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Comparative study of biochemicals and antioxidant activities of two wild edible mushrooms *Russula gnathangensis* and *Ramaria thindii* from Sikkim Himalayas, India

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ABSTRACT

Wild edible mushrooms are utilized by ethnic people in rural communities of India as food and medicinal purposes. The aim of the present study was to estimate biochemical constituents and antioxidant activities of two wild edible mushrooms *viz., Russula gnathangensis* and *Ramaria thindii* isolated from Sikkim Himalayas, India. Methanolic extract of oven dried mushroom powders were used for the estimation of polyphenol, flavonoid, tannin, lycopene and β-carotene contents and determination of antioxidant activities though protein and carbohydrate contents were estimated using aqueous extracts. All the chemical constituents were measured on dry wt. basis. Protein contents of *R. thindii* and *R. gnathangensis* were 185.8 μg/mg and 164.7 μg/mg, respectively. *R. thindii* showed 191.7 μg/mg carbohydrate content whereas *R. gnathangensis* showed 140.5 μg/mg. Polyphenol and flavonoid contents of *R. thindii* were 7.9 μg/mg and 1.0 μg/mg, respectively whereas *R. gnathangensis* showed 4.25 μg/mg polyphenol and 0.75 μg/mg flavonoid contents. Tannin contents of *R. thindii* and *R. gnathangensis* were 2.35 μg/mg and 1.46 μg/mg, respectively. *R. thindii* showed better lycopene content but both the species showed similar amount of β-carotene. Both the species showed high amount of antioxidant activities. These wild mushrooms may fulfill the nutritional requirement of the local ethnic people if properly conserved.

Keywords: Antioxidant activity, biochemical constituents, ethnic people, wild mushrooms

Edible mushrooms are utilized by rural communities of India as food and medicine from time immemorial though the knowledge is not well documented. In recent days, wild unexplored mushrooms are attracted by scientific communities for new sources of food as well as for various neutraceuticals. In general, mushrooms provide delicious dishes, as they are quite rich in protein, carbohydrate, fibre and have a low fat content (Gogavekar *et al*., 2014; Gupta *et al.,* 2018). Edible mushrooms contained large amount of phenolic compounds which act as antioxidants due to their scavenging capability of free radicals through single-electron transfer. Consumption of antioxidant rich foods augment the antioxidant systems of the body thus prevent the cellular oxidative damage which is related to ageing as well as different diseases like cirrhosis, atherosclerosis, diabetes, cancer etc. (Paul *et al*., 2017; Sharma and Annepu, 2017).

Sikkim Himalayas are very rich in wild mushrooms. Das *et al*. (2018) reported a number of novel wild mushrooms from this area. Some of these mushrooms are edible and very much nutritious

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(Acharya *et al*., 2017), and have medicinal importance also (Sharma and Gautam, 2017). Though wild mushrooms accumulate heavy metals but Himalayan mushrooms particularly coral mushroom (*Ramaria* spp.) do not contained any heavy metal. Mushroom protein also possesses almost all essential amino acids (Rai and Acharya, 2012). The local common people collected them for their food without knowing their exact nutritional importance. Two wild edible mushrooms were recently collected from subalpine Sikkim Himalayas and identified as *Russula gnathangensis*, K. Das, Hembrom & Buyck, *sp. Nov.* (KD 18- 014), a gilled mushroom (Das *et al*., 2018) and *Ramaria thindii*, K. Das, Hembrom, A. Parihar & A. Ghosh, sp. nov. (KD 18- 72), a coral fungus (Rossi *et al.,* 2020). Both the species are edible and traditionally consumed by local people. In the present work the protein, carbohydrate and other biochemical parameters and antioxidant activities of both the newly identified wild mushroom species were reported and the results were compared with the available data from other species of *Russula* and *Ramaria.*

MATERIALS AND METHODS

Mushroom species

Both the mushroom specimens were collected from subalpine Sikkim Himalayas and kindly gifted by Dr. Kanad Das, Scientist, AJC Bose Indian Botanic Garden, BSI, Howrah, India. The gilled mushroom *Russula gnathangensis*, was identified by K. Das, Hembrom & Buyck, *sp. Nov.* (KD 18- 014) (Das *et al.,* 2018) and the coral fungus *Ramaria thindii* was identified by K. Das, Hembrom, A. Parihar & A. Ghosh, sp. nov. as new specimens (Rossi *et al.,* 2020).

Preparation of mushroom extract and analysis

Fresh fruiting bodies of these two mushrooms were kept at 60°C in an oven for overnight till the constant weight was obtained. Oven dried powder (0.5gm) was crushed with 20 mM five millilitre of imidazole buffer (pH 7.8) having 1 mM EDTA, 2 mM PMSF for assay of protein & carbohydrate. One gram mushroom powder was crushed with 50 ml of absolute methanol and kept for overnight. It was centrifuged at 10,000 rpm. After 10 min, supernatant was collected. Then again the pellet was re-suspended in 30 ml of absolute methanol and kept for overnight and the supernatant was collected by same process for two times. Then final volume made up to 5 ml by evaporation on rotary evaporator and the final concentration was 200 mg/ml. All the chemical constituents are measured on the basis of the dry wt. of the mushroom.

Protein content was determined by the method of Lowry *et al.* (1951) using BSA as standard. The sugar content was determined by DNSA reagent according to Miller (1959) using glucose as standard. The quantification of phenolics was determined by the method of Mukhia *et al.* (2014) with slight modifications using Folin-Ciocalteu as reagent and gallic acid as standard. Flavonoid content was measured according to Zhishen *et al.* (1999) using catechol as standard. Tannin content was determined according to Kavitha and Indira (2016) using tannic acid as standard. The ß-carotene and lycopene was calculated according to the method of Nagata and Yamashita (1992) with slight modifications. One hundred mg of methanolic extract was robustly shaken with 10 ml of acetone-hexane mixture (4:6) for one minute and absorbance of the mixture was taken at 453, 505 and 663 nm. Lycopene and ß-carotene contents were measured based on the following formulae:

$$\text{Lycopene } (mg^{-100\ ml}) = -0.0458A_{663} + 0.372A_{505} - 0.0806A_{453};\ \text{ß-carotene } (mg^{-100\ ml}) = 0.216A_{663} - 0.304A_{505} + 0.452A_{453}$$

Antioxidant activity assay

Antioxidant activities were measured by 2, 2´ - azinobis (3 – ethylbenzthiazoline–6– sulphonic acid) i.e. ($ABTS^+$) scavenging antioxidant assay method and (2, 2 – diphenyl – 1 picrylhydrazyl) i.e. DPPH reduction assay method. $ABTS^+$ radical scavenging activity of extract was determined spectrophotometrically by the method of Lee *et al.* (2007). Scavenging activity of the sample was calculated based on percentage inhibition of absorbance at 734 nm against the reagent blank by the following formula:

Inhibition % = $[(A_0 – A_1)/A_0]$ x 100 where A_0 = Absorbance of control and A_1 = Absorbance of the sample

Reduction of the DPPH (2, 2 – diphenyl – 1 picrylhydrazyl) radical was measured according to Sharma and Bhat (2009). Scavenging activity of the sample was calculated based on percentage decolorization of the sample according to following equation:

% inhibition of DPPH activity = $[(A_0 – A_1)/A_0]$ x 100 where, A_0 is the absorbance value of the control reaction or blank sample and A_1 is the absorbance value of tested sample.

Statistical analysis

The experimental results discussed here are the mean ± standard deviation of three replicates. Microsoft® Excel Version 2007 statistical package is used to estimate mean, standard deviation etc.

RESULTS AND DISCUSSION

Russula gnathangensis K. Das, Hembrom & Buyck sp. nov. was first described by Das *et al.* (2018) from Sikkim Himalaya under *Abies densa* forests and *Ramaria thindii* K. Das, Hembrom, A. Parihar & A. Ghosh, sp. nov. was recently reported by Rossi *et al.* (2020) from subalpine Sikkim Himalaya under *Abies densa* also. Both the species are highly cherished edible mushrooms by local inhabitants. As there is no scientific information about the nutritive value of these species till date so, estimation of bio-chemicals and antioxidants activities of both the species was done.

In the present investigation protein, carbohydrate, polyphenol, flavonoid, tannin, lycopene and -β-carotene contents were estimated in both the species. The protein contents of *R. thindii*. and *R. gnathangensis* were 18.58% and 16.47%, respectively (Fig 1). *R. thindii* showed 19.17% soluble sugar content whereas *R. gnathangensis* showed 14.05% soluble sugar (Fig 2). Rai and Acharya (2012) reported that, the protein content of *R. aurea* was 19.3% and the carbohydrate content was estimated to 21.8%. Sharma and Gautam (2017) reported the protein contents (% of dry wt.) of *R. botrytis, R. rubripermanens, R. flava, R. flavescens, R. aurea and R. stricta* as 21.65 ± 0.2, 16.32 ± 3.2, 15.80 ± 1.2, 14.60 ± 0.2, 13.30 ± 3.2 and 10.81 ± 1.2, respectively. The protein content of *R. thindii* matches with the other *Ramaria* species but the carbohydrate content seems very less as the soluble sugar content is a part of the total carbohydrate contents as reported by Sharma and Gautam (2017). The protein content of *R. gnathangensis* was 16.47% whereas total carbohydrate content was 14.05%. Kouassi *et al.* (2016) reported high amount of crude protein (≈38%) and total sugar contents (17-18%) of three *Russula* species i.e. *R. delica, R. lepida* and *R. mustelina.* The high crude protein content of Kouassi *et al.* (2016) was possibly due to their estimation process of nitrogen by Kjeldahl method.

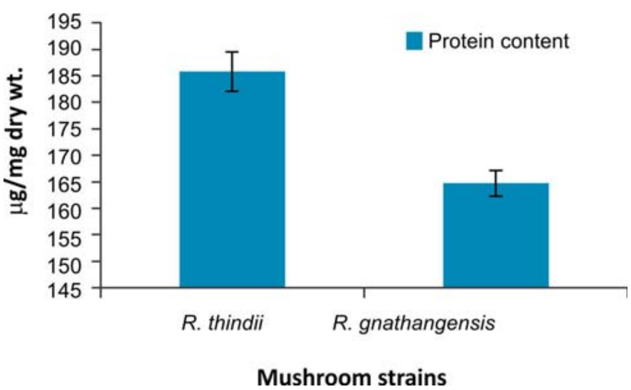

Fig. 1. Bar graph showing the protein content (µg/mg dry wt.) of *R. thindii* and *R. nathangensis*

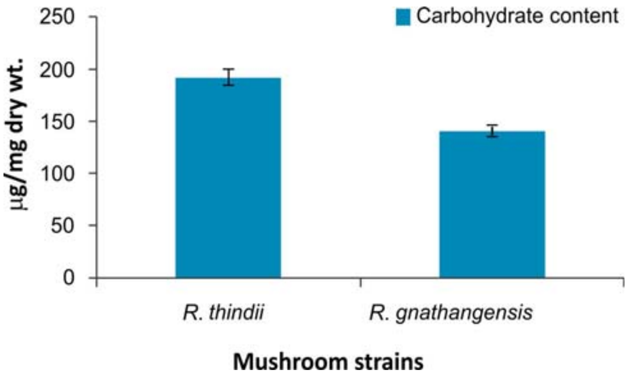

Fig. 2. Bar graph showing the carbohydrate content (µg/mg dry wt.) of *R. thindii* and *R. gnathangensis*

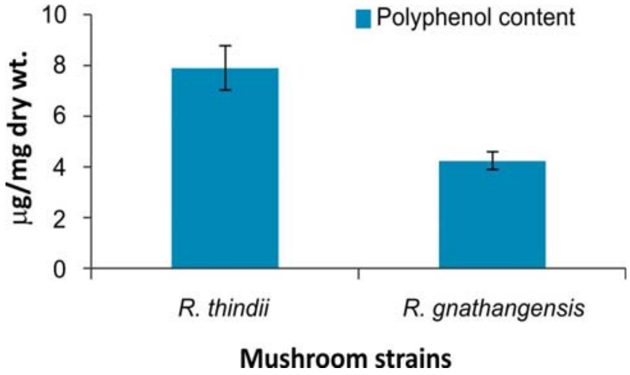

Fig. 3. Bar graph showing the polyphenol content (µg/mg dry wt.) of *R. thindii* and *R. gnathangensis*

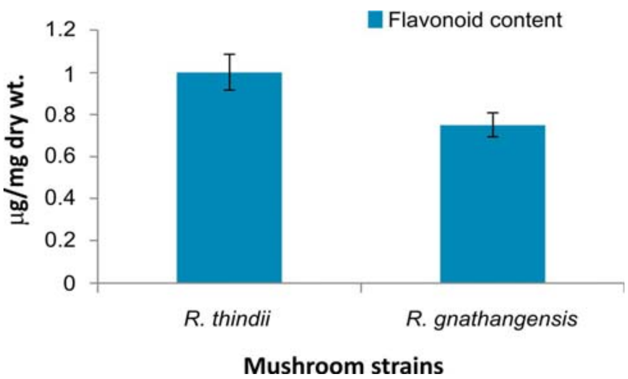

Fig. 4. Bar graph showing the flavonoid content (µg/mg dry wt.) of *R. thindii* and *R. gnathangensis*

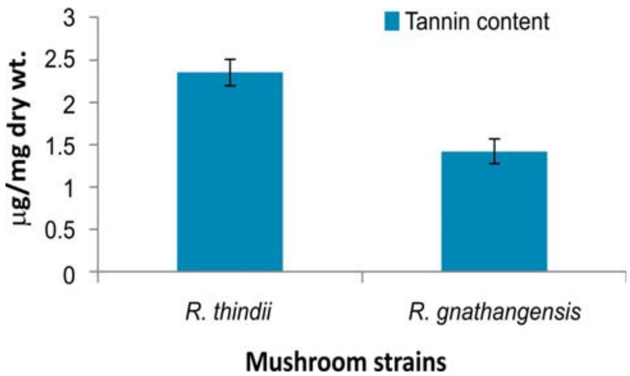

Fig. 5. Bar graph showing the tannin content (µg/mg dry wt.) of *R. thindii* and *R. gnathangensis*

Flavonoids and polyphenols are important constituents of mushroom. Flavonoids are an important nutraceutical found in different mushrooms. They can reduce the risk of tumour formation, coronary heart diseases, menopausal symptoms and other diseases in human which are associated with oxidative damages of nucleic acids, proteins or membranes (Ferguson, 2001; Rice Evans *et al.,* 1996). Here polyphenol and flavonoid contents of *R. thindii* were 7.9 µg/mg and 1.0 µg/mg, respectively whereas *R. gnathangensis* showed 4.25 µg/mg polyphenol and 0.75 µg/mg flavonoid contents (Fig 3 & 4). In present investigation tannin contents of *R. thindii* and *R. gnathangensis* was 2.35 µg/mg and 1.42 µg/mg, respectively (Fig 5). Polyphenol and flavonoid contents of *R. thindii* were better than the *R. gnathangensis*. *R. flava (Schaeff.)* Quél. showed higher amount of total phenolic compounds (10.51 ± 0.47 µg/mg) but lower amount of (0.51 ± 0.01 µg/mg) total flavonoids than *R. thindii* (Gursoy *et al.,* 2010). *R. formosa* also showed very less amount of phenol (3.62 ± 0.05 mg/ml) and flavonoid contents (0.40 ± 0.05 mg/ml) (Ramesh and Pattar, 2010) whereas the phenolic content of six different *Ramaria* species were very high (40.32– 56.35 mg/g) as reported by Sharma and Gautam (2017) than our results of *R. thindii.* Acharya *et al.,* 2017 reported the phenolic content of *R. subalpina* was 17.5 µg/mg and flavonoid content was 2.5 µg/mg. According to Tripathy *et al.* (2016), *R. nigricans* contained slightly higher amount total phenolics (4.55 mg/g) and flavonoids (1.09 mg/g) than *R. gnathangensis.*

In present investigation tannin contents of *R. thindii* was better than the *R. gnathangensis*. The tannin content of *R. gnathangensis* showed lower value than *Russula nigricans* (7.28±0.27 mg/g) and *R. brevipes* (6.81±0.27 mg/g) as reported by Tripathy *et al*. (2016). Though β carotene contents were similar in both the species but lycopene contents were slightly higher in *R. thindii* than *R. gnathangensis*. Lycopene contents of *R. thindii*. and *R. gnathangens*is were 0.0184 µg/mg and 0.0151 µg/mg, respectively whereas the β carotene contents were 0.0290 and 0.0289 µg/mg (Table 1). Acharya *et al*. (2017) reported 0.0239±0.002 µg/mg β- carotene content and 0.0122±0.0016 µg/mg lycopene content of *R. subalpina* which were less than the respective values of *R. thindii* in our observation. Sharma and Gautam (2017) reported Lycopene contents are 0.18-0.49 µg/100g and β carotene contents are 0.51-0.91 µg/100g in six *Ramaria* species which were very less than our results. Here, *R. gnathangens*is showed 0.0151 µg/mg lycopene and 0.0289 µg/mg β carotene whereas Tripathy *et al*. (2016) reported higher amount of lycopene and β- carotene (0.029±0.002 mg/g and 0.056±0.004 mg/g, respectively) in *R. lepida* but very less amount of (0.003±0.02 mg/g) lycopene but higher amount of (0.037±0.02 mg/g) β- carotene in *R. brevipes* (Table 1).

Table 1. lycopene and β- carotene content[a] (µg/mg dry wt.) of *R. thindii* and *R. gnathangensis*

Mushroom species	Lycopene content (µg/mg dry wt.)	β-carotene content (µg/mg dry wt.)
R. thindii	0.0184	0.0290
R. gnathangensis	0.0151	0.0289

[a]One hundred mg of methanolic extract of fruiting bodies was robustly shaken with 10 ml of acetone-hexane mixture (4:6) for one minute and absorbance of the mixture was taken at 453, 505 and 663 nm.

Antioxidant is a major nutrient parameter of any fruits or vegetables. They are the possible protector against oxidative damage by reactive oxygen species

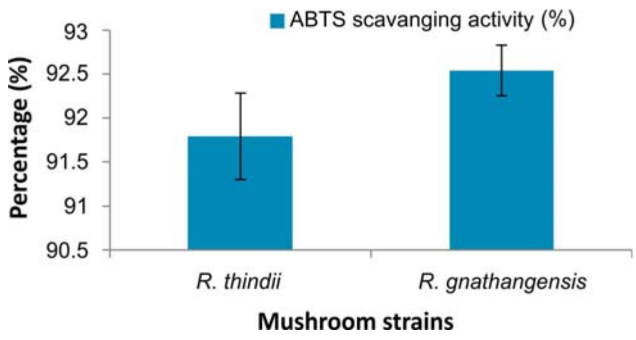

Fig. 6. Bar graph showing the ABTS scavenging activity (%) of *R. thindii* and *R. gnathangensis*

unless otherwise causes a number of diseases like ageing, diabetes, atherosclerosis, cirrhosis even cancer (Paul *et al*., 2017). According to Hatano *et al.* (1989) phenolic compounds played a major role in scavenging activity of these adverse free radicals due to the presence of hydroxyl group. Mushrooms are very rich in phenolic compounds which are the source of their major antioxidant machineries than other antioxidant components like ascorbic acid, tocopherol, β- carotene, lycopene etc. (Chirinang and Intarapichet, 2009; Khatun *et al.*,2015; Pullareddy *et al.,* 2021). According to Kozarski *et al.* (2015) different types of wild or cultivated mushrooms showed considerable antioxidant properties due to the presence of bioactive compounds like polysaccharides, polyphenols, flavonoids, vitamins, carotenoids, etc. In the present investigation the antioxidant values are quite encouraging. In the present investigation antioxidant contents were measured in terms of DPPH and ABTS reduction activities. Both the species showed high

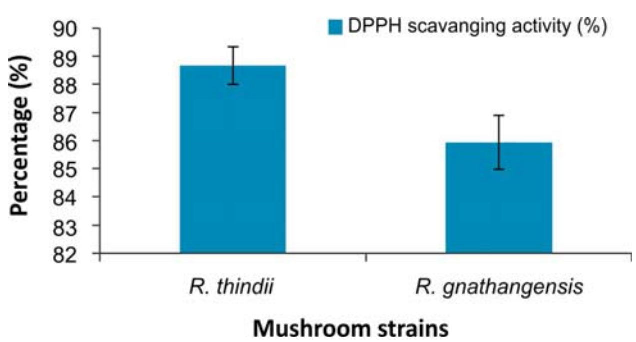

Fig. 7. Bar graph showing the DPPH scavenging activity (%) of *R. thindii* and *R. gnathangensis*

amount of antioxidant activities. *R. thindii* showed 91.79% ABTS radical scavenging activity and 88.67% DPPH radical scavenging activity whereas *R. gnathangensis* showed 92.54% ABTS radical scavenging activity and 85.94% DPPH radical scavenging activity in 1 mg/ml conc. (Fig 6 & 7). Here, both the species showed more or less similar amount of antioxidant activities. *R. flava* (94.78%) *and R. formosa* (94.5%), showed slightly higher electron donating capacity than *R. thindii* (Gursoy *et al*., 2010; Ramesh and Pattar, 2010). *R. gnathangensis* showed much better antioxidant activities than *R. nigricans* (66.60%) whereas comparatively less activities than *R. brevipes* (93.19%) (Tripathy *et al*., 2016).

CONCLUSION

The proximate compositions of mushroom not only vary from species to species but also the nutritional content of a particular mushroom species may varies due to several factors like agro-climatic conditions, developmental stages, harvesting time as well as extraction and determination methods. In the present investigation two different species were collected from the same agro-climatic condition of subalpine region of Sikkim Himalaya under *Abies densa* and measurements of different nutrient parameters were done in identical condition. The present work is the first report of biochemical characterization of these two novel species which explore that both *R. gnathangensis* and *R. thindii* are very much sound in their nutritional constituents as potential alternate food sources and comparable to other species reported by different workers. Here *R thindii* showed better nutritional contents like protein, carbohydrate, flavonoid polyphenol, tannin, lycopene, etc. As per the antioxidant activities are concerned, ABTS scavenging activity is more or less similar in both species whereas DPPH scavenging activity is higher in *R. thindii*. All of these above results indicate that in addition to food source both of these novel species have the potentiality to act in culinary purposes which require thorough study of these species. Awareness should be developed within the common people to conserve these precious but neglected gifts of nature.

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