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"Anti-Oxidant Potential of *Hydrous Indicus* and *Gryllotalpa Orientalis*: Two Widely Consumed Edible Insects of Manipur, A North-Eastern State of India"

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Abstract: North East India is well renowned for its rich biodiversity, and the locals there eat differently from those in other parts of the nation. Numerous people in this area considered insects delicacies and used them in their diets. Manipur is a relatively undiscovered region with limited research on the variety of its wildlife, particularly about insects. Even though entomophagy and entomotherapy have been practiced for a long time, there is currently a lack of comprehensive information regarding the nutraceutical potential of edible insects.Thus far, no research has been done on the antioxidant capacity of *Gryllotalpa orientalis* and *Hydrous indicus*, two edible insects frequently eaten by Manipur's living population. Herein, we aim to investigate the antioxidant potential of *Hydrous indicus* and*Gryllotalpa orientalis*. The antioxidant potential activity includes DPPH, ABTS scavenging, and total antioxidant FRAP assays were studied. The findings show that in the samples of *H.indicus* and*G.orientalis,*as for the antioxidant DPPH activity assay, it was observed that IC50=745μg/ml for *H.indicus*, which is equivalent to IC50 =16.98μg/ml of ascorbic acid, showing*H.indicus*to be a more potent DPPH scavenger than*G.orientalis*. For the ABTS assay, an IC50 value of 253.5μg/ml was recorded for *H. indicus,* which was found to be equivalent to IC50 = 3.552μg/ml of ascorbic acid, while *G.orientalis*did not show any ABTS activity. For the FRAP assay observed in *H. indicus*(IC50=2.242 ± 0.195 μg/ml) and *G.orientalis*(IC50=105.7 ± 0.676 μg/ml), which were found equivalent to IC50=0.8348 ± 0.458 μg/ml of ascorbic acid, thus indicating *H. indicus is* more potent than *G.orientalis*.The study results suggest that *H.indicus*and *G.orientalis*have high antioxidant potential and, therefore, can be sustainable and functional foods in the future. The management of the environment, human health, and food security are thus significantly impacted by these insects.

Keywords: Nutraceutical, Anti-oxidant, Food security, Health and diseases traditional knowledge

Introduction:

Zootherapy is the practice of treating human illnesses with animals or medicines derived from animals. Between 70 and 80 percent of the world's rural population receives primary healthcare from traditional medicine, according to a 1993 World Health Organization (WHO) estimate. It has a long history and is currently widespread in many parts of the world to treat people with various health problems by employing animals and their products, even though modern science has advanced to tremendous heights[1].To develop novel therapeutic options and preventative measures for several unidentified diseases, documentation of the nation's traditional treatment practices utilizing animal-based medications is crucial[1].One of the most extensively embraced dietary habits for humans in Europe is entomophagy [2]. Many societies around the world have traditionally considered insects to be a food group. At least 2,000 different types of insects are consumed as food worldwide [3]

Only a tiny part of the more than 1,000000 insect types known today—around 1,500 to 2,000 species—are considered edible worldwide. People often eat insects that are easy to find. Some of the most popular include termites and dragonflies, making up about 3% of the edible insect population. Flies are around 2%. Grasshoppers, locusts, & crickets are at about 13%. Cicadas, leafhoppers, planthoppers, scale insects, and true bugs account for about 10%. Beetles are a big group, making up about 31% of the edible insects. Caterpillars come in at about 18%. While Bees, wasps, & ants make up around 14%. Other insects add up to roughly 5%. [4]

True water beetle: Its morphology and therapeutic properties:

"Tharaikokpi," known scientifically as *Hydrous indicus* and commonly known as True Water Beetle, has been a part of Manipur's culinary heritage for generations. Belonging to Order Coleoptera and Family Hydrophilidae, Tharaikokpi is typically found in freshwater environments such as ponds, lakes, slow-moving streams, and marshes. The hard-bodied beetles of the Coleoptera order are holometabolous and undergo intricate metamorphosis; their hind wings are membranous and are shielded from the elements during resting by their chitinous elytra. Their distinguishing features are their mandibulate mouthparts, antennae with 11 or fewer antennomeres, worm-like larvae, and the female ovipositor and the male aedeagus retreat into the abdominal apex when not in use. On Earth, beetles are the animal group with the highest species richness.

based on the number of described animal diversity[5]

The taste of Tharaikokpi is a celebrated feature among the locals, who appreciate its versatility in traditional Manipuri dishes. Beyond its flavor,Tharaikokpi is renowned for its nutritional benefits. The inclusion of Tharaikokpi in the diet during the rainy season is particularly beneficial, as it supports the nutritional needs of the community naturally and sustainably.

Mole cricket: Its morphology and therapeutic properties:

"Waheibi or Wahee," known scientifically as *Gryllotalpa orientalis*and a type of Mole cricket, a staple in the diet of Manipur's communities, is deeply ingrained in the local traditions and culinary practices. Belonging to Order Orthoptera and Family Gryllotalpidae, these insects are typically found in moist soil and are known for their burrowing behaviour. The crickets' bodies, which range in size from tiny to medium, are primarily cylindrical and somewhat flattened vertically. The head is spherical, with two big compound eyes just behind the long, thin antennae that emerge from cone-shaped scapes (first segments). There are three ocelli (simple eyes) on the forehead. The pronotum, or initial thoracic segment, is robust, well-sclerotized, and has a trapezoidal form. It has no lateral or dorsal keels or ridges and is smooth. [6]

The prevention of mole crickets relies on tracking the hatch of eggs and precisely timing the administration of insecticides to coincide with peak emergence [7]. The health benefits of incorporating Waheibi into the diet are significant. The soluble fiber in Waheibi also promotes digestive health by enhancing gut flora and preventing digestive disorders.

Nutritional benefit:

These edible insects are nutritious and provide a variety of nutrients to the gut, including zinc, vitamins B12, iron, fiber, and omega-3 fatty acids. They also have a low water and land utilization rate, making them an ideal source of these essential amino acids[3]. In addition, they have a higher efficiency when it comes to converting food into energy and reducing greenhouse gas emissions[8].Insect consumption may also increase the amount of bioactive substances in the human diet, such as physiologically active peptides. Bioactive peptides, or small-sized molecules, have been reported to exert beneficial biological actions such as antihypertensive, antioxidant, anti-inflammatory, and antimicrobial. They are formed by the digestive system or owing to prior in vitro protein digestion [9].

Antioxidant properties:

Antioxidants are substances that, in tiny amounts, either naturally occur in food and the human body, acting to prevent, slow down, or regulate oxidative processes that lead to food deterioration or the onset and development of degenerative illnesses. The method of preventing these antioxidant molecules from oxidizing involves a variety of techniques and actions. [10]

Antioxidant-rich diets are crucial for preventing the development of many illnesses. Herbal antioxidants have a well-established beneficial impact in avoiding organ pathology caused by free radicals [12]. Multiple studies have proven the protective benefits of antioxidant and anti-inflammatory peptides against reactive oxygen species (ROS) and may significantly lower the amount of oxidative stress [2, 15, 16].

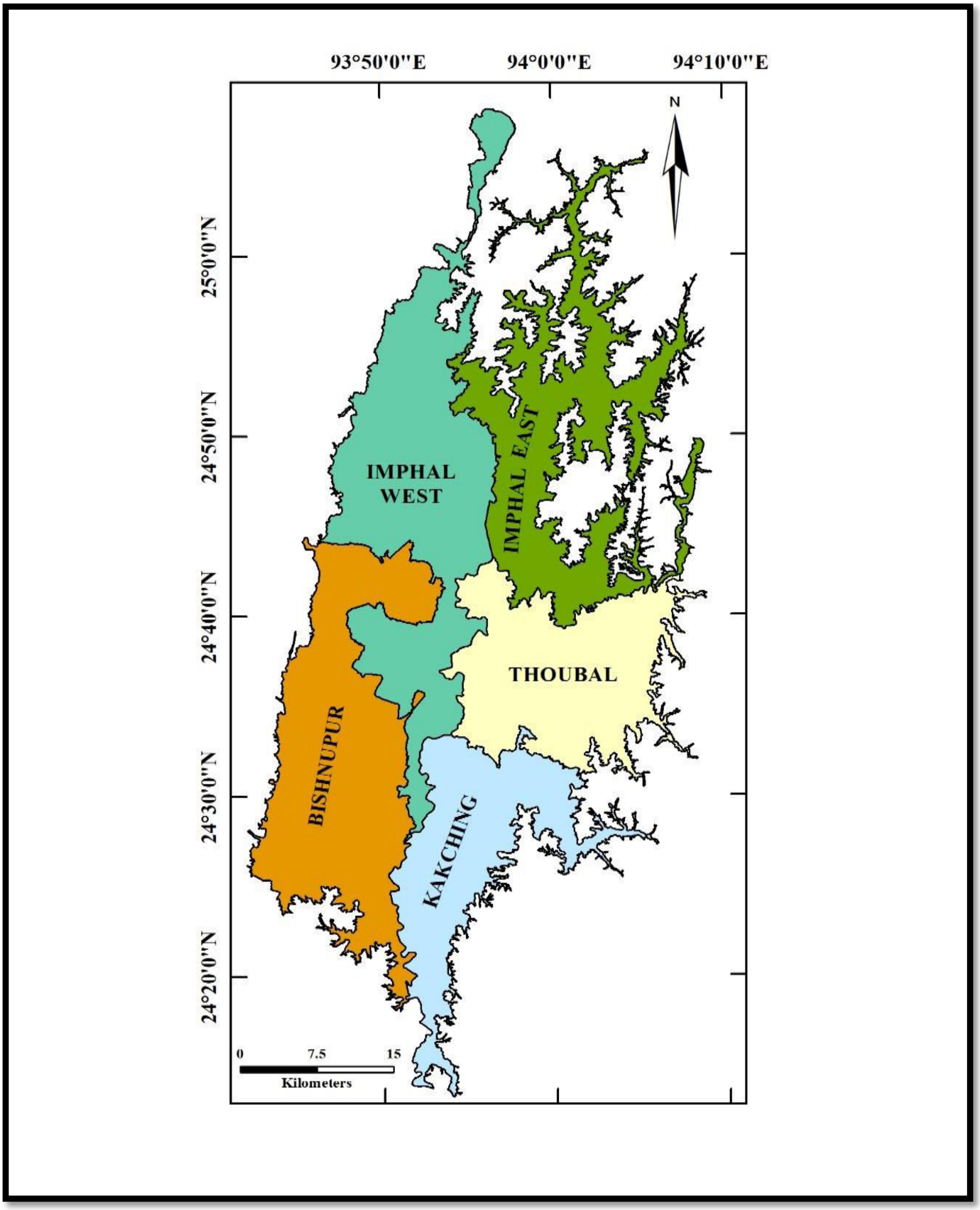
The Manipur ecosystem offers a variety of freshwater edible insects, many of which have long been used as remedies for various illnesses and have yet to be thoroughly researched or investigated for scientific confirmation. Natives of Manipur most frequently eat these two edible insects, locally known as "Tharaikokpi" and "Wahee," respectively. Due to their delicacy and status as excellent protein providers, the Indians eat these two insects frequently. The Manipureans also use these two insects in their traditional medicines, believing them effective in treating disorders such as fever, cough, dog bites, bowel movement issues, sores on the body, abdominal dropsy, urinary retention, etc. Adult *H. indicus* are eaten fried and have both traditional and therapeutic purposes. The fresh insects are crushed and used to treat tumors and dog bites. These eatable insects also provide an economical and practical supply of animal protein, which could enhance their nutritional qualities or bioactivities. [13]
Various studies have investigated and assessed edible insects as a valuable food source. Nevertheless, no data that describes the potential of the heat and grind method on the edible insect's therapeutic characteristics is available. Therefore, in this work, the *Hydrous indicus* (True water beetle) and *Gryllotalpa orientalis* (Mole cricket), which are indigenous to Manipur and called 'Tharaikokpi' and 'Wahee' respectively, were purposefully selected. Thus, this study assessed the effect of heat and grinding processing of two edible insects, *H. indicus* and *G. orientalis*, on beetle and cricket antioxidant activity.

Methodology:

Area of study:

The current investigation was confinding only in Manipur, mainly in the Loushi Pat basin of Kakching district, NambolOinam of Bisnupur district, and some from Imphal East and West. The Loushi Pat was a perennially flooded lake, and NambolOinam has many paddy fields suited for sample sites. Since these insects are seasonal, their data collection is mainly conducted during the rainy season. So, it was collected from March to July with the help of a local practitioner and a nearby professional.

Figure 1:Showing the location of sample collection on the map of Manipur



Preparation of insect's extract:

Firstly, after collection, the samples are cleaned with clean bottled water to get rid of any dirt or dust, and the whole edible portion of their body is oven-dried from 60 °C to 80 °C for a 24-hour period. Secondly, after drying, the samples were weighed and pulverized using an electric ball shaker equipped with a 75 µm screen sieve. Thirdly, the dried insect sample was to be crushed with a mortar and pestle into a fine powder and taken for analysis. [14]. The following is the sequential step for the flow of analysis.

Figure 2:Chart showing the flow of sample analysis

Insect sample collected from sites

Photograph 1: Showing the photograph after collection of the sample *Hydrous indicus*, true water beetle and*Gryllotalpa orientalis*, Mole cricket.

a) *Hydrous indicus* (True water beetle)b) *Gryllotalpa orientalis*(Mole cricket)s

Methods and materials:

Anti-oxidant potential activity:

For the present investigation, it includes DPPH (2, 2-Diphenyl-1-picrylhydrazyl) Scavenging Assay, ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) Radical Scavenging Ability, and total antioxidant FRAP (ferric reducing antioxidant power).

DPPH (2,2-Diphenyl-1-picrylhydrazyl) Scavenging Assay:

To ascertain the extract solution's ability to scavenge 1, 1-diphenyl-2-picryl hydroxyl (DPPH) free radical, 0.1 ml of 0.1 mM DPPH solution was combined with 5µl of an alternative test chemical stock in a 96-well plate. The reaction was set up in triplicate, and blank duplicates with five different doses of chemicals and 0.2 ml of DMSO/methanol were made. The plate was left in the dark for thirty minutes. Using a microplate reader (iMark, BioRad), the decolorization was measured at 495 nm after the incubation. A reaction mixture with 20µl of deionized water was used as a control. The scavenging activity in relation to the control was shown as "% inhibition." Using Graph Pad Prism software, IC-50 was computed[15,16,17].

ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) Radical Scavenging Ability:

To generate the ABTS free radical reagent, APS (2.45 mM) and ABTS (7 mM) solution were mixed and diluted 100 times, resulting in ABTS (SRL-Chem-Cat no.-28042) radicals. In a 96-well plate, combine 200µl of ABTS free radical reagent with 10µl of a separate stock of the standard (Ascorbic Acid-SD Fine-F13A/0413/1106/62) and samples. Then, incubate at room temperature for 10 minutes while keeping the plate dark. Using an iMark microplate reader (BioRad), the absorbance of the decolorization at 750 nm was measured following incubation. Results about the negative control were given. Utilizing Software Graph Pad Prism 6, IC-50 was computed

[18,19,20,21].

Total antioxidant FRAP (Ferric reducing antioxidant power) assay:
For the FRAP assay, 0.04 ml of 0.2 M sodium phosphate (Rankem, Cat. No. S0240) buffer (pH 6.6) and 0.05 ml of 1% potassium ferricyanide [K3Fe(CN)6] (SRL, Cat. No. 15766) solution were mixed with 10µl of various stocks of the test compound and standard (ascorbic acid) (SRL, Cat. No. 23006) concentration. After giving the reaction mixture a thorough vortex, it was incubated for 20 minutes at 50 °C. 0.5 ml of 10% trichloroacetic acid (SRL, Cat. No. 92390) was added to the mixture at the conclusion of the incubation. Next, 50µl of 0.1% ferric chloride (Fischer Scientific – Cat. no. 23585) and 50µl of deionized water were added. Using a microplate reader, the colored solution was read at 750 nm in comparison to the blank[22,23].

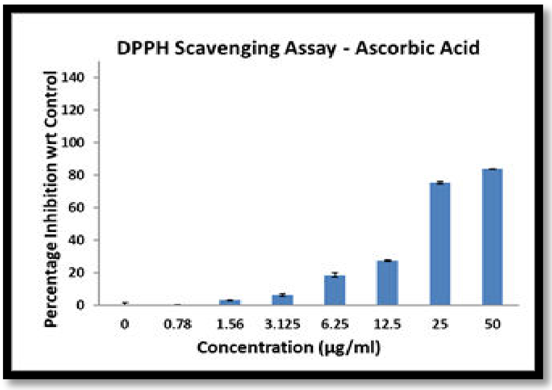
Results:
Evaluation of Anti-Oxidant Potential in the *G. orientalis* and *H. indicus:*
DPPH (2,2-Diphenyl-1-picrylhydrazyl) Scavenging assay:

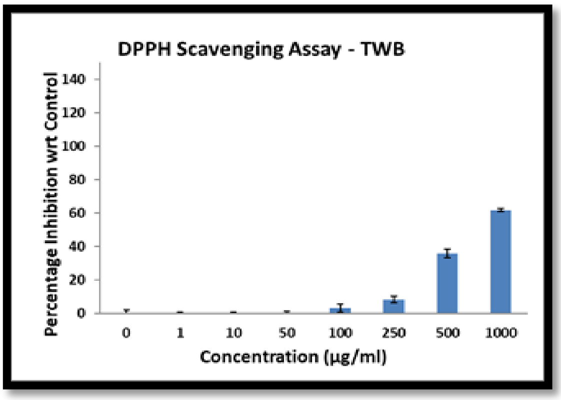
Antioxidant property (DPPH scavenging, i.e., (2,2-Diphenyl-1-picrylhydrazyl)) was observed insample *Hydrous indicus* (True Water Beetle, TWB) (IC50 = 745 ± 0.017 µg/ml) and*Gryllotalpa orientalis* (Mole cricket,FC)(IC50= 16.98 µg/ml) of ascorbic acid (Table1) and bar diagram showing in Figure no.3.

Table1: Table showing the calculated IC_{50} value (µg/ml) of DPPH assay.

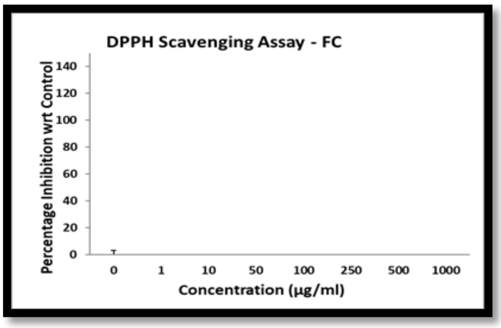
Sample name/Sample code	**IC_{50} value (µg/ml)**
Ascorbic Acid	16.98 ± 0.040
Hydrous indicus (True water beetle, TWB)	745 ± 0.017
Gryllotalpa orientalis (Molecricket, FC)	Not converged

Figure 3:Bar diagram showing the ranging calculated value of DPPH scavenging assay, percentage inhibition wrt control vs. concentration (µg/ml).





a)DPPH Scavenging assay-Ascorbic acid **b) DPPH Scavenging assay-(*Hydrous indicus*,TWB)**



c) DPPH Scavenging assay-(*Gryllotalpa orientalis*,FC)

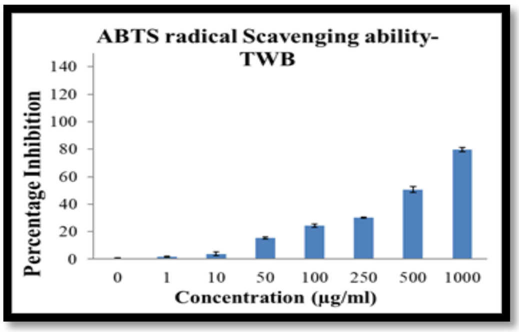
ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) Radical Scavenging Ability:

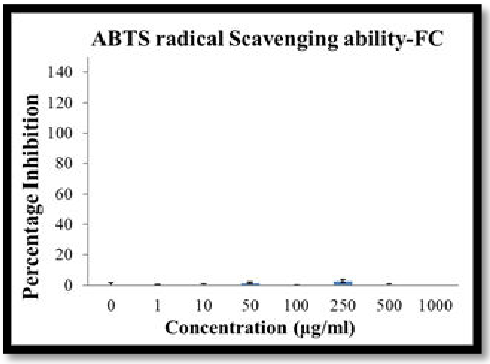
Antioxidant property (ABTS, i.e., 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) was observed in sample *Hydrous indicus* (True Water Beetle, TWB) (IC_{50} = 253.5 µg/ml), which was found equivalent to (IC_{50} = 3.552 µg/ml) of the ascorbic acid (Standard).Sample *Gryllotalpa orientalis* (Mole cricket, FC) did not show antioxidant activity in the ABTS assay (Table2)and bar diagram showing in Figure no.4

Table2: Table showing the calculated IC_{50} value (µg/ml) of ABTS radical Scavenging ability activity.

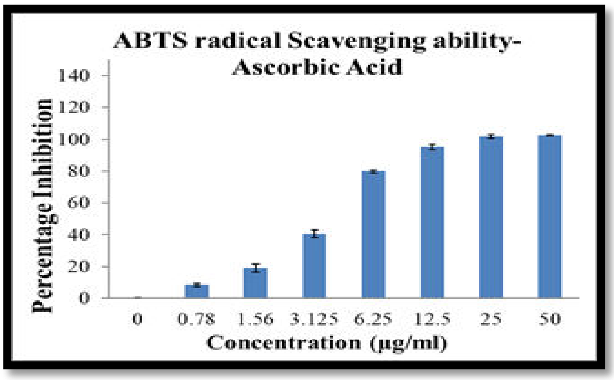
Sample name/Sample code	**IC_{50} value (µg/ml)**
Ascorbic Acid	3.552 ± 0.019
Hydrous indicus (True water beetle, TWB)	253.5 ± 0.090
Gryllotalpa orientalis (Molecricket, FC)	Not Converged

Figure 4: Bar diagram showing the ranging calculated value of ABTS radical Scavenging ability activity, percentage inhibition vs. concentration (µg/ml).





a) **ABTS activity (*Hydrous indicus*,TWB)** b) **ABTS activity -(*Gryllotalpa orientalis*,FC)**



c)**ABTS activity -Ascorbic acid**

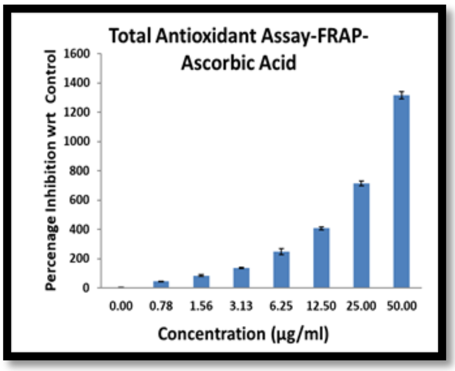
Total antioxidant FRAP (Ferric reducing antioxidant power):

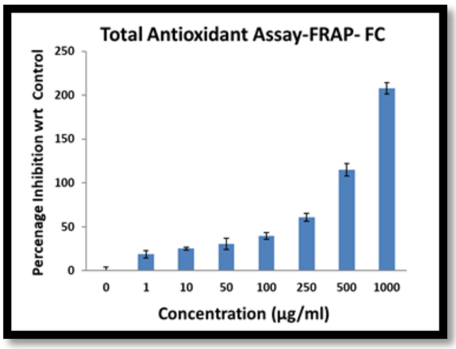
Antioxidant property (FRAP, i.e., ferric reducing antioxidant power) was observed in sample *Hydrous indicus* (true water beetle, TWB) (IC50 = 2.242 ±0.195 µg/ml) and *Gryllotalpa orientalis* (mole cricket, FC) (IC50 = 105.7 ±0.676 µg/ml), which were found equivalent to (IC50 = 0.8348 ± 0.458 µg/ml) of ascorbic acid. Sample *Hydrous indicus* (true water beetle, TWB) was found more active than sample *Gryllotalpa orientalis* (mole cricket, FC) (Table3) and bar diagram showing in Figure no.5.

Table3: Table showing the calculated IC_{50} value (µg/ml) of FRAP activity.

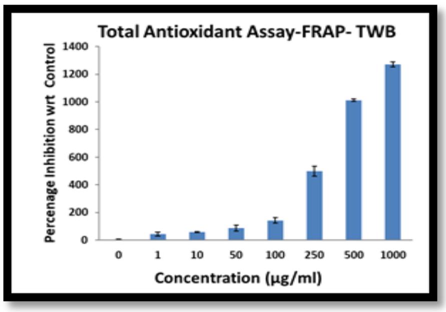
Sample name/Sample Code	**IC_{50} Value (µg/ml)**
Ascorbic Acid	0.8348 ± 0.458
Hydrous indicus (True water beetle, TWB)	2.242 ± 0.195
Gryllotalpa orientalis (Mole cricket,FC)	105.7 ± 0.676

Figure 5: Bar diagram showing the ranging calculated value of FRAP activity, percentage inhibition wrt control vs. concentration (µg/ml).





a)FRAP activity-Ascorbic acidb)FRAP activity-(*Gryllotalpa orientalis*,FC)



c)FRAP Activity-(*Hydrous indicus*,TWB)

Discussion:

Entomophagy has gained significant traction in European countries and has long been a common practice in northeastern India. The capacity to reliably provide supplements, minerals, vitamins, and fatty acids of the highest caliber without adversely affecting the environment is well-established; nevertheless, little is known about their potential application as a source of bioactive components[24]. This region of Northeast India has readily available edible insects and invertebrates, which are a good source of antioxidant components because of their efficient classification and eating habits.

To many data, it was discovered that Thai frozen goods sold commercially could be either field cricket (*Gryllusbimaculatus*), silkworm (*Bombyx mori*), or bamboo caterpillar (*Omphisafuscidentalis*) were found to have moderate microbial burdens, which food preparation methods may further decrease. [25].

Another study reported that people often include edible insects in their meals and consume them dried, roasted, boiled, fried,or as a relish. The preparation techniques for edible insects are influenced by custom and culture. People use the expertise inherited from previous generations to prepare insects. The stink bug insect,*Encosternumdelergorguei*, is either raw or fried in Limpopo, South Africa; however, it is only consumed dried or fried in Zimbabwe[26].

Our current study directly evaluates the protective nutraceutical and potential efficacy of *Hydrous indicus* and *Gryllotalpa orientalis.* Analysis of the result includes antioxidant and free radical scavenging potentials. *H.indicus*has a very high antioxidant activity in FRAP with an IC50 value of 2.242 ± 0.195 µg/m (Table3). Interestingly, *G.orientalis* did not converge or find any result in the antioxidant potential of ABTS and FRAP activity. The insect *G.orientalis*has also not converged or found any result in ABTS and DPPH activity of antioxidant potential.

One of the reports included an analysis and presentation of the antioxidant report, which involved comparing the results with standard ascorbic acid. The five aquatic edible insects were *Lethocerus indicus* (Lepeletier and Serville), *Lactotrephes maculates* (F.) (Nepidae), *Hydrophilus olivaceous* (F.), *Cybistertripunctatus* (Olivier), and *Crocothemis service* (Drury). The assay used DPPH free radical scavenging methods with the methanol extract. For *C. tripunctatus*, the IC50% ranged from 110 µg/mL to 880 µg/mL, but for *C. servilia*, it was lower. The species exhibiting higher antioxidant activities were those with lower IC50% values. Consequently,*C.tripunctatus*possessed the best antioxidant properties of all the species. Compared to the other four species, *C.servilia* had an IC50% of 880µg/mL, which is higher but not a significant antioxidant property. This study contrasts *C.servilia*, whose IC50% is 880 µg/mL, and *H. indicus*, whose IC50% is 745 µg/mL; *H. indicus*has more DPPH antioxidant activity than *C.servilia.* [27]

Moreover, another study reported the highest level of antiradical activity against DPPH of baked cricket *Gryllodessigillatus* hydrolysate peptide fraction (IC50 value: 10.9 μg/mL). In contrast, a maximum antiradical activity against ABTS free radicals was offered by the hydrolysate of raw mealworm, the *Tenebrio Molitor* (IC50 value: 5.3 μg/mL). Among derived peptides of hydrolysates containing boiled locust *Schistocerca gregaria*, Fe2+ chelation capacity proved to be highest (IC50 value 2.57 μg/mL); and also, the crude *G.sigillatus* gastral juice possessed the most potent reducing power. In these reports against DPPH activity, *H. indicus* with susceptible concentrations of 745 μg/mL and *Gryllodessigillatus* with an IC50 of 10.9 μg/mL compared; *Gryllodessigillatus* was found more effective than *H.indicus* in terms of DPPH antiradical activities. Even against other active ABTS containing *H.indicus* rated at IC50 of 253.2μg/mL and *Tenebrio molitor* of IC50 value: 5.3μg/mL. It was discovered that *H.indicus* was less ABTS free radical scavenging than *Tenebrio molitor*. Finally, regarding FRAP activity *H. indicus* with an IC50 value of 2.242 μg/mL was ranked higher than *Schistocerca gregaria* IC50 2.57 μg/mL.The most marked inhibitory effect on lipoxygenases and cyclooxygenase-2 has antioxidant and anti-inflammatory activity.

With IC50 values of 3.13 μg/mL and 5.05 μg/mL, respectively, the peptide fraction from a protein preparation from the locust *S.gregaria* demonstrated the most noteworthy inhibitory effect against lipoxygenase and cyclooxygenase-2. Also, in these cyclooxygenase-2 inhibitory activities, locust *S.gregaria* has an IC50 value of 5.05 μg/mL, and*H.indicus* and *G.orientalis* have IC50 values of 13.2 μg/mL and 22.22 μg/mL. Locust *S.gregaria* has more cyclooxygenase-2 inhibitory anti-inflammatory activity than *H. indicus.* [2].

Subsequent analysis revealed that the rich content of short peptides, phenolic compounds, short-chain hydroxyl, and carboxylic fatty acids, and several antioxidant-type vitamins A and E are all accountable for the antioxidant properties of royal jelly. Hydroxyl radical scavenging activity of these peptides has been demonstrated[28]

Regarding one modality, while performing antioxidant tests on guava fruit extracts using its methanolic extract, it was established that the ABTS, DPPH, FRAP, and ORAC assay gave similar results. High levels of ascorbic acid and total phenolics correlate closely with the FRAP method, which was also highly repeatable, simple, and fast. Hence, it would be reasonable to use the process in question to determine the antioxidant potential of guava fruit extract. [29]

The findings of this research are thus, at the very least, very encouraging as far as the observation concerning the consumption of these two edible insects is concerned, which can be looked at as possible functional foods in the foreseeable future, and such can be important sources for discovering new drugs in the future. Additionally, the use of insects and products made from them can change the state of the diversity of insects. [30]

Conclusion:

By 2050, it is predicted that the world's food demand will have increased by more than 50%, posing a challenge to agricultural systems everywhere. The detrimental effects of raising cattle to to manufacture traditional dairy and meat goods on the environment have resulted in the suggestion that eating cultivated edible insects could help alleviate the food problem [31]. The current study's result offers novel insights into the components essential to Manipur's two most commonly consumed edible insects: their capacity to scavenge free radicals and act as antioxidants. The analysis results demonstrate that *H.indicus*possesses significant antioxidant and free radical scavenging potential activity compared to *G.orientalis.* The study's results suggest that *H.indicus*and *G.orientalis* have high antioxidant potential and thus can be sustainable and functional foods. As a result, these insects significantly impact environmental management, health, and food security. Further, our upcoming in vivo research may include animal models, which will confirm the current findings. Thus, the current study's findings offer compelling evidence that eating edible insects could one day be considered a dietary supplement source and a valuable bioresources for developing new medications.

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