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RESEARCH ARTICLE

Exploring *Apis mellifera* L. Venom's Antioxidant Power in Various Solvents: Unveiling its *In Vitro* Potential

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ABSTRACT

Apis mellifera L. venom contains bioactive components with antioxidant properties. Diluted in various polar solvents, the venom is utilized for therapeutic purposes. This study aims to determine the *in vitro* antioxidant activities (AOA) of standard crude venom (SV) and venom from breeders (BV) by dissolving them in distilled water, saline, and PBS at concentrations of 1.95-500 µg.ml^{-1}. Radical scavenging activity (DPPH) and metal chelating activity (MCA) assays were employed for AOA assessment. SV dissolved in distilled water exhibited higher RSA (73.26±11.24%) than BV (34.60±21.08%), with no difference between SV, ascorbic acid (AA), and Trolox RSA's. BV's RSA was lower than AA (75.07±15.59%) and Trolox (84.02±1.63%). BV's MCA (30.31±24.06%) exceeded AA (8.93±16.08%). SV in saline showed higher RSA (63.83±9.73%) than BV (46.99±18.31%), lower than AA (71.63±4.14%) and Trolox (79.01±6.94%). MCAs of SV (85.42±4.65%) and BV (85.53±7.19%) surpassed Trolox (55.06±30.92%). No difference existed between RSA's of SV (37.16±16.54%) and BV (38.47±17.24%) in PBS, both lower than AA (71.48±3.66%) and Trolox (72.87±6.05%). Optimal RSA and MCA were observed at different solvents and concentrations, indicating the use of 500 µg.ml^{-1} (1.95 µg.ml^{-1} BV for RSA) venom dissolved in saline for optimal AOA. PBS or distilled water usage resulted in decreased AOA.

Keywords: Antioxidant activity, Apitoxin, Bee venom, Metal chelating activity, Radical scavenging activity, Venom solubility

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Apis mellifera L. Zehirinin Çeşitli Solventlerdeki Antioksidan Gücü: *In Vitro* Potansiyelin Ortaya Çıkarılması

ÖZ

Apis mellifera L. zehiri, antioksidan özelliklere sahip biyoaktif bileşenler içermektedir. Çeşitli polar çözücülerde seyreltilen zehir, terapötik amaçlar için kullanılmaktadır. Bu çalışma, standart ham zehir (SV) ve üreticiden temin edilen zehir (BV) örneklerinin *in vitro* antioksidan aktivitelerini (AOA) belirlemeyi amaçlamaktadır. Bu amaçla örnekler, 1.95-500 µg.ml^{-1} konsantrasyon aralığında distile su, fizyolojik tuzlu su ve PBS içinde çözülmüştür. Antioksidan aktivitelerin değerlendirilmesi için serbest radikal giderme aktivitesi (DPPH) ve metal şelasyon aktivitesi (MCA) analizleri kullanılmıştır. SV, distile suda çözündüğünde BV'ye göre daha yüksek RSA (73.26±11.24%) sergilemiş, SV, askorbik asit (AA) ve Trolox RSA'ları arasında fark bulunmamıştır. BV'nin RSA'sı, AA (75.07±15.59%) ve Trolox (84.02±1.63%) RSA'larından düşük bulunmuştur. BV'nin MCA'sı (30.31±24.06%), AA (8.93±16.08%) değerini aşmıştır. SV, tuzlu su içinde çözüldüğünde BV'ye göre daha yüksek RSA (63.83±9.73%) sergilemiş, AA (71.63±4.14%) ve Trolox (79.01±6.94%) RSA'larından düşük bulunmuştur. SV (85.42±4.65%) ve BV (85.53±7.19%) örneklerinin MCA değerleri, Trolox (55.06±30.92%) değerini aşmıştır. SV (37.16±16.54%) ve BV (38.47±17.24%) örneklerinin PBS içindeki RSA değerleri arasında fark bulunmamış, her ikisi de AA (71.48±3.66%) ve Trolox (72.87±6.05%) değerlerinin altında kalmıştır. Optimal RSA ve MCA değerleri farklı çözücü ve konsantrasyonlarda gözlemlenmiş, bu durum 500 µg.ml^{-1} (BV için RSA'da 1.95 µg.ml^{-1}) konsantrasyonda fizyolojik tuzlu su içinde çözünen zehirin optimal AOA için kullanılmasına işaret etmektedir. PBS veya distile su kullanımı ise AOA değerlerinde azalmaya neden olmuştur.

Anahtar kelimeler: Antioksidan aktivite, Apitoksin, Arı zehiri, Metal şelasyon aktivitesi, Serbest radikal giderme aktivitesi, Zehir çözünürlüğü

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INTRODUCTION

The European honeybee, *Apis mellifera* L., stands as a focal point of extensive research, specifically within the realm of apitherapy, and holds the distinction of being the most investigated subspecies. Renowned for its utilization in apitherapeutic products, this species has a longstanding tradition of being harnessed for its diverse array of healing elements, including honey, pollen, propolis, royal jelly, and venom (Eze et al., 2016; Şenel and Demir 2018). These natural substances have been employed for therapeutic purposes for millennia, rooted in empirical knowledge, and have transcended into contemporary practice under the guidance of certain clinicians. Notably, applications targeting chronic inflammatory ailments such as arthritis have been prevalent. Moreover, the attention garnered by the species extends to its applications in antimicrobial, anti-inflammatory, anti-cancer, and wound healing contexts (Han et al. 2012; Gupta and Stangaciu 2014).

Within the diverse matrix of bee products, the venom of the European honeybee has emerged with heightened prominence, propelled by its biologically active constituents, particularly peptides (Mehdi et al. 2022). The increasing recognition of these bioactive components underscores their potential in the treatment of various ailments (Zhang et al. 2018). However, the transformative journey from traditional bee-product therapies to their acceptance within evidence-based medicine hinges upon the establishment of rigorous scientific foundations and empirical validation of venom's role in disease treatment and prevention (Hwang et al. 2015; Denk and Fidan 2021).

This study bridges this gap by delving into the *in vitro* antioxidant activity (AOA) of *Apis mellifera* L. venom. We explore the optimal AOA attributes of the venom using three commonly employed solvents—distilled water, physiological saline, and phosphate-buffered saline (PBS). These solvents were chosen due to their prevalence in scientific research.

Within the context of this study, we conduct a comparative statistical analysis of the antioxidant capacity of venom samples sourced from standard *Apis mellifera* L. venom (SV) and venom obtained directly from beekeepers (BV), employing well-established *in vitro* AOA markers such as the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay and metal chelating activity (MCA) assay. The investigation further encompasses a diverse range of venom concentrations, from 1.95 to 500 $\mu g.ml^{-1}$, for each solvent, including both venom samples and standard preparations. The outcomes are meticulously analyzed and compared, shedding light on the venom's potential antioxidant properties.

By unraveling the intricate properties of *Apis mellifera* L. venom and investigating its antioxidant potential, this study takes strides towards advancing our comprehension of the therapeutic prowess of bee-derived substances. Furthermore, it endeavors to provide the scientific substantiation needed to foster the integration of venom into evidence-based medical practices for disease management and prevention.

MATERIAL and METHODS

Venom Acquisition and Preparation:

The SV was obtained as a commercially available HPLC-grade preparation from Sigma (Sigma-Aldrich, Darmstadt, Germany). The BV was sourced from a local beekeeper engaged in beekeeping activities in the İzmir/Foça region (38.6704° N, 26.7579° E). Venom collection from the beekeeper was carried out mid-April 2021 using a venom collection device. Both SV

and BV were stored in crude and powdered forms at -80 °C until further use.

Stock Solution Preparation:

To create stock solutions, 5 mg of each venom was weighed and dissolved in distilled water, physiological saline (0.9% NaCl solution), and PBS (pH:7.4). Subsequently, 8 dilution solutions were prepared from each stock solution at a 1:2 ratio, yielding concentrations ranging from 1.95 to 500 $\mu g.ml^{-1}$. The same dilution methodology was applied to prepare dilution solutions of two standard antioxidants, ascorbic acid (AA) and polar vitamin E analogue ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid; Trolox), which are commonly used for AOA measurements.

Antioxidant Activity Analysis:

The study encompassed the comparative analysis of AOA in SV and BV using DPPH assay and MCA assay. AOA measurements were also conducted for AA and Trolox as reference antioxidants. The experimental design aimed to elucidate the potential variations in antioxidant capacities across different venom samples and reference compounds.

The DPPH assay is a widely employed method for assessing antioxidant activity. DPPH is a stable free radical molecule at room temperature, displaying a characteristic purple color in the solution. It serves as an electron acceptor, reflecting its capacity to capture free electrons from suitable antioxidants (Munteanu and Apetrei 2021). The principle underlying the DPPH assay lies in the conversion of DPPH to its reduced form, DPPH-H, through the transfer of a hydrogen ion (H^+) from antioxidants possessing hydrogen-donating (H-donating) capabilities. This reduction process leads to a color change in the solution from deep purple to a lighter shade of yellow, a reaction that can be quantified spectrophotometrically at 520 nm. The degree of color change inversely correlates with the concentration of the reduced DPPH radical, forming the basis for evaluating the scavenging potential of antioxidants (Pinto et al. 2021).

To perform the DPPH assay, a DPPH working solution is prepared with an optical density of 0.968 at 520 nm. The procedure is carried out in a dark and standard room temperature environment. For each sample (SV, BV, AA, Trolox), 50 µl of the sample is mixed with 450 µl of the DPPH working solution. The samples are then incubated for 30 minutes to allow the interaction between antioxidants and DPPH. As a control solution, DPPH alone is used. After the incubation period, the absorbance of all solutions, including the control, is measured at 520 nm using a spectrophotometer (Shimadzu Corp. Kyoto, Japan). The term radical scavenging activity (RSA) is used in the context of DPPH assay to describe the ability of an antioxidant to neutralize or scavenge free radicals present in the solution (Dontha 2016; Pinto et al. 2021).

The RSA percentage, indicative of the antioxidant efficacy, is calculated using the following formula:

$$RSA(\%)=[(Abs_{control}-Abs_{sample})\times(Abs_{control})^{-1}]\times 100$$

Where:

$Abs_{control}$ represents the absorbance of the control solution (containing only DPPH),

Abs_{sample} represents the absorbance of the sample solution (containing DPPH, the SV, the BV, and the standard antioxidant dilutions).

Antioxidants play a crucial role in terminating or delaying oxidative processes by chelating catalytic metal ions (Dontha 2016). Due to the functional groups capable of metal binding, antioxidants have been reported to exhibit effective iron-chelating capabilities (Gulcin and Alwasel 2022).

The MCA assay involves the competition between antioxidants and 3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine-p,p'-disulfonic acid monosodium salt hydrate (FerroZine™, Sigma-Aldrich, Darmstadt, Germany) for binding with Fe^{2+} ions. This assay sheds light on the capacity of antioxidants to form complexes with metal ions, inhibiting their participation in oxidative reactions. The principle behind this assay centers on the fact that as antioxidants vie for binding to Fe^{2+} ions, a decrease occurs in the formation of a reddish-colored complex, resulting in a measurable change in absorbance (Dontha 2016; Gulcin and Alwasel 2022).

To conduct the MCA assay, 100 µl of each sample (SV, BV, AA, Trolox, EDTA) is mixed with 50 µl of a 2 mM $FeCl_2.4H_2O$ solution. The mixture is incubated at room temperature for 5 minutes. Following this incubation, 100 µl of a 5 mM FerroZine solution is added to the mixture. The final volume is adjusted to 3 ml using distilled water. The mixture is then incubated for an additional 10 minutes at room temperature. Subsequently, the absorbance of the solution is measured at 562 nm using a spectrophotometer (Dontha 2016; Gulcin and Alwasel 2022).

The MCA is quantified as the percentage of metal-chelating activity, which can be calculated using the formula:

$$MCA(\%)=[(Abs_{control}-Abs_{sample})\times(Abs_{control})^{-1}]\times 100$$

Where:

$Abs_{control}$ represents the absorbance of the control solution (containing only Fe^{2+} and FerroZine),

Abs_{sample} signifies the absorbance of the sample solution (containing the SV, the BV, and the standard antioxidant dilutions).

Statistical Analysis:

After verifying the fulfillment of the normality assumption through both the Shapiro-Wilk and Kolmogorov-Smirnov tests, we conducted a two-tailed unpaired t-test on the dataset. This approach enabled us to evaluate the statistical significance of distinctions between the two groups while confirming the conditions required for parametric testing. We employed the two-tailed unpaired t-test method, executed via the SPSS program (v20, IBM Corp., New York, United States), to assess the statistical significance among different samples. To support our analysis, we calculated the group means and standard deviations.

RESULTS

The findings related to the RSA assay are presented in Figure 1, Figure 2, and Figure 3.

DPPH Assay Findings for Standard Venom:

The SV exhibited a remarkable RSA with the highest percentage recorded at 91.63%. The solvent and concentration combination that yielded the most effective antioxidative response was distilled water at 500 $\mu g.ml^{-1}$, indicating optimal solubility. Conversely, the lowest RSA percentage of 3.10% was observed, attributed to the solvent and concentration combination of PBS at 7.81 $\mu g.ml^{-1}$.

DPPH Assay Findings for Venom from Beekeeper:

The venom obtained from the beekeeper demonstrated a noteworthy RSA percentage of 73.86%.The solvent and concentration combination that resulted in the highest RSA was distilled water at 1.95 $\mu g.ml^{-1}$, reflecting optimal solubility and potent radical scavenging ability. Conversely, the lowest RSA value of 15.50% was observed for the solvent and concentration combination of PBS at 250 $\mu g.ml^{-1}$.

The RSA percentages for Different Solvents:

The percentages of RSA for various solvents are presented in Table 1. When comparing the average RSA percentages of venom dilutions in distilled water, it was observed that the RSA of SV dissolved in this solvent was higher than that of BV ($p<0.001$). No significant difference was noted between the RSA of SV and the RSA of AA and Trolox ($p<0.05$). However, the RSA of BV was notably lower than that of both AA and Trolox ($p<0.001$).

In the case of venom dissolved in physiological saline solution, the RSA of SV was observed to be higher than that of BV ($p<0.05$). However, both SV ($p<0.05$) and BV ($p<0.05$, compared to AA; $p<0.001$, compared to Trolox) exhibited lower RSA values compared to AA and Trolox.

For venom dissolved in PBS, there was no significant difference between the RSA of SV and BV ($p<0.05$). However, the RSA values for both venoms were lower than those of AA and Trolox ($p<0.001$).

The findings related to the MCA assay are presented in Figure 4, Figure 5, and Figure 6.

MCA Assay Findings for Standard Venom:

In the MCA assay conducted on the SV, the highest MCA percentage recorded was 91.63%. The solvent and concentration combination that exhibited the highest MCA was saline at 500 $\mu g.ml^{-1}$. On the other hand, the lowest MCA percentage of 1.04% was observed. This limited activity was linked to the solvent and concentration combination of distilled water at 15.63 $\mu g.ml^{-1}$.

MCA Assay Findings for Venom from Beekeeper:

The highest MCA percentage was recorded at 93.63%. The solvent and concentration combination that resulted in the highest MCA was similar to the SV, saline at 500 $\mu g.ml^{-1}$. However, the lowest MCA percentage of 8.67% was observed, attributed to the solvent and concentration combination of distilled water at 15.63 $\mu g.ml^{-1}$.

The MCA Percentages for Different Solvents:

The percentages of MCA for different solvents are displayed in Table 2. When comparing the average MCA percentages of venom dilutions in distilled water, distinct observations were made. The MCA of SV dissolved in distilled water did not exhibit significant statistical differences from the MCA of BV ($p<0.05$). However, the MCA of both venoms was notably lower than that of Trolox ($p<0.05$). Furthermore, the MCA of BV surpassed that of AA ($p<0.05$).

For venoms dissolved in physiological saline solution, no significant statistical differences were observed in the MCA between SV and BV ($p<0.05$). Notably, the MCA values of both venoms exceeded that of Trolox ($p<0.05$).

When venom and standards were dissolved in PBS, no significant statistical differences were found among the MCA values ($p<0.05$).

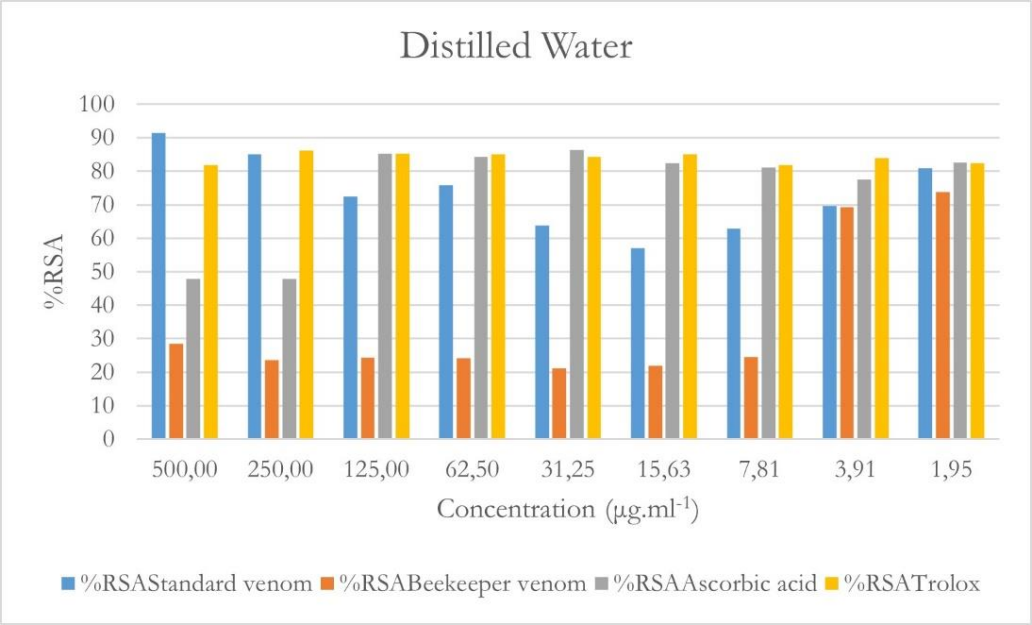

Figure 1. Concentration-RSA Percentage Graph of Venom Samples and Standard Antioxidants diluted in Distilled Water

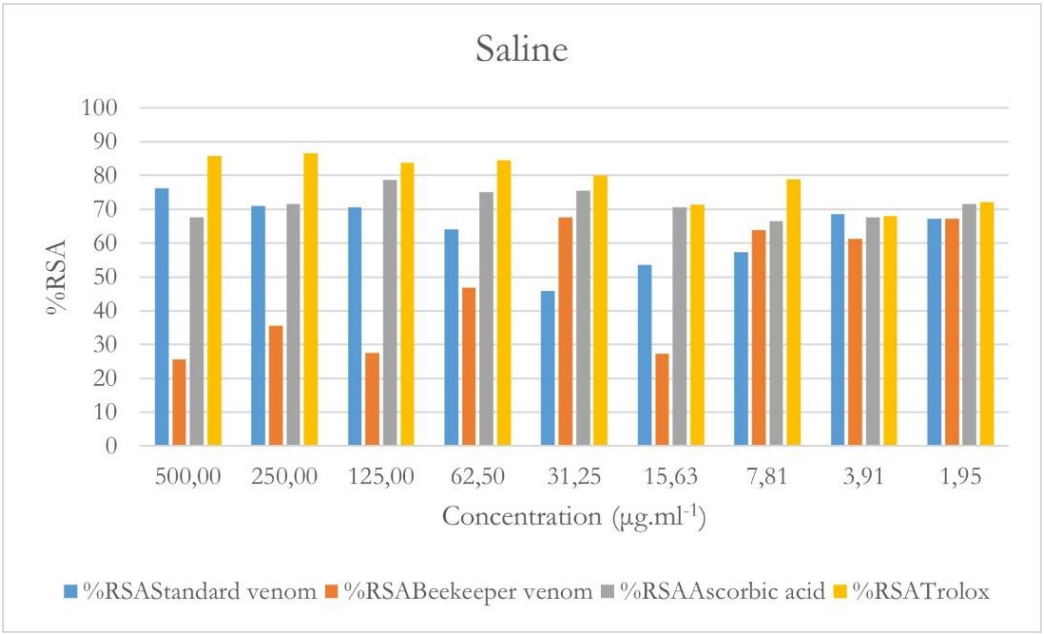

Figure 2. Concentration-RSA Percentage Graph of Venom Samples and Standard Antioxidants diluted in Saline

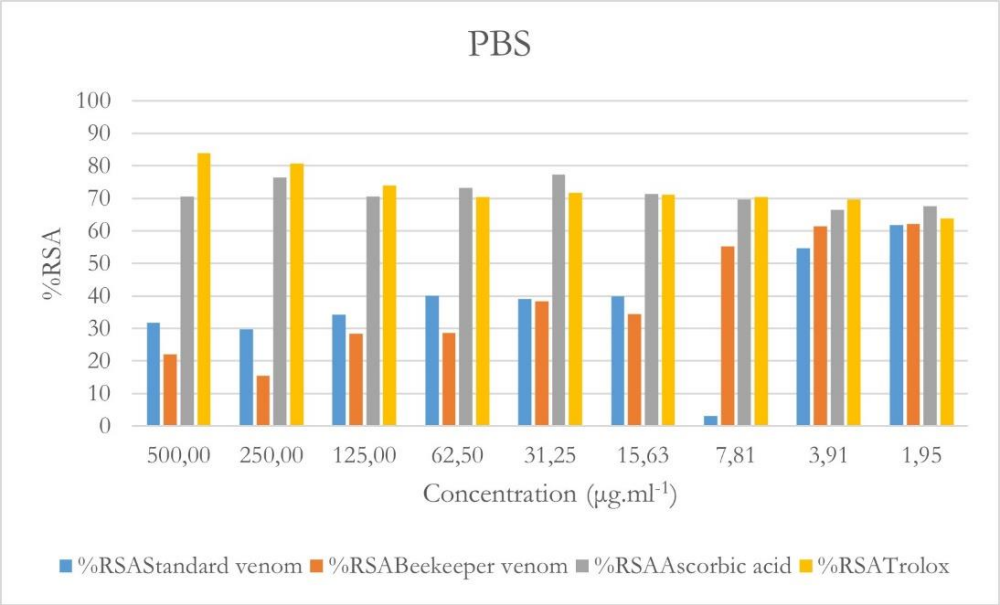

Figure 3. Concentration-RSA Percentage Graph of Venom Samples and Standard Antioxidants diluted in PBS

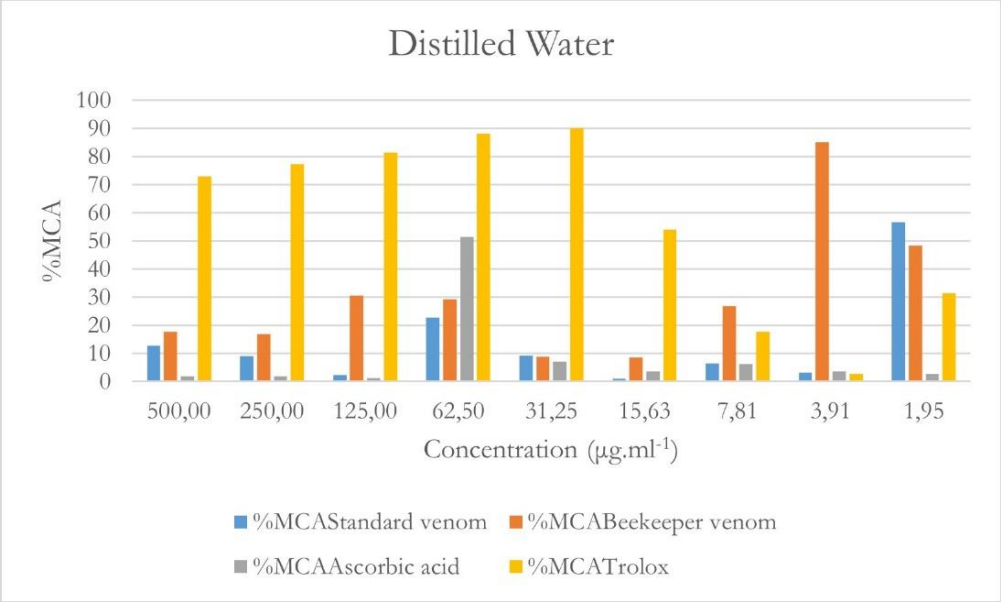

Figure 4. Concentration-MCA Percentage Graph of Venom Samples and Standard Antioxidants diluted in Distilled Water

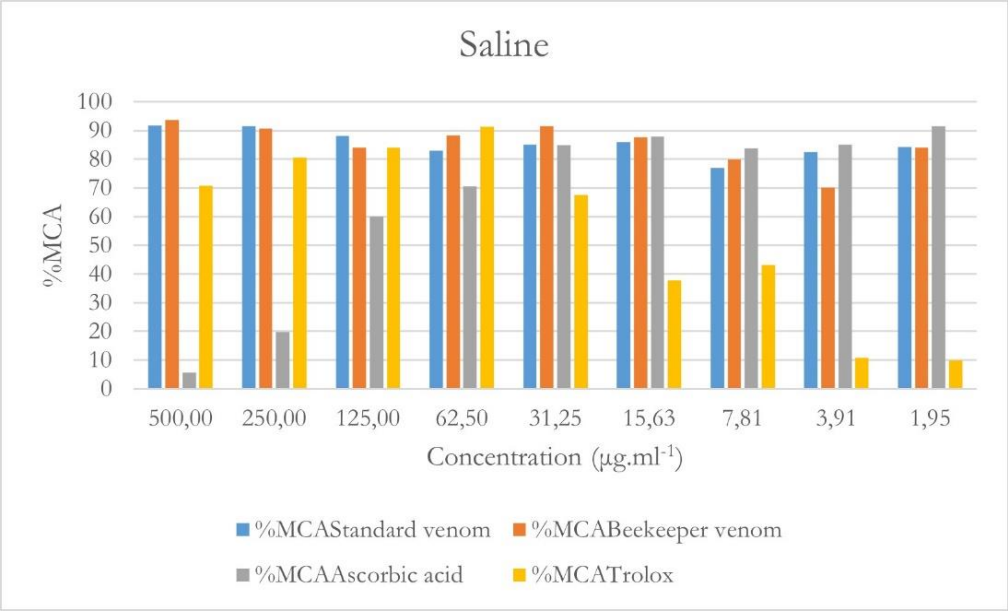

Figure 5. Concentration-MCA Percentage Graph of Venom Samples and Standard Antioxidants diluted in Saline

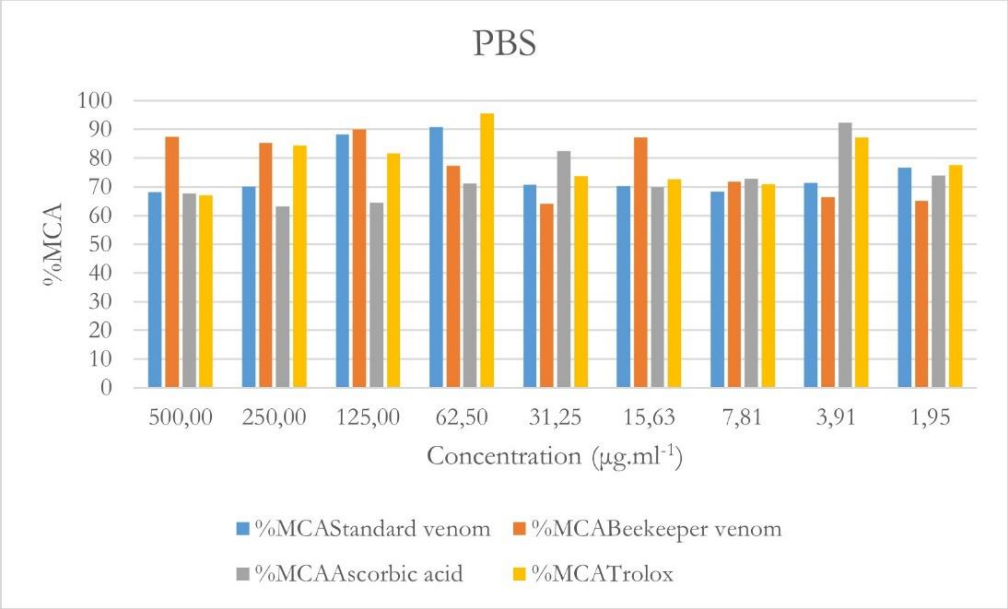

Figure 6. Concentration-MCA Percentage Graph of Venom Samples and Standard Antioxidants diluted in PBS

Table 1. Average RSA percentages of venom dilutions and standard antioxidants in different solvents

	Distilled water	**Saline**	**PBS**
Standard venom (SV)	73.26±11.24[a]	63.83±9.73[a]	37.16±16.54[a]
Beekeeper venom (BV)	34.60±21.08[b]	46.99±18.31[b]	38.47±17.24[a]
Ascorbic acid (AA)	75.07±15.59[a]	71.63±4.14[c]	71.48±3.66[b]
Trolox	84.02±1.63[a]	79.01±6.94[c]	72.87±6.05[b]

The letters "a", "b", and "c" in the respective columns of the table represent significant statistical differences between groups ($p<0.05$).

Table 2. Average MCA percentages of venom dilutions and standard antioxidants in different solvents

	Distilled water	Saline	PBS
Standard venom (SV)	13.75±17.36[a, b]	85.42±4.65[a]	74.95±8.65
Beekeeper venom (BV)	30.31±24.06[a]	85.53±7.19[a]	77.16±10.58
Ascorbic acid (AA)	8.93±16.08[b]	65.45±31.67[a, b]	73.09±9.22
Trolox	57.35±32.55[c]	55.06±30.92[b]	78.98±9.05

The letters "a", "b", and "c" in the respective columns of the table represent significant statistical differences between groups ($p<0.05$).

DISCUSSION

Bee products are chemically complex, and the dissolution using solvents of varying polarity can influence the composition of the analyzed extract. Hydrophilic components tend to dissolve more readily in polar solvents such as alcohols, while hydrophobic ones, like hydrocarbons, show a greater affinity for non-polar solvents. This phenomenon has been observed in various studies (Martinello and Mutinelli 2021). The components of venom also possess hydrophilic and hydrophobic characteristics; certain enzymes like melittin, apamin, and phospholipase A2 are recognized as amphipathic polycationic peptides (Lee et al. 2016). Hence, variations in AOA based on the solvent medium are likely to be observed.

In our study, the DPPH assay results provided insights into the distinctive antioxidant capacities of the SV and BV. The SV exhibited a noteworthy RSA with the highest percentage, revealing its potent antioxidant capability. Conversely, the lowest RSA percentage was attributed to the solvent and concentration combination of PBS at 7.81 $\mu g.ml^{-1}$, indicative of reduced solubility and a corresponding reduction in antioxidant potential. Similarly, BV demonstrated a significant RSA percentage, underscoring its substantial antioxidant effectiveness. The lowest RSA value observed for BV was linked to the solvent and concentration combination of PBS at 250 $\mu g.ml^{-1}$, suggesting compromised solubility and diminished radical scavenging potential within this specific context.

In line with our findings, a study by Frangieh et al. (2019) evaluated AOA using DPPH assay on *Apis mellifera syriaca* crude venom. They demonstrated dose-dependent AOA of the venom, with a lower RSA compared to ascorbic acid, their standard antioxidant (Frangieh et al. 2019). Similarly, Sobral et al. (2016) employed DPPH assay to assess AOA on five bee venom samples obtained from *Apis mellifera iberiensis*. They found comparable antioxidant effects of diluted venom in distilled water, along with AA and Trolox, standard antioxidants (Sobral et al. 2016). Our results aligned with these studies, as we observed a similar pattern of RSA for SV diluted in distilled water across the range of 15.63-500 $\mu g.ml^{-1}$, showing antioxidant effects statistically indistinguishable ($p<0.05$) from AA and Trolox, though this trend was not evident in BV. Notably, the highest RSA for BV was observed at the lowest concentration (1.95 $\mu g.ml^{-1}$).

Contrasting observations were highlighted in a study by Somwongin et al. (2018), which assessed AOA using DPPH assay on venom samples diluted in PBS from *Apis cerena*, *Apis florea*, *Apis dorsata*, and *Apis mellifera*. Extracts from *Apis dorsata* exhibited the highest AOA, even comparable to AA, a recognized antioxidant compound (Somwongin et al. 2018). In contrast, our results indicated that both SV and BV diluted in PBS displayed lower RSA compared to standard antioxidants. Furthermore, it can be noted that the points at which the RSA-related AOA of SV and BV samples diluted in PBS were observed to be

the highest are at the lowest doses (1.95 μg.ml^{-1}).The results obtained from the MCA assay shed light on the distinct metal chelation capacities of the SV and BV. In the MCA assay conducted on SV, the highest MCA percentage emphasized its robust metal-chelating capability. Conversely, the lowest MCA percentage was linked to the solvent and concentration combination of distilled water at 15.63 μg.ml^{-1}, indicating reduced solubility and subsequent compromised metal chelation potential. Similarly, BV exhibited a notable MCA percentage, consistent with its effective metal-chelating efficacy. The solvent and concentration combination of saline at 500 μg.ml^{-1} demonstrated optimal solubility for efficient metal chelation, while the solvent and concentration combination of distilled water at 15.63 μg.ml^{-1} resulted in diminished activity due to solubility-related limitations.

In general, the MCA, reducing power (RP), and Ferric reducing/antioxidant power (FRAP) methods employ different fundamental principles to measure antioxidant capacity, focusing on different compounds. While MCA is based on the assumption that metal ions are chelated to prevent metal-catalyzed oxidation (Gulcin and Alwasel 2022), RP and FRAP methods assess AOA through reducing capacity (Sobral et al. 2016; Somwongin et al. 2018). Although scientific literature on MCA measurement for *Apis mellifera* venom is scarce, the AOA of the venom has been evaluated using RP (Sobral et al. 2016) and FRAP (Somwongin et al. 2018) assays, measuring their ferric ion reducing activities. It can be concluded that all three methods suggest that *Apis mellifera* venoms exhibit AOA by chelating and/or reducing metal ions.

The impact of solvent choice and concentration on the manifestation of antioxidant and metal chelation activities is a pivotal observation. While SV and BV demonstrated diverse responses across various solvents, a consistent pattern was discernible. Optimal AOA was consistently achieved within a physiological saline solution, as evidenced by both the DPPH and MCA assays. This study further delineated distinct trends in the AOA of Apis mellifera venom across different solvent systems and concentrations. Notably, the use of 500 μg/mL (equivalent to 1.95 μg/mL BV in terms of RSA) of venom dissolved in saline emerged as the prime strategy for maximizing RSA and MCA. Conversely, the employment of PBS or distilled water resulted in diminished AOA outcomes. This underscores the pivotal roles of solvent selection and concentration in harnessing the venom's complete antioxidative potential.

In the broader context of research, it is well-established that the powdered form of bee venom is often dissolved in distilled water, physiological saline solution, or PBS for both *in vitro* and *in vivo* applications. These findings underscore the significance of solvent and concentration optimization when assessing and harnessing the antioxidative and metal chelation potential of bee venoms. Overall, this study contributes to the understanding of the nuanced interactions between bee venom, different solvents, and their antioxidative and metal chelation capacities.

CONCLUSION

Drawing insights from the findings of our study, several important conclusions can be drawn:

1. Polar solvents have a notable impact on the *in vitro* AOA levels of bee venom. Our results emphasize the significance of solvent selection in evaluating the antioxidative potential of bee venom.
2. When considering application methods for assessing the optimal AOA of bee venom in an *in vivo* context, both topical and other parenteral applications may benefit from dissolving bee venom in physiological saline

solution. This solvent choice could potentially enhance the antioxidative efficacy of the venom.

3. Our investigation has highlighted the variability in solvent and concentration preferences for different AOA measurements, suggesting that these factors play a key role in biological applications. Future research endeavors could explore additional AOA measurement methods in conjunction with the two methods evaluated in this study.

In conclusion, our study sheds light on the intricate relationship between solvent selection, concentration, and the antioxidative potential of bee venom. These findings have implications for the development of therapeutic applications involving bee venom and underscore the importance of tailoring solvent and concentration choices based on the desired outcomes. Further investigations into other AOA measurement methods and their responses to various solvents could provide a more comprehensive understanding of bee venom's antioxidative capabilities.

Conflict of interest: The authors have no conflicts of interest to report.

Authors' Contributions: BD contributed to the project idea, acquisition of data, data analysis, and writing the original draft, as well as designing and executing the study. All authors have read and approved the finalized manuscript.

Ethical approval:"This study is not subject to the permission of HADYEK in accordance with the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees" 8 (k). The data, information and documents presented in this article were obtained within the framework of academic and ethical rules."

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Explanation: We presented this study as an oral presentation at the 12th International Scientific Research Congress in Delhi, India, in 2022.

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