Oecophylla smaragdina (Hymenoptera: Formicidae) and *Odontotermes* sp. (Isoptera: Termitidae) a potential source of antioxidant: the two most preferred edible insects of Arunachal Pradesh, India

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Abstract

Antioxidant potential of weaver ant *Oecophylla smaragdina* (adult & brood) and termite *Odontotermes* sp the two common species of insects used as food by tribes of Arunachal Pradesh and elsewhere in India. Our findings highlight the antioxidant potential of these two insects. DPPH[•] scavenging activity IC₅₀ (μg/mL) ranged from 59.56 (weaver ant adult) to 66.30 (termite). Termite species scored higher ABTS^{•+} scavenging activity (IC₅₀: μg/mL), Ferric reducing power (TPEE μg/g) and phenolics (mg GAE/g) (18.70, 36.60 and 626.92) than weaver ant adult (52.57, 211.21 and 369.69) and weaver ant brood (33.34, 114.32 and 486.04). On the other hand, weaver ant adult scored higher flavonoids (mg RTE/g) (663.43) than its brood (387.19) and termite species (58.04). Weaver ant brood contained substantial amounts of phenolics and flavonoids, comparatively higher than phenolics of weaver ant adult and flavonoids of termite. These two insects may serve as an ideal dietary food supplement for handling oxidative stress and as a replacement for some conventional food products. However, further study is needed to find out the bioactive compound at the individual species level.

1 Introduction

Antioxidants are substances that can prevent or slowdown the damage to cell caused by free radicals, produced in the body as a reaction to environmental or other pressures [1]. In the past decade, antioxidant compounds have gained acceptance among the general public for their ability to neutralize reactive and damaging forms of free radicals [2]. Although insects are known for their diverse nutritional properties, a high percentage of protein and/or high content of fatty acids (mono and polyunsaturated), minerals, and vitamins [3], however, the contribution of insects as a functional food (i.e.; to provide both basic nutrition and health benefit) is still too scarce. Henry (2010) suggested that it is essential to evaluate and assess the functional benefits of food to provide evidence-based science to substantiate health benefits [4]. Keeping this in mind the present investigation was aimed to evaluate the antioxidant potential of most preferred edible insects i.e.; weaver ant and termite [5, 6].

Among the tribe of Arunachal Pradesh, entomophagy is a traditional and culturally accepted practice. The interest in the use of insects as food in this state has been reported in several earlier studies [6–10] and has revealed 102 edible species of insects belonging to 13 different orders taken as food by one or the other 26 major tribes of Arunachal Pradesh [11]. Two kinds of edible insects, weaver ants (*Oecophylla smaragdina* Fabricius 1775) and termites (*Odontotermes* sp.) are appreciated by virtually all tribes of the region. We found that the main reason behind the wide

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acceptance of these two insects apart from their taste and flavours that they were locally abundant throughout the year (weaver ant) and they could be gathered in large amounts during their swarming periods (termites). Weaver ant and termite have been known for a long time to be popular food insects not only in Arunachal Pradesh but also in many parts of the world [12–15] [8–11 including other parts of India [11]. Given this widespread acceptance of these two kinds of insects as human food, except for our investigation on nutrient content analyses [6], no detailed information on antioxidant activity of the Indian species has been published to date except a very preliminary investigation by Raza et al. [5]. Ever since, weaver ants, as well as termites, depend on nearby food sources that are influenced by climatic factors, the mineral content of the soil and vegetation in their respective geographical areas, etc. Therefore, the present investigation is the extension of our earlier study on taking into account of species collection only from a selected area of Arunachal Pradesh. Our study on the antioxidant potential of these insects, nevertheless, demonstrates that these two insects might serve as an ideal dietary food supplement for handling oxidative stress and as a replacement of some conventional food products.

2 Material and methods

2.1 Sample collection and identification

Odontotermis sp. (termite) and, *Oecophylla smaragdina* (weaver ant) were collected from Emchi village (Papumpare district, Arunachal Pradesh). Termite was collected at night, and weaver ant (adult and brood) was collected from the nest on the mango tree. The sexes of the specimens were not separated, because both are equally appreciated as food. Both 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 *Oecophylla smaragdina* Fabricius 1775 (Hymenoptera, Formicidae) and *Odontotermes* sp. (Isoptera, Termitidae). Once in the laboratory the sampled insects were washed thoroughly in double distilled water and brought them to dryness and stored at -20°C. All the analysis were performed within a months' time.

All the solvents and chemicals used in the study were of analytical grade. The analyses were done for the 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, phenolics and flavonoids.

2.2 Preparation of insect extracts

10 g each of chopped insect samples was homogenized in 50 mL of buffered methanol (1 mM PBS buffer pH 6.8; 1: 1) kept in orbital shaker for 48 h. The resultant extract was centrifuged at 7000 rpm for 30 min at 4°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°C to dryness and dissolved in buffered methanol for further analysis.

2.3 Analyses

2.3.1 ABTS^{•+} free-radical scavenging activity

ABTS radical scavenging activity was determined by following the modified method Moore and Yu [16]. Briefly, by addition of 30 µL of diluted insect extract from seven different final concentrations to 270 µL of ABTS working solution by using 96 well microplate. After 6 min of incubation in dark at 37°C, the absorbance was determined at 734 nm. Trolox was used as standard and ABTS antiradical activity was expressed as IC₅₀ value (µg/mL).

ABTS stock solution (7.4 mM) was added to (2.6 mM) potassium persulfate solution in equal quantities and kept for 16–24 h at room temperature in the dark to yield a dark-coloured solution containing ABTS radical and working reagent diluted to an initial absorbance of about 0.7 ± 0.02 at 734 nm.

2.3.2 DPPH[•] free-radical scavenging activity

DPPH radical scavenging activity was determined according to the method described by Blois, [17] with slight modifications. Briefly, the reaction mixture contained 200 μ L of extract diluted in methanol at different concentrations and 100 μ L of (0.8 mM DPPH in 95% methanol). After being incubated at room temperature 37°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 IC₅₀ value (μ g/mL).

2.3.3 Ferric-Reducing power (FRP)

Ferric-Reducing power (FRP) was done using the method from Oyaizu [18] with minor modification as described by Jayaprakasha et al. [19]. The extract (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°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 μ L) was added with 800 μ L of Millipore water, followed by adding 100 μ 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 along with α -Tocopherol standard. The results are expressed as α -Tocopherol equivalent TPEE (μ g/g).

2.3.4 Phenolics

Phenolics in extracts was determined by the modified Folin-Ciocalteu method described by Singleton et al. [20]. Briefly, 500 μ L of the extract was mixed with 1.0 mL of Folin-Ciocalteu reagent (previously diluted with Millipore water 1:10 v/v), and after 5 min of incubation, 1.0 mL of sodium carbonate reagent (10% Sodium chloride solution in Millipore water) was added. The tubes were vortexed for the 30 s and allowed to stand for 25 min at 37°C for colour development, and absorbance was measured at 760 nm. Gallic Acid was used as standard and expressed as Gallic Acid equivalent (GAE mg/100 g).

2.3.5 Flavonoids

Flavonoids were estimated using aluminium chloride as described by Chang et al.[21]. Briefly, 1.0 mL of insect extract was added to 1.0 mL of (10% w/v) aluminium chloride and vortexed for the 30 s. 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 Rutin equivalent (mg RTE/100 g).

2.4 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 was carried out through Graphpad Prism. 9.0.0 (California Corporation Inc., CF, USA), and Microsoft Excel 2016 were used for graphical representation.

3 Result and discussion

Oecophylla smaragdina, the weaver ant when required leafy nests that the weaver ants construct are plucked from their host tree for consumption after sorting them out from the nest. Both larval and adult stages are preferred and are eaten either raw or turned into “chutney” by mixing them with spicy ingredients. On the other hand, *Odontotermes* sp. usually collected when it is abundant i.e.; during the swarming phase in rainy season, and is eaten either raw or roasted. Therefore, it is evident that compounds meant for antioxidant capacity are consumed without any loss of activity when consumed as fresh/raw. Our results (Table 1; Figs. 1, 2, 3, 4) show that the antioxidant pattern of studied insects varied according to taxonomy and life stages.

From the analysis Table 1 and Fig. 1, it appears that termites better inhibit free radicals with low inhibitory concentrations (IC₅₀) of 18.70 for ABTS followed by weaver ant brood (33.34) and weaver ant adult (52.57). In turn, antiradical activity against DPPH was noted in weaver ant adult and its brood (59.55 and 60.93) with slightly lower in termite (66.38). These values are either superior or similar to some edible insects, common fruits, and vegetable, though, in some cases

Table 1 ABTS^{•+}, DPPH[•] free radical scavenging activity (IC₅₀ µg/mL), ferric-reducing power (α-TPEE µg/g), phenolics (mg GAE/100 g) and flavonoids (mg RTE/100 g) of weaver ant (*O. smaragdina*) and termite (*Odontotermes sp.*)

species	Edible Stages	ABTS ^{•+} Radical Scavenge (IC ₅₀ µg/mL)	DPPH [•] Radical Scavenge (IC ₅₀ µg/mL)	Ferric-Reducing Power (µg TPEE/g)	Phenolics (mg GAE/100 g)	Flavonoids (mg RTE/100 g)
<i>O. smaragdina</i>	Adult	52.57 ± 0.99	59.56 ± 1.02	14.39 ± 0.76	369.69 ± 3.93	663.43 ± 20.76
<i>O. smaragdina</i>	brood	33.34 ± 0.89	60.93 ± 1.03	10.62 ± 0.59	486.04 ± 3.99	387.18 ± 22.55
<i>Odontotermes sp.</i>	Adult	18.70 ± 0.70	66.38 ± 1.05	36.60 ± 0.51	626.92 ± 2.83	58.04 ± 5.19

Values are presented as mean ± SD (n = 3) for each dataset

* (ABTS^{•+}): 2, 2-azinobis-3-ethylbenzothiazoline-6-sulfonate; (DPPH[•]): 2, 2-Diphenyl-1-picrylhydrazyl; α-TPEE: α-Tocopherol equivalent/g; GAE: Gallic Acid equivalent/100 g; RTE: Rutin equivalent/100 g

*FRP assays were 500 µg/mL

Fig. 1 Inhibition of **a** ABTS^{•+}, **b** DPPH[•] free-radical of *O. smaragdina* and *Odontotermes sp.* extracts

Fig. 2 Comparative accounts of DPPH[•] (IC₅₀ µg/mL) of *O. smaragdina*, *Odontotermes* sp., conventional food of plant origin (fruit and vegetables), common edible insects (Values other than those of *O. smaragdina* and *Odontotermes* sp., adapted from Zielinska et al. [22], Chatsuwan et al. [23], Islary et al. [24], Tsiba et al. [25] and Saiki et al. [26])

Fig. 3 Comparative accounts of ABTS^{•+} (IC₅₀ µg/mL) of *O. smaragdina*, *Odontotermes* sp., conventional food of plant origin (fruit and vegetables), common edible insects (Values other than those of *O. smaragdina* and *Odontotermes* sp., adapted from Zielinska et al. [22], Chatsuwan et al. [23], Islary et al. [24] and Pyo et al. [27])

better scavenging activity could be noticed (Figs. 2, 3). Differences in radical scavenging activity between weaver ant and termite in the present study and other reports from edible insects might be for their protein quality in respective species. Chatsuwan et al. [23] postulated that protein from different species might give different antiradical scavenging activities (ABTS, DPPH, and FRP). The strong inhibition of free radicals could be justified by the relatively high concentrations of phenolics and flavonoids quantified in this study. Phenolic compounds are known to be powerful compounds with the ability to reduce free radicals [25].

Flavonoids (Fig. 4) in the weaver ant adult was significantly ($P < 0.05$) higher (663.43) than its brood (387.18) while least was observed in the termite species (58.04 mg RTE/100 g). These values (Fig. 2), except for termites, are higher than or similar to most of the fruits and vegetables with few exceptions [28]. Nevertheless, with the value 58.0,

Fig. 4 Comparative accounts of flavonoids (mg RTE/100 g) of *O. smaragdina*, *Odontotermes* sp., some conventional food of plant origin (fruit and vegetables) (Values other than those of *O. smaragdina* and *Odontotermes* sp., adapted from Singh et al. [28])

flavonoids—in termite is higher than vegetables such as *Amaranthus*, *S. nigrum*, *B. campestris*, *S. oleracea* (values between 27.52 to 59.70) and fruits such as *A. gigantum*, *A. muricata*, *A. ginseng*, *A. albobolaceum*, *F. capensis*, (values between 23.36 to 36.86) as reported by Tsiba et al. [25].

Phenolics (mg GAE/g) (Fig. 5) was significantly higher ($P < 0.05$) in the termite species (626.92) followed by the weaver ant brood (486.04) and its adult (369.69). These values are superior to as reported for some edible insects and comparable to most of the fruits and vegetables, except for few.

The results obtained advocate that weaver ant (*O. smaragdina*) and termite (*Odontotermes* sp.) can both be recommended as a source of natural antioxidants on par with or superior to conventional fruits and vegetables to avoid oxidative stress such as degenerative diseases [31] and provide protection against oxidation at the cellular level by interfering in enzyme activity [32].

Fig. 5 Comparative accounts of phenolics (mg GAE/100 g) of *O. smaragdina*, *Odontotermes* sp., conventional food of plant origin (fruit and vegetables), common edible insects (Values other than those of *O. smaragdina* and *Odontotermes* sp., adapted from Singh et al. [28], Di-Mattia et al. [29] and Flores et al. [30])

4 Conclusion

Weaver ant (*O. smaragdina*) and termite (*Odontotermes* sp.) can play a role against the diseases caused by oxidative stress to human and can provide an added value as bioactive ingredients for nutraceutical or (can be labeled as) “entomocetical”. This study also reflects, since time immemorial, how logically the tribes/people had chosen these insects as food.

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Authors' Contribution MR conducted the experiment, data analysis and interpretation. SDT contributed in sample collection and experimentation. JC designed and executed the experiment and manuscript drafting. All authors read and approved the final manuscript.

Data availability The dataset generated for this study are available on request to the corresponding author.

Declarations

Competing interests The authors declare no competing interests.

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