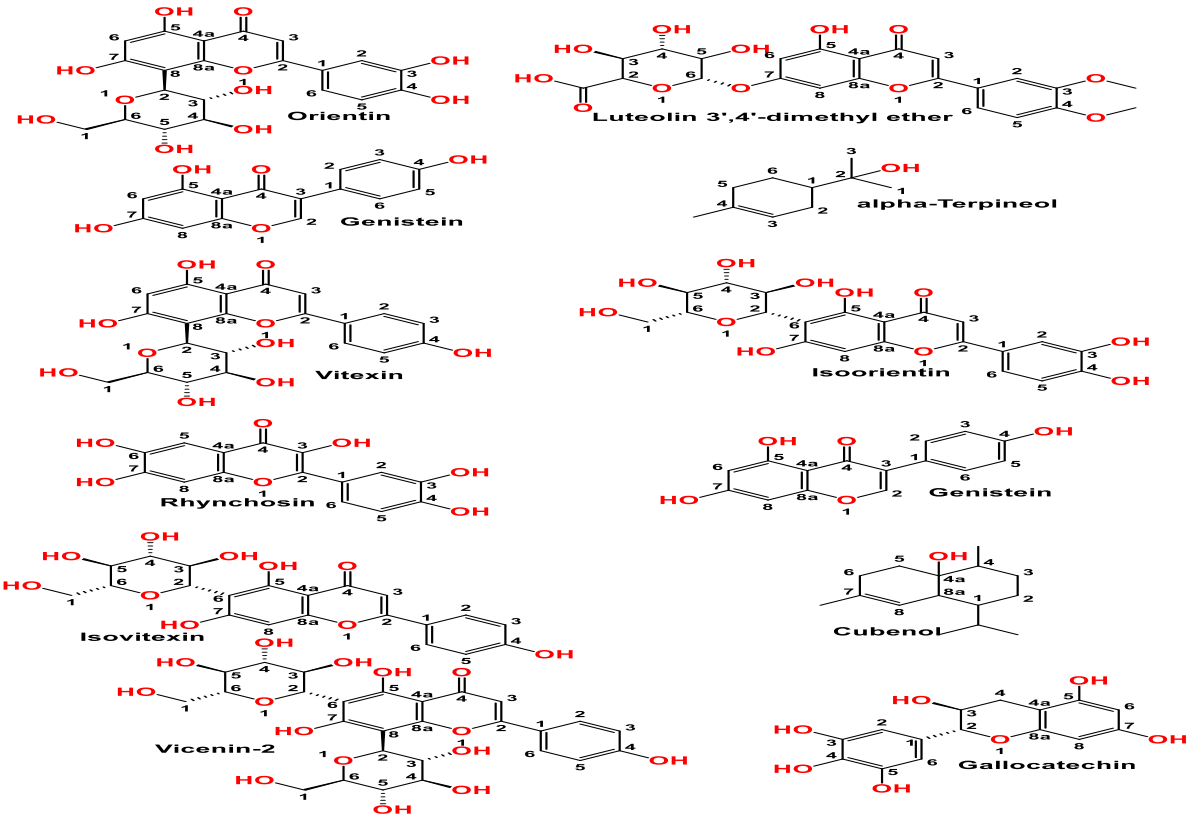

Figure 1. 2D Representation Of Various Ligands From *Rhynchosia Cana*

ADMET analysis

ADMET of ligands refers to their pharmacokinetic properties, which must be investigated to determine their role inside the system. The ADMET inheritance of ligands was investigated using admetSAR. [39, 40].

Results

Phytochemical screening

Preliminary phytochemical screening of various extracts of *R. cana* extracts revealed the presence of several phytoconstituents listed in Table 1.

Table 1. Preliminary Phytochemical Analysis Of Various Extracts Of *Rhynchosia cana*

Test for Specific Phytoconstituent	PERC	EARC	MERC
Alkaloids	-	-	+
Flavonoids	-	+	+
Tannins	-	+	+
Steroids	+	-	+
Volatile oils	+	-	+
Saponins	-	-	+
Fats and Fixed oils	+	-	+
Proteins	-	-	+
Carbohydrates	-	-	+
Acidic Compounds	-	-	+

" +" Present "-" Absent
PERC: Petroleum ether extract of *Rhynchosia cana;*
EARC: Ethyl acetate extract of *Rhynchosia cana*;
MERC: Methanol extract of *Rhynchosia cana*

In-vitro anti-oxidant activity

DPPH radical scavenging activity

The DPPH scavenging function was used to monitor the capacity of the different *Rhynchosia cana* extracts to mop up free radicals. The IC_{50} values of PERC (360.88), EARC (162.38), MERC (62.08), and ascorbic acid (38.97) were obtained using the linear regression equation. Figure 2 and Table 2 show the percentage inhibition of various extracts of *R. cana* and reference standard over a range of concentrations.

$$IC_{50} = (0.5\text{-}b)/a$$

Hydrogen peroxide radical scavenging activity

The hydrogen peroxide radical scavenging potential of various extracts of *R. cana* was evaluated using the hydrogen peroxide (H_2O_2) scavenging method. The results are shown in Figure 2 and Table 2.

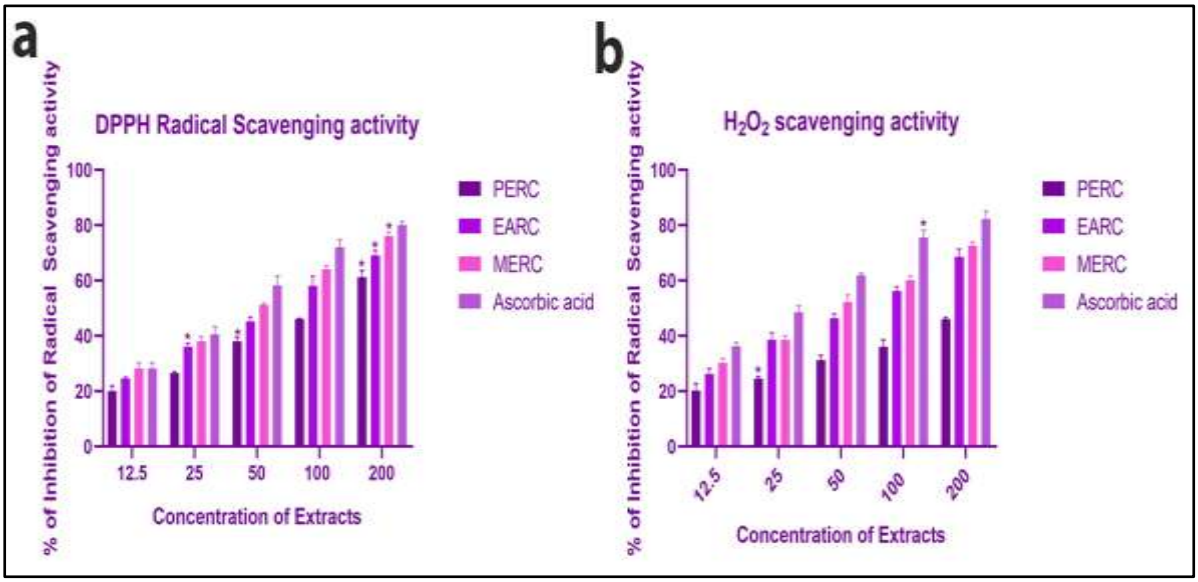

Figure 2. Anti-Oxidant Activity Of Various Extracts Of *R. Cana* (A) DPPH Scavenging Activity (B) Hydrogen Peroxide Scavenging Activity. *Values are expressed as mean±SEM (n=3).* One-way ANOVA followed by multiple Tukey's comparison test. *$p < 0.05$, as compared to ascorbic acid.

Table 2. Dose-dependent DPPH free radical scavenging and H_2O_2 radical scavenging activity of different extracts of *R. cana*

Method	Treatment	% Inhibition of Radical Scavenging activity					IC_{50} (µg/ml)
		12.5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml	
DPPH free radical scavenging (% Inhibition)	PERC	20.15±1.84	26.51±0.55	38.12±1.48*	46.15±2.61	61.25±2.43*	360.88
	EARC	24.52±0.63	36.15±1.24*	45.22±1.54	58.15±3.51	69.12±1.86*	162.38
	MERC	28.22±2.11	38.15±1.66	51.22±0.69	64.15±1.26	76.12±1.52*	62.08
	Ascorbic acid	28.21±1.52	40.51±2.84	58.22±3.51	72.15±2.71	80.12±1.36	38.97
H_2O_2 scavenging activity (% Inhibition)	PERC	20.21±2.62	24.51±0.84*	31.22±1.82	36.15±2.51	46.12±0.55	440.08
	EARC	26.33±1.85	38.61±2.51	46.42±1.61	56.31±1.44	68.62±2.51	276.03
	MERC	30.22±1.63	38.51±1.52	52.33±2.51	60.14±1.56*	72.52±1.44	75.77
	Ascorbic acid	36.23±1.36	48.51±2.44	61.84±0.89	75.61±2.66	82.32±2.81	37.45

***Values are expressed as mean±SEM (n=3).* One-way ANOVA followed by multiple Tukey's comparison test. *p < 0.05, as compared to ascorbic acid.**

In-vitro anthelmintic activity

On *Pheretima posthuma* worms, the antihelmintic function of different extracts was calculated. The results indicate that the degree of anthelmintic activity is directly proportional to the concentration of the extract, which ranges from 5 to 20 mg/mL. The paralysis time and death time was enlisted in Table 3 and represented in Figure 3.

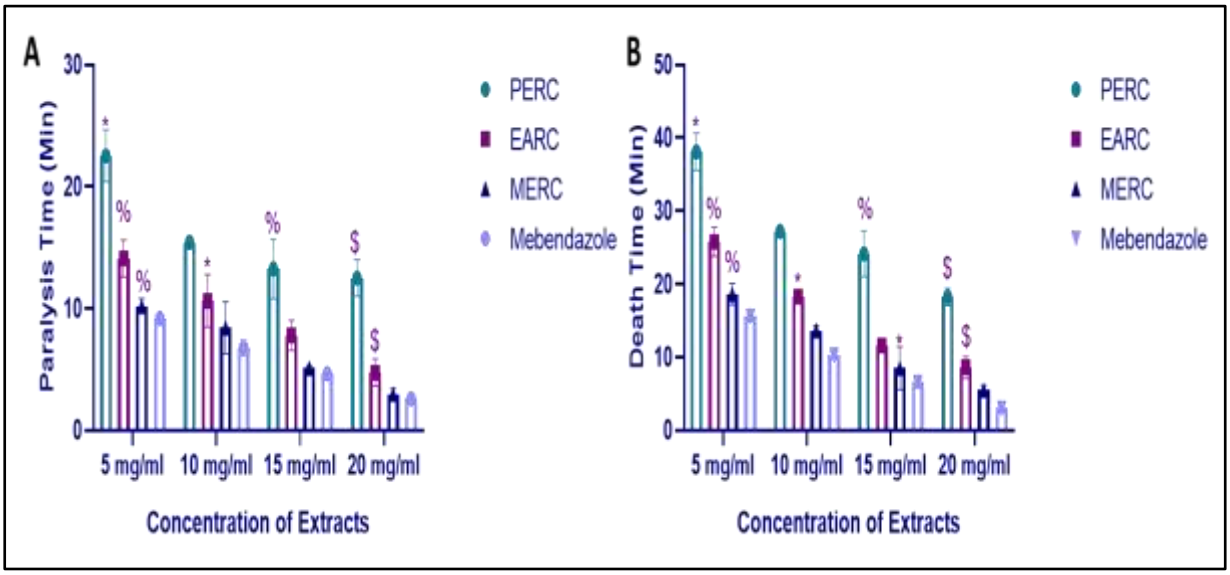

Figure 3. The results are presented as a mean±standard deviation, (n=3). One-way ANOVA was used to evaluate the results, followed by Dunnett's examination.(a) Paralysis time for various extracts of *Rhynchosia cana* with standard Mebendazole. (b) Death time for various extracts of *Rhynchosia cana* with standard Mebendazole.*, p<0.05, %, p<0.01 and $, p<0.001 versus Standard.

Table 3.Anthelmintic activity of various extracts of *R. cana* against *Pheretima posthuma*

Treatment / Dose	Paralysis Time	Death Time
Control (Water)	-	-
Mebendazole (5 mg/ml)	9.21±0.32	15.62±0.11
Mebendazole (10 mg/ml)	6.81±0.64	10.32±0.62
Mebendazole (15 mg/ml)	4.65±0.21	6.55±0.22
Mebendazole (20 mg/ml)	2.62±0.15	3.02±0.18
PERC (5 mg/ml)	22.54±2.12*	38.12±2.51*
PERC (10 mg/ml)	15.45±0.51	27.22±0.36
PERC (15 mg/ml)	13.26±2.44%	24.15±3.15%
PERC (20 mg/ml)	12.51±1.51$	18.25±1.19$
EARC (5 mg/kg)	14.12±1.52%	25.81±1.98%
EARC (10 mg/kg)	10.63±2.15*	18.32±0.64*
EARC (15 mg/kg)	7.81±1.23	11.66±0.32
EARC (20 mg/kg)	4.74±1.12$	8.63±1.51$
MERC (5 mg/kg)	10.22±0.61%	18.63±1.44%
MERC (10 mg/kg)	8.45±2.11	13.68±0.66
MERC (15 mg/kg)	5.11±0.23*	8.51±2.95*
MERC (20 mg/kg)	2.98±0.49$	5.51±0.66$

Values are expressed as mean±SEM (n=3). Dunnett's test after a one-way ANOVA was used for evaluating the paralysis and death time, *, $p<0.05$, %, $p<0.01$ and $, $p<0.001$ versus Standard. PERC= Petroleum ether extract of *R. cana*; EARC= Ethyl acetate extract of *R. cana*; MERC: Methanol extract of *R. cana*

GC-MS Analysis

Eleven peaks were shown in the GC-MS chromatogram analysis of the methanolic extract of *R. cana* (Figure 4), indicating 11 phytochemical constituents. Eleven phytocompounds were characterised and defined about the constituents' mass spectra and the NIST library (Table 4).

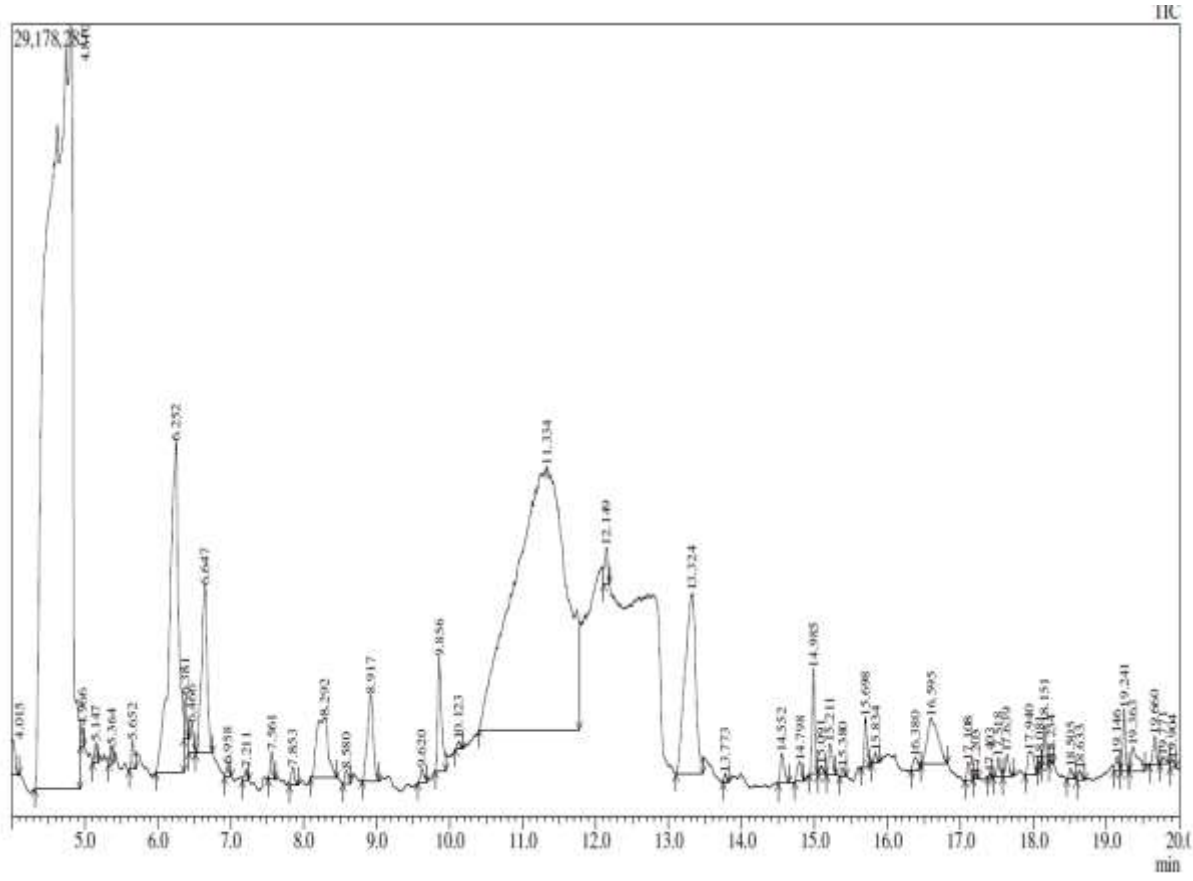

Figure 4.GC-MS Spectral analysis of Methanol Extract of *Rhynchosia cana* (MERC)

Table 4. Biologically active compounds derived from *R. cana*

S. No	Retention Time	Compound name	Canonical Smiles	Area (%)	Molecular formula	Molecular weight
1.	6.252	Orientin	C1=CC(=C(C=C1C2=CC(=O)C3=C(O2)C(=C(C=C3O)O)C4C(C(C(C(O4)CO)O)O)O)O)O	8.68	$C_{21}H_{20}O_{11}$	448.4
2.	6.647	Luteolin 3',4'-dimethyl ether	COC1=C(C=C(C=C1)C2=CC(=O)C3=C(C=C(C=C3O2)OC4C(C(C(C(O4)C(=O)O)O)O)O)O)OC	1.89	$C_{23}H_{22}O_{12}$	490.4
3.	8.292	Genistein	C1=CC(=CC=C1C2=COC3=CC(=CC(=C3C2=O)O)O)O	10.98	$C_{15}H_{10}O_5$	270.24
4.	8.917	alpha-Terpineol	CC1=CCC(CC1)C(C)(C)O	5.53	$C_{10}H_{18}O$	154.25
5.	11.334	Vitexin	C1=CC(=CC=C1C2=CC(=O)C3=C(O2)C(=C(C=C3O)O)C4C(C(C(C(O4)CO)O)O)O)O	48.23	$C_{21}H_{20}O_{10}$	432.4
6.	12.149	Isoorientin	C1=CC(=C(C=C1C2=CC(=O)C3=C(O2)C=C(C(=C3O)C4C(C(C(C(O4)CO)O)O)O)O)O)O	2.85	$C_{21}H_{20}O_{11}$	448.4
7.	13.324	Rhynchosin	C1=CC(=C(C=C1C2=C(C(=O)C3=CC(=C(C=C3O2)O)O)O)O)O	10.14	$C_{15}H_{10}O_7$	302.23
8.	15.698	Isovitexin	C1=CC(=CC=C1C2=CC(=O)C3=C(O2)C=C(C(=C3O)C4C(C(C(C(O4)CO)O)O)O)O)O	2.37	$C_{21}H_{20}O_{10}$	432.4
9.	16.595	Cubenol	CC1CCC(C2C1(CCC(=C2)C)O)C(C)C	10.32	$C_{15}H_{26}O$	222.37
10.	17.940	Vicenin-2	C1=CC(=CC=C1C2=CC(=O)C3=C(C(=C(C(=C3O2)C4C(C(C(C(O4)CO)O)O)O)O)C5C(C(C(C(O5)CO)O)O)O)O)O	6.02	$C_{27}H_{30}O_{15}$	594.5
11.	19.241	Gallocatechin	C1C(C(OC2=CC(=CC(=C21)O)O)C3=CC(=C(C(=C3)O)O)O)O	2.32	$C_{15}H_{14}O_7$	306.27

Drug likeliness

The physicochemical properties of 11 active compounds, i.e., Orientin, Luteolin 3',4'-dimethyl ether, Genistein, alpha-Terpineol, Vitexin, Isoorientin, Rhynchosin, Isovitexin, Cubenol, Vicenin-2 and Gallocatechin were investigated using the DruLito program. Except for four compounds, the remaining compounds followed Lipinski's law. (Table 5).

Table 5. Physicochemical Properties of Active Compounds and Accordance with the Rule of Drug-likeliness

Ligand	MW	Logp	Alogp	HBA	HBD	TPSA	AMR	nRB	nAtom	nAcidic	RC	nRigidB	nAromRing	nHB	No. of Violations
Orientin	448.4	-0.36	-3.22	11	0	35.53	114.1	3	32	0	4	32	2	11	2
Luteolin 3',4'-dimethyl ether	490.4	0.55	-2.65	12	0	80.29	124.4	6	35	0	4	32	2	12	2
Genistein	270.24	1.043	-0.39	5	0	26.3	78.92	1	20	0	3	21	2	5	0
alpha-Terpineol	154.25	2.369	1.122	1	0	0	47.92	1	11	0	1	10	0	1	0
Vitexin	432.4	-0.71	-2.66	10	0	35.53	112.5	3	31	0	4	31	2	10	0
Isoorientin	448.4	-0.36	-3.22	11	0	35.53	114.1	3	32	0	4	32	2	11	0
Rhynchosin	302.23	2.263	-1.24	7	0	26.3	83.44	1	22	0	3	23	2	7	1
Isovitexin	432.4	-0.71	-2.66	10	0	35.53	112.5	3	31	0	4	31	2	10	0
Cubenol	222.37	4.054	1.364	1	0	0	66.87	1	16	0	2	16	0	1	0
Vicenin-2	594.5	-2.55	-5.09	15	0	44.76	144.9	5	42	0	5	41	2	15	2
Gallocatechin	306.27	1.2	-1.5	7	0	9.23	82.67	1	22	0	3	23	2	7	0

Molecular Docking Studies

Molecular docking was used to identify potential anti-oxidants and anthelmintic candidates against various targets using phytoconstituents obtained from *R. cana*. These 11 compounds were docked to the target enzymes, and their docking results were assessed. The top three scored compounds with multiple targets were an excellent representation of anti-oxidant and anthelmintic activity. Refer to Table 6 for detailed molecular interactions of the ligand with the targeted proteins.

Studies on Molecular Interactions

BIOVIA Discovery Studio was used to predict the rigid docking effects. The best binding sites for protein-ligand interaction were identified in Tables 7-12 and Figures 5-9.

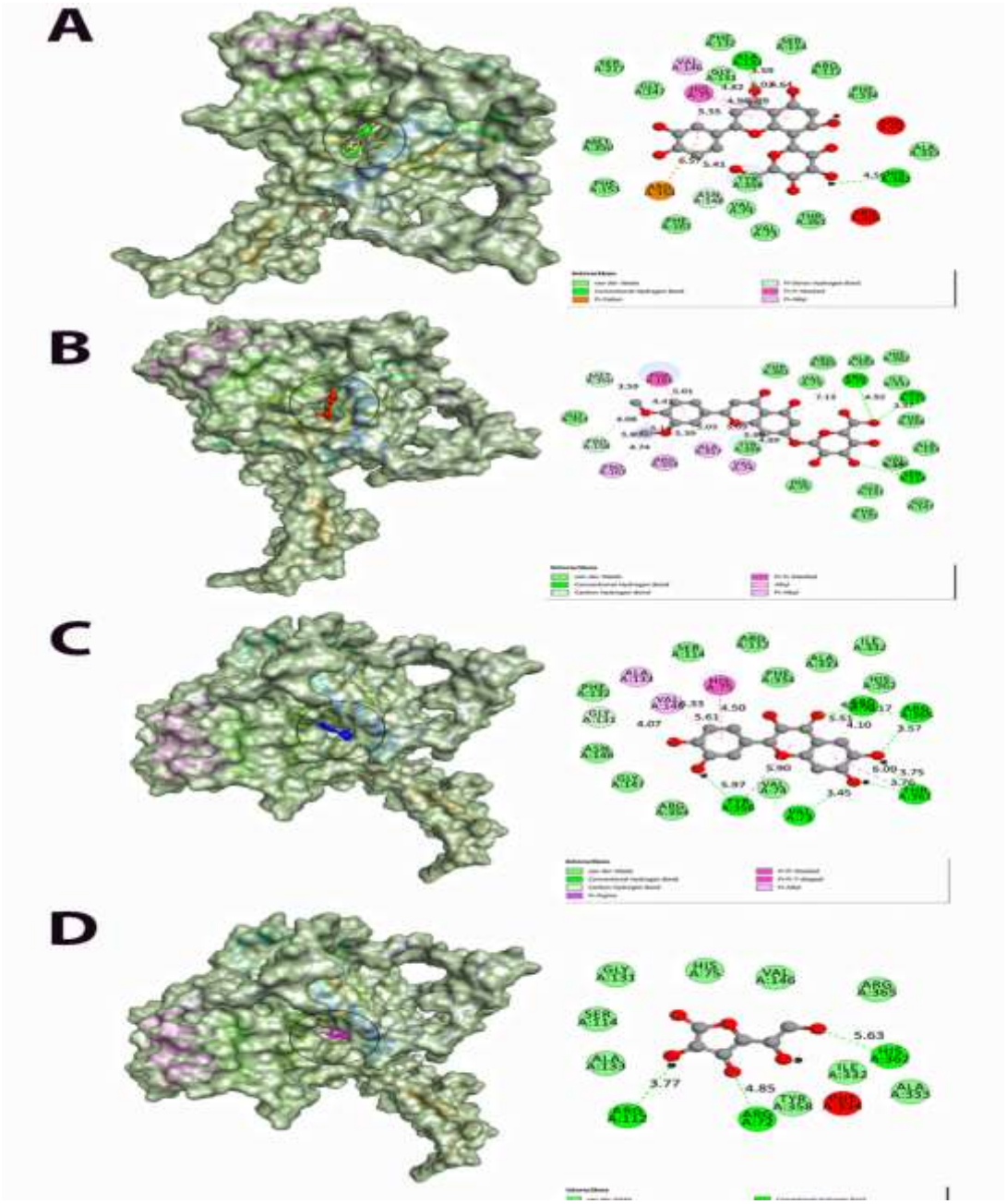

Figure 5. Molecular docking (molecular surface view) and 2D representation of interactions between various ligands with the Human Erythrocyte Catalase (PDB ID: 1DGH). (A) Orientin, (B) Luteolin-3',4'-dimethyl ether, (C) Rhynchosin, and (D) Ascorbic acid

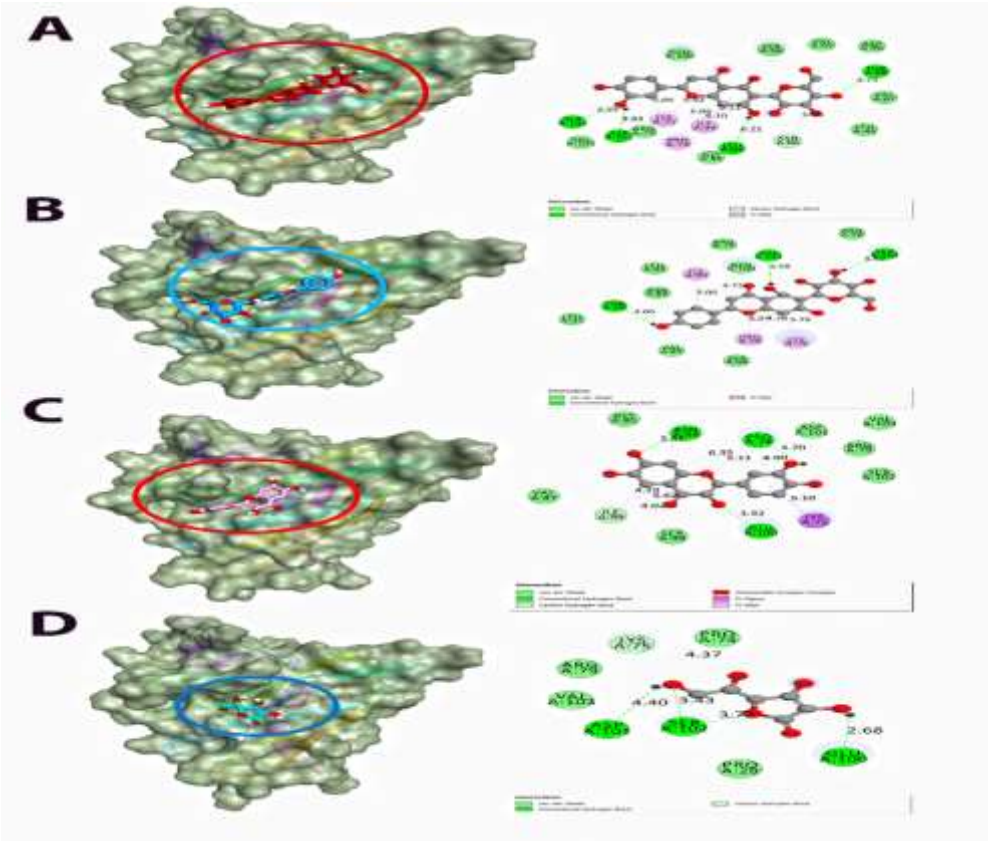

Figure 6. Molecular docking (molecular surface view) and 2D representation of interactions between various ligands with the Human SOD1 complexed with isoproterenol. (A) Isoorientin, (B) Isovitexin, (C) Rhynchosin, and (D) Ascorbic acid

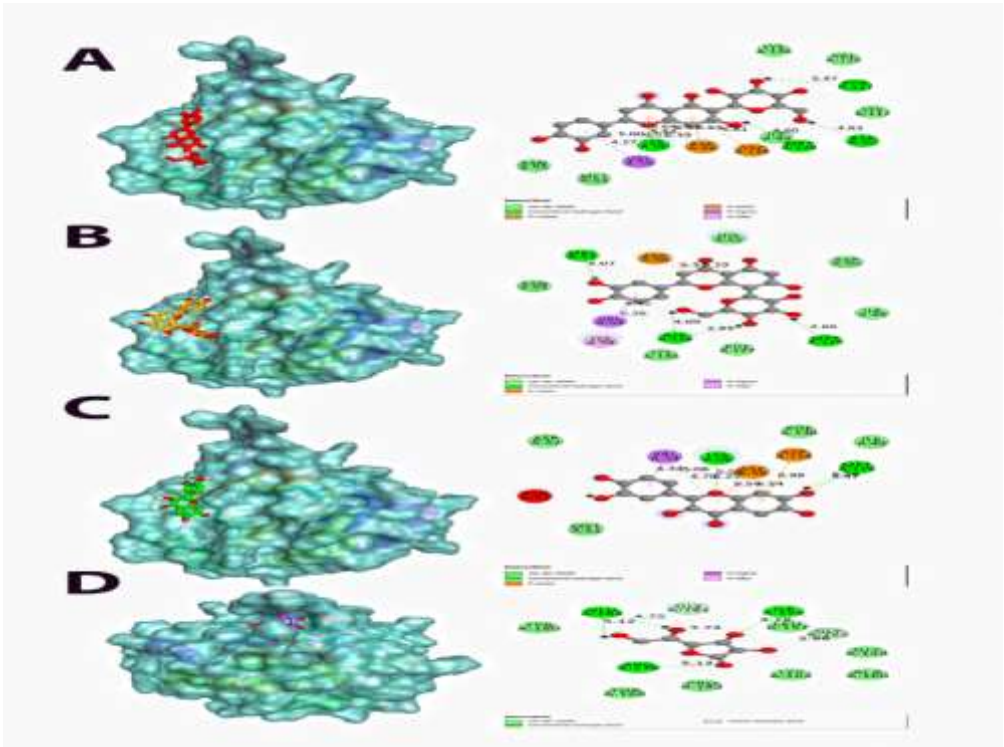

Figure 7. Molecular docking (molecular surface view) and 2D representation of interactions between various ligands with the Human GPX4 complex with GXpep-1 (PDB ID: 5H5Q). (A) Isoorientin, (B) Orientin, (C) Rhynchosin, and (D) Ascorbic acid

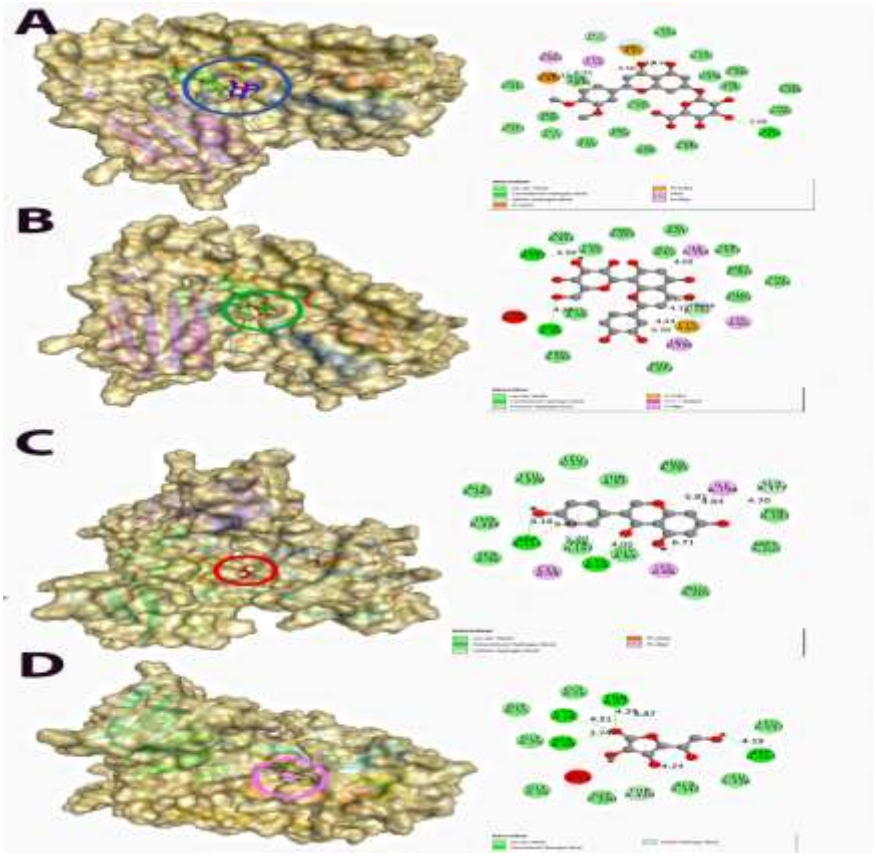

Figure 8. Molecular docking (molecular surface view) and 2D representation of interactions between various ligands with the Human Glutathione Reductase in complex with a Xanthene Inhibitor (PDB ID: 1XAN). (A) Luteolin-3',4'-dimethyl ether, (B) Orientin, (C) Genistein, and (D) Ascorbic acid.

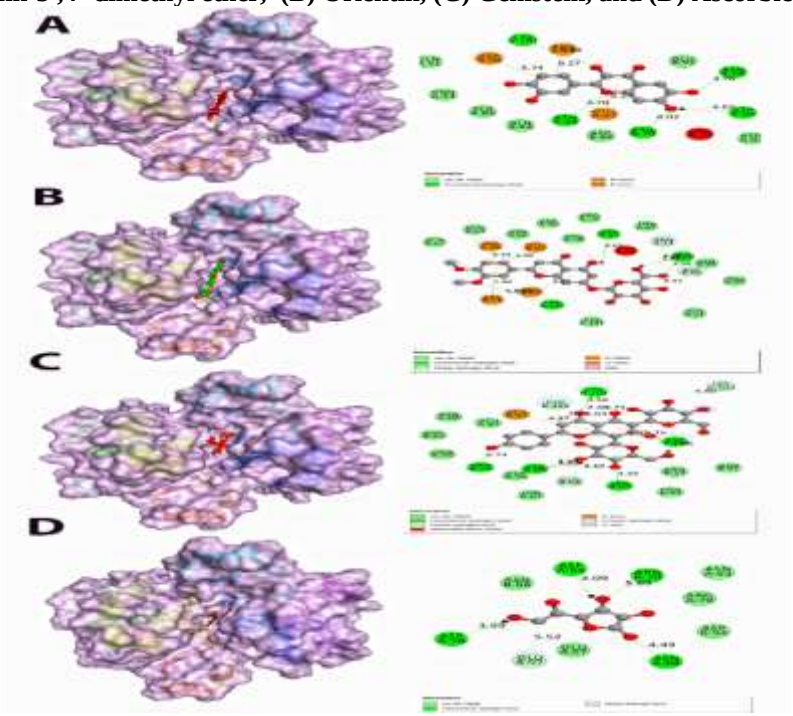

Figure 9. Molecular docking (molecular surface view) and 2D representation of interactions between various ligands with Glutathione S-Transferase complexed with Sulfasalazine (PDB ID: 13GS). (A) Rhynchosin, (B) Luteolin-3',4'-dimethyl ether, (C) Vicenin-2, and (D) Ascorbic acid

Table 6. Molecular Docking of Selected Compounds from *R. cana* with various targets associated with oxidation and helminthiasis.

Ligands	Binding affinities (Kcal/mol)					
	1DGH	**5YTU**	**5H5Q**	**1XAN**	**13GS**	**1OJ0**
Gallocatechin	-8.8	-6	-6.1	-7.4	-7.2	-7.1
Rhynchosin	-9.8	-6.8	-6.1	-8.1	-8.5	-6.2
Genistein	-9.6	-5.9	-5.5	-8.2	-7.1	-5.8
α-Terpineol	-6.6	-4.2	-4.9	-5.2	-4.8	-5.4
Cubenol	-8.6	-5	-5	-6.4	-5.8	-5.2
Vitexin	-8.1	-6.2	-6.1	-7.6	-6.7	-4.5
Orientin	-11.3	-6.3	-6.2	-8.7	-7.1	-4
Isovitexin	-7.8	-7	-5.6	-8.1	-7.3	8.7
Isoorientin	-8.1	-7.4	-7.5	-8	-7.3	9.6
Vicenin-2	-7.8	-6.3	-2.4	-7.9	-7.5	22.8
Luteolin-3',4'-dimethylether	-10.5	-6.8	-5.6	-9	-8.4	-4
Ascorbic acid	-6.2	-4.6	-5.7	-6.5	-5.8	-
Mebendazole	-	-	-	-	-	-5.4

Table 7. Interactions of catalase (PDB ID: 1DGH amino acid residues with ligands at receptor sites.

Ligands	Binding Affinity, ΔG (Kcal/mol)	Amino acids involved and Distance (A°)		
		Hydrogen Binding Interactions	**Hydrophobic Interactions**	**Electrostatic Interactions**
Orientin	-11.3	HIS A:362 (4.56), ALA A:133 (3.59)	HIS A:75 (4.96, 5.55), VAL A:146 (4.82), ALA A:133 (4.64), ASN A:148 (5.41)	ARG A:354 (6.57)
Luteolin-3',4'-dimethylether	-10.5	ARG A:72 (4.92), ARG A:112 (3.37), SER A:114 (4.49)	PHE A:161 (4.41, 5.01), MET A:350 (3.59), PRO A:158 (4.08), PRO A:162 (4.74), ARG A:354 (5.14, 5.39), ALA A:357 (5.03, 5.09), VAL A:74 (4.89, 5.90), ARG A:72 (7.13)	-
Rhynchosin	-9.8	TYR A:358 (5.97), VAL A:73 (3.45), THR A:361 (3.76), ARG A:365 (2.17)	ALA A:133 (6.33), VAL A:146 (5.61), HIS A:75 (4.50), TYR A:358 (5.90), ARG A:72 (4.10, 5.51), THR A:361 (3.76), GLY A:131 (4.07)	-
Ascorbic acid	-6.2	ARG A:72 (4.85), ARG A:112 (3.77), HIS A:362 (5.63)	-	-

Table 8. Interactions of superoxide dismutase (PDB ID: 5YTU) amino acid residues with ligands at receptor sites.

Ligands	Binding Affinity, ΔG (Kcal/mol)	Amino acids involved and Distance (A°)		
		Hydrogen Binding Interactions	Hydrophobic Interactions	Electrostatic Interactions
Isoorientin	-7.4	SER A:102 (3.59), ASP A:101 (3.62), LEU A:84 (6.22), THR A:88 (3.73)	LYS A:75 (5.05, 5.32), ILE A:99 (6.10), PRO A:74 (5.06, 6.10), ASN A:86 (5.45)	-
Isovitexin	-7	THR A:88 (4.00), ASP A:101 (4.58), SER A:102 (3.50)	ILE A:99 (4.72, 5.06), PRO A:74 (4.78, 6.07), LYS A:75 (5.79)	-
Rhynchosin	-6.8	PRO A:74 (4.70), ASN A:86 (3.85), GLU A:100 (3.92)	ILE A:99 (4.02, 4.29, 5.62), PRO A:74 (4.90, 5.11, 6.35)	-
Ascorbic acid	-4.6	GLU A:100 (2.68), ASP A:101 (4.40), SER A:102 (3.43, 3.72)	LYS A:75 (4.37)	-

Table 9. Interactions of glutathione-S-transferase (PDB ID: 5H5Q) amino acid residues with ligands at receptor sites.

Ligands	Binding Affinity, ΔG (Kcal/mol)	Amino acids involved and Distance (A°)		
		Hydrogen Binding Interactions	Hydrophobic Interactions	Electrostatic Interactions
Isoorientin	-7.5	PHE A:127 (5.47), ASP A:50 (4.83), MET A:129 (4.60), LYS A:58 (5.52)	VAL A:54 (4.27, 4.57, 6.19), LYS A:58 (5.00)	ASP A:48 (6.55, 5.63), ASP A:128 (6.81), LYS A:58 (5.52)
Orientin	-6.2	ASP A:128 (2.93, 4.09), MET A:129 (4.69), MET A:53 (5.07)	VAL A:54 (4.45), LYS A:58 (6.26)	ASP A:48 (6.29, 6.31))
Rhynchosin	-6.1	LYS A:58 (5.56), MET A:129 (5.28, 5.37)	VAL A:54 (4.34, 4.70, 5.06), LYS A:58 (5.06)	ASP A:48 (6.54), ASP A:128 (6.99)
Ascorbic acid	-5.7	ILE A:156 (3.78), GLU A:184 (4.75, 5.12), ARG A:179 (5.13)	GLY A:181 (3.74), GLY A:155 (3.86)	-

Table 10. Interactions of glutathione reductase (PDB ID: 1XAN) amino acid residues with ligands at receptor sites.

Ligands	Binding Affinity, ΔG (Kcal/mol)	Amino acids involved and Distance (A°)		
		Hydrogen Binding Interactions	Hydrophobic Interactions	Electrostatic Interactions
Luteolin-3',4'-dimethylether	-9	VAL A:370 (3.68)	ALA A:342 (5.23, 6.75), CYS A:58 (4.50), GLY A:62 (4.01)	ASP A:331 (5.76), CYS A:63 (4.68, 6.15)
Orientin	-8.7	SER A:30 (4.34), LEU A:337 (3.59)	LEU A:338 (6.10), LYS A:66 (5.44), CYS A:63 (4.17), ILE A:198 (4.99)	CYS A:63 (4.13)
Genistein	-8.2	CYS A:63 (4.05), ASP A:331 (4.18)	CYS A:58 (5.20), LYS A:66 (6.71), ILE A:198 (4.84, 6.81), SER A:177 (4.20),	ASP A:331 (6.93)
Ascorbic acid	-6.5	SER A:30 (3.74), CYS A:58 (4.51), THR A:57 (4.29), ASP A:331 (4.19)	THR A:57 (3.87), THR A:339 (4.24)	-

Table 11. Interactions of glutathione peroxidase (PDB ID: 13GS) amino acid residues with ligands at receptor sites.

Ligands	Binding Affinity, ΔG (Kcal/mol)	Amino acids involved and Distance (A°)		
		Hydrogen Binding Interactions	Hydrophobic Interactions	Electrostatic Interactions
Rhynchosin	-8.5	ASN A:66 (4.70), ARG A:70 (6.02), CYS A:101 (5.93), ASN B:66 (3.76), ASP A:94 (4.93)	-	ASP B:98 (5.74), GLU A:97 (5.29), ARG A:13 (6.27)
Luteolin-3',4'-dimethylether	-8.4	CYS B:101 (5.86), SER A:165 (2.61, 3.58), ASP B:94 (4.60)	CYS B:101 (5.86), ARG A:13 (4.24)	ARG A:13 (7.48), ASP A:98 (5.33), GLU A:97 (5.38), GLU A:97 (4.81)
Vicenin-2	-7.5	ARG B:70 (5.74), ASN B:66 (3.57), SER B:65 (3.25), ASP A:98 (4.96), CYS A:101 (4.56)	L;YS A:102 (4.90), CYS B:101 (5.71, 7.30), CYS B:101 (5.63, 7.13)	GLU B:97 (4.87)
Ascorbic acid	-5.8	ASN A:66 (4.43), ARG B:70 (5.69), ASP A:94 (4.09), ASP A:98 (3.99)	GLU A:97 (5.52)	-

Table 12. Interactions of β- tubulin (PDB ID: 1OJ0) amino acid residues with ligands at receptor sites.

Ligands	Binding Affinity, ΔG (Kcal/mol)	Amino acids involved and Distance (A°)		
		Hydrogen Binding Interactions	Hydrophobic Interactions	Electrostatic Interactions
Gallocatechin	-7.1	SER A:138 (3.73), GLY A:140 (2.75), LEU A:139 (4.89), VAL A:169 (3.57), ASN A:204 (5.12), ASN A:226 (4.67)	CYS A:12 (5.76), VAL A:169 (6.00), GLU A:181 (4.23, 7.60)	-
Rhynchosin	-6.2	GLY A:140 (4.64), ASN A:204 (5.45)	CYS A:12 (4.86), VAL A:169 (5.42, 5.56), ILE A:16 (5.97)	-
Genistein	-5.8	ASN A:204 (4.85), ASN A:226 (3.61)	CYS A:12 (4.73, 5.18), VAL A:169 (5.35), ILE A:16 (6.18)	MET A:233 A (5.21, 5.50, 5.58)
Mebendazole	-5.4	HIS A:6 (5.65), GLN A:134 (4.72), SER A:165 (3.66)	PHE A:167 (3.99), VAL A:229 (6.54)	MET A:233 (5.21, 5.50, 5.58)

ADME/T analysis by ADMETSAR

The ADMET properties of ligands were determined using admetSAR (Table 13). Both substances demonstrated adequate human intestinal absorption (HIA) and blood-brain barrier penetration (BBB). None of them has been determined to be carcinogenic. None of the compounds was found to be AMES-negative. The following Table 13 summarises the HIA, BBB, and LD_{50} tests performed on compounds.

Table 13.ADME/T Analysis of various phytoconstituents from *R. cana*

Ligand	Absorption					Distribution	Metabolism								Toxicity			
	BBB	HIA	Caco2	P-glyco protein Substrate	Renal Organic Cation Transporter	Subcellular localization	CYP450 2C9 Substrate	CYP450 2D6 Substrate	CYP450 3A4 Substrate	CYP450 1A2 Inhibitor	CYP450 2C9 Inhibitor	CYP450 2D6 Inhibitor	CYP450 2C19 Inhibitor	CYP450 3A4 Inhibitor	AMES Toxicity	Carci-nogens	Fish Toxicity (mg/L)	Rat Acute Toxicity (mol/kg)
Orientin	0.6742	0.9442	0.9163	0.8777	0.8909	0.5728	0.8041	0.8767	0.6032	0.8355	0.9071	0.9476	0.9240	0.8310	0.7232	0.9553	0.9771	2.3664
Luteolin 3',4'-dimethyl ether	0.9068	0.6737	0.6036	0.6546	0.9436	0.6622	0.8072	0.9161	0.5871	0.8108	0.8959	0.9307	0.8909	0.7521	0.8536	0.9469	0.7843	2.7471
Genistein	0.6785	0.9877	0.7002	0.5000	0.9075	0.7606	0.7672	0.9105	0.6821	0.9254	0.8949	0.9232	0.8881	0.7960	0.9638	0.9276	0.4316	0.8689
α-Terpineol	0.9568	0.9941	0.7505	0.5466	0.8024	0.4268	0.8255	0.8650	0.6082	0.8390	0.6034	0.9322	0.7049	0.8411	0.9133	0.7414	0.6781	1.5603
Vitexin	0.6472	0.9442	0.9163	0.5836	0.8909	0.5728	0.8041	0.8767	0.6032	0.8355	0.9071	0.9476	0.9240	0.8310	0.7232	0.9553	0.9771	2.3664
Isoorientin	0.6472	0.9442	0.9163	0.5836	0.8909	0.5728	0.8041	0.8767	0.6032	0.8355	0.9071	0.9476	0.9240	0.8310	0.7232	0.9553	0.9771	2.3664
Rhynchosin	0.5116	0.9833	0.8367	0.5510	0.9242	0.7742	0.8088	0.9110	0.6630	0.9249	0.8949	0.9230	0.6945	0.7054	0.5905	0.9390	0.2432	3.1831
Isovitexin	0.6472	0.9442	0.9163	0.5836	0.8909	0.5728	0.8041	0.8767	0.6032	0.8355	0.9071	0.9476	0.9240	0.8310	0.7232	0.9553	0.9771	2.3664
Cubenol	0.9404	1.000	0.8378	0.7280	0.7956	0.4847	0.8351	0.7109	0.9032	0.9160	0.9784	0.9555	0.8607	0.8508	0.9032	0.9160	0.2472	2.4872
Vicenin-2	0.6871	0.9156	0.9096	0.6277	0.8732	0.5454	0.8119	0.8714	0.6173	0.8801	0.9182	0.9445	0.9102	0.8760	0.6401	0.9478	1.1546	2.1951
Gallo-catechin	0.5331	0.9654	0.8956	0.5972	0.9458	0.4440	0.8227	0.8771	0.6345	0.9046	0.9071	0.9231	0.9041	0.8309	0.7658	0.9539	0.9422	1.8700

Discussion

As a possible source of new medicinal agents, plant-derived natural products have gained interest. Because of their potent pharmacological functions, low toxicity, and cost-effectiveness, the therapeutic properties of plants have been examined. In addition, several of the currently available treatments come from natural ingredients, reflecting the significance in the drug development phase of medications having biological origins. Therefore, it is necessary to research medicinal plants to determine the active ingredient in natural products for curing diseases and then synthesise the defined active compounds in the laboratory [41-43]. With this insight, the herb, *R. cana, In vitro* approaches have been studied to assess anti-oxidants; anthelmintic function utilising *P. posthuma* accompanied by an *In silico* molecular docking and ADME/T analysis research.

Free radicals, also known as reactive oxygen species (ROS), are produced in the body during biological metabolism. ROS contributes to multiple human diseases, including diabetes and cancer. Anti-oxidant molecules have the potential to regulate certain free radicals or neutralise them. The most important route is to scavenge free radicals under which phenolic compounds interrupt free radical chain reactions. *R. Cana* has been shown to have a more significant potential for neutralising ROS. With the rise in plant concentration suggesting dose-dependent function, anti-oxidant activities have been improved. Extracts could be able to limit their ability as a measure of possible anti-oxidants. By breaking the free radical chain by contributing hydrogen atoms, the diminishing ability of the extracts can serve as an indicator of potential anti-oxidant activities.

When a ligand binds to a binding site in any conformation, molecular docking scores represent the energy of interactions in the binding area. A ligand's conformation is a three-dimensional structure or arrangement of its atoms or pharmacophoric groups that can be used to determine the interaction energy. The following table 6 summarises the top phytochemicals that function as unique protein ligands.

The highest docking value of -11.3 kcal/mol with orientin was found for the catalase among the GC-MS eluted phytoconstituents. The other compounds, Luteolin-3',4'-dimethylether, Rhynchosin and ascorbic acid, have interacted well with the catalase target with binding energies of -10.5, -9.8 and -6.2 kcal/mol, respectively (Table 7 Fig. 5). Isoorientin was found to be effective against superoxide dismutase and glutathione-S-transferase enzymes with binding scores of -7.4 kcal/mol, whereas Ascorbic acid was found to be -4.6 kcal/mol. The ligand Luteolin-3',4'-dimethyl ether was effective with a docking score of -9.0 kcal/mol regarding the glutathione reductase enzyme, whereas ascorbic acid was found to be -6.5 kcal/mol. Rhynchosin displayed a docking score of -8.5 kcal/mol with the target glutathione peroxidase, whereas ascorbic acid was found to be -5.8 kcal/mol. By observing the *In silico* analysis, the phytoconstituents of *R. cana* were effective against free radicals and could be a potent anti-oxidant source compared to ascorbic acid.

Additionally, we evaluated the molecular docking of several compounds to demonstrate the molecular interaction between the compounds and the protein, which enables us to depict the behaviour of the molecules in the coupling site of the targeted proteins and to illustrate the biochemical process underlying the anthelminthic activity. As a consequence of the results (as shown in Table 12), it is determined that Gallocatechin (-7.1 kcal/mol), Rhynchosin (-6.2 kcal/mol), and Genistein (-5.8 kcal/mol) all had substantial docking scores similar to the reference medication Mebendazole (-5.4 kcal/mol). All three compounds had a higher docking score than the conventional medication Mebendazole. Docking analysis indicates that these compounds, particularly Gallocatechin, may be a promising candidate for a novel anthelmintic drug.

GC-MS analysis revealed important phytocompounds in *R. cana* (Figure 5 and Table 4), including the major Gallocatechin, Rhynchosin, Genistein, α-Terpineol, Cubenol, Vitexin, Orientin, Isovitexin, Isoorientin, Vicenin-2 and Luteolin-3',4'-dimethyl ether, which have been detected in previous studies with R. cana [44]. It is understood that these compounds have crucial anti-oxidant efficacy. [44]. As oxidative stress is implicated in the pathophysiology of several human diseases, anti-oxidant substances are pretty significant. [45].

The effective dose for anthelmintic activity was calculated to be 20 mg/ml MERC for our research, with 2.98 and 5.51 min for the time of paralysis and death, respectively. It took more time to paralyse and death for the rest of the concentration. The extracted power was inversely proportional to the time required to paralyse the worms. Studies demonstrate pretty clearly that the methanol extract has a significant anthelmintic effect. As indicated in folk medicine, the above observations support the usage of an anthelmintic.

To demonstrate the molecular relationship between compounds and proteins, we also examined the molecular docking of selected compounds, which enables us to represent the behaviour of these molecules at the coupling site of targeted proteins and clarify the biochemical mechanism of anthelminthic action. It is inferred from the findings (as seen in Table 12) that Gallocatechin (-7.1 kcal/mol) and Rhynchosin (-6.2 kcal/mol) displayed substantial docking scores similar to those of Mebendazole (-5.4 kcal/mol), the reference drug. A relatively better docking score than the standard medication, Mebendazole, is Gallocatechin and

Rhynchosin. It is clear from the outcome of the docking study that these compounds, particularly Gallocatechin, maybe a good candidate for a new anthelmintic agent.

The ADME and Toxicity analyses revealed that all compounds met Lipinski's rule of five for being recognised as having drug-like potential in terms of improved pharmacokinetic properties with fewer adverse effects.

Conclusion

We can assume from the above discussion that this plant can play a prominent role in the anti-oxidant and anthelmintic activity. Our molecular docking analysis showed that Rhynchosin, Luteolin-3',4'-dimethyl ether, isoorientin, orientin, and Gallocatechin could be valuable sources for developing novel anti-oxidants and anthelmintic agents. However, further research is needed to elucidate their primary molecular mechanism of action, safety, toxicity, and bioavailability.

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