



Bioactive potential of the wild edible mushroom *Ramaria versatilis*

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Abstract

The scrub jungles of the southwestern India support different mushrooms of economic significance. The coral mushrooms belong to the genus *Ramaria* distributed worldwide and many species are edible, medicinal and ectomycorrhizal. *Ramaria versatilis* occurring in scrub jungles were analyzed for biochemical profile and antioxidant potential. Qualitative tests of uncooked samples showed presence of saponins, flavonoids, alkaloids, terpenoids and coumarins, whereas the cooked samples possess saponins, alkaloids, terpenoids, cardiac glycosides and coumarins. Quantitative assessment revealed significantly higher quantities of total phenolics as well as vitamin C in uncooked than cooked samples. The total antioxidant activity, ferrous ion-chelation capacity and DPPH radical-scavenging activity were also significantly high in uncooked samples. Occurrence, substrates, mycorrhizal association and edibility of different *Ramaria* occurring in the Western Ghats region have been reviewed with comparison of nutritional and antioxidant potential of *R. versatilis* with other *Ramaria* spp.

Key words – Antioxidant activities – bioactive compounds – ectomycorrhizae – scrub jungles

Introduction

Cultivated as well as wild mushrooms meet the requirement of nutrition, medicine and bioactive compounds (Hobbs 1995, Smith et al. 2002, Boa 2004, Karun & Sridhar 2017). More than 2,000 mushroom species worldwide have been regarded as safe for human consumption (Maihara et al. 2012). They have potential to serve as alternative to plant- and animal-derived nutritional as well as medicinal source. Edible mushrooms endowed with adequate quantities of proteins, fibre and essential amino acids with low amount of lipids (Sanmee et al. 2003, Kavishree et al. 2008, Pavithra et al. 2018). In addition to nutritional components, mushrooms provide many primary metabolites (e.g. oxalic acid and peptides) as well as secondary metabolites (e.g. steroids, terpenes and quinones) (Alves et al. 2012).

The Himalayas and Western Ghats are the major hotspots of mushroom diversity. The Western Ghats sustain edible, ectomycorrhizal and medicinal mushrooms (Farook et al. 2013, Karun & Sridhar 2014, Senthilarasu 2014, Senthilarasu & Kumaresan 2016). Similar to the Western Ghats, the scrub jungles, mangroves and coastal sand dunes of southwest India are also known for many economically valuable macrofungi (Sridhar 2018a). The diversity and distribution of macrofungi during southwest monsoon is the highest in Western Ghats region followed by scrub jungles, coastal sand dunes and mangroves (Sridhar 2018b).

Clavarioid mushrooms belong to the genus *Ramaria* is one of the diverse genera of the order Gomphales consisting 336 species (18 genera) worldwide with 616 names in Index Fungorum

(Kirk et al. 2008, Giachini & Castellano 2011). Under Nordic *Ramaria* project, Bendiksen et al. (2015) indentified 46 species of *Ramaria* based on morphological and molecular data. Over 20 *Ramaria* spp. grew in nutrient-poor coniferous forests. Among the clavarioid mushrooms, the Indian subcontinent offers 51 species of *Ramaria* with a highest record of 19 species in the State of Uttarakhand (Verma & Pandro 2018).

Proximal components, minerals, fatty acids, amino acids and bioactive profile of traditionally edible 12 species of *Ramaria* occurring in Northwestern Himalayas have been evaluated by Sharma & Gautam (2017). Edible *Ramaria aurea* consist of significant quantities of proteins, carbohydrates, amino acids, minerals and fibre as promising dietary supplement (Rai & Acharya 2012). Another edible *Ramaria botrytis* consists of unsaturated fatty acids, tocopherol and other bioactive compounds (e.g. flavonoids, ascorbic acid, β -carotene and lycopene) (Barros et al. 2008). Edible *Ramaria subalpina* possesses significant antioxidant activity owing to presence of flavonoids, ascorbic acid, β -carotene and lycopene (Acharya et al. 2017). *Ramaria* spp. in Mexico consist of 53 edible and 40 ectomycorrhizal species, among them 16 species possess dual traits as edibility as well as ectomycorrhizal association (González-Ávila et al. 2013).

During expedition in scrub jungles of southwestern India, one of the edible mushrooms *Ramaria versatilis* was found on soil around the basins of many tree species including coconut (*Cocos nucifera*) trees with relative abundance 0.6–1.7% (Greeshma et al. 2016, Dattaraj et al. 2020). *Ramaria versatilis* is also one of the ethnically edible wild mushrooms in Nepal, Western Himalayas (Christensen et al. 2008). This preliminary account records selected nutritional and bioactive components of uncooked and cooked *R. versatilis* occurring in the scrub jungles of South Western India in comparison with other *Ramaria* spp. as well as other edible wild mushrooms.

Materials & Methods

Mushrooms collection and treatments

Ramaria versatilis Quél was frequent on the soil in scrub jungles in the basins of tree species during the southwest monsoon. The basidiomata have profuse dichotomous branching with light-brown to dark-brown shades. Sufficient quantities of fruit bodies of *R. versatilis* from three locations by random sampling in the scrub jungles were collected in sterile polythene bags. They were rinsed in water to eliminate debris followed by blotting. Each replicate was grouped in to two parts, the first part served as uncooked sample (control), the second part was pressure-cooked with low quantity of water (similar to leafy vegetables). The samples were dried at $55\pm 2^\circ\text{C}$ in an hot-air oven until attaining constant weight. After drying, the samples were powdered by a hand grinder to get fine to coarse powder. The labeled samples were preserved in air-tight containers until further analysis.

Qualitative tests

Uncooked and cooked 5 g replicate samples of *R. versatilis* were extracted with 50 ml distilled water in rotary shaker (150 rpm) (24 hr) (Banu & Catherine 2015). After the extracts were centrifuged, supernatants were assessed for presence of bioactive components (phenols, tannins, phlobatannins, cardiac glycosides, saponins, terpenoids, coumarins, flavonoids and alkaloids) by following different methods (Trease & Evans 1989, 2002, Safowora 1993, Herborne 1998, Parekh & Chanda 2007, Das et al. 2010, Soares et al. 2013, Pandey & Tripathi 2014).

Phenols – To 1 ml extract, 2 ml distilled water was added followed by addition of a few drops of 10% aqueous FeCl_3 . Formation of blue or green color reveals presence of phenols.

Tannins – To 2 ml extract, 2 ml distilled water and 2–3 drops of aqueous 1% FeCl_3 were added. Formation of brownish-green or green or blue-black color discloses presence of tannins.

Saponins – To 5 ml extract, 5 ml distilled water was added, heated, cooled to room temperature and shaken vigorously to develop foam. Formation of froth indicates occurrence of saponins. For emulsion test, to 5 ml extract, 5 ml distilled water was added, after vigorous shaking,

the froth developed was mixed with 3 drops of olive oil and repeated shaking for the formation of emulsion as an indication of presence of saponins.

Flavonoids – To 2 ml extract, 2 ml methanol was added and heated, followed by addition of 2–3 drops of concentrated HCl. Development of red or orange color is an evidence for presence of flavonoids.

Alkaloids – To 2 ml extract was treated with 5 ml 1% HCl, placed in steam bath for 10 min followed by filtering. 1 ml filtrate was treated with 5–6 drops of Mayer's reagent (solution 1: 0.355 g HgCl₃ dissolved in 60 ml distilled water; solution 2: 5 g KI dissolved in 20 ml distilled water; volume was made up to 100 ml with distilled water after mixing solution 1 and 2). Formation of cream-colored precipitate shows presence of alkaloids.

Terpenoids – To 5 ml extract was mixed with 2 ml chloroform followed by addition of 1 ml concentrated H₂SO₄ on the sides of the test tubes. Appearance of reddish-brown color in the interface shows presence of terpenoids.

Cardiac glycosides – To 5 ml extract, 2 ml mixture glacial acetic acid containing ferric chloride (1 volume of 5% FeCl₃ + 99 volume of glacial acetic acid) was added followed by 1 ml concentrated H₂SO₄. Appearance of brown ring at the interface reveals positive test for cardiac glycosides.

Coumarins – To 2 ml extract was treated with 2–3 ml 10% aqueous sodium hydroxide and formation of yellow color indicates presence of coumarins.

Phlobatannins – To 2 ml extract was boiled with 2 ml 1% HCl for formation of red precipitate as positive for presence of phlobatannins.

Quantitative tests

Total phenolics – Total phenolic content of uncooked and cooked mushroom was assessed according to Rosset et al. (1982). 50 mg samples was extracted with 5 ml of 50% aqueous methanol and 5 ml distilled water in water bath (95±1°C) for 10 min, centrifuged at 1500 rpm and the supernatant was drawn. The leftover material was re-extracted, pooled extracts and made up to 10 ml with distilled water. Aliquots of 0.1 ml were made up to 1 ml by distilled water followed by addition of 5 ml 2% Na₂CO₃ (in 0.1 N NaOH). After incubation up to 10 min at room temperature, 0.5 ml Folin-Ciocalteu's reagent (1:2 v/v) was added, mixed and absorbance value was measured at 725 nm. Tannic acid served as standard and the results expressed as mg tannic acid equivalents (TAEs) per gram mushroom dry mass (mg TAEs/g).

Vitamin C – Vitamin C content of mushroom samples was estimated as per the method by Roe (1954) with a slight modification. Five gram mushroom samples were extracted with 25 ml of trichloroacetic acid (TCA) (5%). Aliquots of 0.5 ml of samples were made up to 1 ml using 5% TCA followed by addition of 1 ml chromogen reagent (0.6% copper sulfate, 5 ml + 5% Thiourea, 5 ml + 2.2% 2,4-dinitrophenylhydrazine, 90 ml). After boiling the mixture (10 min), cooled to room temperature, 4 ml of 65% H₂SO₄ was added and incubated up to 30 min at room temperature. Using ascorbic acid as standard, the absorbance value was measured at 540 nm and vitamin C content was expressed in mg ascorbic acid equivalents (AAEs) per gram mushroom dry mass (mg AAEs/g).

Antioxidant tests

Total antioxidant activity – Total antioxidant activity (TAA) of mushroom flour was evaluated according to Prieto et al. (1999) with a few modifications. Aqueous 0.2 ml mushroom extracts were treated with 2 ml reagent mixture (H₂SO₄, 0.6 M + sodium phosphate, 28 mM + ammonium molybdate, 4 mM). After incubation of mixtures at 95°C for 90 min, cooled to room temperature and absorbance value was measured at 695 nm against distilled water as blank. The TAA was expressed as μM equivalent of ascorbic acid equivalents per gram mushroom flour (μM AAEs/g).

Ferrous ion-chelation capacity – Ferrous ion-chelating capacity (FCC) was assessed based on Hsu et al. (2003). One ml aqueous extracts were treated with 0.1 ml 2 mM FeCl₃ and 0.2 ml 5

mM ferrozine. The volume was made up to 5 ml with distilled water. The absorbance was measured at 562 nm after incubation of mixture for 10 min at room temperature. The reagents devoid of sample served as control.

Ferrous ion-chelating capacity (%) = $[1 - (A_{S562}/A_{C562})] \times 100$ (where Ac, absorbance of control; As, absorbance of sample).

DPPH radical-scavenging activity – The aqueous extracts were assessed for radical-scavenging activity by following Singh et al. (2002). Different concentrations of extract (0.2–1.0 mg) were made up to 1 ml with distilled water. 4 ml 0.01 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) was added followed by incubation at room temperature up to 20 min. The absorbance value was measured at 517 nm and reagents without sample served as control.

Free radical-scavenging activity (%) = $[(A_{C517} - A_{S517}) / (A_{C517})] \times 100$ (Ac, absorbance of the control; As, absorbance of the sample).

Data analysis

The significant difference in total phenolics, vitamin C and antioxidant activities among uncooked and cooked samples of *R. versatilis* was determined by *t*-test (StatSoft 2008).

Results & Discussion

Ramaria in the Western Ghats

The literature survey reveals that 10 species of *Ramaria* are known from the Western Ghats region (Table 1). Reports on *Ramaria* spp. are available from the states of Maharashtra (3 spp.), Karnataka (7 spp.) and Kerala (7 spp.). All these *Ramaria* spp. except for *R. eumorpha* have been listed to have mutualistic association (ectomycorrhizal) with several tree species in Mexico and other parts of the world (González-Ávila et al. 2013). Except *R. eumorpha* and *R. zippelii*, the rest of the species are edible in Mexico. Although diverse *Ramaria* spp. have been assessed for their morphology, phylogeny and bioactive components in the Western Ghats, their nutritional and mutualistic association have not been assessed.

Table 1 *Ramaria* spp. reported from the Western Ghats region (?, to be defined; *, González-Ávila et al. 2013).

	Common name	Location	Substrate (Reference)	Mutualism*	Edibility*
<i>Ramaria apiculata</i> (Fr.) Donk	Green-tipped coral mushroom	Radhanagari (Maharashtra)	Soil (Thite et al. 1976, Patil & Thite 1977)	Mycorrhizal	Edible
		Ammayambalam (Kerala)	Soil & humus (Mohanan 2011)		
<i>Ramaria botrytis</i> (Pers.) Bourdot	Pink-tipped coral or Cauliflower mushroom	Panhala (Maharashtra)	Humus-rich soil (Patil et al. 2016-17)	Mycorrhizal	Edible
		Shimoga (Karnataka)	Soil (Swapna et al. 2008)		
<i>Ramaria eumorpha</i> (P. Karst.) Corner	Rose-bud mushroom	Chandhakkunnu (Kerala)	Soil & humus (Mohanan 2011)	?	?
<i>Ramaria flava</i> (Schaeff.) Quél.	Yellow coral mushroom	Soil (Karnataka)	Soil (Karun & Sridhar 2016)	Mycorrhizal	Edible
		Chandhakkunnu (Kerala)	Soil & humus (Mohanan 2011)		
<i>Ramaria formosa</i> (Pers.) Quél.	Yellow-tipped coral mushroom	Dandeli (Karnataka)	Soil (Ramesh & Pattar 2010)	Mycorrhizal	Edible
		Chandhakkunnu (Kerala)	Soil & humus (Mohanan 2011)		

Table 1 Continued.

	Common name	Location	Substrate (Reference)	Mutualism*	Edibility*
<i>Ramaria gracilis</i> (Pers.) Quél.	Coral mushroom	Soil (Karnataka) Peechi (Kerala)	Soil (Karun & Sridhar 2016) Soil & humus (Mohanan 2011)	Mycorrhizal	Edible
<i>Ramaria pallida</i> (Schaeff.) Ricken	Colic coral mushroom	Konaje (Karnataka) Sidhanpocket (Kerala)	Soil (Karun & Sridhar 2014, Pavithra et al. 2016a, Dattaraj et al. 2020) Humus-rich soil (Mohanan 2011)	Mycorrhizal	Edible
<i>Ramaria stricta</i> (Pers.) Quél.	Strict-branch coral mushroom	Koodlu Theertha (Karnataka)	Soil (Prakash & Colney 2019)	Mycorrhizal	Edible
<i>Ramaria versatilis</i> Quél.	?	Konaje (Karnataka) Chandhakkunnu (Kerala)	Soil (Greeshma et al. 2016, Dattaraj et al. 2020) Soil & humus (Mohanan 2011)	Mycorrhizal	Edible
<i>Ramaria zippelii</i> (Lév.) Corner	?	Mahabaleshwar (Maharashtra)	Soil (Senthilarasu 2013)	Mycorrhizal	?

Bioactive components

Qualitative tests of uncooked samples revealed occurrence of saponins, flavonoids, alkaloids, terpenoids and coumarins, whereas cooked samples consist of saponins, alkaloids, terpenoids, cardiac glycosides and coumarins. However, both samples devoid of phenols, tannins and phlobatannins. As saponins, flavonoids, alkaloids and terpenoids are known for antibacterial activity (Machumi et al. 2010, Aboh et al. 2014), probably these compounds may defend the mushrooms against bacterial attack in their habitats. Many edible *Ramaria* spp. (*R. aurea*, *R. botrytis*, *R. cystidiophora*, *R. flava*, *R. flavescens*, *R. formosa*, *R. rubripermanens* and *R. stricta*) are known for antimicrobial potential against pathogens (bacteria, mycobacteria and fungi) (Barros et al. 2008, Ramesh & Pattar 2010, Centko et al. 2012, Sharma & Gautam 2017). Besides, these compounds are also possessing antioxidant potential (Poumale et al. 2013, Nithya et al. 2016). The cardiac glycosides (or aglycones) are known to raise the capability of blood pumping by heart muscle (Aldred 2008). The coumarins have many bioactive potential (e.g. antioxidant, antimicrobial, antiinflammatory and antidiabetic) (Poumale et al. 2013). The qualitative assessment of *R. versatilis* revealed occurrence of typical bioactive components presented in medicinal plant species, thus serve as potential alternative therapeutic source. Thermal and other processing methods may influence the quantity as well as loss of some bioactive components of edible mushrooms, whereas some compounds those are not present in raw mushrooms may perceive after processing. However, some bioactive components may be below detectable level by qualitative or quantitative analysis.

Quantitative tests for total phenolics and vitamin C showed worthy result. The total phenolics was significantly higher in uncooked than cooked samples ($p < 0.05$) (Fig. 1a). The total phenolics in uncooked as well as cooked *R. versatilis* is higher than other edible mushrooms of scrub jungles (e.g. *Astraeus hygrometricus*, *Amanita* sp. and *Auricularia auricula*) (Karun et al. 2016, Pavithra et al. 2016b, Greeshma et al. 2018). The total phenolics content of uncooked samples is comparable to uncooked *Lentinus squarrosulus*, *Termitomyces clypeatus* and *T. umkowaan* (Karun et al. 2016, Ghate & Sridhar 2017). The total phenolics content not decreased drastically in *R. versatilis* on cooking as in *L. squarrosulus*, *T. clypeatus* and *T. umkowaan*. Conventional pressure-cooking

employed in the present study will not destroy total phenolics, thus cooked *R. versatilis* have potential to quench the free radicals.

The vitamin C content was significantly higher in uncooked than cooked samples ($p < 0.05$) (Fig. 1b). Vitamin C is one of the common constituents in many *Ramaria* spp., and its content in uncooked and cooked *R. versatilis* in our study are higher than eight *Ramaria* spp. studied by previous researches (Barros et al. 2008, Ramesh & Pattar 2010, Acharya et al. 2017, Sharma & Gautam 2017). Similarly, the vitamin C content in *R. versatilis* is higher than many uncooked and cooked edible mushrooms occurring the scrub jungles (*A. hygrometricus*, *A. auricula* and *L. squarrosulus* and *T. umkowaan*) (Karun et al. 2016, Pavithra et al. 2016b, Ghate & Sridhar 2017). However, vitamin C content is comparable with *T. clypeatus*, while lower than *Amanita* sp. occurring in scrub jungles (Ghate & Sridhar 2017, Greeshma et al. 2018).

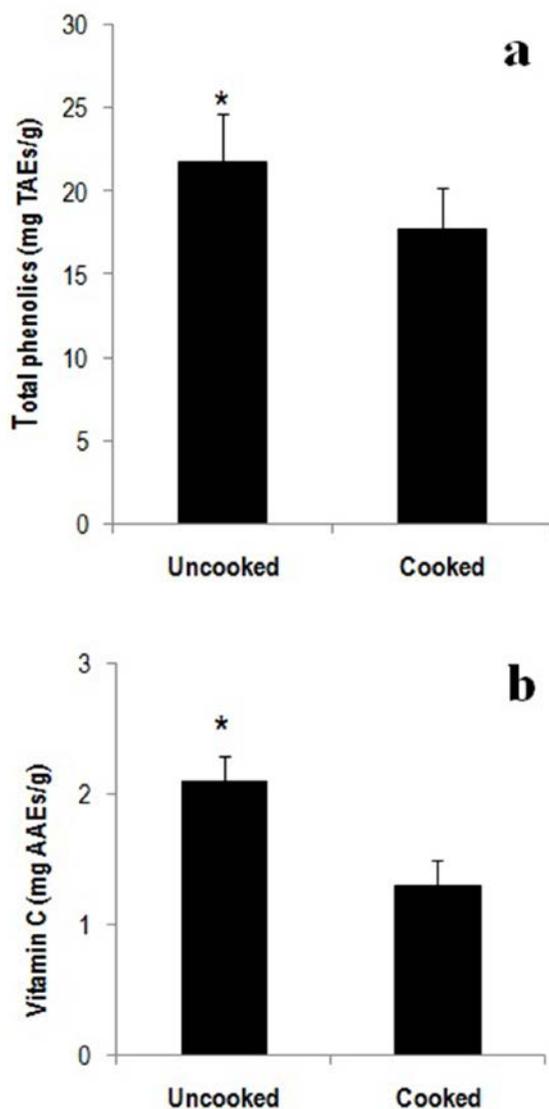


Fig. 1 – Total phenolics (a) and vitamin C (b) in uncooked and cooked *Ramaria versatilis*; mean \pm SD, n=3; *t*-test: * $p < 0.05$).

Antioxidant potential

The TAA of *Ramaria versatilis* was significantly higher in uncooked than cooked samples ($p < 0.05$) (Fig. 2a). The FCC was also significantly high in uncooked samples ($p < 0.01$) (Fig. 2b). Similar to TAA and FCC, the DPPH radical-scavenging activity was higher in uncooked than cooked samples, it was significant at 0.2, 0.8 and 1 mg/ml ($p < 0.05$) (Fig. 2c). In all the three tests,

uncooked samples possess better antioxidant potential than cooked samples. The TAA of *R. versatilis* was comparable with other edible mushrooms in scrub jungles (*Amanita* sp., *A. hygrometricus*, *T. clypeatus* and *T. umkowaan*), while lower than *A. auricula* (Karun et al. 2016, Pavithra et al. 2016b, Ghate & Sridhar 2017, Greeshma et al. 2018). The FCC is higher than *Amanita* sp. and *A. auricula*, while comparable to *A. hygrometricus*, *L. squarrosulus*, *T. umkowaan*, but lower than *T. clypeatus*. The DPPH radical-scavenging activity is lower than *Amanita* sp. and *A. hygrometricus*, while it is higher than *A. auricula*, *T. umkowaan*, *L. squarrosulus* and *T. clypeatus*. *Ramaia subalpina* also possess good antioxidant activity similar to *R. versatilis* (Acharya et al. 2017). *Ramaria formosa* possess lower DPPH radical-scavenging activity than *R. versatilis* (Ramash & Pattar 2010). Among the six *Ramaria* spp. evaluated by Sharma & Gautam (2017), the DPPH radical-scavenging activity of *R. versatilis* is comparable with three *Ramaria* spp. (*R. botrytis*, *R. flava* and *R. rubripermanes*), while lower than other three *Ramaria* spp. (*R. aurea*, *R. Flavescens* and *R. stricta*).

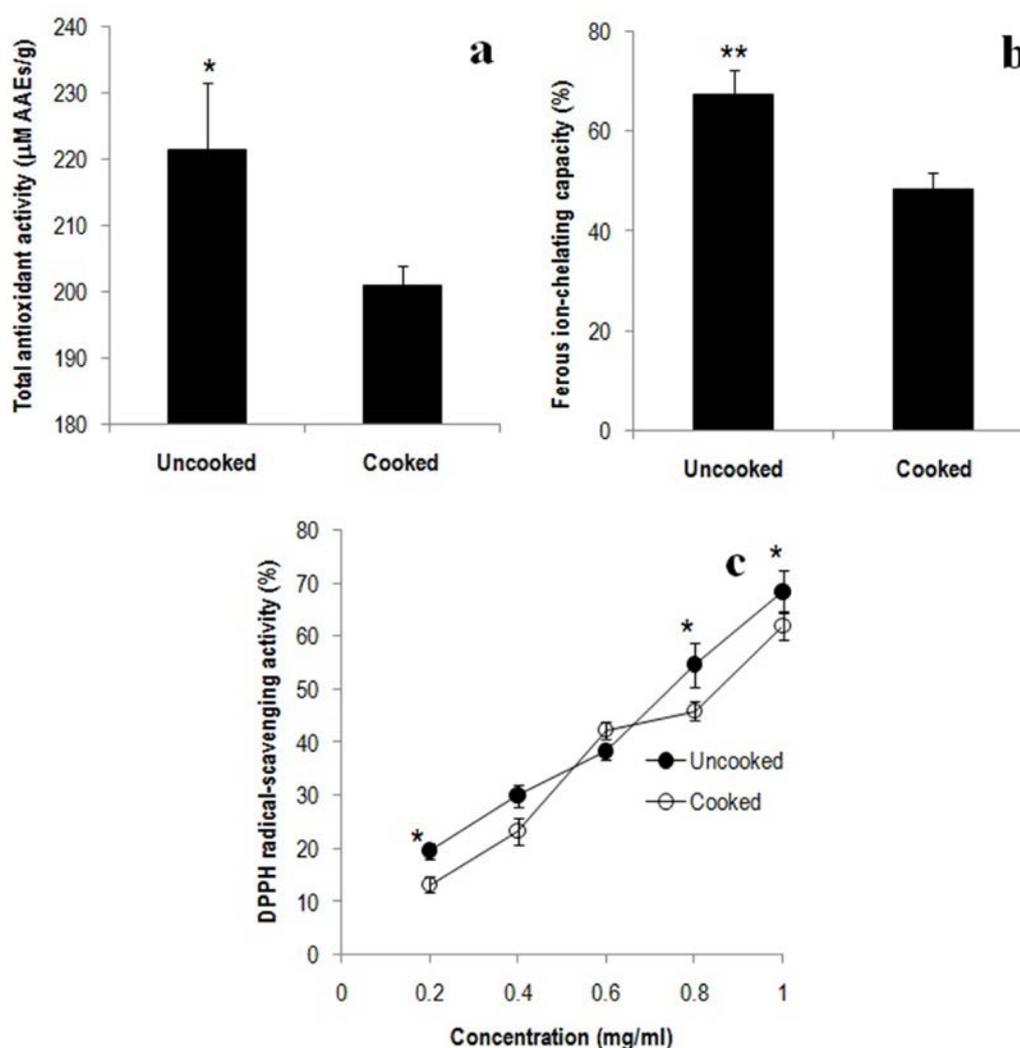


Fig. 2 – Total antioxidant activity (a), ferrous ion-chelating capacity (b) and DPPH radical-scavenging activity (c) of uncooked and cooked *Ramaria versatilis* (mean±SD, n=3; t-test: *p<0.05, **p<0.01).

Conclusions

Aqueous extract of uncooked and cooked wild edible mushroom *Ramaria versatilis* occurring in the scrub jungles of the southwest India contains many bioactive components similar to plant

species. The total antioxidant, ferrous ion-chelation and DPPH radical-scavenging activities are comparable or higher than many edible wild mushrooms occurring in the scrub jungles as well as other *Ramaria* spp. In the scrub jungles, *R. versatilis* grows in the basins of tree species including the basins of coconut (*Cocos nucifera*). Coconut being one of the important plantation crops of southwestern India, it is likely that *R. versatilis* has ectomycorrhizal association. Further precise studies on nutritional components, bioactive potential and ectomycorrhizal association of *R. versatilis* in the scrub jungles will be highly rewarding.

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