enhance haemolytic activity posed by *Acanthaster planci* spines with 99.5% severity[16].

Manganese was detected the fourth most abundant metal ion in *Naja naja karachiensis* venom. The manganese content is not reported from most of the venoms except from the Elapidae family. Manganese is found in minute amount when compared to the other cobra venoms such as *N. naja* (200 µg/g) and *N. naja atra* (13 µg/g)[9,10]. Literature review reveals that manganese is involved in activation of 5′-ND, PDE, NADase and AT(D)Pase activities. Nevertheless, it is reported to neutralize caseinolytic activity posed by acutolysin D[11,12,15].

Copper was the least abundant metallic inorganic element detected in cobra venom. It has not been documented before in different types of venoms except in a few species of Crotalidae such as *A. acutus* (175 µg/g) and *S. milarius barbouri* (200 µg/g)[9,10]. Copper is found to activate PDE and AA-NADase. However, it diminishes haemolytic, caseinolytic (acutolysin D) and insecticidal (clavata) activity[11–13,15,16].

Phosphorus is the only non-metallic inorganic constituent detected in this cobra venom. Phosphorus content might be due to degradation of normal tissue components present in snake venom glands[4]. Phosphorus apparently lacks physiological/pathophysiological function(s) to impart snake venom toxicity.

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Screening and comparison of two edible macrofungi of *Auricularia* spp.

Auricularia is a genus comprising edible macro-fungi. It grows on fresh wood or decaying tree trunks. *Auricularia* spp., family Auriculariaceae, locally known as Uchina, is consumed as a dish widely by patients in course of therapy by local traditional healers in Manipur, India. The two species *Auricularia delicata* (Mont.) Henn. and *Auricularia polytricha* (Mont.) Sacc. are found in the hilly swampy forest. It is a group of edible type of mushrooms. Our survey indicated that Uchina (UCHI-RAT, NA-EAR in Manipuri language) is the common name of the species of *Auricularia* clubbed together as one locally. It is primarily used for treating diarrhoea, dysentery, diabetes, hypertension, constipation and liver pain in the folk medicine of Manipur[1,2], the Maiba Maibae system. *A. delicata* has been studied extensively for its artificial production, physiological properties and nutritional value[3–6]. But there has been no report on studies of antioxidant compounds of these species from Manipur. Mushrooms have been studied widely for various bioactive compounds and isolation of polysaccharides, phenolics, proteins, etc.[7]. Many other mushrooms such as *Lentula edodes*, *Grifola frondosa* and *Tricholoma lobayense* have been reported of having hepatoprotective effect against paracetamol-induced liver injury[8]. The folklore use of the above species for healing diseases of liver, literature report on high antioxidant compounds[9] and antioxidant property of chlorogenic acid which are bioavailable in humans and its antiinflammatory activities[10] prompted

us for screening for high antioxidant compound chlorogenic acid. Here we report a HPLC method for screening traditional medicinal use and intra-species comparison of *A. delicata* and *A. polytricha* regarding the chlorogenic acid and elemental content with elemental analysis with EDX analysis.

Uchina (*Auricularia* spp.) was collected from 24°37′28.7″N 093°44′44.1″E, 901 msl at Bishnupur district of Manipur, India, and identified at RHMD (Raw Material Herbarium and Museum) NISCAIR-CSIR, New Delhi. A sample of each of *A. delicate* (IBSD/M-206) and *A. polytricha* (IBSD/M-205) are deposited in IBSD Herbarium. All the chemicals, *n*-hexane, petroleum ether, chloroform, methanol and ethyl acetate were of analytical grades and purchased from Merck. Extractions were performed by using polarity order of solvents such as hexane, ethyl acetate, chloroform, alcoholic, hydro-alcoholic and aqueous fractions. The extracts were concentrated using vacuum evaporator. Chlorogenic acid was purchased from Sigma-Aldrich. The HPLC studies were carried out with Waters instrument (1525 Model) using PDA detector (2996 Model), column Lichocart (C-18) (250 × 4.6, 5 µm) using HPLC grade solvents (Merck). EDX analysis was performed on FIE QUANTA EDAX instrument.

The ethyl acetate extracts of the two species were injected in a High Performance Liquid Chromatography (HPLC) (Waters, 1525 Model) with PDA detector using C-18 column (Lichrosphere®) (250 × 4.6, 5 µm) with gradient mobile phase consisting of 0.4% acetic acid and methanol at 1.0 ml/min flow rate. For 10 min the elution was carried out in 1 : 1 with 0.4% acetic acid : methanol and then 15 min on the ratio 4 : 6, at 20 min the methanol concentration was 100%. Finally the gradient was at initial stage at 25 min. The separation was carried out at room temperature at wavelength 254 nm. The content of chlorogenic acid was established by spiking the standard chlorogenic acid into the sample. Chlorogenic acid was tested in chloroform and hexane extract. Standard chlorogenic acid is injected followed by ethyl acetate extracts of the two species without the standard for estimation. Then a known amount of standard was added for confirmation of chlorogenic acid. Figures 1 and 2 show chromatograms of standard and *A. polytricha* ethyl acetate extract respectively. Chlorogenic acid content was calculated from the peak area of the sample against the area of the standard used. The analytical method was validated as per IHC rules (International Conference on Harmonization) P NORMS. The chlorogenic acid peak was confirmed by spiking with standard chlorogenic acid (Figure 3).

Fresh mushrooms were dried in shade at ambient temperature and powdered. The analysis was performed by EDX (energy dispersion X-ray) technique. The patterns are shown in Figures S1 and S2 (see Supplementary Information online).

The fruiting bodies of *A. delicata* and *A. polytricha* were air-dried. Hexane, ethyl acetate, chloroform and methanol extract were prepared for both the species.

Chlorogenic acid (Figure 4) has been reported for high oxygen radical absorbance capacity (ORAC) value. Chlorogenic acid was detected and estimated in

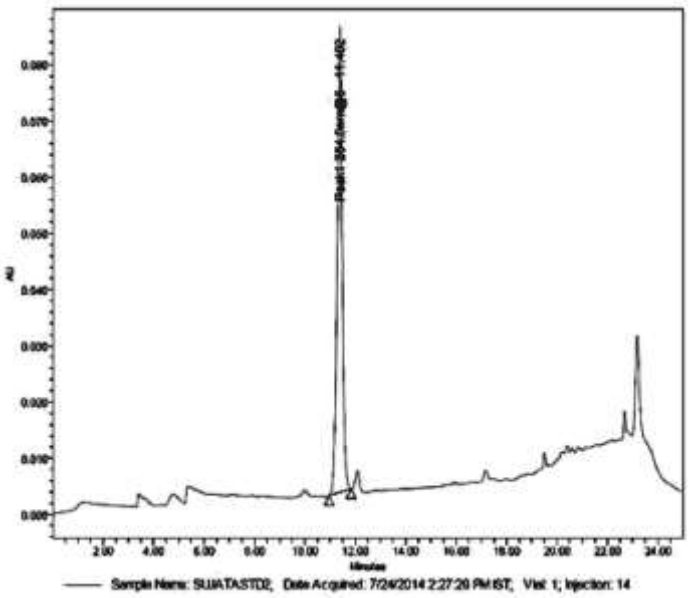

Figure 1. Chromatogram of standard chlorogenic acid.

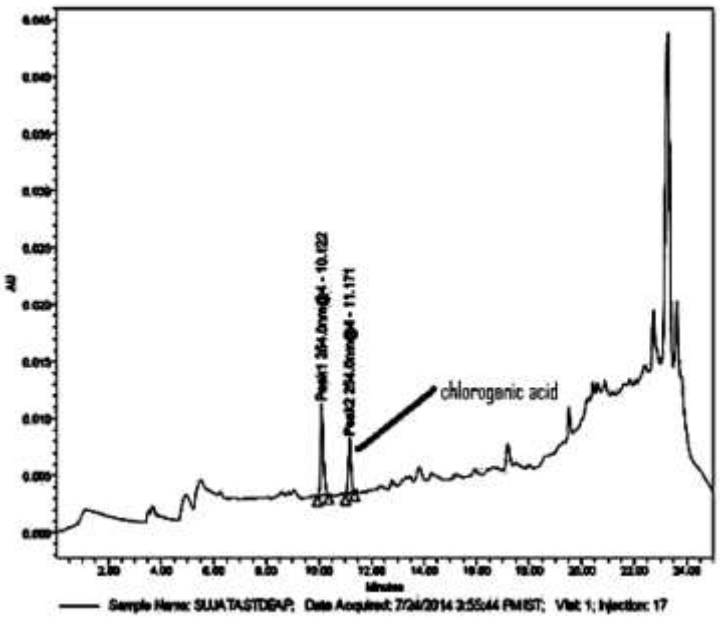

Figure 2. Chromatogram of ethyl acetate extract of *Auricularia polytricha*.

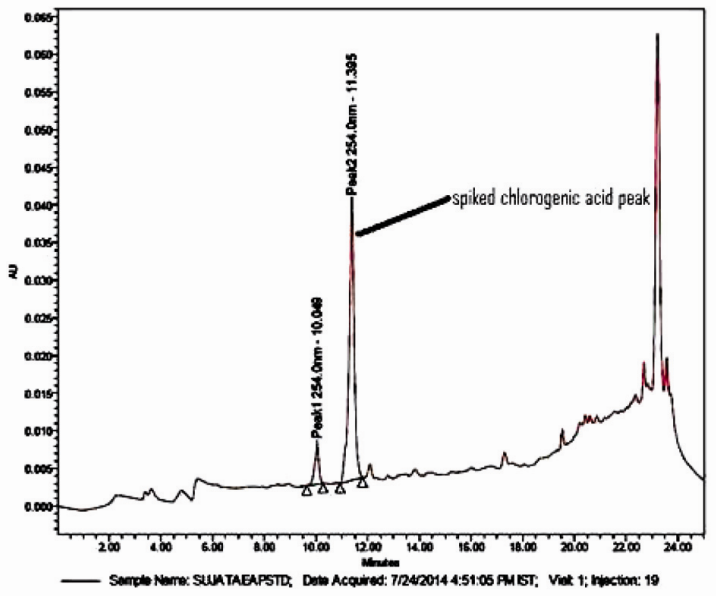

Figure 3. Chromatogram of standard added ethyl acetate extract of *Auricularia polytricha*.

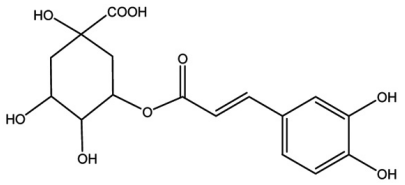

Figure 4. Chlorogenic acid.

the ethyl acetate extract with internal standard method. Chlorogenic acid was absent in hexane and chloroform extract. Chlorogenic acid was analysed using High Performance Liquid Chromatography (HPLC) (Waters, 1525 Model) with PDA detector using C-18 column (Lichrosphere®) (250 × 4.6, 5 µm) with gradient mobile phase consisting of 0.4% acetic acid and methanol at 1.0 ml/min flow rate in the two ethyl acetate extracts. For 10 min the elution was carried out on 1 : 1 with 0.4% acetic acid : methanol and then 15 min in the ratio 4 : 6, at 20 min the concentration of methanol was 100%. Finally the gradient was at initial stage at 25 min. The results showed 0.11% of chlorogenic acid in *A. polytricha* and 0.02% in *A. delicata*. The chromatogram of the standard is given in Figure 1. The chromatogram of the ethyl acetate extract of *A. polytricha* is shown in Figure 2. The quantity of the compound was estimated from the peak area of the standard and extract. The chromatogram of the ethyl acetate extract along with the extract spiked with the standards is shown in Figure 1 for confirmation of presence of chlorogenic acid. Chlorogenic acid was not present in chloroform and hexane extract. The content of cholorogenic acid which has antioxidant activity, in the species supports the folklore healing system.

The trace elements in an organism play important role as a source of nutrients and its utility in controlling many ailments. K plays a major role in controlling the heart diseases. Ca helps in the maintenance of bone and prevents bone diseases. Fe sources are required for controlling anemia[11,12]. Elements present in a sample can be identified and estimated by EDX technique[13]. Elemental analysis of these two macrofungi has been performed by EDX. Table 1 shows results of the EDX pattern. The same kind of elements was present but with different compositions. C, N, O, Na, Mg, Al, Si, P, S, Cl, K, Ca and Fe were identified and estimated. K was found in abundance in *A. delicata* (3.84%) while it was 2.16% in *A. polytricha*. Ca was higher in *A. polytricha* (1.79%) and it was only 0.55% in *A. delicata*. Silicon was 6.23% in *A. polytricha* and only 3.51% in *A. delicata*. Hence the two species showed marked differences in the elemental composition. The folklore use of these two species for treating hypertension, dysentery and diarrhea has been supported by the presence of appreciable amount of potassium and calcium in the species[11,12].

Table 1. Elemental analysis of *A. delicata* and *A. polytricha*

Element	*A. polytricha* (wt%)	*A. delicata* (wt%)
C	46.65	55.41
N	6.75	8.18
O	31.65	24.78
Na	0.26	0.08
Mg	0.82	0.72
Al	1.67	1.18
Si	6.23	3.51
P	0.80	0.53
S	0.12	0.30
Cl	0.05	0.12
K	2.16	3.84
Ca	1.79	0.55
Fe	1.06	0.80
Total	100	100

In conclusion, these two species showed distinguishable differences in quantities and chlorogenic acid and elements present such as Si, K and Ca. The results showed the difference in the concentration of bioactive and antioxidant compound and elements at molecular level between the two species from the same genus *Auricularia.* An HPLC method for identifying and estimating chlorogenic acid has been developed. The folklore use as medicine is thus screened for the presence of chlorogenic acid. It is under further investigation for liver healing utility.

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Malaria risk mapping: a study of Visakhapatnam district

Malaria is a vector-borne disease and endemic in degraded environments, especially in tropical and subtropical ecosystems[1]. Globally, malaria is the third leading cause of death among infectious diseases in children under five years of age[2]. Even though the number of malaria deaths declined by 48% during 2000–2015, ~0.4 million deaths occurred globally in 2015 (ref. 3). The intensity of malaria transmission depends on factors related to the parasite (*Plasmodium* species), the vector (mosquitoes), the human host and the environment. Thus, malaria risk is not uniform throughout a given region[4]. The local heterogeneity of the disease motivated the epidemiologists to generate maps for understanding the local disease ecology, which is necessary for targeting the preventive measures. Although efforts were made to map the malaria risk for over a century, proper understanding of malaria distribution has been achieved only during the past few decades, stemmed from the necessity to develop better public health tools[5]. At present, geospatial technologies are extensively used globally to identify the malaria risk zones for targeting malaria eradication programmes[6].

We used statistical techniques for malaria risk mapping in a study of Visakhapatnam district. Covering an area of 11,161 sq. km in the north coastal Andhra Pradesh, Visakhapatnam district exhibits two distinct physiographic regions – the eastern coastal region and the western hilly region (Figure 1). The coastal region covers 44% (4928 sq. km) and lies below 100 m elevation with only a few isolated hills rising above 300 m. The hilly region, which forms a part of the Eastern Ghats, is above 600–1500 m elevation and covers 56% (6233 sq. km) area of the district. The annual rainfall in the coastal region is 1178 mm and in the hilly region it is 1322 mm. The coastal region experiences semi-arid climate with relatively higher mean annual temperature (26–28°C) than in the dry subhumid hilly region (26°C). The district is divided into 43 revenue 'mandals' (tehsils): 32 in the coastal region and 11 in the hilly region. Out of the 4.29 million (2011 Census) population of the district, the coastal region accounts for 3.69 million (86%) and the remaining 0.60 million (14%), mostly scheduled tribes, are from the hilly region. The average density of the population in the district is 343 persons per sq. km, with higher density of 829 per sq. km in the coastal region against 112 per sq. km in the hilly region.

Of the five malaria-causing species of the parasite, *Plasmodium – P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi – P. falciparum* is responsible for a majority of malaria deaths globally[7], while, among other four types of the parasite, *P. vivax* is widespread in the temperate and subtropical zones causing large-scale morbidity[8]. Although malaria prevails almost throughout the year, *P. falciparum* malaria is most common during July–January, whereas *P. vivax* malaria proliferates during February–June[9]. As mosquitoes (the carriers of malaria-causing parasites) need stagnant water to breed, the incidence of malaria is mainly associated with rainy season when the mosquitoes proliferate. However, periods immediately following heavy rainfall are associated with low malaria incidence because of temporary flushing of mosquito breeding grounds[10].

Data on malaria cases recorded during 1999–2011 at 72 Primary Health Centres spread over 43 mandals showed that both *P. falciparum* and *P. vivax* malaria are prevalent in Visakhapatnam district although with significant spatial variations. Of the total 160,970 malaria cases, 109,312 cases were from the hilly region and 51,658 from the coastal region. The *P. falciparum* cases were relatively high (94,031) in the hilly region than those in the coastal region (13,717). But, *P. vivax* malaria cases were higher (37,941) in the coastal region than those in the hilly region (15,281). However, the annual average share of *P. falciparum* cases in the hilly (coastal) region was 1.20% (0.03%) over a range of 0.38–2.42% (0–0.39%) and that of *P. vivax* cases was 0.19% (0.08%) over a range of 0.01–0.54% (0–0.24%) (Figure 1), clearly indicating the relatively higher rate of incidence of both types (*P. falciparum* and *P. vivax*) of malaria in the hilly region.

The malaria risk analysis was carried out to identify malaria intensity levels among various mandals in Visakhapatnam district. For this purpose, mandal-wise yearly data on malaria cases during 1999–2011 were analysed using location quotient (LQ) method, which is a ratio of proportions that illustrates the relative incidence of malaria by mandal. The formula for deriving LQ of a mandal is

$$\mathrm{LQ} = \frac{N_{\mathrm{mandal}}/P_{\mathrm{mandal}}}{N_{\mathrm{district}}/P_{\mathrm{district}}}$$

where N_{mandal} and N_{district} represent number of malaria cases in the mandal and the district respectively, and P_{mandal} and P_{district} represent the total population of