**Evaluation of Antioxidant Properties in Three Commercially Important Edible Insects from Manipur**

**ABSTRACT**

 The study evaluates the antioxidant activity of three edible insect species (*Anoplophora glabripennis, Lethocerus indicus* and *Vespa magnifica*) collected from different market of Manipur, India using DPPH assay, performed in methanol and ethanol solvents. Results indicate variations in antioxidant potential across samples and solvents. *L. indicus* demonstrated the strongest radical scavenging activity, particularly in ethanol, with high inhibition percentages of 82.64% and low IC50 values (62.22 µg/mL). In contrast, *A. glabripennis* showed moderate activity in methanol (RSA%=82.69%; IC50 = 549.71 µg/mL) and relatively weak performance in ethanol (RSA %=61.55%; IC50 = 1414.89 µg/ml), while *V. magnifica* exhibited moderate antioxidant capacity in ethanol (RSA%=83.90%; IC50=277.5 µg/mL) but weaker activity in methanol (RSA%=74.06%; IC50=600 µg/mL). The findings of the present study suggest that *A. glabripennis, L. indicus* and *V. magnifica* have antioxidant potential and holds significant promise as a sustainable food source with potential applications in health sciences.

Key words: Edible insects, Antioxidant property, Entomophagy, IC50, Scavenging Activity

**INTRODUCTION**

The practice of eating insects is widespread across the world. Such consuming of insects have been habituated in many countries of Africa, South America, and Southeast Asian countries (Baiano, 2020). The natives of these countries have consume edible insects as the delicious food items and a good source of proteins in many restaurants and markets. According to United Nation, the global population is projected to reach 9 billion by 2050, and the demand for food will increase accordingly. The use of edible insects as a food source has gained maximum attention as a potential solution to meet the increasing demand for food, while reducing the environmental impact (Premalatha et al., 2011). So far relevant literature are concerned, around 2,205 insect species from 24 distinct orders have been identified as edible species (Omuse et al., 2024). The edible insects primarily belong to the insect orders Orthoptera, Heteroptera, Hemiptera, Coleoptera, Hymenoptera, and Lepidoptera. Among these, the order Coleoptera has the highest representation (32%), with up to 705 species. The orders Hymenoptera (15.5%) and Lepidoptera (15.2%) follow, with 341 and 335 species, respectively. The orders Orthoptera (14.1%) and Hemiptera (11.4%) include 310 and 251 species, respectively. Other insect orders, such as Isoptera, Odonata, Diptera, and Dictyoptera*,* each represents less than 5% of the total (Omuse et al., 2024).

Studies on the consumption of insects as food have identified numerous edible species in India, with approximately 298 species consumed by various tribes (Chakravorty, 2014; Gahukar, 2018). Among these, the coleopteran species were found to be the most commonly consumed, followed by Orthoptera, Hemiptera, Hymenoptera, Odonata, Lepidoptera, Isoptera and Ephimeroptera (Chakravorty, 2014). Since time immemorial, people of various ethnic backgrounds residing in Manipur have been capturing and consuming a wide range of insect species. These insects are also sold in some local markets in Manipur, and serve as a source of income for many local communities (Lokeshwari & Singh, 2019). Insects such as *Belostoma* sp., *Lethocerous* sp., *Hydrophilus* sp*., Locust* sp., larvae and pupa of silkworm, bees and cricket species have been commonly sold in the popular market as delicious and costly items. In Manipur, 73 edible insect species belonging to 9 orders, under 29 families have been identified. The order Hemiptera has the highest representation with up to 21 species, while the orders Odonataand Hymenoptera have 17 and 10 species, respectively. The orders Orthopteraand Lepidoptera each have 4 species, while the orders Isoptera and Dictyoptera, as well as the order Ephemeroptera*,* are represented by only one species each (Thangjam et al., 2020; Babu & Singh, 2021). Various researchers predicted that the sustainable source of protein for the enormous increase of human population in future would be the insect proteins (Tang et al., 2019; Zhao et al., 2021). In addition to their nutritional value, edible insects are also used for their medicinal properties. For example, termites are believed to have anti-inflammatory and anti-microbial properties while *Locust migratoria* are used to treat coughs and asthma (Singh, 2014). In Manipur, insects such as silkworm pupae and grasshoppers are used in traditional medicine to treat various ailments (Singh, 2014).

Antioxidants are compounds that protect cells from oxidative stress generated by free radicals, which are unstable molecules that can harm cells and lead to aging and development of chronic diseases like heart disease, cancer, rheumatoid arthritis and neurodegenerative disorder (Erhirhie & Paul, 2019). Edible insects have been found to have a variety of bioactive compounds with antioxidant properties, making them not only a nutritious food source but also potentially beneficial in preventing oxidative damage in the body (Sarmah et al., 2022; D’Antonio et al., 2023; Kowalski et al., 2023; Park et al., 2023). Therefore, the objective of this study was to evaluate the antioxidant properties of three commercially important edible insect species of Manipur namely *Anoplophora glabripennis, Lethocerus indicus* and *Vespa magnifica*.

**MATERIALS AND METHODS**

The present investigation was designed to evaluate the antioxidant properties of three different edible insect species, viz., *Anoplophora glabripennis, Lethocerus indicus* and *Vespa magnifica* bought from three different markets of Manipur i.e, Nagaram Village (24° 49’ N latitude and 93° 57’ E longitude), Thangal Bazar (24° 48’ N latitude and 93° 56’ E longitude), and Bishnupur Bazar (24° 37’ N latitude and 93° 45’ E longitude). After the collection, the samples were washed and clean of dust before sun-dried. After drying each insect samples were crushed with mortar and pestle into a fine powder. The insect powder was then stored in airtight plastic containers for further analysis.

The study involved assessing the antioxidant activity of three samples using the 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) assay, following standard protocols. Initially, ascorbic acid was prepared in methanol to serve as the standard solution at varying concentrations (200, 400, 600, 800, and 1000 µL). Concurrently, a DPPH solution was prepared by dissolving 6 mg of DPPH in 100 mL of methanol. The prepared standard solutions were mixed with the DPPH solution, incubated in the dark at room temperature for 30 min, and their optical density (O.D.) measured at 517 nm using UV-Vis spectrophotometer.

For the test samples, 2 mg/mL solution of each was prepared, and different volumes (100, 200, 500, and 1000 µL) of the sample extracts were mixed with 3 mL of DPPH solution. These mixtures were incubated at room temperature in the dark for 30 min, followed by O.D. measurements at 517 nm using UV-Vis spectrophotometer. The DPPH scavenging activity was calculated using the equation:

Where, AB is the absorbance of the blank; AA is the absorbance of the sample.

A calibration curve was plotted to determine the antioxidant activity based on the % DPPH scavenged.

**RESULTS AND DISCUSSION**

This section presents the findings of the antioxidant potential of three edible insect species, *Anoplophora glabripennis, Lethocerus indicus* and *Vespa magnifica*, using DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay. The DPPH assay is a widely used method to evaluate the antioxidant capacity of various substances by measuring their ability to scavenge the stable DPPH radical (Erhirhie & Paul, 2019). Oxidative stress, which contributed to the development of various human diseases, can be reduce by dietary antioxidants. Edible insects, beyond their nutritional value, offer a promising source of such natural antioxidants. In this study the antioxidant activity was assessed by measuring the scavenging effect of the samples on DPPH radical in both methanol and ethanol solvents. The results are presented as percent radical scavenging activity (% RSA) and IC50 values, which represent the concentration of the sample required to scavenge 50 % of the DPPH radicals (Table 1, 2 and 3).

Table 1: DPPH radical scavenging activity of standard (Ascorbic Acid)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Ascorbic Acid volume (µL) | Concentration (mL) | Absorbance (517nm) | RSA% | IC50 (µg/mL) |
| Solvent | DPPH Blank | 0.614 |   | 0.56 |
| 100 | 200 | 0.557 | 22.02 ± 0.42 |
| 200 | 400 | 0.463 | 35.23 ± 0.44 |
| 300 | 600 | 0.347 | 51.47 ± 0.04 |
| 400 | 800 | 0.225 | 68.46 ± 0.21 |
| 500 | 1000 | 0.136 | 81.01 ± 0.42 |

Table 2: DPPH radical scavenging activity and IC50 values of Test sample in methanol solvent

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Sample | Concentration (2000 µg/mL) | Absorbance (517 nm) | % Inhibition | IC50(Sample)µg/mL |
| *Anoplophora glabripennis* | DPPH Blank | 0.714 | 0 | 549.71 |
| 200 | 0.602 | 15.73 ± 0.13 |
| 400 | 0.395 | 44.75 ± 0.55 |
| 1000 | 0.244 | 65.79 ± 0.41 |
| 2000 | 0.124 | 82.69 ± 0.47 |
| *Lethocerus indicus* | 200 | 0.373 | 47.83 ± 0.83 | 224.41 |
| 400 | 0.246 | 65.61 ± 0.49 |
| 1000 | 0.147 | 79.47 ± 0.32 |
| 2000 | 0.075 | 89.45 ± 0.45 |
| *Vespa magnifica* | 200 | 0.434 | 39.29 ± 0.20 | 600.00 |
| 400 | 0.395 | 44.70 ± 0.67 |
| 1000 | 0.275 | 61.55 ± 0.48 |
| 2000 | 0.185 | 74.06 ± 0.61 |

Fig 1: DPPH Scavenging in methanol solvent a) Ascorbic Acid; b) *A. glabripennis* ; c) *L. indicus* and, d) *V. magnifica*

Table 3: DPPH radical scavenging activity and IC50 values of Test sample in ethanol solvent

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Sample | Concentration (2000 µg/ml) | Absorbance (517 nm) | % Inhibition | IC50(Sample)µg/ml |
| *Anoplophora glabripennis* | DPPH Blank | 0.714 | 0 | 1414.89 |
| 200 | 0.632 | 11.48 ± 0.28 |
| 400 | 0.597 | 16.42 ± 0.51 |
| 1000 | 0.416 | 41.81 ± 0.63 |
| 2000 | 0.275 | 61.55 ± 0.42 |
| *Lethocerus indicus* | 200 | 0.311 | 56.46 ± 0.13 | 62.22 |
| 400 | 0.276 | 61.41 ± 0.72 |
| 1000 | 0.159 | 77.71 ± 8.13 |
| 2000 | 0.124 | 82.64 ± 0.18 |
| *Vespa magnifica* | 200 | 0.435 | 39.15 ± 0.28 | 277.5 |
| 400 | 0.235 | 67.15 ± 0.63 |
| 1000 | 0.175 | 75.46 ± 0.37 |
| 2000 | 0.115 | 83.90 ± 0.26 |

Fig 2: DPPH scavenging in ethanol solvent. a) Ascorbic Acid; b) *A. glabripennis* ; c) *L. indicus* and, d) *V. magnifica*

The DPPH radical scavenging activity results highlight the antioxidant potential of the tested samples in methanol (Table 2; Fig. 1) and ethanol solvents (Table 3, Fig. 2). In methanol, *A. glabripennis* demonstrated a maximum inhibition of 82.69% at a concentration of 2000 µg/mL, with an IC50 value of 549.71 µg/mL. This indicates moderate antioxidant activity. *L. indicus* exhibited stronger activity in methanol, achieving 89.45% inhibition at 2000 µg/mL and an IC50 value of 224.41 µg/mL, suggesting it has better radical scavenging potential compared to *A. glabripennis*. Conversely, *V. magnifica* showed weaker antioxidant activity in methanol, with a maximum inhibition of 74.06% and an IC50 value of 600.00 µg/mL, indicating it is less effective than both *A. glabripennis* and *L. indicus* in this solvent.

In ethanol, the results revealed enhanced antioxidant activity for most samples. *A. glabripennis* achieved a maximum inhibition of 61.55% at 2000 µg/mL, with an IC50 value of 1414.89 µg/mL, indicating relatively poor antioxidant potential in this solvent. In contrast, *L. indicus* displayed the strongest activity in ethanol, with an impressive 82.64% inhibition at 2000 µg/mL and an IC50 value of just 62.22 µg/mL. This makes *L. indicus* the most potent antioxidant among all samples tested. *V. magnifica* also showed improved performance in ethanol compared to methanol, achieving 83.90% inhibition at 2000 µg/mL and an IC50 value of 277.5 µg/mL, indicating moderate antioxidant potential in this solvent. Overall, ethanol appears to enhance the antioxidant activity of most samples, with *L. indicus* emerging as the most potent antioxidant in both solvents. While *V. magnifica* performs moderately well in ethanol, it remains less effective than *L. indicus*. *A. glabripennis*, on the other hand, shows moderate activity in methanol but poor activity in ethanol. These findings suggest that *L. indicus*, particularly in ethanol, holds the most promise for applications requiring high radical scavenging activity.

The present findings are in agreement with the findings of Sarmah et al. (2022), which reports 87.29 % DPPH inhibition in *Lethocerus indicus*. Similarly, Mwanza et al. (2024) also reported varied antioxidant activities, 91.1 – 92.7 % and 55 – 60 % in methanol extract of *C. aurata* larvae and *O. rhinoceros* as compared to the present findings. Moreover the antioxidant activity of (Beetle larvae) *Passalus punctiger* (Passalidae)was recorded 87.43 % as reported by Kibet et al. (2024), slightly higher than *Anoplophora glabripennis* as found in the present studies. Whereas antioxidant activity of *Titoceres jaspideus* (Cerambycidae) (67.87%) was found to be lower than the present findings (Kibet et al., 2024). Also, the IC50 value (0.701mg/ml of adults and 0.813 mg/ml of larvae) of *Vespa magnifica* as reported by Sheileja et al., (2023) was slightly higher than the present study. The antioxidant capacity of edible insects varies significantly across different species, dietary factors, processing methods and developmental stages (Čaloudová et al., 2023; Kowalski et al., 2023).Chen et al. (2024) observed higher antioxidant activity in early-stage red palm weevil larvae compared to later stages. Sheileja et al. (2023) also compare the antioxidant property of *Vespa magnifica* in larval and adult stage and found that the IC50 value of adults (0.701 mg/ml) was lower than that of larvae (0.813 mg/ml), indicating that adult Hymenopterans have stronger antioxidant properties compared to their larval stage. Furthermore, it becomes apparent from the study that different solvents can significantly influence the antioxidant capacity of the samples which is supported by the findings of Zhou & Yu, (2004) on the antioxidant activities of wheat and wheat-based food products which demonstrated different values depending on the extraction method and solvent used. The findings from this study, along with previous research, highlight the potential of edible insects as sustainable and functional foods with significant antioxidant properties. Further research is needed to isolate and identify the specific compounds responsible for the antioxidant activities and explore their applications in health sciences.

**CONCLUSION**
This findings revealed that all the three edible insect species namely Anoplophora glabripennis, Lethocerus indicus, and Vespa magnifica shows notable antioxidant activity, with L. indicus showing the highest antioxidant activity especially in ethanol extract. This study also highlight the promising role of theses edible insects as functional foods and sustainable sources of natural antioxidants with potential health benefits. **Future studies could focus on further isolating specific bioactive compounds and evaluating the therapeutic applications of theses edible insects.**

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