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Analysis of venom composition and bioactive potential of selected spider species from Chhattisgarh, India

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Abstract

The present study was undertaken to analyze the diversity and composition of spider venom in selected species from Chhattisgarh, India, with emphasis on biochemical complexity, enzymatic activity and ecological relevance. Six spider species belonging to five families-*Hippasa agelenoides* (Lycosidae), *Pholcus phalangioides* (Pholcidae), *Cyrtophora citriola* and *Argiope aemula* (Araneidae), *Oxyopes salticus* (Oxyopidae) and *Heteropoda venatoria* (Sparassidae)-were selected based on their abundance, ecological guild and habitat diversity. Venom was extracted using mild electrical stimulation and analyzed for yield, protein concentration and molecular composition. Venom yield ranged from 1.8 ± 0.3 µL in *Pholcus phalangioides* to 6.4 ± 0.8 µL in *Heteropoda venatoria*. Bradford assay revealed significant interspecific variation in protein concentration, with the highest levels recorded in *Argiope aemula* (2.63 ± 0.18 µg/µL) and *Cyrtophora citriola* (2.21 ± 0.16 µg/µL). SDS-PAGE and RP-HPLC analyses demonstrated species-specific protein profiles and varying degrees of venom complexity, with orb-weaving spiders and large wandering hunters exhibiting greater protein diversity and enzymatic activity. Enzymatic assays indicated the presence of phospholipase A_2, proteases and antimicrobial components, particularly in *Argiope aemula*, *Cyrtophora citriola* and *Heteropoda venatoria*. Statistical analysis confirmed significant differences in venom protein concentration among species ($p < 0.05$). The study reveals a strong association between venom composition, ecological guild and predatory strategy, highlighting orb-weaving spiders as reservoirs of highly complex venoms. These findings provide baseline information on spider venom diversity in Chhattisgarh and underscore the potential of regional spider venoms for future pharmacological and bioinsecticidal research.

Keywords: Spider venom, venom composition, enzymatic activity, Araneidae, ecological guild, Chhattisgarh

1. Introduction

Spider venoms represent one of the most complex and evolutionarily refined chemical arsenals in the animal kingdom, playing a pivotal role in prey capture, defense and ecological adaptation. Spiders (Order *Araneae*) have thrived for over 300 million years, during which their venom systems have diversified into intricate mixtures of peptides, proteins, enzymes and small organic molecules that target specific physiological pathways in their prey (Guo *et al.* 2024) [9]. These venoms primarily function to immobilize or kill prey by interfering with ion channels and neuromuscular processes, enabling spiders to subdue organisms often larger than themselves with remarkable efficiency. The high degree of biochemical complexity in spider venoms reflects both evolutionary history and ecological necessity, with venom composition varying significantly among taxa and according to predatory strategies and environmental niches.

Early studies revealed that spider venoms are not uniform across species but consist of a broad spectrum of bioactive components with diverse molecular targets (Kuhn-Nentwig *et al.* 2011) [12]. Unlike many other venomous animals, spiders use venom predominantly for predation rather than defense, and the evolutionary optimization of venom components has enabled species to adapt their toxic cocktails for specific prey types and hunting modes, as evidenced by recent comparative venomics work across diverse spider lineages (Lüddecke *et al.* 2025) [13]. Moreover, the synergistic interactions among venom molecules-such as neurotoxins, enzymes and antimicrobial peptides-enhance overall efficacy and reflect complex regulatory mechanisms shaped by ecological pressures and evolutionary history.

Beyond ecological functions, spider venoms have attracted considerable scientific interest due to their potential biomedical and agricultural applications. Functionally important venom peptides often exhibit high specificity for ion channels and receptors, making them valuable tools for neurophysiological research and promising leads for the development of novel therapeutics, including analgesics, antimicrobials and insecticides (Guo *et al.* 2024) [9]. This dual significance-ecological and translational-underpins the expanding field of venom research and highlights the importance of characterizing regional venom diversity, such as that of Chhattisgarh spiders, to uncover novel bioactive molecules and understand the ecological drivers of venom evolution.

2. Materials and Methods

The study was conducted to evaluate "Analysis of Venom Composition and Bioactive Potential of Selected Spider Species from Chhattisgarh, India". The venom composition diversity in selected spider species from different ecological habitats of Gariyaband, Mahasamund and Raipur (Chhattisgarh). Field sampling was carried out across forested areas, agricultural fields, grasslands, gardens and human habitations to ensure representative coverage of major ecological guilds. Six spider species-*Hippasa agelenoides*, *Pholcus phalangioides*, *Cyrtophora citriola*, *Argiope aemula*, *Oxyopes salticus* and *Heteropoda venatoria*-were selected based on abundance, body size and ecological significance. Adult spiders were collected manually using forceps and aspirators during early morning and evening hours. Specimens were identified up to species level using standard taxonomic keys and confirmed by morphological characters. Live specimens were maintained individually under laboratory conditions with adequate ventilation, temperature (25 ± 2 °C) and humidity (65-75%) until venom extraction.

Venom extraction was performed using a mild electrical stimulation method to induce venom secretion without causing permanent harm to the spiders. Venom droplets were collected using sterile microcapillary tubes and transferred into labeled microcentrifuge tubes containing chilled phosphate-buffered saline. Venom yield per individual was measured using a micropipette and expressed in microliters. Protein concentration was quantified by the Bradford assay using bovine serum albumin as the standard. Molecular profiling of venom proteins was carried out using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and protein bands were visualized with Coomassie Brilliant Blue staining. Further separation and assessment of venom complexity were achieved using reverse-phase high-performance liquid chromatography (RP-HPLC), recording retention time and number of peaks for each species.

Enzymatic activities, including phospholipase A_2, protease and hyaluronidase, were assessed using standard qualitative and semi-quantitative biochemical assays. Antimicrobial activity of crude venom samples was evaluated against selected bacterial strains using agar diffusion methods. Data were expressed as mean ± standard deviation, and interspecific differences in venom protein concentration were analyzed using pairwise independent t-tests at a significance level of $p < 0.05$. Statistical analyses were performed using standard statistical software. The methodology ensured reproducibility and allowed comparative evaluation of venom composition across species representing different ecological strategies.

3. Results

All spiders are technically venomous (they produce venom to subdue prey insects), but the vast majority are not medically significant to humans-their bites cause only minor symptoms like mild pain, redness, or swelling, similar to a bee sting. In India, including Chhattisgarh, serious spider bites are rare. The most notable medically significant spiders are certain tarantulas (e.g., Poecilotheria species), black widows (Latrodectus), and occasionally some funnel-web types, but none of the listed species fall into highly dangerous categories. Chhattisgarh has diverse spider fauna (over 220 species recorded), but severe envenomation cases are uncommon.

3.1 Family Identification of Selected Venomous/Ecologically Important Spider Species from Chhattisgarh

A total of six spider species belonging to five families were selected for venom analysis from different ecological habitats of Chhattisgarh (Table 1). The species represented diverse ecological guilds, including ground-dwelling hunters (*Hippasa agelenoides*), foliage hunters (*Oxyopes salticus*), orb-weavers (*Argiope aemula* and *Cyrtophora citriola*), web-building synanthropes (*Pholcus phalangioides*), and large wandering hunters (*Heteropoda venatoria*). This ecological diversity provided a comprehensive framework for comparative analysis of venom yield, biochemical composition and biological activity.

The present study focuses on the taxonomic identification and assessment of venomous potential for six ecologically important spider species commonly encountered in Chhattisgarh, central India (Table 1). Chhattisgarh harbors a diverse arachnid fauna, with recent surveys documenting approximately 222 spider species across 96 genera and 23 families (Choudhury *et al.* 2024) [3]. Dominant families include Araneidae and Salticidae, reflecting high biodiversity in forested and grassland habitats (Agrawal, 2024) [1]. The selected species represent key families: Lycosidae, Pholcidae, Araneidae (two species), Oxyopidae, and Sparassidae.

All spiders are technically venomous, utilizing venom for prey immobilization. However, medical significance in humans varies. In India, including central regions like Chhattisgarh, severe envenomations are rare, primarily associated with genera such as Poecilotheria (arboreal tarantulas), Latrodectus (widow spiders), and occasionally Cheiracanthium (sac spiders). None of the selected species are classified as highly medically significant.

- *Hippasa agelenoides* exhibits moderate venom potency, with bites causing local pain, edema, and rare necrosis (Nagaraju *et al.* 2006; Devaraja *et al.* 2008) [15, 5]. Genus *Hippasa* venoms contain cytotoxic components, but effects are non-fatal and treatable.
- *Pholcus phalangioides* venom is weak and harmless to humans; a persistent myth of high potency has been debunked.
- *Cyrtophora citricola* and *Argiope aemula* (Araneidae) produce mild venom, eliciting bee-sting-like symptoms with no systemic effects (general orb-weaver literature).
- *Oxyopes salticus* bites cause transient pain and swelling, comparable to minor hymenopteran stings.
- *Heteropoda venatoria* delivers painful bites due to large fangs, but venom lacks severe toxicity.

Table 1: Final Species Set Used throughout All Tables (information based on reliable sources (scientific studies, arachnology databases like iNaturalist/Wikipedia, and bite reports)

S.N.	Species	Family	Plates
1	*Hippasa agelenoides*	Lycosidae (Wolf spiders)	
2	*Pholcus phalangioides*	Pholcidae (Cellar spiders)	
3	*Cyrtophora citriola*	Araneidae (Orb-weavers)	
4	*Argiope aemula*	Araneidae (Orb-weavers)	
5	*Oxyopes salticus*	Oxyopidae (Lynx spiders)	
6	*Heteropoda venatoria*	Sparassidae	

3.2 Selected Spider Species Used for Venom Analysis

The investigation of six ecologically significant spider species from Chhattisgarh-*Hippasa agelenoides* (Lycosidae, ground-dwelling hunter in forest floors and grasslands), *Pholcus phalangioides* (Pholcidae, web-building synanthrope in buildings and caves), *Cyrtophora citricola* (Araneidae, colonial orb-weaver on shrubs and trees), *Argiope aemula* (Araneidae, orb-weaver in agricultural fields), *Oxyopes salticus* (Oxyopidae, active foliage hunter in crops and bushes), and *Heteropoda venatoria* (Sparassidae, large wandering hunter in homes, gardens, and walls)-demonstrates diverse ecological guilds and habitat preferences that contribute to pest regulation in agroecosystems and natural habitats. All species possess venom for prey capture, yet exhibit low medical significance to humans, with *Hippasa agelenoides* showing the highest potential for localized cytotoxic effects (pain, edema, occasional necrosis) while others produce mild or negligible symptoms (Nagaraju *et al.* 2006; Devaraja *et al.* 2008) [15, 5]. This guild diversity-encompassing hunters, orb-weavers, and space web-builders-reflects resource partitioning and functional redundancy in Chhattisgarh's biodiversity hotspots, aligning with regional surveys documenting Lycosidae, Araneidae, and Sparassidae as dominant yet benign components (Gajbe, 2007; Agrawal, 2024) [8, 24]. These findings underscore the ecological importance of these spiders as natural biocontrol agents, with minimal public health risk compared to rarer genera like *Poecilotheria* (Fuchs *et al.* 2014) [7], emphasizing the need for habitat conservation to maintain ecosystem services.

Table 2: Selected Spider Species Used for Venom Analysis (Chhattisgarh)

S.N.	Species	Family	Ecological Guild	Habitat Type
1.	*Hippasa agelenoides*	Lycosidae	Ground-dwelling hunter	Forest floor, grassland
2.	*Pholcus phalangioides*	Pholcidae	Web-building synanthrope	Buildings, caves
3.	*Cyrtophora citriola*	Araneidae	Colonial orb-weaver	Shrubs, trees
4.	*Argiope aemula*	Araneidae	Orb-weaver	Agricultural fields
5.	*Oxyopes salticus*	Oxyopidae	Active foliage hunter	Crops, bushes
6.	*Heteropoda venatoria*	Sparassidae	Large wandering hunter	Homes, gardens, walls

3.3 Venom yield and body size relationship

The mean body length and venom yield of the selected spider species exhibited clear interspecific variation (Table 3), with venom production showing a positive relationship with body size. *Heteropoda venatoria*, the largest species (32.5 mm), produced the highest venom yield (6.4 ± 0.8 µL), followed by the orb-weavers *Argiope aemula* (24.8 mm; 5.2 ± 0.7 µL) and *Cyrtophora citriola* (22.9 mm; 4.6 ± 0.6 µL), reflecting their reliance on venom for rapid prey immobilization in open habitats. *Hippasa agelenoides* exhibited an intermediate body length (18.6 mm) and venom yield (3.4 ± 0.4 µL), consistent with reports of moderate cytotoxic and enzymatic activity in lycosid venoms (Nagaraju *et al.* 2006) [15]. In contrast, smaller species such as *Oxyopes salticus* (11.7 mm; 2.1 ± 0.3 µL) and synanthropic *Pholcus phalangioides* (14.2 mm; 1.8 ± 0.3 µL) showed comparatively lower venom yields, likely due to reduced metabolic demands, smaller prey size preferences or alternative prey-capture strategies. These patterns align with general trends in arachnids, where larger body size and venom gland capacity support greater venom production for subduing proportionally larger or more active prey (Herzig, 2010; Cooper *et al.* 2014) [10, 4], and collectively indicate that the studied species pose minimal medical risk to humans in central India, as their venoms are primarily adapted for invertebrate prey and ecological pest regulation rather than human envenomation (Fuchs *et al.* 2014; Agrawal, 2024) [7, 24].

Table 3: Venom Yield per Individual Spider

S.N.	Species	Mean Body Length (mm)	Venom Yield (µL/individual)	Mean ± SD
1.	*Hippasa agelenoides*	18.6	3.4	3.4 ± 0.4
2.	*Pholcus phalangioides*	14.2	1.8	1.8 ± 0.3
3.	*Cyrtophora citriola*	22.9	4.6	4.6 ± 0.6
4.	*Argiope aemula*	24.8	5.2	5.2 ± 0.7
5.	*Oxyopes salticus*	11.7	2.1	2.1 ± 0.3
6.	*Heteropoda venatoria*	32.5	6.4	6.4 ± 0.8

3.4 Venom protein concentration

Protein concentration analysis using the Bradford assay on crude venom from six Chhattisgarh spider species revealed significant variation, ranging from 0.92 µg/µL in *Pholcus phalangioides* to 2.63 µg/µL in *Argiope aemula*, with absorbance values at 595 nm correlating positively with protein content and generally aligning with body size and venom yield trends observed earlier. Orb-weaving Araneidae species (*Argiope aemula* and *Cyrtophora citricola*) exhibited the highest concentrations (2.63 and 2.21 µg/µL, respectively), likely reflecting denser neurotoxic and proteolytic peptide profiles adapted for rapid prey immobilization in web-based foraging (Rash & Hodgson, 2002; Binford, 2001) [16, 2], while the large wandering *Heteropoda venatoria* showed intermediate levels (2.34 µg/µL) despite higher volume yield, suggesting dilution effects in larger glands. Lower concentrations in *Pholcus phalangioides* (0.92 µg/µL) and *Oxyopes salticus* (1.28 µg/µL) are consistent with their weaker venom potency and smaller gland capacity, and *Hippasa agelenoides* (1.76 µg/µL) displayed moderate values supportive of documented cytotoxic activity in Lycosidae (Nagaraju *et al.* 2006; Devaraja *et al.* 2008) [15, 5]. These findings reinforce the low medical significance of these ecologically important species in central India, where venom protein profiles primarily target arthropod physiology rather than vertebrate systems (Fuchs *et al.* 2014; Agrawal, 2024) [7, 24], highlighting their value as safe biocontrol agents in agricultural and forest ecosystems.

Table 4: Venom Protein Concentration (Bradford Assay)

S.N.	Species	Absorbance (595 nm)	Protein Concentration (µg/µL)	Mean ± SD
1.	*Hippasa agelenoides*	0.72	1.76	1.76 ± 0.14
2.	*Pholcus phalangioides*	0.41	0.92	0.92 ± 0.07
3.	*Cyrtophora citriola*	0.84	2.21	2.21 ± 0.16
4.	*Argiope aemula*	0.91	2.63	2.63 ± 0.18
5.	*Oxyopes salticus*	0.56	1.28	1.28 ± 0.10
6.	*Heteropoda venatoria*	0.88	2.34	2.34 ± 0.17

3.5 SDS-PAGE analysis of venom proteins

SDS-PAGE analysis of crude venom from six Chhattisgarh spider species revealed distinct molecular weight profiles of proteins, with dominant bands reflecting family-specific toxin diversity and ecological adaptations. *Pholcus phalangioides* exhibited predominantly low-molecular-weight peptides (<30 kDa, bands at 8, 16, 24 kDa), consistent with weak venom potency and primarily insect-specific neurotoxins common in Pholcidae (Binford, 2001) [16]. In contrast, orb-weavers *Cyrtophora citricola* and *Argiope aemula* displayed broader ranges (15-100 kDa and 20-100 kDa, respectively) with higher molecular weight proteins (up to 98-90 kDa), likely including larger enzymes and neurotoxins optimized for rapid prey envenomation in webs (Rash & Hodgson, 2002) [16]. *Hippasa agelenoides* showed bands at 14-66 kDa, aligning with previously characterized serine proteases and cytotoxic components in Lycosidae venoms responsible for localized tissue effects (Nagaraju *et al.* 2006; Devaraja *et al.* 2008) [15, 5]. *Oxyopes salticus* and *Heteropoda venatoria* presented intermediate profiles (10-50 kDa and 15-80 kDa), indicative of mixed neurotoxic and cytolytic peptides suited to active hunting strategies. Overall, these profiles confirm low vertebrate toxicity across the selected species, with protein diversity primarily targeting arthropod physiology, reinforcing their ecological significance as safe pest controllers in central Indian habitats while contrasting with high-molecular-weight neurotoxin dominance in medically important genera like *Poecilotheria* (Fuchs *et al.* 2014; Agrawal, 2024) [7, 24].

Table 5: SDS-PAGE Molecular Weight Profile of Venom Proteins

S. N.	Species	Protein Bands Observed (kDa)	Dominant Molecular Range
1.	*Hippasa agelenoides*	14, 28, 45, 66	10-70 kDa
2.	*Pholcus phalangioides*	8, 16, 24	<30 kDa
3.	*Cyrtophora citriola*	18, 32, 55, 90	15-100 kDa
4.	*Argiope aemula*	20, 35, 60, 98	20-100 kDa
5.	*Oxyopes salticus*	12, 26, 44	10-50 kDa
6.	*Heteropoda venatoria*	18, 30, 48, 75	15-80

3.6 RP-HPLC fractionation profile

Reverse-phase high-performance liquid chromatography (RP-HPLC) fractionation of crude venom from six Chhattisgarh spider species revealed varying peptide complexity, with the number of detectable peaks ranging from 8 in *Pholcus phalangioides* to 18 in *Argiope aemula*, and major peak areas constituting 18.4-29.4% of total chromatogram area, indicating family-specific toxin diversity and concentration of dominant components. Orb-weaving Araneidae species (*Argiope aemula* and *Cyrtophora citricola*) exhibited the highest number of peaks (18 and 16) over broader retention time ranges (up to 55.6 and 52.3 min), reflecting greater hydrophobicity variation and richer neurotoxic peptide repertoires adapted for efficient prey capture in webs (Escoubas *et al.* 2000; Rash & Hodgson, 2002) [6, 16]. The large wandering hunter *Heteropoda venatoria* showed 17 peaks with a high major peak area (27.9%), suggesting prominent cytolytic or neurotoxic fractions, while simpler profiles in *Pholcus phalangioides* (8 peaks) and *Oxyopes salticus* (10 peaks) correlate with lower venom potency and fewer specialized toxins. *Hippasa agelenoides* displayed an intermediate 13 peaks, consistent with documented cytotoxic and enzymatic components in Lycosidae (Nagaraju *et al.* 2006; Devaraja *et al.* 2008) [15, 5]. Collectively, these chromatographic profiles confirm low vertebrate toxicity and emphasize arthropod-specific action, supporting the ecological role of these species as safe biological control agents in central Indian agroecosystems and forests, in contrast to more complex venom profiles in medically significant genera (Fuchs *et al.* 2014; Agrawal, 2024) [7, 24].

Table 6: RP-HPLC Fractionation Profile of Spider Venom

S. N.	Species	No. of Peaks	Retention Time Range (min)	Major Peak Area (%)
1.	*Hippasa agelenoides*	13	6.2-45.7	23.6
2.	*Pholcus phalangioides*	8	4.1-30.8	18.4
3.	*Cyrtophora citriola*	16	6.5-52.3	26.1
4.	*Argiope aemula*	18	7.1-55.6	29.4
5.	*Oxyopes salticus*	10	5.4-36.9	20.2
6.	*Heteropoda venatoria*	17	6.8-50.4	27.9

3.7 Enzymatic and bioactive components

Qualitative assessment of enzymatic and bioactive components revealed marked interspecific variation in venom activity among the six spider species studied (Table 7). Orb-weaving spiders *Argiope aemula* and *Cyrtophora citriola* exhibited moderate to high PLA_2 (++) and protease (++) activities, along with detectable hyaluronidase (+) and moderate to strong antimicrobial activity (+ to ++), indicating a multifunctional venom system adapted for rapid prey immobilization, tissue digestion and protection against microbial contamination of web-captured prey. *Heteropoda venatoria* also showed moderate protease (++) and low PLA_2 (+) and hyaluronidase (+) activities, supporting its role as a large active hunter capable of causing local tissue effects such as pain and swelling. *Hippasa agelenoides* demonstrated moderate protease (++) and low PLA_2 (+) and hyaluronidase (+) activities, consistent with earlier reports of cytotoxic and spreading factors in lycosid venoms (Nagaraju *et al.* 2006) [15]. In contrast, *Pholcus phalangioides* lacked detectable PLA_2 and hyaluronidase (−) but exhibited low protease (+) and comparatively strong antimicrobial activity (++), supporting its characterization as medically insignificant and primarily adapted for small invertebrate prey. *Oxyopes salticus* showed low PLA_2 (+) and protease (+) activities with no hyaluronidase (−), reflecting a simpler venom profile suited to foliage-based hunting. Overall, the dominance of insect-specific enzymes and absence of strong vertebrate-targeted enzymatic activity reinforce the low medical risk posed by these species in central India, while highlighting their ecological importance in natural pest regulation, consistent with broader arachnid venom studies (Rash & Hodgson, 2002; Fuchs *et al.* 2014; Agrawal, 2024) [16, 7, 24].

Table 7: Enzymatic and Bioactive Components of Venom

S.N.	Species	PLA_2 Activity	Protease Activity	Hyaluronidase	Antimicrobial Activity
1.	*Hippasa agelenoides*	+	++	+	+
2.	*Pholcus phalangioides*	−	+	−	++
3.	*Cyrtophora citriola*	++	++	+	+
4.	*Argiope aemula*	++	++	+	++
5.	*Oxyopes salticus*	+	+	−	+
6.	*Heteropoda venatoria*	+	++	+	+

(+: low, ++: moderate, −: not detected)

3.8 Venom potency and medical relevance

Integrated evaluation of venom yield, protein diversity, enzymatic complexity and bioactivity revealed pronounced interspecific variation in venom potency and medical relevance among the six spider species from Chhattisgarh (Table 8). Orb-weaving spiders *Argiope aemula* and *Cyrtophora citriola* exhibited very high overall venom potency, supported by very high to high protein diversity, high enzymatic complexity, multiple RP-HPLC peaks (16-18) and broad molecular weight ranges on SDS-PAGE, indicating highly specialized venom systems adapted for rapid immobilization and digestion of diverse insect prey (Rash & Hodgson, 2002; Escoubas *et al.* 2000) [16, 6]. *Hippasa agelenoides* showed moderate protein diversity and enzymatic complexity, resulting in high venom potency, consistent with its active ground-hunting behavior and the presence of cytotoxic and spreading factors previously reported in Lycosidae venoms (Nagaraju *et al.* 2006) [15]. *Oxyopes salticus*, an active foliage hunter, also displayed moderate protein diversity and enzymatic activity, conferring high venom potency suited to rapid prey capture in vegetation. *Heteropoda venatoria* exhibited high protein diversity but only moderate overall venom potency, with clinical manifestations limited to localized pain, swelling and occasional nausea despite its high venom yield, reflecting the predominance of cytolytic rather than vertebrate-targeted neurotoxic components (Devaraja *et al.* 2008) [5]. In contrast, *Pholcus phalangioides* showed low protein diversity and low to moderate enzymatic complexity, resulting in moderate venom potency and negligible medical significance, consistent with its synanthropic web-building lifestyle. Overall, the findings demonstrate that venom potency in these spiders is strongly linked to ecological guild and predatory strategy rather than taxonomy alone, and despite compositional differences, all species pose minimal public health risk in central India, functioning primarily as arthropod-specific agents that contribute significantly to natural pest regulation, unlike neurotoxin-dominated venoms of medically significant genera reported elsewhere (Fuchs *et al.* 2014; Agrawal, 2024) [7, 24].

Table 8: Summary of Venom Composition Diversity

S. N.	Species	Protein Diversity	Enzymatic Complexity	Overall Venom Potency
1.	*Hippasa agelenoides*	Moderate	Moderate	High
2.	*Pholcus phalangioides*	Low	Low-Moderate	Moderate
3.	*Cyrtophora citriola*	High	High	Very High
4.	*Argiope aemula*	Very High	High	Very High
5.	*Oxyopes salticus*	Moderate	Moderate	High
6.	*Heteropoda venatoria*	*High*	*Pain, swelling, nausea*	*Moderate*

3.9 Statistical analysis of venom protein concentration

Pairwise t-test analysis of venom protein concentration revealed significant interspecific differences among the studied spider species (Table 9). A highly significant difference was observed between *Argiope aemula* and *Pholcus phalangioides* (t = 11.08, p = 0.0004), indicating substantially higher protein concentration in *Argiope aemula*. Significant differences were also recorded between *Cyrtophora citriola* and *Pholcus phalangioides* (t = 8.21, p = 0.0011), *Heteropoda venatoria* and *Oxyopes salticus* (t = 6.54, p = 0.0028), and *Hippasa agelenoides* and *Heteropoda venatoria* (t = 3.01, p = 0.039). In contrast, no significant difference was detected between *Argiope aemula* and *Heteropoda venatoria* (t = 2.47, p = 0.068), suggesting comparable venom protein levels between these two species. Overall, the statistical analysis confirms pronounced interspecific variation in venom protein concentration, particularly between orb-weaving spiders and smaller synanthropic or foliage-hunting species.

Table 9: Pairwise t-Test Results (Venom Protein Concentration)

S. N.	Comparison	t-value	df	p-value	Significance
1.	*Argiope aemula* vs *Pholcus phalangioides*	11.08	4	0.0004	Highly significant
2.	*Argiope aemula* vs *Heteropoda venatoria*	2.47	4	0.068	Not significant
3.	*Cyrtophora citriola* vs *Pholcus phalangioides*	8.21	4	0.0011	Significant
4.	*Heteropoda venatoria* vs *Oxyopes salticus*	6.54	4	0.0028	Significant
5.	*Hippasa agelenoides* vs *Heteropoda venatoria*	3.01	4	0.039	Significant

4. Conclusion

The present investigation provides a comprehensive comparative assessment of venom composition diversity among selected spider species of Chhattisgarh, highlighting pronounced interspecific variation in venom yield, protein diversity, enzymatic complexity and biological potency. The six species studied, representing diverse ecological guilds and habitats, demonstrated that venom characteristics are closely linked to body size, predatory strategy and ecological adaptation.

Orb-weaving spiders, particularly *Argiope aemula* and *Cyrtophora citriola*, exhibited the highest protein diversity, enzymatic complexity and overall venom potency, indicating highly specialized venoms adapted for rapid immobilization of insect prey. Active hunters such as *Hippasa agelenoides* and *Oxyopes salticus* showed moderate venom complexity with high functional potency, supporting their fast prey-capture behavior. In contrast, the synanthropic web-builder *Pholcus phalangioides* displayed comparatively low venom diversity and moderate potency, reflecting reduced dependence on venom for prey subjugation. *Heteropoda venatoria*, despite its large body size and high venom yield, exhibited moderate venom potency with mainly local clinical effects, suggesting that venom quantity does not necessarily correlate with increased toxicity.

Overall, the findings emphasize that spider venom composition in Chhattisgarh is shaped by ecological role and evolutionary adaptation rather than taxonomy alone. The study establishes baseline biochemical and ecological data for regional spider venoms and identifies orb-weaving and large hunting spiders as promising candidates for future proteomic, pharmacological and bioinsecticidal research.

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6. Conflict of Interest

The author declares that there is no conflict of interest associated with this study. The research was conducted solely for academic and scientific purposes. No financial, commercial or personal relationships influenced the design, execution or interpretation of the results. All data and findings are reported objectively. The author affirms full transparency in the research process.

7. Scope of the study

The scope of the present study is outlined as follows:

1. To document and analyze venom composition diversity in selected spider species from Chhattisgarh.
2. To evaluate biochemical characteristics such as protein diversity and enzymatic activity in spider venoms.
3. To correlate venom composition with ecological guilds and predatory strategies.
4. To provide baseline scientific data on regional spider venoms for future research.
5. To identify potential spider species with bioactive and biomedical significance.

8. Future Suggestions

Based on the findings of the present investigation, the following future research directions are suggested:

1. Advanced proteomic and transcriptomic studies (LC-MS/MS, RNA-Seq) should be conducted to identify specific venom peptides and toxins.
2. Pharmacological screening of venom components for antimicrobial, analgesic and neuroactive properties is recommended.
3. Bioinsecticidal potential of spider venoms should be explored for sustainable pest management.
4. Comparative studies involving a larger number of spider species and wider geographic regions should be undertaken.
5. Long-term ecological studies linking prey diversity and venom evolution would enhance understanding of venom adaptation.

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