Phytochemical Profiling and Acute Oral Toxicity Study of Hydro-Ethanolic Extract of Whole Plant of *Artemisia annua* in Wistar albino rat

ABSTRACT

Aims: To evaluate the phytochemical constituents, and acute oral toxicity assessment of the hydro-ethanolic extract of the whole plant of *Artemisia annua* (family Asteraceae), a plant contains several pharmacologically active compounds, the most prominent being artemisinin, a hydrophobic sesquiterpene lactone responsible for its potent antiparasitic effects.

Methodology: In the present study, whole-plant material of *A. annua* was procured, authenticated, and processed into fine powder using a pulverizer. The powder was subjected to cold hydro-ethanolic maceration technique, and the resulting extract was evaporated and stored in airtight vials for subsequent phytochemical analyses and acute oral toxicity assessment.

Results: Hydro-ethanolic extraction yielded 6.45% crude extractability, indicating efficient recovery of phytochemicals under the extraction conditions. The extract was greenish-brown, solid and sticky, bitter in taste, and pleasant in odour, consistent with known organoleptic characteristics of *A. annua* extracts. Qualitative phytochemical screening of extracts prepared in twelve different solvents revealed the presence of alkaloids, flavonoids, saponins, tannins, sterols, sesquiterpene lactones, reducing sugars, glycosides, and phenolics, reflecting a broad spectrum of bioactive constituents. These compounds, particularly sesquiterpene lactones, contribute to the plant's documented antiparasitic activity against *Theileria*, *Babesia*, *Trypanosoma*, *Eimeria*, and gastrointestinal nematodes, highlighting its importance in veterinary parasitology. Acute oral toxicity study of the hydro-ethanolic extract was assessed in Wistar albino rats following OECD guideline 420. Rats received 300, 2000, and 5000 mg/kg body weight of hydro-ethanolic extract of *A. annua* and were observed for 14 days. No mortality, behavioural abnormalities, or clinical signs of toxicity were observed at any dose.

Conclusion: These findings demonstrated that the hydro-ethanolic extract of *A. annua* is non-toxic and well-tolerated, supporting its safe use and potential as a natural antiparasitic agent in veterinary medicine.

Keywords: *Artemisia annua*, Artemisinin, Phytochemical analysis, Wistar rat, Acute toxicity

1. INTRODUCTION

Artemisia annua L. is a medicinal plant of major pharmacological importance, widely studied for its rich secondary metabolite profile. Recent investigations confirm that the plant contains sesquiterpene lactones (artemisinin and its precursors), flavonoids, phenolic acids, and volatile terpenoids that contribute to its antimalarial, antioxidant, anti-inflammatory, and antimicrobial activities (Swamikannu *et al*., 2025). Molecular studies have shown that transcription factors regulating glandular trichome development directly influence artemisinin biosynthesis, linking plant morphology with

metabolite production (Yuan *et al*., 2025). Furthermore, a doctoral thesis demonstrated that environmental factors and chemotype variation significantly affect artemisinin yield, emphasizing the importance of optimized cultivation strategies (Kam, 2020).

Recent phytochemical profiling studies of *Artemisia annua* L. have expanded our understanding of its complex secondary metabolite composition beyond artemisinin alone. Comprehensive analyses using gas chromatography–mass spectrometry (GC-MS), high-performance liquid chromatography–high resolution mass spectrometry (HPLC-HRMS/MS), and near-infrared spectroscopy have identified a diverse array of metabolites, including monoterpenes, sesquiterpenes, flavonoids, phenolic acids, and coumarins in *A. annua* and related species (Abdallah *et al*., 2025; Trifan *et al*., 2022; Abate *et al*., 2021). A recent comparative profiling study employing integrated GC-MS, HPTLC, and NIR spectroscopy detected over 48 volatile compounds in *A. annua* essential oils, along with chemical markers such as artemisinin, coumarins, phenolic acids, and flavonoids that facilitate species discrimination and quality control (Abdallah *et al*., 2025).

Multi-omics metabolic analysis using LC-MS and GC-MS has also uncovered extensive metabolite networks, revealing alterations in flavonoids, terpenoids, and other secondary metabolites related to developmental and genetic factors (Qin *et al*., 2024). Additionally, LC-HRMS/MS profiling across multiple *Artemisia* species documented 15 phenolic acids, 26 flavonoids, and 14 sesquiterpene lactones, highlighting the broad phytochemical diversity within the genus and the antioxidant and bioactive potential of *A. annua* extracts (Trifan *et al*., 2022). These contemporary profiling efforts are crucial for standardizing extracts, guiding metabolic engineering, and enabling novel bioactivity research.

Acute oral toxicity assessment is a critical component of preclinical safety evaluation, determining the potential adverse effects and median lethal dose (LD_{50}) of herbal extracts when administered orally. Studies conducted on *A. annua* L. following OECD acute toxicity guidelines have demonstrated a wide margin of safety. Hydroethanolic extracts administered orally to rodents at doses up to 2,000–5,000 mg/kg body weight produced no mortality or severe clinical signs, indicating that the extract can be classified as practically non-toxic (Lagarto, 2020). A doctoral thesis further confirmed the absence of acute toxic manifestations and significant body-weight changes following high oral doses, supporting the safe oral use of *A. annua* in experimental models (Kam, 2020).

Hence, considering the above facts, the present research study was conducted to study phyto-chemical analysis after extraction of *Artemisia annua* and acute toxicity studies in Wistar albino rat.

2. MATERIALS & METHODS

The research work was carried out in the Department of Veterinary Clinical Medicine, Ethics and Jurisprudence and the Department of Veterinary Pharmacology and Toxicology of College of Veterinary and Animals Sciences, MAFSU, Parbhani. The acute toxicity studies were performed in CPCSEA-registered animal house facility after approval of Institutional Animal Ethics Committee (IAEC) of COVAS, Parbhani.

2.1 Extraction of *A. annua*

A. annua was procured from a local Ayurvedic medicine shop and authenticated by an expert taxonomist. The whole plant material was processed into a fine powder using a pulverizing machine. Freshly prepared powder (20 g) was subjected to cold hydro-ethanolic extraction by immersing it in 100 ml of hydro-ethanolic solvent (40% distilled water and 60% ethanol) in a tightly stoppered flask. The mixture was kept at room temperature for 48 hours with continuous agitation at 150 rpm in an orbital shaker. After extraction, the contents were filtered through muslin cloth, and the residue was rinsed with a small volume of the same solvent and re-filtered. The combined filtrate was further filtered through Whatman's No. 1 filter paper and exposed to ultraviolet light for 12 hours. The filtrate was then transferred to a pre-weighed Petri dish and allowed to evaporate at room temperature to remove the solvent. The concentrated extract was stored in airtight screw-cap vials in a desiccator until further use. The hydro-ethanolic whole-plant extract of *A. annua* was used for subsequent experiments (Fig. 1-2).

2.2 Phytochemical analysis of *A. annua*

For phytochemical analysis, *A. annua* plant material was extracted using twelve different solvents of varying polarity, namely acetic acid, acetone, benzene, chloroform, distilled water, ethyl acetate, ethanol, hexane, hydro-ethanol, methanol, petroleum ether, and xylene. The resulting extracts were qualitatively screened for the presence of major phytochemical constituents following standard procedures described by Roberts *et al*., (1981) and Harborne (1984).

For the estimation of phytochemical constituents, a series of qualitative tests were performed on the *Artemisia annua* extracts. Carbohydrates were assessed using Molisch's test, while reducing sugars were detected using Fehling's solution and Benedict's reagent. Monosaccharides were determined by Barfoed's test, and hexose sugars were evaluated using Seliwanoff's and cobalt chloride tests (Evans, 2009; Silva *et al*., 1998). Proteins and amino acids were identified through Biuret, Millon's, xanthoproteic, and ninhydrin tests (Sawhney and Singh, 2000). Tannins and phenols were detected using ferric chloride and lead acetate tests (Evans, 2009). Glycosides were assessed by Legal's test, Keller-Killiani test, and Borntrager's test (Evans, 2009). Saponins were detected using the foam (frothing) test (Sofowora, 1996). Phytosterols and triterpenoids were evaluated through the Salkowski and Liebermann-Burchard tests (Evans, 2009; Sofowora, 1996). Alkaloids were identified using Mayer's, Wagner's, Hager's, and Dragendorff's tests. Flavonoids were detected using sodium hydroxide, lead acetate, and ferric chloride tests (Ansari, 2006; Silva *et al*., 1998). Anthraquinones and phenolics were examined using Borntrager's and ferric chloride tests, respectively (Evans, 2009). These tests collectively provided a comprehensive qualitative profile of the bioactive constituents present in *A. annua* (Fig. 3).

2.3 Acute oral toxicity studies of *A. annua*

The acute oral toxicity study of *A. annua* extract was conducted in Wistar rats in accordance with OECD 420 guideline (Acute Oral Toxicity—Fixed Dose Procedure) (OECD, 2002). Healthy Wistar rats of either sex, weighing between 180 - 220 g, were used for the study. The rats were procured from the CPCSEA-registered small laboratory animal facility, Resource Section, College of Veterinary and Animal Sciences (COVAS), Parbhani. Prior to the initiation of the study, the experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of COVAS, Parbhani. The extract of *A. annua* was administered orally to the rats at doses of 300, 2000, and 5000 mg/kg body weight. Following administration, the animals were closely observed for the first 24 hours for any immediate signs of toxicity and mortality. Subsequently, they were monitored daily for a period of 14 days to record any delayed toxic effects. Observations included general behaviour, agility, feed and water intake, locomotor activity, muscular tremors, convulsions, and other clinical signs of toxicity. The overall health and well-being of the rats were continuously assessed to determine the safety profile of the hydro-ethanolic extract of *A. annua* (Fig. 4).

3. RESULTS AND DISCUSSION

Hydro-ethanolic solvent is widely recognized as one of the most effective solvents for extracting bioactive compounds from crude plant material; therefore, in the present study, it was selected for the extraction of the whole plant of *A. annua* to ensure optimal recovery of phytochemicals. Using this method, 100 g of *A. annua* powder yielded an average of 6.45 g of extract, corresponding to a percent extractability of 6.45%, indicating efficient recovery of phytochemicals under the applied extraction conditions. The hydro-ethanolic extract was greenish-brown in colour, solid and sticky in consistency, bitter in taste, and pleasant in odour, consistent with the known organoleptic characteristics of *A. annua* extracts (Table 1).

Qualitative phytochemical screening indicated that the hydro-ethanolic solvent extracted the widest range of phytochemical constituents, with strong positive reactions (+++) for flavonoids, tannins, sesquiterpene lactones, and glycosides. Ethanol also showed broad extraction ability across multiple compound classes. Non-polar solvents such as hexane and petroleum ether selectively extracted sterols and sesquiterpene lactones, while showing minimal extraction of polar compounds. Chloroform effectively extracted alkaloids, saponins, and sterols. In contrast, distilled water and acetic acid exhibited limited extraction, primarily yielding phenolics and flavonoids. Overall, screening using twelve different solvents confirmed the presence of alkaloids, flavonoids, saponins, tannins, sterols, sesquiterpene lactones, reducing sugars, glycosides, and phenolics in *A. annua* (Table 2).

In accordance with OECD 420 guideline, oral administration of *Artemisia annua* extract at dose levels of 300, 2000, and 5000 mg/kg body weight produced no mortality or treatment-related clinical signs of toxicity in Wistar rats. All animals survived and remained clinically normal throughout the 14-day observation period, with no evidence of acute toxic effects.

The selection of a hydro-ethanolic solvent for extracting *A. annua* in the present study aligns with findings that solvent polarity markedly influences phytochemical recovery. Mixed hydro-alcoholic systems, such as ethanol–water, enhance the extraction of a broad range of secondary metabolites compared with single-solvent systems, supporting efficient recovery of phenolics and flavonoids that contribute to antioxidant potential. For example, methanol and ethanol extracts of *A. annua* leaves exhibited higher total phenolic and flavonoid contents and stronger antioxidant activity across multiple assays, demonstrating the effectiveness of alcohol-based solvents in phytochemical extraction (Gavarić

et al., 2025). Such phenolic and flavonoid compounds are recognized for their radical-scavenging and health-promoting properties. Other studies have noted that extraction solvent choice significantly affects the qualitative and quantitative profile of phytoconstituents and associated biological activities in *Artemisia* species, with hydro-alcoholic mixtures often providing broader phytochemical coverage than purely polar or non-polar solvents (Liu *et al*., 2023). These findings corroborate the rich phytochemical profile observed in the hydro-ethanolic extract of *A. annua* in the current work (Iqbal *et al*., 2012).

The solvent-dependent variation in phytochemical profiles observed in this study aligns with current research demonstrating that solvent polarity critically influences the extraction of secondary metabolites in *A. annua*. Hydro-ethanolic and ethanolic solvents efficiently solubilize a broad spectrum of polar and mid-polar constituents, including flavonoids, phenolic acids, and sesquiterpene lactones, which are often associated with antioxidant and anti-inflammatory activities (Abate *et al*., 2021; Chebbac *et al*., 2023). Ethanolic extraction at different concentrations has been shown to enhance the recovery of artemisinin, flavonols, phenolic acids, and low-molecular phenolics compared with aqueous or less polar solvents, consistent with the strong qualitative results (+++) recorded here (Abate *et al*., 2021). Studies comparing solvent efficacy also report that non-polar solvents preferentially extract lipophilic compounds such as sterols and certain terpenoids, while highly polar solvents yield predominantly phenolics and flavonoids, further validating the solvent-dependent profiles in the present screening (Iqbal *et al*., 2012; Siddiqui *et al*., 2018; Trifan *et al*., 2022).

The bioactive sesquiterpene lactones in *Artemisia annua*, especially artemisinin, contribute not only to antimalarial activity but also exhibit inhibitory effects against other protozoan parasites. Artemisinin and related compounds have demonstrated *in vitro* activity against *Trypanosoma cruzi* and *Trypanosoma brucei* rhodesiense, indicating potential trypanocidal properties beyond Plasmodium species (Mishina *et al*., 2007). Additionally, *A. annua* and its phytochemicals have shown promising anticoccidial effects; dietary supplementation with *A. annua* leaf powder significantly reduced oocyst output and lesion scores in coccidiosis models in broiler chickens and lambs, highlighting its utility against *Eimeria* infections in livestock (Liu *et al*., 2025; Coroian *et al*., 2022). Recently, *A. annua* has demonstrated potential effects against sub-clinical bovine tropical theileriosis (Siddiqui *et al*., 2026). These findings support the broad antiparasitic potential of *A. annua* constituents and underscore the

need for further research to elucidate mechanisms and optimize therapeutic applications in veterinary parasitology.

The absence of mortality and treatment-related clinical signs following oral administration of *Artemisia annua* extract up to 5000 mg/kg body weight indicates a high margin of safety under acute exposure conditions. Similar findings have been reported in earlier toxicological evaluations, where hydro-ethanolic and ethanolic extracts of *A. annua* produced no lethality or significant behavioural, neurological, or systemic toxicity in rodents at doses up to 5,000 mg/kg body weight, suggesting an LD_{50} greater than this limit (Lagarto *et al*., 2020; Siddiqui *et al*., 2018). Additionally, comprehensive safety assessments of *A. annua*–derived preparations have demonstrated minimal acute toxicity, supporting their classification as practically non-toxic when administered orally (Ferreira *et al*., 2010). The present findings are consistent with OECD guideline 420 criteria and support the continued preclinical development of *A. annua* extracts for pharmacological applications (OECD, 2002).

The results of the present study revealed that *Artemisia annua* extract contains a wide range of phytoconstituents, which may collectively contribute to its diverse pharmacological activities. The presence of multiple bioactive classes suggests possible synergistic interactions that could enhance therapeutic efficacy. However, the current findings are limited to qualitative assessment, and comprehensive quantitative analysis is warranted. Further studies are therefore required to evaluate different extracts from various plant parts of *A. annua* and to systematically investigate their pharmacological activities through *in-vitro* and *in-vivo* models.

4. CONCLUSIONS

Artemisia annua contains a diverse range of bioactive phytochemicals, with hydro-ethanolic extraction yielding the most comprehensive profile. Acute oral toxicity in Wistar rats (OECD 420) showed the extract is safe up to 5,000 mg/kg body weight with no adverse effects. These results support its therapeutic potential, warranting further clinical and pharmacological studies.

ETHICAL APPROVAL

The research work protocol has been approved by the Institutional Animal Ethics Committee (IAEC), College of Veterinary and Animal Sciences, Parbhani and Board of Studies of Maharashtra

Animal and Fishery Sciences University, Nagpur and the experiment was performed according to internationally accepted standards of ethical guidelines for animal use and care.

Disclaimer (Artificial Intelligence)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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Table 1. Physical characteristics and extraction yield of hydro-ethanolic extract of *Artemisia annua* plant

Extraction Method	Solvent	Yield	Extractability Efficiency	Physical State	Colour	Taste	Odour	Consistency with *A. annua*
Crude/ Cold Extraction	Hydro-ethanol	6.45 %	Efficient recovery under the applied conditions	Solid and sticky	Greenish-brown	Bitter	Pleasant	Matches reported organoleptic characteristics

Table 2. Qualitative Phytochemical analysis of extract of *Artemisia annua* plant

Solvent	Active Principles											
	Alkaloids	Flavonoids	Anthraquinone	Amino Acid	Protein	Saponin	Tannins	Sterol	Sesquiterpene Lactones	Reducing Sugar	Glyosides	Phenolics
Acetic acid	-	+	-	-	-	+	+	-	+	-	-	++
Acetone	-	+	-	-	-	-	-	-	++	-	-	+
Benzene	-	-	-	-	-	+	-	++	++	-	++	+
Chloroform	+	-	-	-	-	++	-	+++	++	-	+	++
Ethyl acetate	-	+	-	-	-	-	-	+	+	-	-	+
Ethanol	++	++	-	-	-	-	++	+	++	-	+	++
Hexane	-	-	-	-	-	+	-	+++	+++	-	+	-
Hydro-ethanol	++	+++	-	-	-	++	+++	++	+++	-	+++	+++
Methanol	+	++	-	-	-	+	+	++	+	-	+	++

Petroleum ether	-	-	-	-	-	++	-	+++	+++	-	+	-
Xylene	-	++	-	-	-	+	++	-	++	-	-	++
Water	-	+	-	-	-	-	+	-	-	-	-	+

- Nil; + Mild; ++ Moderate; +++ Abundance

Fig. 1: Grinded *A. annua* whole plant

Fig. 2: Extracted A. annua

Fig. 3: Phytochemical analysis of *A. annua*

Fig. 4: Administration of *A. annua* extract