

# Antioxidant Potential of *Brachytrupes orientalis*, *Ducetia japonica* the Orthopterans and *Mantis* sp. Mantidae: The Valued Edible Insects among the Tribes of Arunachal Pradesh, India

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## ABSTRACT

A new global interest in entomophagy, the practice of eating insects with low ecological impact, has historically been used by humans for food or medicinal use. It is not only healthier; it is also the product of a generation of harmonious co-existence between tribes and environmental resources and human insect interaction that, most of the time, is little known. Although the nutritional value of edible insects is widely recognized and insects have therapeutic value, few studies have been focused on the antioxidant potential of edible insect extracts.

The present study was undertaken to analysis of antioxidants parameter: ABTS (2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid radical cation), DPPH (2,2-di(4-tert-octylphenyl)-1-picrylhydrazyl radical), Ferric Reducing-Power (FRP), Total Antioxidant Activity (TAC) and also to establish the possible correlations between phenolics and flavonoid content of respective extract from *Brachytrupes orientalis* (mole cricket), *Ducetia japonica* (bush-cricket) the orthopterans and ootheca of *Mantis* sp. (mentids) extract and were compared to conventional fruit vegetable, and other reported insects. The results of this study demonstrate that mantids otheeca, comprises stronger free-radical scavenging activity ABTS•+(C<sub>50</sub> µg/m) and DPPH (IC<sub>50</sub> µg/ml) TAC (µg BHTE q/g) FRP (µg TPEE q/g) 19.76 ± 0.37; 46.38 ± 0.95; 37.12 ± 0.41 and 47.02 ± 0.93 respectively compared to bush-cricket (37.12 ± 0.41; 47.02 ± 0.93, and mole cricket (26.43 ± 0.37, 63.32 ± 1.04). Similarly TAC (µg BHTE q/g) and FRP (µg TPEE q/g) was higher in mantids otheeca (25.59 ± 0.25 and 18.98 ± 0.18) than orthopterans (0.15 ± 0.04, 1.31 ± 0.01 mole-cricket; 0.59 ± 0.083, 3.29 ± 0.04, bush-cricket). In terms of total phenols (GAE q/100 g), again mantis otheeca scores higher value (345.63 ± 3.51) yet, with the value 78.59 ± 2.54 and 75.67 ± 6.47 bush-cricket and mole cricket contain substantial amount of phenol. However, total flavonoid (mg RTE q/100 g) is higher in bush-cricket (37.78 ± 2.18) followed by mantids otheeca (35.08 ± 0.62) and comparatively lower in mole cricket (28.06 ± 0.26). TPC showed a strong positive correlation with antioxidants assayed. Hence, these insects in general and mantis in particular could play a role in the diseases caused by oxidative stress, inhibiting the development of various human diseases. Hence, our study gives a direction towards the isolation and identification of novel bioactive compounds responsible for antioxidant activity.

**Keywords:** Entomophagy; Mantids; Antioxidant; Oxidative stress; Human diseases

## INTRODUCTION

Insects have served as a food source for humanity since the first bipedal human ancestor came down from trees and started walking with the savannahs. Insects are known for their proteins, minerals, and high amounts of essential amino acids. The insect's protein is considered as an environment source of protein, frequently labeled "eco-protein". Food oracles have highlighted the future move towards high protein diet and the emergence of insects as protein sources; this in combination with a projected doubling in global demand for protein and lack of suitable land for agriculture expansion, has led to a renewed focus on

the use of insects in the human food chain. Interestingly, today, insect eating is rare in the western world, but remains a significant source of food for people in other culture. However, in recent times, global media channels have increasingly brought to European citizens the concept of eating insects themselves.

According to the FAO, 1900 species of insects are consumed by more than 2 billion people in more than 80 countries across Asia, Africa, and the America. As far as India is concerned, perusal of the literature has revealed scanty and fragmentary information about edible insects in India, except for a few rural tribes of North East India. However,

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one of the regions in NE India where large numbers of insects are still appreciated as human food is Arunachal Pradesh [1-5]. In Arunachal Pradesh, which is a biodiversity hotspot endowed with rich and diverse insect fauna and among the ethnic people of the state, entomophagy is a traditional and culturally accepted practice? The interest in the use of insect as food in this state has been expressed in several earlier reports [6] and revealed more than 102 edible insect species belonging to 13 different orders taken as food by one or the other 26 major tribes of Arunachal Pradesh.

Besides being described by many insect enthusiasts as a tasty food commodity of high nutritive value, research has reported that several insect species possess health enhancing properties, directly through their cancer-fighting fibrous components or indirectly as part of medicinal compounds [1,7-9].

In some African countries, children are fed with flour made from dried caterpillars to curb malnutrition, while pregnant and nursing women, as well as people who are anemic, are encouraged to eat caterpillars that are high in protein, calcium, and iron [10-12] or, as in the case of the larvae of the *Cirina fonda*, contain a greater amount of phosphorus, potassium, and magnesium than the larvae of the *Cirina* [13].

It is, of course, necessary to follow up how efficient the treatment of diseases or whether specific chemical stemming from the insects exert measurable and quantifiable beneficial physiological effects. The suggested that it is essential to evaluate and assess functional benefits of food to provide evidence based science to substantiate health benefit [14].

To follow up the process it is essential to evaluate the active component present in insects. Insect with bioactive properties for human health is an emerging field for the edible insect. Together with their nutritional value, evidence till date show that edible insects contain a huge diversity of other micronutrients and bioactive compounds such as antioxidant phenolic, flavonoids different from insect fat and protein yet to be discovered. In-fact, such bioactive compounds are being reported recently, although studies are still scarce in this regards [15-18].

Antioxidants are a group of naturally occurring biomolecules present in a large array of food items ranged from animal to plants. As a source of naturally producing substance, antioxidants perform fantastic job to scavenge free radical and repaired the cellular damage cause by these radicals in biological system.

On the basis of their biological function, antioxidant is defined as bio-molecular substances, present at low concentrations compared to reactive oxygen species (oxidize substrate) that neutralize free radicals or delay their actions in physiological system [19]. Antioxidants are substances that can prevent or slowdown the damage to cell caused by free radical, produced in the body as a reaction to environmental or other pressure. In the past decade, antioxidant compounds have gained acceptance among the general public for their ability to neutralise reactive and damaging forms of free radicals.

The mole cricket, *Brachytrupes orientalis* (Gryllidae), Bush Cricket, *Ducetia japonica* (Tettigoniidae) and mainly Praying Mantis sp. (Mentoidae) are the common species of orthoptera and mantodae used as food by tribal people of Arunachal Pradesh (NE India).

Our research to date has shown that Arunachal tribes consume comparatively greater number of orthopterans than do other insect consuming tribals in India [20,21]. These insects are appreciated by all members of the ethnic community of Arunachal Pradesh [1,6]. Besides these orthopterans, a species of praying mantis is also appreciated by

tribes of Arunachal Pradesh [22,23]. It is for this reason the present study focuses on these species of Insects. These popular insects are hand collected when these insects emerge in large number during the season from the surrounding bushes of village and town as well as agricultural fields (*D.japonica* ), or with the aid of light trap and/or by pouring water in the burrows (*B. orientalis*) and from rice field during harvesting of rice plant where the egg case of *Mantis* sp. remain fixed to the rice stem.

After the adult insects have their wings and appendages are removed from *B. orientalis* and *D. japonica* they can be eaten either raw or as "chutney" (by crushing the insect and mixing them with spicy ingredients), sometime they are boiled with vegetable, are fried or roasted over open fire. For consumption of mantids the oothecae is being appreciated better than the adults. The eggs are placed in cases, oothecae which can contain several hundred eggs each [24]. They (oothecae) are plucked from the rice stem when the women/children engage in harvesting the paddy in the field but some time they make collection of these egg cases for further consumption at home by other members of the family. Egg case of mantids are chewed directly or fried with little oil before consumption.

From our earlier report it was revealed that, Mantid Nymphs/eggs but not adults are eaten because the latter are believed to possess worms inside their stomach but only occasionally adult form are consumed [23,24]. The locals collect these insects for their personal consumption not obsessive as a nutritional supplement or to ward of starvation, rather cherished for their test and regarded as a delicacy since time immemorial. Our earlier investigation on the analysis of the nutrient composition of *B.orientalis* and *D. japonica* has demonstrated that these insects are valued as a source of useful fatty acid, minerals and protein with high quantity and quality.

However their antioxidant potential has never been scrutinized scientifically. The present study was aimed at evaluating the antioxidant potential of these insects to explore the possibility of their use as a base for formulating new food/feed products with nutritional and functional value to be promoted among the tribe of the state of Arunachal Pradesh or elsewhere in India.

In this paper we present the results of analysis of bioactive components that act as antioxidants in *B.orientalis*, *D. japonica* and oothecae of *Mantis* sp.in their raw state. The present study shows the potential of these orthopteran species, *B.orientalis*, *D. japonica* and ootheca of *Mantis* sp. as a source of antioxidant for human health.

## MATERIALS AND METHODS

### Sample collection and identification

Mole cricket (*Brachytrupes orientalis* ) was collected during summer night of June to July from the bushes/burrows, Bush Cricket (*Ducetia japonica* ), during morning hours of May to August from surrounding shrubs, bushes, paddy fields and praying mantis (*Mantis* sp.) oothecae were collected from their egg laying sites like paddy stems and bushes during the early winter season September to November from Emchi and Yazali village, Papum Pare district of Arunachal Pradesh. The sexes of the specimens were not separated, because both are equally appreciated as food.

All the specimens were taken to the laboratory of Rajiv Gandhi University in chilled freeze-boxes. The insects were taxonomically identified in the laboratory, confirmed by the Zoological Survey of India, Kolkata as *Brachytrupes orientalism*, *Ducetia japonica*

and oothecae of *Mantis* sp. Once in the laboratory the sampled insects were washed thoroughly in double distilled water and brought them to dryness and stored at  $-20^{\circ}\text{C}$ . All the analysis was performed within a months' time. All the solvents and chemicals used in the study were of analytical grade. The analyses were done for the antioxidant parameter: ABTS (2, 2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid radical cation), DPPH (2, 2-di (4-tert-octylphenyl)-1-picrylhydrazyl radical), FRP (Ferric Reducing-Power), TAC (Total Antioxidant Capacity), phenolic and flavonoids.

### Extraction procedure

10 g each of chopped insect samples was homogenized in 50 mL of buffered methanol (1 m/M PBS buffer pH 6.8; 1:1) kept in orbital shaker for 48 hours. The resultant extract was centrifuged at 7000 rpm for 30 minutes at  $4^{\circ}\text{C}$ . The supernatant was collected and the precipitate was re-dissolved to get the second batch of supernatant and the combined supernatant was concentrated under vacuum evaporator at  $40^{\circ}\text{C}$  to dryness and dissolved in buffered methanol for further analysis.

### Analyses

**ABTS free-radical scavenging activity:** ABTS radical scavenging activity was determined by following the modified method. Briefly, by addition of 30  $\mu\text{l}$  of diluted insect extract from seven different final concentrations to 270  $\mu\text{l}$  of ABTS working solution by using 96 well micro plates. After 6 min of incubation in dark at  $37^{\circ}\text{C}$ , the absorbance was determined at 734 nm. Trolox were used as standard and ABTS antiradical activity was expressed as  $\text{IC}_{50}$  value ( $\mu\text{g}/\text{ml}$ ).

ABTS stock solution (7.4 m/M) was added to (2.6 m/M) potassium per sulfate solution in equal quantities and kept for 16-24 h at room temperature in the dark to yield a dark-colored solution containing ABTS radical and working reagent diluted to an initial absorbance of about  $0.7 \pm 0.02$  at 734 nm.

**DPPH free-radical scavenging activity:** DPPH radical scavenging activity was determined according to the method described by Blois 1958 with slight modifications. Briefly, the reaction mixture contained 200  $\mu\text{L}$  of extract diluted in methanol at different concentrations and 100  $\mu\text{L}$  of 0.8 mM DPPH in 95% methanol. After being incubated at room temperature  $37^{\circ}\text{C}$  for 30 min in dark, the absorbance was measured at 517 nm. For control, distilled water was used instead of the sample. For standard, BHT was used and expressed as  $\text{IC}_{50}$  value ( $\mu\text{g}/\text{ml}$ ).

**Ferric-reducing power (FRP):** Ferric-Reducing Power (FRP) was done using the method from Oyaizu 3 with minor modification as described by Prasad using a tocopherol as standard and expressed as TPE ( $\mu\text{g}/\text{g}$ ). The samples (1.0 ml) were mixed with 1.0 ml of 1% Potassium ferricyanide (pH 6.8 0.1 M phosphate buffer) and incubated at  $60^{\circ}\text{C}$  for 30 min. After incubation, 1.0 ml 10% TCA was added and centrifuged at 5000 g for 5 min an aliquot of supernatant (200  $\mu\text{l}$ ) was added with 800  $\mu\text{l}$  of Millipore water, followed by adding 100  $\mu\text{l}$  of 1%w/v Ferric chloride to initiate the reaction. The reaction was incubated for 10 min at room temp in dark conditions then absorbance read at 700 nm. The results are expressed as  $\alpha$ -tocopherol equivalent TPEE ( $\mu\text{g}/\text{g}$ ).

**Total Antioxidant Capacity (TAC):** The total antioxidant activity of the methanolic extract was determined by using the phosphomolybdenum method [25]. An aliquot of 0.1 to 0.5 mL of methanolic extract (1 mg/mL) was mixed with 4 ml of the

phosphomolybdenum reagent (0.6 M sulphuric acid, 28 m/M sodium phosphate, and 4 m/M ammonium molybdate). After being incubated in a water bath at  $95^{\circ}\text{C}$  for 90 minutes, cooled and the absorbance was read at 695 nm. The antioxidant activity of the sample was determined using a standard curve of ascorbic acid and expressed as ascorbic acid equivalents ASCE ( $\mu\text{g}/\text{g}$ ) of fresh sample.

**Total phenolics:** Total Phenolic content in extracts was determined by the modified Folin-Ciocalteu method [26]. Briefly, 500  $\mu\text{l}$  of the extract was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with Millipore water 1:10 v/v), and after 5 minutes of incubation, 1.0 ml of sodium carbonate reagent (10% Sodium chloride solution in Millipore water) was added. The tubes were vortexed for the 0 sec and allowed to stand for 25 minutes at  $37^{\circ}\text{C}$  for color development, and absorbance was measured at 760 nm. Gallic Acid was used as standard and expressed as Gallic Acid equivalent (GAE mg/100 g).

**Total flavonoids:** Total flavonoids were estimated using aluminum chloride as described by Chang [27]. Briefly, 1.0 ml insect extract added to 1.0 ml of (10% w/v) Aluminum chloride and vortexed for the 30s. After 5 min, 0.5 ml of (5% w/v) sodium nitrate was added and the final volume was made to 5.0 ml using Millipore water and absorbance was measured at 510 nm. Rutin was used as standard and expressed as Ritin equivalent (mg RTE/100 g).

**Data and statistical analysis:** Each sample was analysed in triplicate ( $n=3$ ) and data is reported as mean  $\pm$  SD throughout the analysis. Data was analysed using the one way analysis of variance (ANOVA). Software analysis carried out through Graph pad Prism. 9.0.0 (California Corporation Inc., CF, USA), and Microsoft Excel 2016 were used for graphical representation.

## RESULTS AND DISCUSSION

Oxidative stress has been associated in several diseases including rheumatoid arthritis cardiovascular diseases, neurodegenerative diseases, diabetics and aging [28,29]. Natural antioxidants such as phenolics flavonoids compounds may offer resistance against the oxidative stress by scavenging the free radicals, inhibiting lipid peroxidation and by other mechanism [30]. Therefore the present study was undertaken with the aim to show the antioxidant potentials of the studied edible insects.

Edible insects are well-thought-out as the main solution to the problem to encounter the growing global demand for animal protein that is sought-after for its nutritional value. Given that the nutritional value of edible insects is widely recognized [31] and having therapeutic value [2] very few studies have been focused in relation to the antioxidant prospective of edible insect extract.

In the present study the analyses were done using widely known methods for evaluation of antioxidants parameter: ABTS (2, 2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid radical cation), DPPH (2, 2-di(4-tert-octylphenyl)-1-picrylhydrazyl radical), Ferric Reducing-power (FRP), Total Antioxidant Activity (TAC) and also to establish the possible correlations between phenolics and flavonoid content of respective extract from *Brachytrupes orientalis*, *Ducetia japonica* the orthopterans and ootheca of *Mantis* sp. extract and were compared to conventional fruit vegetable, and other reported insects, presented in Tables 1 and 2 and Figures 1-5.

**Table 1:** ABTS•+, DPPH• free radical scavenging activity (IC50 µg/ml), total antioxidant activity (BHTE µg/g), reducing power (α-TPEE µg/g), total phenolic content (mg GAE/100 g) and total flavonoids content (mg RTE/100 g) of *mantids sp.* (ootheca), *B. orientalis* and *D. japonica*. Values are presented as mean ± SD (n=3) for each dataset.

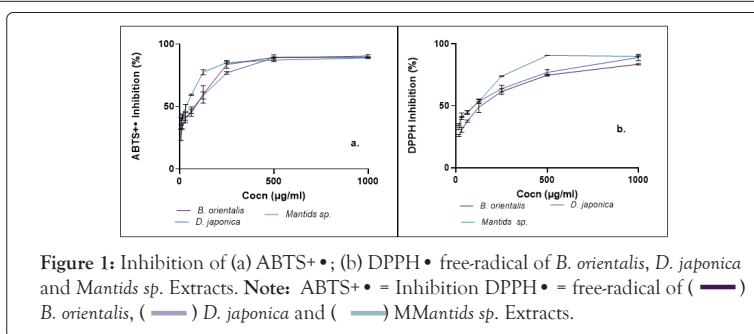
| Species              | Common name    | Edible stages        | ABTS* IC50 (µg/ml) | DPPH*        | TAC          | FRP           | Phenolic (mg GAEq/100 g) | Flavonoids (mg RTEq/100 g) |
|----------------------|----------------|----------------------|--------------------|--------------|--------------|---------------|--------------------------|----------------------------|
|                      |                |                      |                    | IC50 (µg/ml) | µg BHTEq/g   | µg TPEEq/g    |                          |                            |
| <i>B. orientalis</i> | Cricket        | Adult                | 26.43 ± 0.37       | 63.32 ± 1.04 | 0.15 ± 0.04  | 1.31 ± 0.01   | 75.67 ± 6.47             | 28.06 ± 0.26               |
| <i>D. japonica</i>   | Bush-Cricket   | Adult                | 37.12 ± 0.41       | 47.02 ± 0.93 | 0.59 ± 0.08  | 3.29 ± 0.04   | 78.59 ± 2.54             | 37.78 ± 2.18               |
| <i>Mantids sp.</i>   | Praying Mantis | Egg-case/<br>Otheeca | 19.76 ± 0.37       | 46.38 ± 0.95 | 25.59 ± 0.25 | 18.98. ± 0.18 | 345.63 ± 3.51            | 35.08 ± 0.62               |

Note: (ABTS•+): 2, 2-azinobis-3-ethylbenzothiazoline-6-sulfonate; (DPPH•): 2, 2-Diphenyl-1-picrylhydrazyl; and BHTE: Butylated hydroxytoluene equivalent/g; α-TPEE: α-Tocopherol equivalent/g; GAE: Gallic Acid equivalent/100 g; RTE: Rutin equivalent/100 g

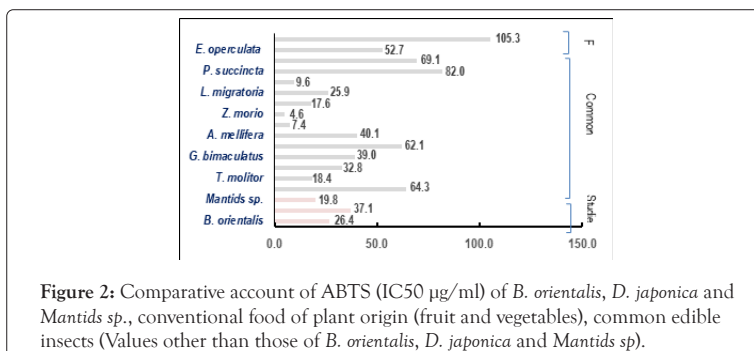
\*The concentrations used for TAC and RP assays were 500 µg/ml; for phenolics 500 µg/ml and for flavonoids 4.0 mg/ml

**Table 2:** Correlation coefficient among ABTS•+, DPPH• free radical scavenging activity, total antioxidant activity, reducing power, total phenolic content and total flavonoids content of *mantids sp.* (ootheca), *B. Orientalis* and *D. Japonica*.

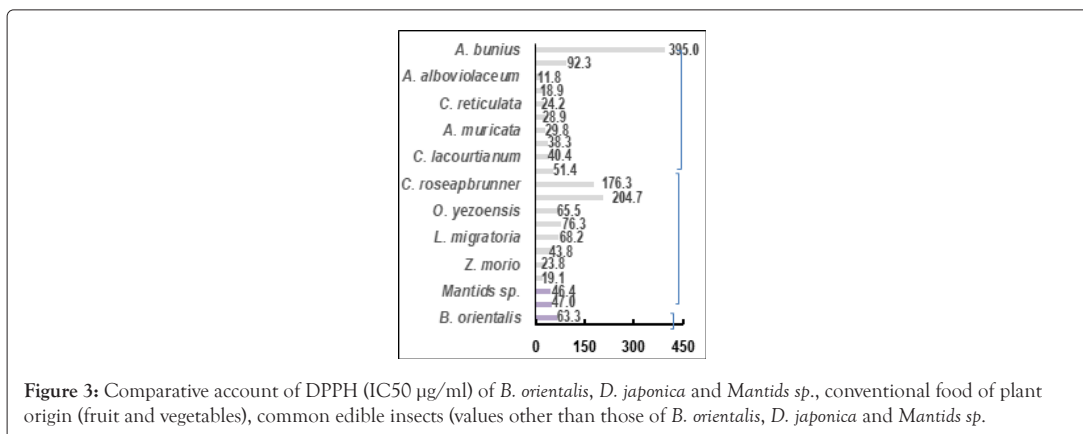
|            | ABTS+   | DPPH•   | TAC    | FRP    | Phenols | Flavonoids |
|------------|---------|---------|--------|--------|---------|------------|
| ABTS+      | -       | -       | -      | -      | -       | -          |
| DPPH•      | -       | -       | -      | -      | -       | -0.9375    |
| TAC        | -0.7828 | -       | 1      | 0.9961 | 0.9996  | -          |
| FRP        | -0.7255 | -       | 0.9961 | 1      | 0.9982  | -          |
| Phenols    | -0.7655 | -       | 0.9996 | 0.9982 | -       | -          |
| Flavonoids | -       | -0.9375 | -      | -      | -       | -          |



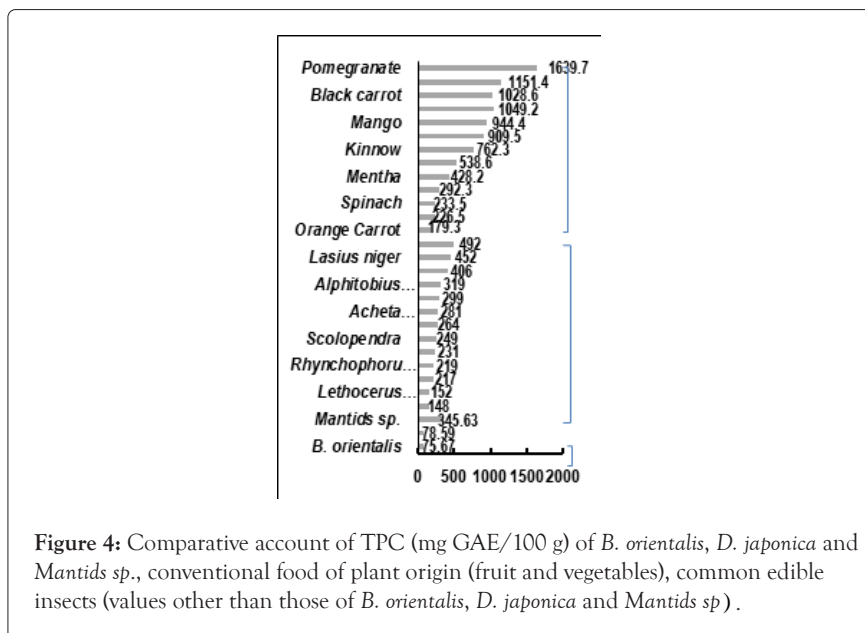
**Figure 1:** Inhibition of (a) ABTS+•; (b) DPPH• free-radical of *B. orientalis*, *D. japonica* and *Mantids sp.* Extracts. Note: ABTS+• = Inhibition DPPH• = free-radical of ( — ) *B. orientalis*, ( — ) *D. japonica* and ( — ) *Mantids sp.* Extracts.



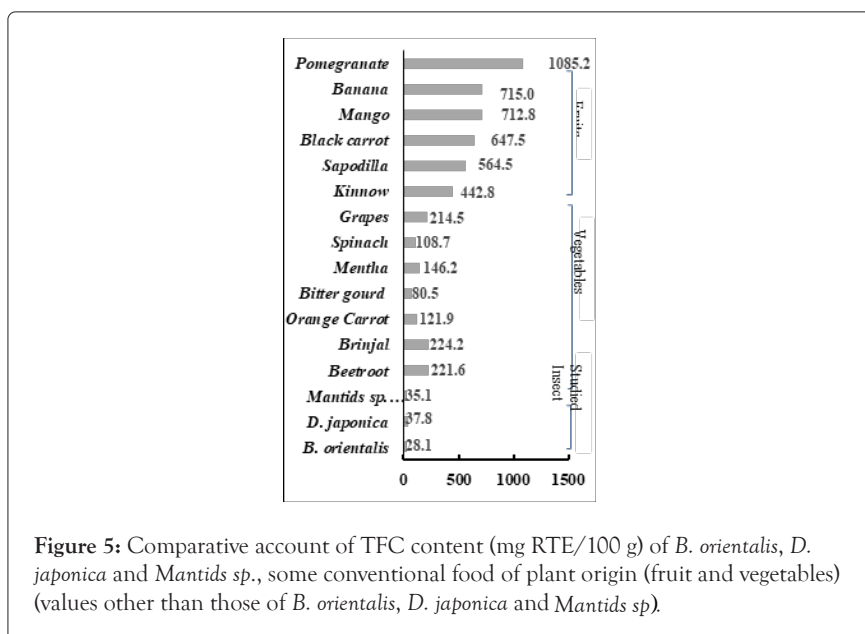
**Figure 2:** Comparative account of ABTS (IC50 µg/ml) of *B. orientalis*, *D. japonica* and *Mantids sp.*, conventional food of plant origin (fruit and vegetables), common edible insects (Values other than those of *B. orientalis*, *D. japonica* and *Mantids sp.*).



**Figure 3:** Comparative account of DPPH (IC50 µg/ml) of *B. orientalis*, *D. japonica* and *Mantids sp.*, conventional food of plant origin (fruit and vegetables), common edible insects (values other than those of *B. orientalis*, *D. japonica* and *Mantids sp.*).



**Figure 4:** Comparative account of TPC (mg GAE/100 g) of *B. orientalis*, *D. japonica* and *Mantids sp.*, conventional food of plant origin (fruit and vegetables), common edible insects (values other than those of *B. orientalis*, *D. japonica* and *Mantids sp.*).



**Figure 5:** Comparative account of TFC content (mg RTE/100 g) of *B. orientalis*, *D. japonica* and *Mantids sp.*, some conventional food of plant origin (fruit and vegetables) (values other than those of *B. orientalis*, *D. japonica* and *Mantids sp.*).

## ABTS, DPPH, FRP and TAC

Free radicals and other oxidants have highly toxic molecules, self-styled unstable and highly reactive molecule (Reactive Oxygen Species/ROS) produced due to aerobic metabolism and environmental stress. These free radicals involve in the regulation of various mechanisms and intercellular signalling at a permissible level. Over generation of free radical leads to oxidation that causes injurious stress to bio-molecular system and the accumulation of tissue damage. Once they are formed, the chain reaction starts. However, biological system is equipped with to encounters free radicals by prevention, interception and repair through production of antioxidants.

From the analysis, it appears that extract of oothecae of *Mantis sp.* better inhibit free radicals with low inhibitory concentrations ( $IC_{50}$ ) of 19.76 for ABTS followed by 26.43 in mole cricket and 37.12 in bush cricket. In turn, with a value of 63.32, 47.02 and 45.38 antiradical activity against DPPH was highest in extract of otheeca of mantids with similar scavenging activity in bush cricket and in

mole cricket. The variation in DPPH and ABTS may not be unusual for different species in this study having different proportion of constituent in them liable to effect antioxidant scavenging activity. Oghenesuwwe proposed that insects, like any other living system, contain bioactive compounds, natural antioxidant enzymes, and several other chemical compounds that can mediate or affect the antioxidant activity of the sample [32]. He postulated that protein from different species might give different scavenging activity on DPPH and ABTS [33]. Similarly, reported that DPPH and ABTS scavenging capability depend on protein content and ABTS assay is sensitive to amino acids and peptides compared to DPPH assay in radical scavenging activities. Besides Tsiba, et al. [34] proposed that phenolic compounds are known to be powerful compounds with ability to reduce free radicals. Having high protein content, at least in mole cricket and bush cricket [2,6] considerable DPPH and ABTS scavenging capability of the studied insect may not be unusual even could be justified by the relatively high content total phenols and flavonoids quantified in this study but the mechanism needs to be further investigated.

The DPPH and ABTS scavenging capability of the insects in the present study is either superior to some edible insects such as cockroach, locust, 2 species of grasshopper, for DPPH; super-worm, stone fly cockroach for ABTS or lower than the DPPH of Madagascar cockroach, stone fly, super worm and ABTS of Honey bee, 2 species of grasshopper, rhinoceros beetle but equivalent to meal worm larvae, Madagascar cockroach and locust for ABTS; DPPH of a species of grasshopper [35-37]. Both ABTS and DPPH are much better than the wild fruits from NE India like *E. operculata* and *A. bunius* in the studied insects [38].

Among the studied insects, FRP value is significantly ( $P < 0.05$ ) higher in ootheca of praying mantis (18.98) followed by bush cricket (3.29) and lowest in mole cricket (1.31). It is to be noted that antioxidant activity is a complex process usually happens through several mechanisms and is influenced by many factors e.g.; DPPH and ABTS assay are based on electron and hydrogen atom transfer, whereas FRP assay is based on electron transfer reaction [39]. The result indicates that extract of insects in the present study can be used as compound able to donate electrons and thus showing antioxidant activity.

TAC considered as the cumulative effect of all antioxidant present in the sample. This assay is based on reduction of molybdenum  $+6/+5$  by the extract. Although TAC assay revealed that studied insects showed TAC of 25.59 in ootheca of mantid, 3.29 in bush cricket and 1.31 in mole cricket indicating the strong antioxidant potential of these insects. Nevertheless, it has been reported that TAC assays do not indicate/measure total antioxidant capacity, rather in biological fluids contains numerous compounds with chain breaking antioxidant activity, including urate, thiol, flavonoids to name a few, the major contributor TAC assays is urate [40,41]. However, in the present study it is revealed that overall with increasing trend of TAC among the studied insect, not only there is increase in the trend of phenolic content among the respective insects but also an increasing trend of scavenging potential/activity with respective insects.

A strong positive correlation with significant value ( $p < 0.05$ ) was observed between the antioxidant assay by TAC ( $r = 0.9962$ ), FRP ( $r = 0.9996$ ) and the phenolic content TPC ( $r = 0.9982$ ). Whereas ABTS is negatively correlated to FRP with moderate value of  $r$ , ( $r = -0.7255$ ), and to TAC ( $r = -0.7828$ ) as well as TPC ( $r = -0.7655$ ) respectively. It point toward that phenolic content is largely contribute to the antioxidant activities of the studied insect, and therefore it could play an important role in the beneficial effects of these important insects. The present findings were in accordance with other researchers report, several studies have found that phenolic compounds are major antioxidants constituents in plants and there is direct relationship between their antioxidative activity and total phenolic content [42-45].

### Phenolic and flavonoids

Phenolic compounds are mainly a class of secondary metabolites of plant having diverse functions including protection from herbivores, resistance against microbial pathogens. Similarly, Flavonoids consist of a large group of poly-phenolic compound universally present in plant. The antioxidant property of flavonoids mediated by various mechanisms of enzyme inhibition involved in free radical generation. Given the biochemical and functional diversity of plant phenolic and flavonoids, herbivorous insects that consume from leaves and other plant tissues gets sequestered to them.

The present study shows that as a major constituent of secondary metabolites, phenolic and flavonoids are present in the insect of this study. The total Phenolic content (GAE/g) is significantly higher in the *Mantis sp.* (345.63) compared to mole and bush cricket (75.67 to 78.59). On the other hand, flavonoid content, varied between 28.06 (mole cricket) to 37.78 (Bush cricket) and *Mantis sp.* (35.08). Overall, the phenolic and flavonoid of the studied insects observed to be comparable with other edible insects such as mealworm beetles crickets and other conventional food of plant and animal origin (Figure 4 and 5). Phenolic and flavonoid content in edible insect has also been reported earlier e.g. Pyo, et al. [36] reported 1.3 to 15.6 mg/g phenol, 0.1 mg/g to 5.7 mg/g, favonoid in three coleopteran sp 2 orthoptera (*gryllidae*) and one species of hymenoptera; 299 to 492 (mg GAE 100 g) phenolic in cricket, grasshopper silkworm, evening cicada African caterpillars. Similarly, Favonoid content in mole cricket, bush cricket and mantids species in the present study, falls within the range of flavonoid content reported for vegetable like *Amaranthus*, *S. nigrum*, *B.campestris* *S. oleracea* (values between 27.52 to 59.70) and fruits (*A.gigantum*, *A.muricata*, *A. genis*, *A.alboviolaceum*, *F.capensis*, (23.36 to 36.86) reported by Tsiba, et al. [46] but lower than the flavonoids for fruits and vegetable e.g. kinnow orange, mango, beetroot carrot etc. reported by Singh, et al [47]. Our results show that the phenolics and flavonoids content of edible insects in this study is different, may be due to taxonomical difference and dietary habits in them. Ghosh reported the variation in amino acids and fatty acid in different developmental stages of honey bee and speculated different feeding habit is accountable for such variation [48]. It was also pointed out that insects feeding on plant or plant exudates or animal resources can efficiently converting otherwise unavailable plant and animal resources into edible form, can serve as human food or food additives [49-59]. The overall antioxidant potential among the insects in the present study the prospective of *Mantid* species seems to be considerable and may be correlated with the therapeutic value of *Mantid sp* [20] but it is premature for such proclamation but further investigation needs to be continued.

### CONCLUSION

The bush cricket, mole cricket and mantid species represent a potential source of unexplored redox ingredients with low ecological impact with an antioxidant efficiency related to their taxonomy and eating habit. Analyses of anti-oxidant activities of these three insect, the bush cricket, mole cricket and mantid species have revealed that these insects have their own biological functionalities. Of the insects studied, mantid species had stronger antioxidant activity than showing better DPPH, ABTS scavenging activities, TAC, FRA as well as TPC but moderate TFC value compared to bush cricket, mole cricket. TPC showed stronger a strong positive correlation with antioxidant assayed. Hence these insects in general and mantis in specific could play a role against the diseases caused by oxidative stress inhibiting the development of various human diseases hence our study give a direction towards, isolation and identification of novel bioactive compounds responsible for antioxidant activity.

These insect extract might be an additional way to impulse other alternative presentation of insects-based food for human consumption and to provide an added value to the edible insects by the production of bioactive ingredients for nutraceutical or (can be labeled as) "entomocutical" or food purpose. The compiled results revealed that these insects' species historically been used by human for food or medicinal use, is not only healthier, it is also the

product of generation of harmonious co-existence between tribe and environmental resource and human insect interaction that most of the time, is little known. More evidence is needed in order to understand if the practice of eating insects might contribute to modulate oxidative stress in human and the identification of their bioactive ingredients. Further studies are in progress to quantify other bioactive compounds.

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## CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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