Pharmacogn. Res., 2025; 17(1):143-153.
https://www.phcogres.com

Original Article

Study of *in silico* and *in vitro* Anthelmintic Activity of Ethanolic Extract of *Artemisia pallens* Wall Ex. DC

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ABSTRACT

Background: Helminths, a disease that affects all ages, are being treated with medicinal herbs such as *Artemisia pallens* Wall. ex DC, a traditional plant material with phytomedicinal properties**. Objectives:** The study assesses the anthelmintic activity of ethanolic plant material extract against common gastrointestinal helminths and explores its potential as an alternative or complementary treatment for parasitic infections. **Materials And Methods:** Indian adult earthworm *Pheritima posthuma* was employed to screen anthelmintic activity. Worms were exposed to different concentration 10 mg/mL, 20 mg/mL, 50 mg/mL, 80 mg/mL and 100 mg/mL concentrations of extract and standard drug, albendazole. Molecular docking for the observation of compounds which had higher activities towards the NADH-Fumarate reductase enzyme. From the database, the compounds with higher docking fitness scores determined using the Auto Dock software version 1.5.6. Further, the ADME/T profiling was done on Swiss ADME Analysis and also on pkCSM software tool. **Results:** Preliminary phytochemical analysis of EEAP indicated the presence of alkaloids, flavonoids, tannins, phenols, terpenoids. The anthelmintic potential of *A. pallens* extract, which showed strong antihelminthic activity in combination with adult *Pheritima posthuma* that produced paralysis and death at an earlier period as compared to albendazole, while the molecular docking study found good binding scores in the range of -5.15 kcal/mol to -9.6 kcal/mol with the fumarate reductase enzyme**. Conclusion:** The study confirmed the potential anthelmintic activity of *Artemisia pallens*. Wall extract, with compounds showing favorable binding scores indicating potential for forthcoming clinical investigations.

Keywords: Anthelmintic, *Artemisia pallens*, AutoDock, *Pheretima posthuma*, Fumarate reductase.

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Received: 28-09-2024;
Revised: 14-11-2024;
Accepted: 03-12-2024.

INTRODUCTION

According to WHO, medicinal plants are those whose organs contain parts that could be used for therapeutic purposes or as a basis for the production of potent drugs.[1] Since ancient times, people have used plants for healing, which remains the bedrock of many present-day medicines.[2] Indeed, hundreds of years ago, lots of medicinal drugs currently in use originated from extracts of medicinal plants.[3] People using such traditional medicine do not know the science behind it but know that some medicinal plants work as medicines. The more we know about the human body, the more we gain appreciation for these healing properties of plants, and therefore their incredible potential for various health issues, because many of these have health-enhancing compounds either alone or together.[4] That being said, because synthetic drugs are of lesser effectiveness and many people face challenges with their use, it has brought back an interest in natural remedies.[5] In general, herbal products were treated as medicinal products if they made claims of a therapeutic or prophylactic indication for many illnesses to come; some components of plants may exhibit pharmacological properties.[6]

Given this backdrop there is a conviction that helminth infections are more frequent in humans than in any other organism especially with growing world population.[7] The most common form of infection by worms in human body parts is referred to as helmintic disease.[8] The worms are usually found in other parts of the body not just the digestive system and it usually inhabits in the liver and any other essential organ in the body. These preparations from medicinal plants for the prevention and treatment of and against gastrointestinal parasites originates from ethnomedicine.[9] Indeed many of these plants have been confirmed in laboratory tests to bear some real anthelmintic properties; either by using the whole plant or by administering the extracts to sick hosts.[10] Synthetic anthelmintics and larvicides lead to drug resistance in parasitic infections owing to the high requirement of naturally available cure which is free from side effects.[11] Apart from this, this disease is highly morbid and contains serious economic problems and since these types of infestations exist in certain

DOI: 10.5530/pres.20251960

regions it also affects these areas.[12] The plant-based treatments are already determined to be active in the modern research but at the same time more research is needed in looking for natural rather than the artificially synthesized compound which would be cheaper and less toxic.[13]

The genus Artemisia is famous for its bitterness; it comprises about 300 species.[14] Among these, *Artemisia pallens* Wall. Ex. DC, or more commonly termed as Davana, is an aromatic herb known to be generally found in the Nilgiri hills of India.[15] It belongs to the family of *Asteraceae*. This herb is a medicinally important plant and used in Indian traditional medicinal system as remedy for diabetes mellitus with antihelmintic, antipyretic and tonic properties.[16] This includes, besides its potential pharmacological qualities of antimicrobial, antinociceptive, antispasmodic, hepatoprotective, antimalarial, anthelmintic, antipyretic, and antimalarial, anticancer, antidepressant, antioxidant, antibacterial, wound healing, and antiprotozoal actions, as well as the obstacles.[17]

NADH-fumarate reductase is an enzyme crucial to the electron transport of anaerobic respiration in many helminth parasites, and this, in turn, makes it a potential drug target for anthelmintics.[18] These molecules should thus impair nafuredin-like compounds, thereby inhibiting energy production by the parasite. This inhibition causes a death effect of the parasite; therefore, it shows considerable anthelmintic activity.[19] Targeting this enzyme, researchers are making an effort to produce drugs that are very much specific to the parasite and diminish the side effects to the host, which can cure infection caused by helminths quite effectively.[20]

The present work is an attempt to explore the anthelmintic activity of ethanolic extract of the aerial portion of the *A. pallens* plant material that performs *in silico* anthelmintic activity by fumarate reductase as well as against adult Indian earthworms, *Pheretima posthuma.*

MATERIALS AND METHODS

Collection of Plant Materials

Artemisia pallens Wall is an herbaceous plant material which grows in sandy loam soil throughout the temperate Himalayas in India. The ariel parts of the plant were collected from flower shop in the Cuddalore district, during the month of March. And it was identified and confirmed by Prof. R. Selvanathan, Senior Research Executive, Department of Botany, Centre de Phyto Botanicals Research, Villianur, Puducherry.

Preparation of Plant Powder

The leaves collected were cut into small portions and allowed to dry in air for more than 15 days. Then the dried material was coarsely powdered by a mixer grinder. The obtained fine powder used in further research.[21]

Preparation of Plant Extract

The coarse powder of the plant material was packed directly into the Soxhlet apparatus or in a thimble of firm filter paper or fine muslin. The diameter of the thimble corresponds to the internal diameter of the socket extractor. The condenser and distillation flask were connected so as to make the extraction setup ready. This is initialized by adding 250 mL of solvent as 95% ethanol at the bottom of the flask to heat up so that it can be vaporized in the sample thimble. The vapor then cools at the top of the condenser, falls back down in droplets, and brings down the phytochemicals with it. Fresh activated porcelain pieces were added to the flask to avoid bumping of solvent.[22] This method resulted in an improved yield compared to the traditional maceration extraction. This cycle was repeated as many times as possible without changing the solvent, so as to get efficient extraction. It shall be done for a period of 72 hr. After that, the extract was placed in the water bath at around 60°C which can be evaporated into concentrated residue.[23]

Phytochemical Analysis

Qualitative phytochemical tests was conducted on the ethanolic extract of plant material in accordance with Pharmacopoeia recommendations to discover secondary metabolites that contribute to its medicinal properties.[24] The standard procedure followed the initial phytochemical screening conducted on the plant extract to ascertain the bioactive compounds in the EEAP.[25]

Thin Layer Chromatography (TLC)

The phytochemical constituents of EEAP were then evaluated using the Thin Layer Chromatography analysis. The extract was applied to thin layer chromatography plates (7.5x2.5 cm, 0.5 mm thick, stationary phase of silica gel G) and developed in solvents in a beaker (developing apparatus). After the solvent traveled to the top of the plate, the sheets were withdrawn and air dried.[26] At that point, the plates were examined for the number of spots and recorded, under a UV transilluminator at 254 nm. On the thin layer chromatography plates, diverse spots were observed, and the same formula was used to determine the retention factor.[27]

GC-MS Analysis

The ethanol extract of the plant *Artemisia pallens* was screened using Agilent 8890 Gas chromatography that is equipped with a 5977 MSD and an Agilent 7693 Automatic Liquid Sampler (ALS). Separation of the compound was performed on the column composed of (5%-phenyl)-methyl polysiloxane-based Agilent J&W HP-5ms-Ultra Inert column with 30m × 0.25 µm × 250 µm in dimension. Separate the components using helium for inlet as carrier gas at constant flow rate of 1 mL/min injection volume 1 µL was used (split ratio of 15:1) temperature of injector constant, but for source and oven temperature was programmed from 75°C for 0.5min and later increased to 180°C by a rate of 5°C/min, then again increased by 5°C/min up to 300°C ending with a 5 min.

The mass spectra detector was taken with fixed electron energy of 70 ev; scan interval of 1.5 s and fragments from 50 to 600 Da. The spectrums of the components were compared with database of spectrum of known components stored in the GC-MS NIST (2008) library.[28,29]

Determination of Anthelmintic Activity

Collection of Worms

The adult *Pheretima posthuma* was harvested from our local area in a moisture-laden environment, thoroughly rinsed in tap water to eliminate foreign materials and particulate debris. It should also be noted that the worms used in the procedures are supposed to be of nearly equal size so that variability within the assay is reduced.

Anthelmintic Activity

Earthworms were also sampled and grouped; each group comprising of five worms. A series of different concentrations were prepared in distilled water which consisted of 10, 20, 50, 80, and 100 mg/mL of the ethanolic extract of *A. pallens* and standard (Albendazole), in distilled water of 10 mL.[30] Earthworms were washed in regular water prior to being placed in 10 mL of specific Petri dishes. The time of introduction was noted, and their movement was observed. The duration of paralysis was recorded when there was no movement, except in cases where the worms were vigorously shaken. After noticing that the worms remained still even when shaken forcefully or placed in warm water at 50°C, the time until death was then recorded.[24]

In silico Docking Study

Molecular docking was performed utilizing Auto Dock Vina version 1.5.6 to evaluate the interaction between the compounds found in ethanolic extract of *A. pallens* leaf extracts (ligands) and a soluble fumarate reductase in yeast (protein).

Ligand Preparation

The phytoconstituents identified through GC-MS analysis served as ligands for the docking studies. Their three-dimensional structures were retrieved from the PubChem database in SDF format, which was then converted to PDB format using Openbabel software.

Protein Preparation

The crystal structure of (PDB 5GLG) novel function of Osm1, a soluble fumarate reductase in yeast was obtained from Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCS PDB). In the docking preparation, the protein structure and its active site were dehydrated for the removal of water molecules and ligand molecules. Polar hydrogen atoms and Kollman charges were added with the help of AutoDock version 1.5.6. The complex was saved in the pdbqt format for docking.[31]

In silico Pharmacokinetic/ADMET Analysis

The optimal compound was identified through docking results to evaluate the ADMET (absorption, distribution, metabolism, excretion, and toxicity) profile, which is an essential criterion for the drug-like assessment of chemical substances. For predicting ADME property; Swiss ADME software were used and also toxicity analysis was carried out using pkCSM software.[32,33]

Statistical Analysis

The values are represented as mean±SD; via student's T test. $p<0.05$ was taken into statistically significant.

RESULTS AND DISCUSSION

Extraction of The Plant Material

The aerial parts of the plant material *Artemisia pallens*. The walls were collected and verified by the botanist. The solvent is separated from the extract through concentration of the two in a water bath as they are heated. Residue is obtained and, the percentage yield of ethanolic extract were found to be around 9.22 W/W. Then the extracted solutions were utilized for preliminary phytochemical screening (Table 1).

Phytochemical Analysis

Preliminary phytochemical analysis of ethanolic extracts showed the presence of flavonoids, tannins, terpenoids, phenols and alkaloids (Table 2).

Thin Layer Chromatography (TLC)

Ethanolic extract of *A. pallens* with the solvent system ethyl acetate: ethanol (5:5) Ratio. In the detection of flavanoid, spots were identified whose retention factor (R_f value) were found to be 0.97 (Table 3).

Table 1: Determination of Extractive Value of *Artemisia pallens*.

Sl. No.	Type of extractive value	Percentage w/w
1.	Ethanol	9.22% W/W

Table 2: Phytochemical screening.

Phytoconstituents	Observations
Alkaloid	+
Proteins and Amino Acids	-
Tannins	+
Phenols	+
Flavonoids	+
Saponins	-
Terpenoids	+

(+)-Indicates the presence of chemical constituents.(-)-Indicates the absence of chemical constituent.

Table 3: Data showing the R_f values of TLC studies on the EEAP.

Sl. No.	Sample	Solvent System	Ratio	TLC Study For	R_f Value
1.	Ethanolic extract of *A pallens*	Ethyl acetate: Ethanol	5:5	Flavonoid	0.97

EEAP-Ethanolic extract of *Artemisia pallens* Wall; Rf- Retention factor.

Table 4: Compounds Obtained From GC-MS Analysis.

Sl. No.	Phytocompound	Retention time [R_t]	Area%	Molecular formula	Molecular weight
1	Dimetridazole	22.150	0.25	$C_5H_7N_3O_2$	141.12
2	L-Pipecolic Acid	13.262	1.28	$C_6H_{11}NO_2$	129.16
3	Vinyl trans cinnamate	11.787	0.31	$C_{11}H_{10}O$	174.20
4	Oleamide	19.304	1.52	$C_{18}H_{35}NO$	281.48
5	Gallocatechin	20.099	0.59	$C_{15}H_{14}O_7$	306.27
6	Dl Octapamine	14.637	0.41	$C_8H_{12}CINO_2$	189.64
7	Catechin	20.099	0.59	$C_{15}H_{14}O_6$	290.27
8	Dihydrojasmone	19.090	1.29	$C_{11}H_{18}O$	166.26
9	Angelicin	11.787	0.31	$C_{11}H_6O_3$	186.16
10	8-Methylnanoic Acid, Ethyl Ester	26.296	4.53	$C_{10}H_{20}O_2$	200.31
11	4-Epipallensin	29.251	6.67	$C_{15}H_{20}O_4$	264.31
12	9,12,15-Octadecatrienal	30.367	6.46	$C_{18}H_{30}O$	262.43
13	Pallensin	29.251	6.67	$C_{15}H_{10}O_4$	264.31
14	Trans 13-Octadecenoic Acid	19.304	1.52	$C_{18}H_{34}O_2$	282.46
15	4-Methyl-4 Vinyl Butyralactone	4.410	2.38	$C_7H_8O_2$	126.15

GC-MS Analysis

The phytochemical components present in the ethanol extract of *A. pallens* were examined using GC-MS, as illustrated below in the Figure 1. This analysis revealed 15 phytoconstituents such as dimetridazole, L-Pipecolic Acid, vinyl Trans Cinnamate, Oleamide, gallocatechin, DL octapamine, catechin, dihydrojasmone, angelicin, 8-Methylnanoic Acid, Ethyl Ester, 4-Epipallensin, 9,12,15-Octadecatrienal, pallensin, Trans 13-Octadecenoic Acid, 4-Methyl-4 and vinyl Butyralactone with their retention time, peak area, molecular weight and molecular formula was mentioned below in the (Table 4).

Anthelmintic Assay

This study suggests that some of the plants used in the past to treat infections caused by intestinal worms can be effective. The results obtained in the laboratory models confirm the ethnomedial use of these plants to treat intestinal worms. 50±0.06 For death time, the result was 24:03±0.03. The experimental findings from laboratory models support the traditional use of these plants as anthelmintics. The results of the anthelmintic activity are presented in Table 5. A review of the data shows that the extract of *A. pallens* at a dosage of 100 mg/mL resulted in the highest levels of paralysis time 17:50±0.06, with a corresponding death time as 24:03±0.03. In comparison, the standard drug Albendazole at a dosage of 25 mg/mL also induced paralysis at 31:43±0.09, which dies at 40:06±0.06 (Figure 2 (a), (b)).

In silico Docking Study

In this study, 15 compounds were identified through GC-MS analysis. In the case of NADH fumarate reductase, the selected compounds acted as ligands and exhibited considerable binding energy. Trans 13-Octadecenoic Acid, 4-Epipallensin, and Catechin were important in finding the compounds with the highest binding energies visualized through Chimera 1.14 software in Figure 4 and had enhanced *in silico* anthelmintic potential relative to Albendazole, which had a binding energy value of -6.74. The antihelmintic effects of the components of the herbal drugs are believed to be due to their bioactive compounds that include alkaloids and flavonoids. These compounds may engage with the newly identified role of Osm1, a soluble fumarate reductase receptor Figure 3, which can either slow the development of anthelmintic diseases or create interference (Table 6).

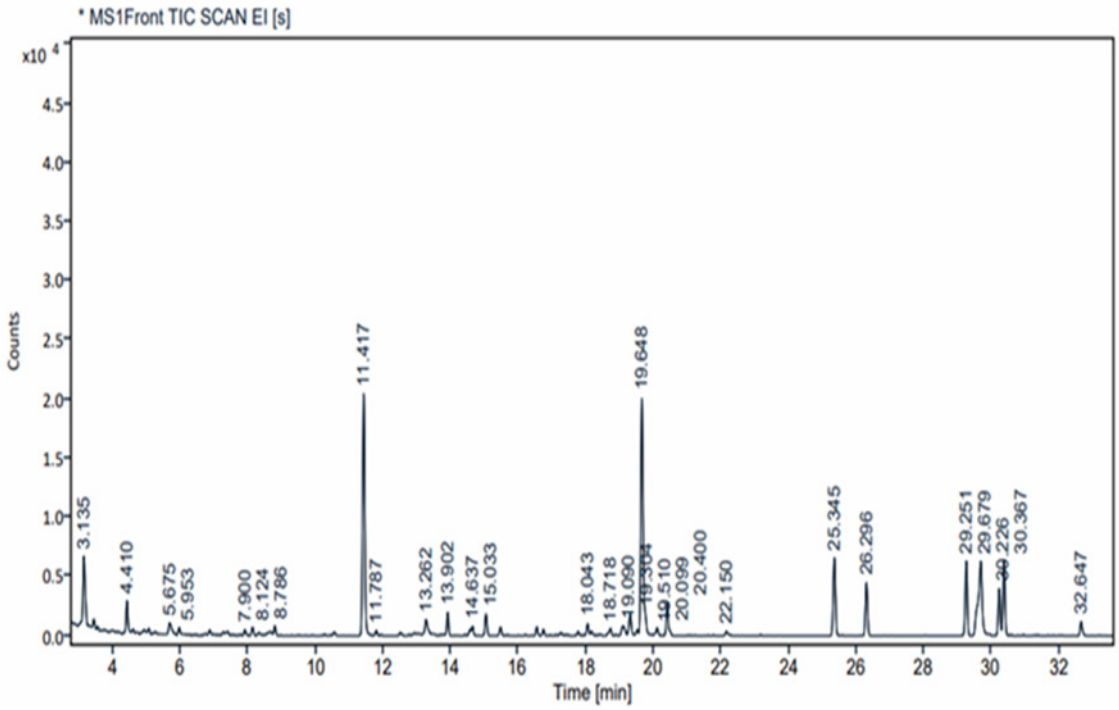


Figure 1: GC-MS chromatogram of bioactive compounds present in the ethanolic extract of *Artemisia pallens* Wall.

Table 5: Anthelmintic activity of EEAP against *Pheritima posthuman*.

Group	Concentration mg/mL	Time in minutes for paralysis/death	
		Paralysis Time in min (Mean & SEM)	Death Time in min (Mean & SEM)
Control Test	-	-	-
1	10	25:48±0.08	31:22±0.04
2	20	23:22±0.10	31:04±0.10
3	50	21:45±0.1	30:20±0.02
4	80	19:50±0.06	27:35±0.16
5	100	17:50±0.06	24:03±0.03
Std. Albendazole	25	31:43±0.09	40:06±0.06

All values are expressed as Mean ± SD, with n=5 in each group. Comparisons were conducted between the standard and treated groups, with a significance level set at $p < 0.005$.

Drug Likeness and Physicochemical Properties

The drug likeness parameters and physicochemical characteristics of the phytochemical constituents of *A. pallens* are detailed in Table 7. All compounds exhibiting the lowest binding energy demonstrated molecular weights between 124.14 and 306.27 g/mol. The obtained TPSA values were within the range between 17.07 and 130.61, and iLogP values changed from 0.23 to 4.34. Hydrogen bond acceptors lied between 1 and 6 while hydrogen bond donors lay between 1 and 6 as 7 compounds had 0. The detected molar refractivity's ranged between 30.74 and 91.07. Lipinski's rule of five indicated no violations for 11 compounds, whereas the remaining 4 compounds exhibited one violation each. An encouraging bioavailability score of 0.55 was noted for all compounds, with the exception of Trans-13-octadecenoic acid, which had a score of 0.85.

Pharmacokinetic Properties

All the compounds had high GI absorption, while dimetridazole and L-Pipecolic acid had no BBB penetration. Pallensin is a substrate of P-glycoprotein P-gp and catechin has been reported to inhibit P-glycoprotein. Some of the identified CYP1A2 inhibitors include oleamide, Angelicin, 9,12,15-octadecatrienal, and Trans-13-octadecenoic acid, some are non-inhibitors. Among them, vinyl trans cinnamate may be a CYP2C19 inhibitor; the others are not inhibitors. Among the compounds, oleamide and 9,12,15-octadecatrienal have been found to inhibit CYP2C9, but other compounds were not inhibitory to the enzyme. All

Table 6: Molecular Interactions of Ligands with Protein [PDB CODE: 5GLG].

Compounds	Binding energy	Ligand Efficiency	H-bond Interaction	H-bond distance (a°)
Dimetridazole	-7.74	0.77	UNK:N:SER78:A UNK:N:SER79:A UNK:O:HIS435:A UNK:O:GLY480:A UNK:O:ARG477:A UNK:O:ARG477:A UNK:O:ARG326:A UNK:O:ARG326:A	2.034 1.912 1.706 1.935 1.793 1.954 2.171 1.891
L-Pipecolic Acid	-5.57	0.62	UNK:O:ARG326:A UNK:O:ARG326:A UNK:O:ARG477:A UNK:O:HIS435:A UNK:O:SER482:A UNK:O:GLY480:A	2.248 2.035 1.77 1.677 2.085 1.999
Vinyl Trans Cinnamate	-6.04	-0.46	UNK:O:LYS76:A UNK:O:ASN253:A	2.106 2.007
Oleamide	-6.69	-0.33	UNK:O:SER79:A	1.994
Gallocatechin	-9.27	-0.42	UNK:O:GLY43:A UNK:O:ASN73:A	2.113 1.884
DL-Octopamine	-5.86	-0.53	UNK:O:GLY43:A UNK:O:GLY46:A UNK:O:ASN73:A UNK:O:ASN73:A	2.149 1.979 1.796 2.162
Catechin	-9.28	-0.42	UNK:O:LYS76:A UNK:O:ASN253:A	2.236 2.191
Dihydrojasmone	-5.82	-0.49	UNK:O:ASN253:A UNK:O:LEU298:A	1.981 2.064
Angelicin	-6.9	-0.49	UNK:O:HIS435:A UNK:O:ARG326:A UNK:O:ARG477:A UNK:O:GLY480:A	1.942 1.921 1.998 2.171
8-Methylnanoic Acid, Ethylester	-6.11	-0.44	UNK:O:HIS435:A UNK:O:ARG326:A UNK:O:ARG477:A UNK:O:ARG477:A	2.215 2.249 1.69 1.975
4-Epipallensin	-9.57	-0.5	UNK:O:HIS435:A UNK:O:ARG326:A UNK:O:ARG477:A	2.075 2.121 1.996
9,12,15-Octadecatrienal	-7.18	-0.38	UNK:O:ASN253:A UNK:O:LEU298:A UNK:O:SER482:A	1.833 2.097 2.172

Compounds	Binding energy	Ligand Efficiency	H-bond Interaction	H-bond distance (a°)
Pallensin	-8.57	-0.45	UNK:O:ARG326:A UNK:O:ARG477:A UNK:O:ARG477:A UNK:O:GLY480:A UNK:O:HIS435:A	2.102 1.649 1.609 1.755 1.967
Trans-13-Octadecenoic Acid	-9.6	-0.48	UNK:O:ARG326:A UNK:O:ARG477:A UNK:O:ARG477:A UNK:O:GLY480:A UNK:O:HIS435:A	2.154 2.215 1.788 2.123 2.248
4-Methyl-4vinyl Butyralactone	-5.15	-0.57	UNK:O:ARG326:A UNK:O:ARG477:A UNK:O:ARG477:A UNK:O:GLY480:A UNK:O:HIS435:A	2.154 2.215 1.788 2.123 2.248
ALBENDAZOLE (Std)	-6.74	-0.37	UNK:O:GLU467:A UNK:O:GLU467:A	2.017 1.808

Table 7: Physicochemical properties and Drug likeness parameter prediction of compounds.

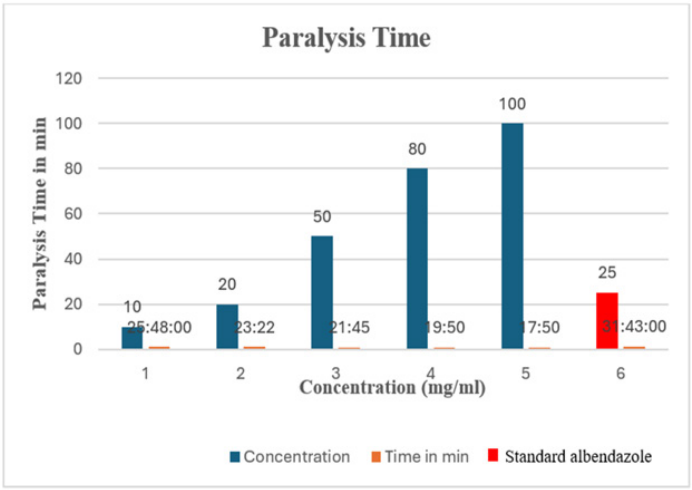
Compound	Molecular Wt g/mol	Tpsa A^2	iLog P	No. Of Hydrogen Bond Acceptor	No. of Hydrogen Bond Donar	Molar Refractivity	Lipinski violation	Bioavailability score
Dimetridazole	141.13	63.64	0.23	3	0	37.28	0	0.55
Pipecolic Acid	129.16	49.33	1.16	3	2	37.33	0	0.55
Vinyl Trans Cinnamate	174.20	26.30	2.48	2	0	51.77	0	0.55
Oleamide	281.48	43.09	4.22	1	1	91.07	1	0.55
Gallocatechin	306.27	130.61	0.98	7	6	76.36	1	0.55
Dl Octapamine	153.18	66.48	1.27	3	3	42.11	0	0.55
Catechin	290.27	110.38	1.47	6	5	74.33	0	0.55
Dihydrojasmone	166.26	17.07	2.67	1	0	52.60	0	0.55
Angelicin	186.16	43.35	2.03	3	0	52.26	0	0.55
8-Methylnanoic Acid, Ethyl Ester	200.32	26.30	4.34	2	0	61.08	0	0.55
4-Epipallensin	264.32	63.30	1.85	4	1	69.83	0	0.55
9,12,15-Octadecatrienal	262.43	17.07	4.06	1	0	87.42	1	0.55
Pallensin	254.24	59.06	0.00	4	1	62.99	0	0.55
Trans 13-Octadecenoic Acid	282.46	37.30	3.93	2	1	89.94	1	0.85
4-Methyl-4 Vinyl Butyralactone	124.14	26.30	3.22	2	0	30.74	0	0.55

Table 8: Pharmacokinetics Property Prediction of Compounds.

Compound Name	GI Absorption	BBB Permeant	P-gp substrate	CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4	Log Kp (Skin permeation) Cm/s
Dimetridazole	High	No	No	No	No	No	No	No	-7.06
L-Pipecolic acid	High	No	No	No	No	No	No	No	-8.73
Vinyl trans cinnamate	High	Yes	No	No	Yes	No	No	No	-5.15
oleamide	High	Yes	No	Yes	No	Yes	No	No	-3.05
Gallocatechin	High	No	No	No	No	No	No	No	-8.17
Dl-octapamine	High	No	No	No	No	No	No	No	-7.87
Catechin	High	No	Yes	No	No	No	No	No	-7.82
Dihydrojasmone	High	Yes	No	No	No	No	No	No	-5.27
Angelicin	High	Yes	No	Yes	No	No	No	No	-5.96
8-methylnanoic acid, ethyl ester	High	Yes	No	No	No	No	No	No	-4.44
4-epipallensin	High	Yes	No	No	No	No	No	No	-7.02
9,12,15-octadecatrienal	High	No	No	Yes	No	Yes	No	No	-3.36
Pallensin	High	Yes	Yes	No	No	No	No	No	-8.66
Trans-13-octadecenoic acid	High	No	No	Yes	No	Yes	No	No	-2.60
4-methyl-4vinyl butyralactone	High	Yes	No	No	No	No	No	No	-6.66

Table 9: Toxicity Analysis.

Compound Name	AMES Toxicity	Oral Rat Acute Toxicity	Minnow Toxicity	Hepatotoxicity	Skin Sensitisation
Dimetridazole	Yes	1.778	1.764	No	Yes
L-Pipecolic acid	No	1.979	2.892	No	No
Vinyl trans cinnamate	No	2.008	0.603	No	Yes
Oleamide	No	1.805	-1.12	No	Yes
Gallocatechin	No	2.492	4.235	No	No
Dl-octapamine	No	2.307	2.773	No	Yes
Catechin	No	2.428	3.585	No	No
Dihydrojasmone	No	1.664	0.844	No	Yes
Angelicin	Yes	2.669	1.546	No	No
8-methylnanoic acid,ethyl ester	No	1.693	0.248	No	Yes
4-epipallensin	Yes	1.868	2.228	No	No
9,12,15-octadecatrienal	No	1.477	-1.297	No	Yes
Pallensin	Yes	2.472	2.588	No	No
Trans-13-octadecenoic acid	No	1.417	-1.438	No	Yes
4-methyl-4vinyl butyralactone	No	1.944	2.278	No	Yes



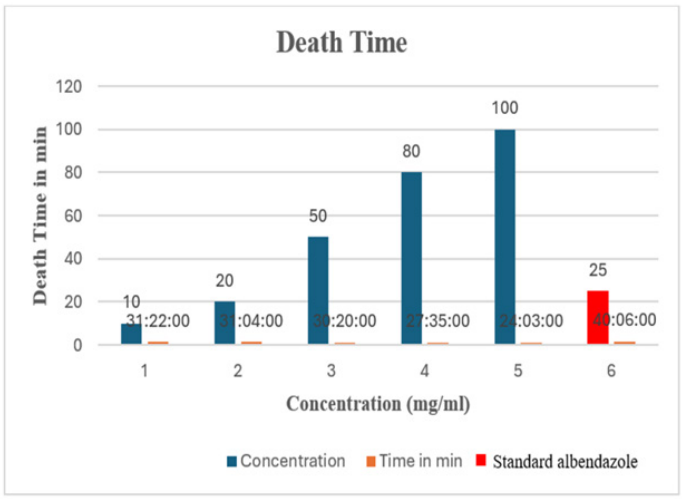
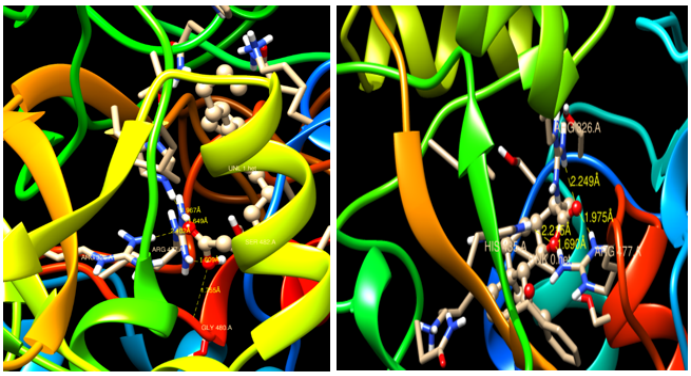
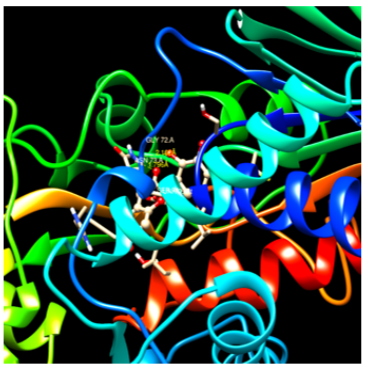


Figure 2: (a) Paralysis time for ethanol extract of *Artemisia pallens* with standard Albendazole. (b) Death time for ethanol extract of *Artemisia pallens* with standard Albendazole.

Figure 3: NADH-Fumarate Reductase Receptor [PDB CODE:5GLG].



(a) Trans 13-Octadecenoic Acid (b) 4-Epipallensin



(c) Catechin

Figure 4: Chimera image of three components having the highest fitting score with the NADH Fumarate reductase receptor.

compounds are not CYP2D6 and CYP3A4 inhibitors. Skin permeability (LogKp) values ranged from -2.60 to -8.73 (cm/s) (Table 8).

Toxicity Prediction

The toxicity of all compounds was evaluated, indicating that dimetridazole, angelicin, 4-epipallensin, and pallensin demonstrate AMES toxicity, whereas the others are not toxic. In rats, the acute oral toxicity falls between 1.417 and 2.492, and for minnows it lies between -1.12 and 4.235. There was no tendency shown in any compound for hepatotoxicity. Compounds like Dimetridazole, Vinyl trans cinnamate, oleamide, DL-octapamine, Dihydrojasmone, 8-methylnanoic acid, ethyl ester, 9,12,15-octadecatrienal, Trans-13-octadecenoic acid, 4-methyl-4vinyl butyralactone exposed skin sensitisation but others exposed no skin sensitisation (Table 9).

CONCLUSION

This study demonstrated that the extraction method used would also extract broad arrays of bioactive compounds within the aerial parts of the plant including some alkaloids, tannins, flavonoids, phenols, and terpenoids with good performance through Soxhlet extraction. Soxhlet extraction was chosen for the extracts since this technique has higher efficiency, used

lesser amount of solvent, demonstrated better ability to preserve heat-sensitive compounds. The biological activity of the ethanolic extract was assessed qualitatively through an anthelmintic activity, which qualitatively assessed its biological activity with bioactive phytochemicals in the extract. Thus, it is convinced that the ethanolic extract of *Artemisia pallens* possesses a remarkable anthelmintic property depending on the dose and noted the maximum efficacy of 100 mg/mL. This anthelmintic activity was higher than the standard control used in the study. Several of the compounds that were reviewed in this study were also analyzed as ligands for fumarate reductase, and had a more favorable affinity than the cocrystallization ligands with positive ADME toxicity mentioned in this study. The study had also performed molecular dynamic simulation studies to validate these findings. Overall, the study demonstrates the promise of *Artemisia pallens* against helminthic infection as organic alternatives, and as a genuinely exciting opportunity and reason to further investigate mechanisms and potential use in herbal medicine for added confidence in their mechanism.

ACKNOWLEDGEMENT

We truly appreciate Sri Balaji Vidyapeeth (Deemed to be University) for offering us all the resources needed to carry out this research work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

WHO: World Health Organization; **EEAP:** Ethanolic extract of *Artemisia pallens* Wall ex. DC; **GCMS:** Gas Chromatography and Mass spectrophotometry; **TLC:** Thin layer chromatography, **ADMET:** Absorption, Distribution, Metabolism, Excretion and Toxicity, **NADH:** Nicotinamide adenine dinucleotide plus hydrogen; **GI:** Gastrointestinal; **TPSA:** Topological polar surface area.

REFERENCES

1. Dagne A, Yihunie W, Nibret G, Tegegne BA. The genus *Commelina*: focus on distribution, morphology, traditional medicinal uses, phytochemistry, and ethno-pharmacological activities: an updated literature review. Heliyon. 2024;10(10):e30945. doi: 10.1016/j.heliyon.2024.e30945, PMID 38784558.
2. Sofowora A, Ogunbodede E, Onayade A. The role and place of medicinal plants in the strategies for disease prevention. Afr J Tradit Complement Altern Med. 2013;10(5):210-29. doi: 10.4314/ajtcam.v10i5.2, PMID 24311829.
3. Abdel-Aziz SM, Aeron A, Kahil TA. Health benefits and possible risks of herbal medicine. In: Garg N, Abdel-Aziz SM, Aeron A, editors. Microbes in food and health. Cham: Springer International Publishing; 2016. p. 97-116. doi: 10.1007/978-3-319-25277-3_6.
4. Petrovska BB. Historical review of medicinal plants' usage. Pharmacogn Rev [Internet]. 2012;6(11):1-5. doi: 10.4103/0973-7847.95849, PMID 22654398.
5. George P. Concerns regarding the safety and toxicity of medicinal plants-An overview. J Appl Pharm Sci. 2011;30(Issue):40-4.
6. International Agency for Research on Cancer. Working group on the evaluation of the carcinogenic risks to humans. Some traditional herbal medicines, some mycotoxins, naphthalene and styrene: this publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon. Lyon: Iarc; Feb 12-19 2002.
7. Awasthi S, Bundy DA, Savioli L. Helminthic infections. BMJ [Internet]. 2003;327(7412):431-3. doi: 10.1136/bmj.327.7412.431, PMID 12933732.
8. Manke MB, Dhawale SC, Jamkhande PG. Helminthiasis and medicinal plants: a review. Asian Pac J Trop Dis. 2015;5(3):175-80. doi: 10.1016/S2222-1808(14)60648-4.
9. Athanasiadou S, Githiori J, Kyriazakis I. Medicinal plants for helminth parasite control: facts and fiction. Animal. 2007;1(9):1392-400. doi: 10.1017/S1751731107000730, PMID 22444894.
10. Sunita K, Kumar P, Khan MA, Husain SA, Singh DK. Anthelminthic/larvicidal activity of some common medicinal plants. Eur J Biol Res. 2017;7(4):324-36. doi: 10.5281/zenodo.1036819.
11. Hotez PJ, Brindley PJ, Bethony JM, King CH, Pearce EJ, Jacobson J. Helminth infections: the great neglected tropical diseases. J Clin Invest. 2008;118(4):1311-21. doi: 10.1172/JCI34261, PMID 18382743.
12. Wang LJ, Cao Y, Shi HN. Helminth infections and intestinal inflammation. World J Gastroenterol [Internet]. 2008;14(33):5125-32. doi: 10.3748/wjg.14.5125, PMID 18777588.
13. Rajeswari VD. Anthelmintic activity of plants: a review. Res J Phytochem. 2014;8(3):57-63. doi: 10.3923/rjphyto.2014.57.63.
14. Suresh J, Singh A, Vasavi A, Ihsanullah M, Mary S. Phytochemical and pharmacological properties of *Artemisia pallens*. Vol. 2(12): 3081-90; 2011. Ijpsr [Internet]. Available from: http://www.ijpsr.com.
15. Pavithra KS, Annadurai J, Pavithra S, Ragunathan R. Phytochemical, antioxidant and a study of bioactive compounds from *Artemisia pallens*. ~ 664 ~ Journal of Pharmacognosy and Phytochemistry. 2018;7(4):664-75.
16. Dongare S. *Artemisia pallens*: an Indian plant with multifarious pharmacological potentials. Int J Med Pharm Sci. 2022;12(2):1. doi: 10.31782/IJMPS.2022.12201.
17. Jha V, Kadam P, Jain T, Bhargava A, Marick A, Saiya B, *et al.* Investigation of physico-chemical properties and evaluation of the biological potential of essential oil extracted from *Artemisia pallens*. JUmm Al-Qura Univ Appll Sci [Internet]. 2023;9(4):494-507. doi: 10.1007/s43994-023-00059-0.
18. Campos-Fernández L, Ortiz-Muñiz R, Cortés-Barberena E, Mares-Sámano S, Garduño-Juárez R, Soriano-Correa C. Imidazole and nitroimidazole derivatives as NADH-fumarate reductase inhibitors: density functional theory studies, homology modeling, and molecular docking. J Comp Chem. 2022;43(23):1573-95. doi: 10.1002/jcc.26959, PMID 35796405.
19. Ōmura S, Miyadera H, Ui H, Shiomi K, Yamaguchi Y, Masuma R, *et al.* An anthelmintic compound, nafuredin, shows selective inhibition of complex I in helminth mitochondria. Proc Natl Acad Sci U S A. 2001;98(1):60-2. doi: 10.1073/pnas.98.1.60, PMID 11120889.
20. Mori M, Nonaka K, Masuma R, Ōmura S, Shiomi K. Helminth electron transport inhibitors Fungi, producer. Springer eBooks; 2018. p. 297-329.
21. Krakowska-Sieprawska A, Kiełbasa A, Rafińska K, Ligor M, Buszewski B. Modern methods of pre-treatment of plant material for the extraction of bioactive compounds. Molecules. 2022;27(3):730. doi: 10.3390/molecules27030730, PMID 35163995.
22. Mahire SP, Patel SN. Extraction of phytochemicals and study of its antimicrobial and antioxidant activity of Helicteres isora L. Clin Phytosci. 2020;6(1). doi: 10.1186/s40816-020-00156-1.
23. Redfern J, Kinninmonth M, Burdass D, Verran J. Using Soxhlet ethanol extraction to produce and test plant material (essential oils) for their antimicrobial properties. J Microbiol Biol Educ [Internet]. 2014;15(1):45-6. doi: 10.1128/jmbe.v15i1.656, PMID 24839520.
24. Amanat M, Tawhid M. Shahid-Ud-Daula AF. Anthelmintic activity *of Zingiber roseum* rhizomes against *Pheretima posthuma*: *in vitro* and *in silico* approach. Int J Sci Res Chem (IJSRCH). 2022 Jan. [Anthelmintic activity of *Zingiber roseum* rhizomes against Pheretima posthuma: *In vitro* and *in silico* approach]
25. Yadav RN, Agarwala M. Phytochemical analysis of some medicinal plants. J Phytol. 2011:3(12).
26. Rudrapal M, Vallinayagam S, Aldosari SM, Khan J, Albadrani HM, Al-Shareeda A, *et al.* Valorization of *Adhatoda vasica* leaves: extraction, *in vitro* analyses and *in silico* approaches. Front Nutr. 2023;10:1161471. doi: 10.3389/fnut.2023.1161471, PMID 37063312.
27. Shukla V, Pala Z, Alok A, Desai N. Screening of Different *Artemisia* spp. from Western Ghats of Maharashtra for an anti-malarial Compound-artemisinin. Am J Plant Sci. 2015;06(09):1619-32.
28. Chandran M. GCMS analysis of phytochemicals from methanol leaf extract of *Artemisia pallens*. J Emerg Technol Innov Res. 2014;1(3):375–88375–88.
29. Bail S, Buchbauer G, Schmidt E, Wanner J, Slavchev A, Stoyanova A, *et al.* GC-MS-analysis, antimicrobial activities and olfactory evaluation of essential Davana (*Artemisia pallens* Wall. ex DC) oil from India. Nat Prod Commun. 2008;3(7):1934578X0800300. doi: 10.1177/1934578X0800300705.
30. Prasanth DS, Aminabee SK, Rao AL, Teja N, Bhargavi K, Monika C, *et al.* Anthelmintic activity of *Mansoa Alliacea* against Pheretima posthuma: *in vitro* and *in silico* approach. Thai J Pharm Sci. 2020;44(3):186-96. doi: 10.56808/3027-7922.2451.

31. Sastry GM, Adzhigirey M, Day T, Annabhimoju R, Sherman W. Protein and ligand preparation: parameters, protocols, and influence on virtual screening enrichments. J Comput Aid Mol Des [Internet]. 2013;27(3):221-34. doi: 10.1007/s10822-013-9644-8 , PMID 23579614.
32. Daina A, Michielin O, Zoete V. SwissADME: a Free web Tool to Evaluate pharmacokinetics, drug-likeness and Medicinal Chemistry Friendliness of Small Molecules. Sci Rep [Internet]. 2017;7(1):42717. doi: 10.1038/srep42717, PMID 28256516.
33. Izatunnafis I, Murti YB, Sudarmanto BS. *In silico* pharmacokinetic and Toxicity Prediction of compounds from *Andrographis paniculata* (Burm. f.) Nees. J Food Pharm Sci:830-8.

Cite this article: Madhivardhana P, Bharathy JV, Karthikeyan SA. Study of *in silico* and *in vitro* Anthelmintic Activity of Ethanolic Extract of *Artemisia pallens* Wall Ex. DC. Pharmacog Res. 2025;17(1):143-53.