Ethnoentomological values of *Oecophylla smaragdina* (Fabricius)

V. V. Vidhu and D. A. Evans*

Department of Zoology, University College, Thiruvananthapuram 695 034, India

Adult worker ants and brood of Oecophylla smaragdina are a delicacy in the food of tribal groups residing in the forests of Wayanadu and Kasaragod districts of Kerala, India. These tribes use the crushed body of worker ants to make a sauce which is sour. Adult worker ants possess high formic acid (FA) content in their abdominal poison gland reservoir. Oecophylla brood has no traces of FA, but possesses all the essential amino acids, especially tryptophan, leucine, threonine, methionine and lysine in high concentration. Brood also has high carbohydrate content and very low lipid content. Carbohydrate, protein and lipid exist in a ratio 5:2.5:1 on wet weight basis. Brood is a rich source of retinol, tocopherol, ascorbic acid, thiamine, niacin and riboflavin, which are present at several times higher concentration than that of the egg of domestic fowl. Among various minerals tested, Na, P, Ca and Mg were present in very high concentration. The tribes made medicated oil using hot extraction of crushed worker ants to treat inflammation of joints and skin infections. Whole-body aqueous extract of worker ants has been shown to have significant antioxidant and anti-arthritic properties. Abdominal gland secretion showed anti-microbial activity against six bacterial and two fungal strains. GC-MS analysis of abdominal glands (Dufour's gland and poison gland) revealed the presence of 39 chemical compounds.

Keywords: Antimicrobial activity, ethnoentomological values, medicated oils, *Oecophylla smaragdina*.

ETHNIC communities in the forests of Wayanadu and Kasaragod districts, Kerala, India follow a peculiar lifestyle, especially in their food habits, incorporating tuberous roots, seeds of bamboo, wild fruits, honey of wild bees and brood of social insects. Entomophagy is common among them and the species consumed are the brood of rock bee *Apis dorsata*, wasp *Vespa orientalis*, ant *Oecophylla smaragdina* and reproductive forms of termite *Odontotermes redimani* and *Odontotermes obesus*. The Asian arboreal weaver ant *Oecophylla smaragdina* is a dominant predatory insect in the tropical ecosystem. Due to their predatory potential, these ants are reported to be used against over 50 species of insect pests from around 12 diverse crops in tropical areas¹. There is much hue and cry in Kasaragod district for the complete ban of endosulfan, which is used for the control of tea mosquito, Helopeltis antonii in sprawling plantations of cashew, Anacardium occidentale². Now it is well understood that O. smaragdina can be successfully utilized for the control of this serious pest of cashew tree³. Tribes regularly collect nests and eat eggs, larvae and pupae of workers and reproductive forms after burning the large leafy nests for a short period to kill the aggressive workers. The tradition of including weaver ants in food and traditional medicine has been reported from various cultures in Thailand, India, Myanmar, Borneo, the Philippines, Papua New Guinea, Australia and Congo⁴. The traditional healers of Bastar region, Chhattisgarh, India use these ants to treat common health problems and as an ingredient in some food items⁵. The present study documents the nutritional and medicinal values of O. smaragdina which have economic significance in preventing malnutrition and health problems among the indigenous human populations.

Materials and methods

Large nests of *O. smaragdina* were collected from the field and put in a glass jar and then anesthetized using chloroform. Adult workers and brood (including egg, larvae and pupae of workers and reproductive forms) were separated and used for quantitative estimation.

Nutritive value of brood and workers

A mix of larvae and pupae of workers (brood), worker ants (major) and egg (whole) of domestic fowl were used for nutrient analysis. The whole-body homogenate of brood and workers in distilled water was used as assay sample. In the case of fowl egg, egg white and yolk were thoroughly mixed and volume and weight were recorded. Sample (brood, adult workers, fowl egg) homogenates were used for the estimation of total carbohydrates⁶ glycogen⁷, total protein⁸, retinols⁹, tocopherol¹⁰, ascorbic acid¹¹, thiamine¹², niacin¹³, riboflavin¹⁴, formic acid¹⁵ and phosphorus¹⁶ by spectrophotometry. Total fat was also estimated¹⁷. Essential amino acids were eluted after paper chromatography and quantified using spectrophotometry

^{*}For correspondence. (e-mail: drevansda@gmail.com)

(Systronics, UV/VIS). Sodium, potassium, calcium, magnesium, manganese, copper, iron and zinc were quantified using atomic absorption spectrometry (PerkinElmer PinAAcle 900H) along with reference standards.

Medicinal value of worker ants

Whole-body homogenate of worker ants in distilled water (100 mg/ml) was prepared and used for assays at various dilutions.

Total antioxidant activity: The total antioxidant activity of samples was evaluated quantitatively by the green phosphomolybdenum complex formation by spectrophotometry using ascorbic acid (10 mg/ml distilled water) as reference standard and the activity was expressed as phosphomolybdenum reduction potential (PRP) in ascorbic acid equivalents¹⁸.

Anti-arthritic activity: The *in vitro* anti-arthritic activity was determined by inhibition of protein denaturation method¹⁹ using bovine serum albumin as substrate. The percentage inhibition of protein denaturation was compared with standard drug diclofenac at various concentrations (250, 500, 1000 μ g/ml).

Antimicrobial assay: Agar-well diffusion method²⁰ was used for detecting antimicrobial properties against different strains of bacteria and fungi. Abdominal glands of 120 worker ants (equal number from three categories) were dissected out, homogenized in DMSO and diluted into three different concentrations in such a way that 100 μ l homogenate contained glands of 3, 6 and 12 worker ants. The homogenate was neutralized to pH 7 prior to assay.

Antibacterial activity: Petri plates containing 20 ml Muller Hinton medium were seeded with 24 h culture of *Eschericia coli*, *Streptococcus mutans*, *Klebsiella pneumonia*, *Pseudomonas aeroginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Vibrio cholerae*. Wells of approximately 10 mm were bored using a well-cutter and samples of 100 μ l from each concentration were added. The plates were then incubated at 37°C for 24 h. The antibacterial activity was assayed by measuring the diameter of the inhibition zone (mm) formed around the well. Gentamycin was used as a positive control.

Antifungal activity: Potato dextrose agar plates were inoculated with *Candida albicans* and *Aspergillus niger*. Wells of approximately 10 mm were bored using a wellcutter and 100 μ l samples of varying concentration were added. The zone of inhibition was measured after 72 h incubation at 24°C and compared with that of clotrimazole standard.

GC-MS analysis and identification of compounds of abdominal gland extract: Abdominal glands from 30 worker ants, viz. 10 numbers of each category such as major, intermediate and minor²¹ were squeezed out of the abdomen by gently pulling the posterior end segments in ice-cold condition. They were immediately crushed in 2 ml ice-cold hexane, centrifuged and supernatant was taken as sample. Gas chromatography and mass spectroscopic (GC-MS) analysis was carried out using Varian CP-3800 (GC), Saturn 2200 (MS) with VF-5 ms, $30 \text{ m} \times 0.25 \text{ mm}$ capillary column. The instrument was set at an initial temperature of 70°C and maintained at this temperature for 2 min. At the end of this period, the oven temperature was increased up to 300°C, at the rate of 5°C/min, and maintained for 9 min. Injection port temperature was ensured as 250°C and helium flow rate was 1 ml/min. The ionization voltage was 70 eV. The samples were injected in split mode as 10 : 1. Mass spectral scan range was set at 60-400 (m/z). Interpretation on mass-spectrum GC-MS was done by referring to National Institute Standards and Technology (NIST) database.

Statistical analysis: Statistical analysis was done using IBM SPSS Statistics 20 software.

Results

The brood of *O. smaragdina* could be seen within large nests on trees such as *Citrus*, *Mangifera*, *Artocarpus*, *Terminalia*, *Anacardium*, monocots such as *Caryota* and climbers such as *Raphidophora* (Figure 1). Carbohydrate, protein and lipid were distributed in the brood in the ratio 5: 2.5: 1 when tested during April to September, which is the peak breeding season of these ants (Table 1). The total carbohydrates content of the brood was ten times higher than that of adult ants and egg of domestic fowl.



Figure 1. Brood of *Oecophylla smaragdina*.

The lipid content of the ant brood was only one-fifth that of fowl's egg (P < 0.05) (Table 1).

All the essential amino acids were present at a higher concentration in ant brood and adult workers compared to fowl egg, except phenyl alanine and histidine (Table 2). Water-soluble vitamins like thiamine, riboflavin, niacin, and ascorbic acid were present at a higher concentration (10.3, 6.5, 42, 12.8 mg/100 g) compared to fowl egg (0.09, 0.56, 0.18, 1.83 mg/100 g) (P < 0.05). Among the fat-soluble vitamins studied, retinol and tocopherol could not be detected in fowl egg, but were present in very high concentration in ant brood (Table 3).

 Table 1. Nutrient content of adults and brood of Oecophylla smaragdina and egg of domestic fowl

	O. smaragdina r		
Parameters	Brood	Adult	Fowl egg
Carbohydrate Protein Fat	$\begin{array}{c} 10.06 \pm 0.7^c \\ 5.4 \pm 0.09^b \\ 2.00 \pm 0.03^a \end{array}$	$\begin{array}{c} 1.10 \pm 0.05^{b} \\ 3.40 \pm 0.05^{a} \\ 3.00 \pm 0.002^{b} \end{array}$	$\begin{array}{c} 0.89 \pm 0.01^{a} \\ 19.56 \pm 0.5^{c} \\ 9.67 \pm 0.5^{c} \end{array}$

Values are expressed in g/100 g fresh tissue. All the values are mean \pm SD, n = 6.

^{a-c}Significance between the groups at 0.05 level by Tukey's test.

 Table 2. Essential amino acids content in adults and brood of O.

 smaragdina and egg of domestic fowl

Amino acid	Brood	Worker	Fowl egg
Phenyl alanine Tryptophan Valine Leucine Isoleucine Lysine Threonine Methionine	$\begin{array}{c} 0.43 \pm 0.01^{a} \\ 3.03 \pm 0.02^{c} \\ 1.10 \pm 0.02^{b} \\ 2.03 \pm 0.05^{b} \\ 1.29 \pm 0.03^{c} \\ 3.00 \pm 0.02^{c} \\ 1.58 \pm 0.13^{b} \\ 2.00 \pm 0.06^{c} \\ \end{array}$	$\begin{array}{c} 0.50 \pm 0.03^{\mathrm{b}} \\ 0.58 \pm 0.01^{\mathrm{b}} \\ 1.75 \pm 0.02^{\mathrm{c}} \\ 3.40 \pm 0.02^{\mathrm{c}} \\ 0.68 \pm 0.03^{\mathrm{a}} \\ 2.34 \pm 0.03^{\mathrm{b}} \\ 1.76 \pm 0.01^{\mathrm{c}} \\ 0.74 \pm 0.02^{\mathrm{b}} \end{array}$	$\begin{array}{c} 0.88 \pm 0.03^{c} \\ 0.20 \pm 0.01^{a} \\ 0.96 \pm 0.03^{a} \\ 0.67 \pm 0.02^{a} \\ 1.24 \pm 0.01^{b} \\ 0.91 \pm 0.02^{a} \\ 0.56 \pm 0.01^{a} \\ 0.38 \pm 0.01^{a} \end{array}$
Histidine	$0.33 \pm 0.02^{\circ}$	$0.32 \pm 0.02^{\circ}$	$0.46 \pm 0.01^{\circ}$

Values are expressed in mg/100 g fresh tissue. All the values are mean \pm SD, n = 6.

^{a-c}Significance between the groups at 0.05 level by Tukey's test.

 Table 3.
 Water-soluble and fat-soluble vitamins content in adults and brood of O. smaragdina and egg of domestic fowl

	O. smaragdina r		
Vitamin	Brood	Adult	Fowl egg
Retinol	4.7 ± 0.2^{b}	0.000 ^a	0.000^{a}
Tocopherol	$17.4 \pm 0.6^{\circ}$	13.5 ± 0.5^{b}	0.000^{a}
Thiamine	$10.3 \pm 0.7^{\circ}$	$6.23\pm0.78^{\text{b}}$	0.09 ± 0.001^{a}
Riboflavin	6.5 ± 0.3^{b}	$7.33\pm0.2^{\rm c}$	0.56 ± 0.003^a
Niacin	$42.0 \pm 12.2^{\circ}$	31 ± 1.6^{b}	0.18 ± 0.001^{a}
Ascorbic acid	$12.8 \pm 0.1^{\circ}$	$10.2\pm0.1^{\text{b}}$	1.83 ± 0.03^{a}

Values are expressed in mg/100 g fresh tissue. All the values are mean + SD n = 6.

^{a-c}Significance between the groups at 0.05 level by Tukey's test.

Among the nine minerals studied, elements such as sodium, potassium, phosphorus, calcium, magnesium, manganese, iron, zinc and copper were present in the brood as well as adults. Adult ants possessed more than double the phosphorus and magnesium content compared to brood, which was five times more than that present in fowl egg (P < 0.05) (Table 4).

GC-MS analysis showed the presence of around 39 compounds. Among them, 13 were in major workers (Table 5 and Figure 2 a), 23 in intermediates (Table 6 and Figure 2 b) and 17 in minors (Table 7 and Figure 2 c).

Abdominal gland extract of worker ants showed antioxidant activity in a dose-dependent manner when tested and compared with ascorbic acid (P < 0.05) as a reference standard (Figure 3). The sample also showed antiarthritic activity when compared with diclofenac as a reference standard (P < 0.05) (Figure 4).

The antimicrobial activity of abdominal gland extracts (combined extracts of three worker castes) of

Table 4. Mineral content of adults and brood of *O. smaragdina* andegg of domestic fowl

Mineral	Brood	Worker	Fowl egg
Na	$600.0 \pm 12.3^{\circ}$	122.80 ± 3.5^{a}	$142.0\pm2.7^{\text{b}}$
K	340.0 ± 10.1^{b}	350.2 ± 10.6^{b}	135.5 ± 2.9^{a}
Р	$426.0\pm10.0^{\text{b}}$	$884.0 \pm 15.9^{\circ}$	240.0 ± 1.8^a
Ca	$163.40 \pm 2.5^{\circ}$	38.87 ± 0.24^a	74.0 ± 1.2^{b}
Mg	46.50 ± 1.02^{b}	$307.5 \pm 2.3^{\circ}$	21.0 ± 0.08^a
Mn	0.64 ± 0.05^{b}	$2.21 \pm 0.03^{\circ}$	0.13 ± 0.001^{a}
Fe	0.80 ± 0.03^{a}	$11.43 \pm 0.06^{\circ}$	$1.22\pm0.02^{\rm b}$
Cu	0.33 ± 0.01^{b}	0.33 ± 0.01^{b}	0.11 ± 0.004^{a}
Zn	$4.22\pm0.2^{\rm b}$	$4.75\pm0.03^{\circ}$	1.59 ± 0.004^a

Values are expressed in mg/100 g fresh tissue. All the values are mean \pm SD, n = 6.

^{a-c}Significance between the groups at 0.05 level by Tukey's test.

 Table 5. GC-MS analysis of abdominal gland extracts of O. smaragdina (major worker)

Compound	Retention time	Amount/ retardation factor counts
Naphthalene	9.898	14,012,267
Formic acid	10.706	77,986,546
Tritetracontane	12.995	297,735
3,5,24-Tritetracontane	13.653	3930
7-Methyl, pentadecane	14.429	33,264
1-Chloro-heptacosane	16.547	378,287
3,3-Dimethyl cyclohexen-1-one	17.405	112,560
3,4-Dimethyl-2-(3-methyl-butyryl) benzoic acid, methylester	17.962	5,221,293
4'-Hydroxy-2,2-dimethylphenone	25.091	26,858
7,9-Di-tert-butyl-1-oxaspiro (4,5) deca- 69-diene-2,8dione	26.070	286,650
7-Methyl hexadecane	14.429	33,264
15-Methyl heptadecanoic acid ester	29.846	59,272
4H-1,3-benzodioxin-4-one	33.416	564,062

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Figure 2. Chromatograms of GC-MS analysis of abdominal gland extracts of *O. smaragdina* workers. *a*, Major workers; *b*, Intermediate workers; *c*, Minor workers.





Figure 3. Antioxidant assay of whole-body homogenate of *O. smaragdina*. Sample concentration 10 mg/ml. Antioxidant activity is proportional to the increase in optical density at 695 nm. All the values are mean \pm SEM, n = 6. Value of each parameter is significantly different in Duncan's multiple range test at 0.05 level.

Figure 4. Anti-arthritic activity of whole-body homogenate of *O. smaragdina*. All the values are mean \pm SEM, n = 6. Value of each parameter is significantly different in Duncan's multiple range test at 0.05 level.

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Figure 5. Antibacterial activity of abdominal gland extract of *O. smaragdina*. 1–3, Increasing concentrations of abdominal gland extract. Concentrate 1, Glands from three ants; concentrate 2, Glands from six ants; concentrate 3, Glands from nine ants. Ab, Antibiotic gentamycin std 100 μ l (40 mg/ml),

Table 6.	GC-MS analysis of abdominal gland extracts of O. smarag-
	dina (intermediate worker)

		Amount/RF
Compound	RT	counts
Naphthalene	7.442	2,805,269
Acetic acid, chloro-, octa decyl ester	8.523	92,201
Tetracontane	8.097	876,541
Tritetracontane	8.428	163,433
Formic acid	9.542	553,258
7-Methyl, hexadecane	10.799	956,280
2-Hexyl 1 decanol	11.188	173,125
1,1-Dimethyl,2,4-bis phenol,	11.548	3,559,240
4-Ethoxy-, benzoic acid, ethyl ester	11.739	484,532
Valeric acid, tridec-2-ynl ester	12.024	245,989
4H-1,3-Benzodioxin-4-one,2-(1,1	12.643	775,506
dimethyl ethyl-6,7,8,8 a tetrahydro		
5 methyl 25-(trans)		
Cadina-1(10),6,8-triene	13.400	250,196
Tert-hexadecanethiol	13.575	1,033,859
1,1-Dimethylethyl,2,4,6-tris phenol	14.995	260,728
4-Hydroxy octadecanoic acid	15.678	720,250
3,7,11-Trim 1-dodecanol	15.679	890,925
7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-	15.822	545,328
69-diene-2,8dione		
2,6,10-Trimethyl tetradecane,	17.520	452,245
15-Methyl heptadecanoic acid ester	17.789	174,700
2-Methyl-1-hexadecanol	18.014	499,941
Glycidol stearate	22.671	449,891
Dibutyl phthalate	23.329	707,140
Bis(2-ethylhexyl)phthalate	23.329	707,140

O. smaragdina was assessed against E. coli, S. mutans, K. pneumonia, P. aeroginosa, S. aureus, S. epidermidis and V. cholerae (Table 8 and Figure 5) and fungal strains, viz. C. albicans and A. niger (Table 9 and Figure 6). The abdominal gland extract showed dose-dependent antimicrobial activity against different species of bacteria and fungi.

Adult worker ants possessed high formic acid (FA) content in their body, but the larvae and pupae had no traces of FA (Table 10). The tribal communities of Kerala use the crushed body of worker ants to make a sauce which is sour. Children of these tribes consume the brood as a delicacy, which has no traces of FA but is a treasure house of all the essential nutrients.

Discussion

The Kattunaikkan tribes of Wayanadu district and Koraga tribes of Kasaragod district, Kerala, practice entomophagy utilizing the brood and adults of *O. smaragdina*. These ants are distributed across the state in both rural and urban areas. Their aggressive bite and simultaneous spray of FA make them a potential nuisance to people, but FA is used by the ants as a major defence chemical which influences their behaviour and physiology^{22,23}. Easy accessibility to these tropical ant colonies facilitates

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Figure 6. Antifungal activity of abdominal gland extract of *O. smaragdina*. 1–3, Increasing concentrations of abdominal gland extract. Concentrate 1, Glands from three ants; concentrate 2, Glands from six ants; concentrate 3, Glands from nine ants. Ab, Clotrimazole std 100 μ l (40 mg/ml).

 Table 7. GCMS analysis of abdominal gland extracts of O. smaragdina (minor worker)

		Amount/RF
Compound	RT	counts
Azulene	7.452	3,711,567
Formic acid ester	9.524	11,258,476
Tritetracontane	10.805	191,935
2,4,6 Tris(1,7 dimethyl ethyl) phenol	11.297	1,321,750
1,1-Dimethyl,2,4-bis phenol,	11.537	38,339,540
1-Hentetracontanol	19.619	1,562,699
7-Methyl,hexadecane	14.092	422,076
1,1-Dimethylethy-1,2,4,6-tris phenol	14.995	399,417
1,2-Benzenedicarboxylic acid, mono (2ethyl hexyl) ester	15.220	2,714,798
13-Methyl pentadecanoic acid, methyl ester	15.538	207,760
7,9-Di-tert-butyl-1-oxaspiro (4,5) deca- 69-diene-2,8-dione	15.818	396,705
15-Methyl heptadecanoic acid ester	17.781	199,901
1-(2-Methylsulfanyl-ethyl-2,8,9 trioxa-5- aza-1sila bicycle (3,3,3) undecane	17.919	646,198
Glycidol stearate	20.066	1,458,171
2,2-Dimethyl oleic acid	22.407	499,092
1,2-Benzene dicarboxylic acid, bis(2 methyl propyl) ester	23.331	4,071,678
4-Amino-7-diethyl amino chromen-2 one	23.680	1,584,919

their collection and consumption by the tribal people. The tribal communities like Kanikkars and Paliyars living in the Western Ghats of Tirunelveli district, Tamil Nadu also practice the tradition of using adult worker ants and brood of *Oecophylla*, *Camponotus*, *Dasymuttila*, *Dorylus* and *Monomorium* as food and medicine²⁴. *O. smaragdina* is used as a condiment with curry by the indigenous people living in the Parambikulam Wildlife Sanctuary, Palaghat district, Kerala²⁵.

The crushed adult workers of *O. smaragdina* are used as a sauce by the tribes. The sour taste of the sauce is attributed to the presence of FA. All the three workers castes of *O. smaragdina*²¹ possess high FA content, but the brood does not possess any traces of FA (Table 10). Being a food of animal origin which is rich in carbohydrate and protein and low in lipid content enhances once again the importance of this social insect as a food supplement. This calls for popularizing entomophagy, if socially acceptable. Just as the mainstream people consume products of animal origin such as milk, meat and egg of higher animals, possessing high lipid content, tribal communities consume natural resources existing in their surroundings, such as ant brood with all essential nutrients and low lipids content. Essential amino acids like tryptophan and lysine are present in high concentration in the brood. These are needed for the production and maintenance of niacin in the human body, which increases the significance of this insect food.

Extracts of the Eurasian ant, *Formica aquilonia* are found to exhibit antioxidant and anti-inflammatory properties²⁶. The analgesic and anti-inflammatory activities of the extracts of *Polirhachis lamellidens* have also been reported²⁷. In the Bastar region, Chhattisgarh, traditional healers consider that regular intake of these ants prevents the attack of rheumatism during old age. They prepare special medicated oils from these ants to treat many common health problems⁵.

It has been reported that one of the features of several non-steroidal anti-inflammatory drugs is their ability to stabilize (prevent denaturation) heat-treated albumin at the physiological pH. The denaturation of tissue proteins is one of the well-documented causes of inflammatory and arthritic diseases. Production of auto antigens is the reason for certain arthritic disease conditions^{28,29}. Presence of significant antioxidant and anti-arthritic activities (P < 0.05) in the abdominal gland extract (Dufour's gland and poison gland) of *O. smaragdina* once again attests the traditional knowledge.

Leafcutter ant species are documented to spread metapleural gland secretions, while grooming themselves,

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Table 8. Antibacterial assay of abdominal gland extract of O. smaragaina				
	Zone of inhibition (mm)			
Bacterial strain	Gentamycin* (100 µl)	Concentrate 1 (100 µl)	Concentrate 2 (100 µl)	Concentrate 3 (100 µl)
Escherichia coli	35 ± 0.09	11 ± 0.08	13 ± 0.04	15 ± 0.05
Streptococcus mutans	27 ± 0.02	12 ± 0.08	09 ± 0.03	14 ± 0.06
Klebsiella pneumoniae	36 ± 0.09	11 ± 0.03	14 ± 0.04	16 ± 0.07
Pseudomonas aeroginosa	31 ± 0.1	09 ± 0.01	11 ± 0.1	14 ± 0.09
Staphylococcus aureus	31 ± 0.1	11 ± 0.02	12 ± 0.1	15 ± 0.07
Staphylococcus epidermidis	41 ± 0.04	15 ± 0.02	16 ± 0.02	18 ± 0.09
Vibrio cholerae	36 ± 0.03	16 ± 0.03	20 ± 0.05	24 ± 0.08

All values are mean \pm SD; n = 6.

*Gentamycin concentration - 40 mg/ml.

Concentrate 1, Glands from three ants; concentrate 2, Glands from six ants; concentrate 3, glands from nine ants.

 Table 9. Antifungal assay of abdominal gland extract of O. smaragdina

	Zone of inhibition (mm)			
Fungal strain	Clotrimazole* (100 µl)	Concentrate 1 (100 µl)	Concentrate 2 (100 µl)	Concentrate 3 (100 µl)
Candida albicans Aspergillus niger	20 ± 0.03 20 ± 0.07	$11 \pm 0.02 \\ 14 \pm 0.02$	10 ± 0.06 15 ± 0.02	12 ± 0.03 17 ± 0.02

All values are mean \pm SD, n = 6. *Standard concentration 40 mg/ml.

Concentrate 1, Glands from three ants; concentrate 2, Glands from six ants; concentrate 3, Glands from nine ants.

Table 10. Formic acid content in O. smaragdina

Worker ants and brood		Formic acid
Workers	Major Intermediate	9.7 ± 0.7 29.1 ± 2.3
	Minor	43.7 ± 3.1
Developing forms	Larva* Pupa*	nil nil

All values are mean \pm SD, n = 6 and are expressed in mg/g fresh tissue. *Larvae and pupae of either worker castes or reproductive forms.

their nest mates, including the queen and their fungal gardens³⁰. Two antibacterial peptides synthesized in the ant, *Myrmecia gulosa* in response to bacterial infection have been characterized³¹. The abdominal gland extract of *O. smaragdina* was found to be effective against various pathogenic bacterial strains such as Gram-negative *Escherichia coli*, *S. mutans*, *K. pneumoniae* and *P. aeroginosa*, Gram-positive *S. aureus* and *S. epidermidis*, and fungal strains such as *C. albicans* and *A. niger*. Unexplored antimicrobial properties of abdominal gland extract of *O. smaragdina* provide a scientific basis for the tradition of using these ants to treat skin infections, inflammation in the joints, rheumatism and other disease conditions.

GC-MS analysis of gland extracts of three types of workers showed the presence of 13 chemical compounds in majors, 23 in intermediates and 17 in minors. Among them, compounds such as alkanes, alkenes, aldehydes, ketones, esters and their derivatives were reported to be pharmacologically significant. Benzoic acid derivatives possess antibacterial, antifungal properties³². Hexadecanoic acid and naphthalene are known to have anti-feedant and insect-repellent activities³³. Phenolic compounds, esters, alkanes, aldehydes, alkenes and ketones are the major volatile compounds which have anti-inflammatory, antiarthritic, antidiabetic, antiulcer, hypolipidemic, antiatherosclerotic, anti-HIV and cytotoxic activities³⁴.

Usefulness of *O. smaragdina* as a food supplement to tribal communities has been validated with the high content of all the essential amino acids and vitamins such as thiamine, riboflavin, niacine, ascorbic acid, tocopherols and retinols with low lipids content. The high FA content in worker ants helps the tribals to use the crushed ants as a sauce. Existence of pharmacologically active compounds with antioxidant, anti-arthritic and antimicrobial activities in the abdominal glands of this ant species helps the traditional healers in making medicated oils with effective remedy against various diseases among tribals, who are away from the reach of modern medicine.

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Received 17 November 2014; revised accepted 13 May 2015