Microanatomical, Physicochemical characterization, and antioxidative activity of methanolic extract of *Oudemansiella canarii* (Jungh.) Höhn

Short title: Physicochemical and pharmacognostic characterization of *O. canarii* (Jungh.) Höhn

ABSTRACT

Objectives: *Oudemansiella canarii*, an edible mushroom highly appreciated throughout the world due to its gastronomic delicacy, is admiringly cultivated. Till date, no extensive work has been reported on the pharmacological and antioxidative aspect of this macrofungus. The present study focuses on the micro-morphological features, confirmation of its identity based on molecular sequence (nrITS rDNA) data, and physicochemical parameters determination such as organoleptic features and fluorescent behaviour.

Materials and Methods: Collected basidiocarps were powdered and used for microscopic and organoleptic evaluation. DPPH radical scavenging method, total antioxidant activity methods and ABTS assay were used for evaluating antioxidant capacities of the methanolic extract. HPLC profile was also recorded to analyse phenolic fingerprint.

Results: The DPPH radical scavenging activity was determined with EC_{50} value of 0.912 µg, total antioxidant activity found to be 15.33 µg ascorbic acid equivalent/mg of extract and ABTS assay revealed 12.91 µm TE/mg of extract antioxidant activity. HPLC chromatogram revealed the presence of 12 peaks. Several parameters were tested for the determination of chemical composition that revealed the existence of major bioactive components in the extract in following order: phenol> flavanoid> ascorbic acid> β-carotene~ lycopene.

Conclusion: The present work suggests that *Oudemansiella canarii* may open a novel prospect as a functional food and antioxidant supplement.

Key words: chromatographic fingerprinting, edible mushroom, fluorescence analysis, internal transcribed spacer, phytochemicals, West Bengal

INTRODUCTION

From millennia mushrooms have a prolong connection with humankind and provide profound biological and economic impact. A latest assessment implicit the existence of around 140,000 species in which only 10% are identified. Recently, scientists have reported that there are at least 7,000 unrevealed macrofungi in world that may exhibit beneficial effects to mankind.[1] Thus, there is a recent trend among mycologists to document edible mushrooms all around the globe.

West Bengal (21°38'-27°10'N latitude and 85°50'-89°50'E longitude) possesses unique phyto-geographical feature with variable altitudinal, climatic and edaphic amalgamations. It is the only state in India that is topographically extends from Himalayas in the north to the Bay of Bengal in the south with regions such as plateau and Ganges delta prevailing in between. These outspread ranges of topographical feature, class of soils and substrata mould the state to be ideal for hosting and blooming rich diversity of mushrooms.[2] In the previous years, our research team has conducted extensive field works and recorded an immense number of wild edible mushrooms from different corners of the state with the help of tribal and ethnic forest dwellers of the regions who prefer those as their daily square meal.[3] Taxonomic and molecular exploration revealed that many of them are new to science,[4,5] new to the record for India[6,7] and an addition to the macrofungal flora of West Bengal.[8,9]

Throughout the globe, mushrooms are well known to the human civilizations because of having nutritional, culinary values, and medicinal prospects. In contemporary terms, they can be regarded as functional foods which can furnish health benefits beyond the traditional nutrients.[10] Mushrooms are known to be rich sources of various bioactive substances like antioxidant,[11-13] antimicrobial,[14,15] immune-modulatory,[16,17] anticancer[18] etc. Despite all the mentioned health promoting effects, this diverse group still lives in dark as more than half of the species remain yet undescribed.

Most of the species belonging to the genus *Oudemansiella* (Basidiomycota, Agaricales, Physalacriaceae) are consumed worldwide.[19] Many *Oudemansiella* species are known to contain bioactive compounds, such as oudenone and lectin (obtained from *O. radicata*),[20,21] mucidin and oudemansin (from *O. mucida*).[22,23] The

edible mushroom *Oudemansiella canarii* (Jungh.) Höhn found to be present in various biomass, where they colonize with several plant species. Morphologically, the taxon is characterized by the presence of a medium to considerably larger pileus with glutinous to viscid surface coloured gray-orange to orange white or with paler to white margin; adnate to shallowly adnexed, white, distant lamellae with 2–3 series of lamellulae; globose to subglobose basidiospores measuring 19–25 × 18–23 µm in diameter; an ixotrichoderm type of pileipellis; well-developed, stalked, pleuro- and cheilocystidia; presence of one- to few-celled caulocystidia with heterogeneous contents; and lignicolous habit.[24] As it is considered an edible mushroom with a good potential of food source, it is highly cultivated artificially on various lignocelluolosic substrates.[25] Solely imperceptible literature reports were found related to their physicochemical, nutritional and medicinal values. However, only one report has been published regarding antifungal and biological activities of *O. canarii*.[26] Therefore, in the present study we documented molecular parameter with phylogeny, physicochemical profile and antioxidant potentiality of *Oudemansiella canarii*.

MATERIALS AND METHODS

Sample collection, microscopic and organoleptic characterization of powdered basidiocarps

Living basidiocarps of *Oudemansiella canarii* were collected from Gangetic plains of West Bengal, India. The basidiocarps were found to grow on dead and decayed woods of dicotyledonous plants. Identification of the specimen was done based on standard literatures.[24,27] Collected basidiocarps were then dried overnight at 40 °C using a field drier. A sample of the voucher specimen was deposited in Calcutta University Herbarium (CUH) following the protocol described by Pradhan *et al*.[28] with the accession number CUH AM26. Remaining basidiocarps were used to make powder and then the powdered sample was hydrated and macerated with 10% KOH and mounted on a glass slide for microscopic observations. For effective results, various stains (lactophenol and cotton blue, Melzer's Reagent, congo red etc.) were used to visualize different cellular structure such as hyphae, basidia and spores. The slides were then viewed under Leica DMLS microscope and images were captured at desired magnification. Different organoleptic characters (viz. colour, odour, taste, nature) of the powdered sample were evaluated.

DNA extraction, PCR and cycle sequencing

Genomic DNA was extracted and the desired region (nrDNA ITS) was amplified using the method as described by Dutta *et al.*[29] with the help of the primer pair ITS1 (forward) and ITS4 (reverse).[30]

Fluorescence Analysis

Fluorescence analysis was performed as per standard protocol.[31] A small amount of sieved powder was kept on a clean grease free microscopic slide and treated with prepared chemical agents including Hager's, Mayer's, Dragendroff's, phloroglucinol and Barfoed's Reagent and were mixed by gently leaning the slide. Then the slide was placed within a UV viewer chamber and viewed against visible, long (365 nm) and short (254 nm) UV radiations. Then the changes in colour on application of these reagents in different radiations were recorded.

Preparation of methanol extract

Initial extraction was done using 100 ml methanol overnight from the dried powdered fruitbodies (5 gm) and then the solution was filtered using a Whatman no. 1 filter paper. The residue was then re-extracted using 30 ml methanol. Volume reduction of the combined methanolic extracts were done by evaporating at 40 °C using a Rotavapor R3 Buchi (Switzerland). The methanolic fraction was then kept at -20 °C in a dark bottle until analysis, for not more than 1 month. Percentage yield and organoleptic features of the extract were recorded.

Quantitative estimation of some important bioactive compounds

Freshly prepared methanolic extract was subjected for several quantitative biochemical assays to investigate the presence and amounts of different phytochemicals. Folin-Ciocalteu reagent was used to estimate the content of total phenolic compounds in the extract[32] with gallic acid as a standard. The results were expressed comparing with µg of gallic acid equivalents/ mg of dry extract. Potassium acetate and aluminium nitrate were used to detect the presence of total flavonoid content.[33] Standard curve was prepared with the help of Quercetin (5–20 µg/ml). The results were expressed as µg of quercetin equivalents per mg of dry extract. β-carotene and lycopene contents were estimated by measuring absorbance at 453,

505 and 663 nm wavelengths following standard protocol.[34] Ascorbic acid was determined by titration against 2, 6-dichlorophenol indophenol dye.[35]

High performance liquid chromatographic (HPLC) profile of methanol soluble extract

HPLC analysis was performed to produce a fingerprinting profile of the extract. 0.2 µm filter was used to filter the methanolic extract and then 20 µl of the filtrate was loaded in HPLC system (Agilent, USA). Separation was performed on an Agilent Eclipse Plus C18 column (100 mm × 4.6 mm, 3.5 µm) with a flow rate of 0.8 ml/min at 25°C. The mobile phase comprised of eluent A (acetonitrile) and eluent B (aqueous phosphoric acid solution, 0.1% v/v). Further a gradient program was utilized for elution: 0-2 min, 5% A; 2-5 min, 15% A; 5-10 min, 40% A; 10-15 min, 60% A; 15-18 min, 90% A. Lately at 280 nm, absorbance of the sample solution was measured.[36]

Antioxidant activity

For evaluation of antioxidant potential of *O. canarii*, the methanolic extract was subjected for several *in vitro* antioxidants activities such as, DPPH radical scavenging assay, ABTS assay and Total antioxidant assay.

DPPH radical scavenging activity

Radical scavenging activity of the extract was achieved using DPPH (2, 2-diphenyl-1-picrylhydrazyl) radicals following Mitra *et al*.[37] 2 ml of the reaction mixture contained several concentrations of the extract along with 0.1 mM methanolic solution of DPPH. After 30 mins of incubation at room temperature in dark, the absorbance was measured against a methanol blank at 517 nm. The EC_{50} value denotes an effective concentration at which 50 % of the DPPH radicals are scavenged. Ascorbic acid was used for comparison. The scavenging ability was calculated using the following equation:

$$\text{Scavenging effect (\%)} = \{(A_0 - A_1)/A_0\} \times 100$$

A_0 was absorbance of the control and A_1 was absorbance in the presence of sample. The percentage (%) of inhibition was plotted against respective concentrations used.

Total antioxidant activity

Measurement of total antioxidant activity is solely based on the reduction of molybdenum (from VI to V) by the sample analyte and thereby formation of phosphate/ molybdenum (V) complex at acidic pH (37). The sample was mixed with reagent solution, prepared with 28 mM sodium phosphate, 0.6 M sulphuric acid and 4 mM ammonium molybdate, and allowed for incubation at 95 ºC for 90 min. The mixture was then cooled down at room temperature. Spectrophotometrically absorbance was recorded for each solution at 695 nm using a blank sample as control. For reference antioxidant, various concentrations (1–30 µg/ml) of ascorbic acid was used and the total antioxidant capacity was expressed equivalent to ascorbic acid (AAE).

ABTS radical scavenging activity

ABTS [2, 2' - azinobis - (3-ethyl-benzo-thiazoline-6 sulfonic acid)] is well regarded as a peroxidase substrate that generates a metastable radical cation when oxidized.[38] The ability to inhibit the accumulation of ABTS radical cation by methanol extract and antioxidant standard, Trolox, was measured spectrophotometrically. ABTS dissolved in methanol to yield 7.4 mM concentration. ABTS radical solution was prepared by reacting ABTS (7.4 mM) with 2.6 mM potassium persulfate solution and permitting the mixture to stand in dark for 12–16 h at room temperature before use. The mother stock was diluted to achieve an absorbance of 0.7 ± 0.02 at 734 nm. Methanol extract at 1 mg/ml concentration was allowed to react with the ABTS working solution for 5 mins and the absorbance was measured.

Statistical analysis

All the assays were done in triplicate and results are expressed by the mean values and standard deviation (SD). For determining the significant difference among samples, results were compared by means of a Student's t-test. The analysis was carried out using Microsoft® Office Excel (Microsoft®, USA), where values of $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Microscopic and organoleptic characterization of the powdered basidiocarps

Dried powder of the fresh basidiocarps that was passed through sieve and further macerated with KOH showed basidiospores and fragmented hyphae (Figure 1). Hyphal system was monomitic in nature with generative hyphae that was 4–6 µm broad, septate, branched, hyaline, thin-walled, clamped. The basidia were measured ranging 64–83 × 11–22 µm in diam., shaped clavate to broadly clavate with 4-spore at the apex. Basidiospores ranged 14–21 × 10.5–18 µm diam., hyaline, globose to sub-globose with hilar appendage, and thick-walled. Melzer's Reaction showed negative result that signifies the non-amyloid nature of the basidiospores.

Sieved powder was used for organoleptic study. The powder was yellowish in colour, with no odour, tasteless and of fibrous in texture.

Molecular analysis

The newly generated sequence of *O*. *canarii* was manually edited using BioEdit sequence alignment editor v.7.0.9.0 (Ibis Biosciences, Carlsbad, USA). The edited DNA sequence of the collected specimen produced 747 bp long stretches that includes the ITS1, 5.8S, ITS2 and 28S ribosomal RNA gene. The edited sequence was then used for BLAST searches in the NCBI Gene Bank database (www.ncbi.nlm.nih.gov).

BLAST analyses with our newly generated sequence showed similarity with the genus *Oudemansiella*, with the highest similarity shown by *O*. *canarii* [AF321476, Identities = 681/698 (98%), Gaps = 7/698 (1%); KR265132, Identities = 690/690 (100%), Gaps = 0/690 (0%)]. So, result of the BLAST search easily help to identify our collected specimen to be *O*. *canarii* (Family- Physalacriaceae). The newly generated sequence of *O*. *canarii* was then deposited to the GenBank database with accession number KU647631.

Fluorescence analysis

The fluorescence test of the powdered sample was carried out for qualitative assessment of crude drug that provides an idea related to their chemical nature. The colours developed by these reagents represent the presence of active constituents. Analysis of powdered drug through fluorescence is vital and very useful pharmacognistic technique for identification of authentic samples and recognizing

adulterates and substituents that help in maintaining the quality, reproducibility and efficacy of natural drugs.[39,40] Here, powdered sample was treated with seven different chemical reagents and the characteristic fluorescence properties or colours were recorded (Table 1).

Quantitative estimation of bioactive compounds

The quantitative estimation of several marked phytochemicals of the methanolic extract was carried out based on the standard protocols as described above. Phenolic compounds are well regarded to be a powerful chain-breaking antioxidant because of the presence of hydroxyl groups that serve by their scavenging ability. It was found that the extract contain phenol as much as 5.38 ± 0.55 µg gallic acid equivalent/ mg of extract. Total flavonoid content was estimated using quercetin as the standard. The extract contained flavonoid as 1.875 ± 0.78 µg quercetin equivalent/ mg of extract. Ascorbic acid content was found to be 1.10 ± 0.42 µg/ mg of the extract. The presence of β-carotene and lycopene content was found to be very negligible amount such as 0.0342 ± 0.004 µg/ mg and 0.0238 ± 0.004 µg/ mg of the extract respectively.

Chromatographic fingerprinting by HPLC

For preliminary determination of chemical constituent presence in any sample, one of the popular and efficient chromatographic technology is HPLC. So, the HPLC analysis was carried out using the methanolic extract of *O*. *canarii*. The UV spectrum analysis of the chromatogram (278 nm) depicts presence of 12 peaks excluding mobile phase peak. The represented chromatogram may serve as a phenolic fingerprint for this mushroom. HPLC chromatogram of methanolic extract of the sample identifies the presence of cinnamic acid (Retention Time 15.358 min) and pyrogallol (Retention Time 15.966 min) based on the standard as provided in our previous study.[15] Chromatographic profile along with the retention time of each peak is presented in Figure 2 and respective areas of each peak are documented in Table 2.

Antioxidant activity

DPPH radical scavenging activity

DPPH assay has been popularly used for testing the free radical scavenging ability of various natural samples. DPPH receives electron or hydrogen to gain stability. Antioxidants, on the other hand could donate electron or hydrogen atom. In methanol solution, DPPH produces violet colour. But when electrons are donated to DPPH, the solution starts losing its colour from purple to yellow and the reduction capacity of DPPH is determined by decrease in its absorbance at 517 nm.[41] The extract showed high effective free radical scavenging activity in the DPPH assay at the rate of 6.53%, 55.85%, and 78.74% at 0.5, 1 and 1.5 mg/ ml concentrations (Figure 3). EC_{50} value was found to be 0.912 ± 0.38 mg/ ml.

Total antioxidant activity

One of the simplest method for evaluating total antioxidant capacity is the phosphomolybdenum method. Total antioxidant capacity of the methanol fraction was investigated and compared against ascorbic acid. The extract revealed antioxidant capacity of 15.33 ± 0.67 µg/ mg ascorbic acid equivalent.

ABTS radical scavenging activity

The ABTS assay is based on the inhibition of the formation of ABTS+, a stable radical cation by one electron oxidants. The methanolic extract of the sample showed 12.91 ± 0.26 µM Trolox equivalents (TE)/ mg of extract antioxidant activity.

CONCLUSION

The present work allocates standards of the macro-fungus, *Oudemansiella canarii*, with the help of pharmacognostic, molecular and chemical characters. Various standardization parameters such as microscopy, molecular characters, physicochemical constants, preliminary mycochemical quantification, HPLC analysis and antioxidant activity were studied, and were reported for the first time for this mushroom. All these combined data put forward this edible mushroom as a gifted source of bioactive molecules like phenolic compounds and an enormous antioxidant compound.

ACKNOWLEDGEMENT

The present work does not include any funder to acknowledge.

CONFLICT OF INTEREST

There is no conflict of interest for the present study.

REFERENCES

1. Thatoi H, Singdevsachan SK. Diversity, nutritional composition and medicinal potential of Indian mushrooms: a review. Afr J Biotechnol 2014;13:523–545.

2. Dutta AK, Acharya K. Traditional and ethno-medicinal knowledge of mushrooms in West Bengal, India. Asian J Pharm Clin Res 2014;7:36–41.

3. Dutta AK, Pradhan P, Basu SK, Acharya K. Macrofungal diversity and ecology of the mangrove ecosystem in the Indian part of Sundarbans. Biodiversity 2013;14:196–206.

4. Acharya K, Dutta AK, Pradhan P. A new variety of *Volvariella pusilla* from West Bengal, India. Mycosphere 2012;3:13–17.

5. Dutta AK, Chandra S, Pradhan P, Acharya K. A new species of *Marasmius* sect. *Sicci* from India. Mycotaxon 2014;128:117–125.

6. Dutta AK, Pradhan P, Roy A, Acharya K. A subtropical agaric new to India. Kavaka 2011;39:37–39.

7. Dutta AK, Pradhan P, Roy A, Acharya K. Agaricales of West Bengal, India. I. Clavariaceae: *Clavaria* and *Scytinopogon*. Ind JAppl Pure Biol 2012;27:53–58.

8. Dutta AK, Chakraborty N, Pradhan P, Acharya K. Phallales of West Bengal, India. II. Phallaceae: *Phallus* and *Mutinus*. Researcher 2012;4:21–25.

9. Acharya K, Pradhan P, Sherpa NL, Dutta AK. *Favolaschia*-a new fungal genus record for Eastern India. Ind For 2014;140:639–640.

10. Sushila R, Dharmender R, Deepti R, Vikash K. Mushrooms as therapeutic agents. Rev bras farmacogn 2012;22:459–474.

11. Khatua S, Mitra P, Halder A, Acharya K. *In vitro* protective ability of *Ramaria aurea* against free radical. J Herbs Spices Med Plants 2015;21:380–391.

12. Pattanayak M, Samanta S, Maity P, Sen IK, Nandi AK, Manna DK, Mitra P, Acharya K, Islam SS. Heteroglycan of an edible mushroom *Termitomyces*

clypeatus: structure elucidation and antioxidant properties. Carbohydr Res 2015;413:30–36.

13. Dasgupta A, Paloi S, Acharya K. Mycochemical analysis and antioxidant efficacy of a wild edible mushroom from the Eastern Himalayas. Res J Pharm Biol Chem Sci 2015;6:943–948.

14. Mallick S, Dey S, Mandal S, Dutta A, Mukherjee D, Biswas G, Chatterjee S, Mallick S, Lai TK, Acharya K, Pal C. A novel triterpene from *Astraeus hygrometricus* induces reactive oxygen species leading to death in *Leishmania donovani*. Future Microbiol 2015;10:763–789.

15. Khatua S, Dutta AK, Acharya, K. Prospecting *Russula senecis*: a delicacy among the tribes of West Bengal. PeerJ 2015;3:810.

16. Maity P, Sen IK, Majia PK, Paloi S, Devi KSP, Acharya K, Maiti TK, Islam SS. Structural, immunological, and antioxidant studies of β-glucan from edible mushroom *Entoloma lividoalbum*. Carbohydr Polym 2015;123:350–358.

17. Samanta S, Nandi A.K., Sen IK, Maity P, Pattanayak M, Devi KSP, Khatua S, Maiti TK, Acharya K, Islam SS. Studies on antioxidative and immunostimulating fucogalactan of the edible mushroom *Macrolepiota dolichaula*. Carbohydr Res 2015;413:22–29.

18. Chatterjee S, Biswas G, Chandra S, Saha GK, Acharya K. Chemopreventive effect of *Tricholoma giganteum* against benzo[a]pyrene-induced forestomach cancer in Swiss albino mice. Int J Pharm Sci Rev Res 2014;26:189–196.

19. Feng Xu, Zhiming Li, Yu Liu, Chengbo Rong, Shouxian Wang. Evaluation of edible mushroom *Oudemansiella canarii* cultivation on different lignocellulosic substrates. Saudi J Biol Sci 2016;23:607–613.

20. Tsantrizos YS, Yang X, McClory A. Studies on the Biosynthesis of the Fungal Metabolite Oudenone. 2. Synthesis and Enzymatic Cyclization of an alpha-Diketone, Open-Chain Precursor into Oudenone in Cultures of *Oudemansiella radicata*. J Org Chem 1999;64:6609–6614.

21. Liu QH, Ng T, Wang HX. Isolation and characterization of a novel lectin from the wild mushroom *Oudemansiella radicata* (Relhan.: Fr.) Sing. Biotechnol Bioprocess Eng 2013;18:465–471.

22. Subik J, Behun M, Smigan P, Musilek V. Mode of action of mucidin, a new antifungal antibiotic produced by the basidiomycete *Oudemansiella mucida*. Biochim Biophys Acta. 1974;343:363–370.

23. Anke T, Hechi HJ, Schramm G. Antibiotics from Basidomycetes. IX. Oudemansin, an antifungal antibiotic from *Oudemansiella mucida* (Schrader ex Fr.) Hochnel (Agaricales). J Antibiot 1979;32:1112–1117.

24. Petersen RH, Desjardin DE, Krüger D. Three type specimens designated in *Oudemansiella*. Fungal Divers 2008;32:81–96.

25. Marcelo JSR, Samia MTT, Vera LRB, Marina C. Cultivation of the edible mushroom *Oudemansiella canarii* (Jungh.) Honn. In Lignocellulosic substrates. Braz J Microbiol 2001;32:211–214.

26. Luiz HR, Betania BC, Ka´ tia, MGM, Carlos AR, Carlos LZ. Antifungal and other biological activities from *Oudemansiella canarii* (Basidiomycota). World J Microbiol Biotechnol 2005;21:983–987.

27. Corner EJH. On the Agaric genera *Hohenbuehelia* and *Oudemansiella*. Part II: *Oudemansiella*, Gard Bull Singapore, 1994:49–75.

28. Pradhan P, Dutta AK, Acharya K. A low cost long term preservation of macromycetes for fungarium. Protocol Exchange 2015

29. Dutta AK, Wilson AW, Antonín V, Acharya K. Taxonomic and phylogenetic study on gymnopoid fungi from Eastern India. I. Mycol Progress 2015;14:79.

30. White TJ, Bruns T, Lee S, Taylor J. PCR Protocols: a Guide to Methods and Applications. In: Innis MA, Gelfand DH, Sninsky DH, White TJ, eds. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Orlando; Florida. 1990:315-322.

31. Kokashi CJ, Kokashi RJ, Sharma M. Fluorescence was powdered vegetable drug in UV radiation. J Am Pharm Assoc 1958;47:715–717.

32. Singleton VL, Rossi JJ. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Viticult 1965;16:44–158.

33. Adebayo EA, Oloke JK, Ayandele AA, Adegunlola CO. Phytochemical, antioxidant and antimicrobial assay of mushroom metabolite from *Pleurotus pulmonarius* -LAU 09 (JF736658). J Microbiol Biotech Res 2012;2:366-374.

34. Nagata M, Yamashita I. Simple method for simultaneous determination of chlorophyll and carotenoids in tomatoes fruit. J Jpn Soc Food Sci 1992;39(10):925–928.

35. Rekha C, Poornima G, Manasa AV, Pavithra DJ, Vijay KHT, Prashith KTR. Ascorbic acid, total phenol content and antioxidant activity of fresh juices of four ripe and unripe citrus fruits. Chem Sci Trans 2012;1:303–310.

36. Mitra P, Mandal NC, Acharya K. Biocomponents and bioprospects of ethanolic extract of *Termitomyces heimii*. Asian J Pharm Clin Res 2015;8:331–334.

37. Mitra P, Mandal NC, Acharya K. Phytochemical characteristics and free radical scavenging activity of ethanolic extract of *Termitomyces microcarpus* R. Heim. Der Pharmacia Lettre 2014:6:92–98.

38. Arnao MB, Cano A, Hernández-Ruiz J, García-Cánovas F, Acosta M. Inhibition by L-ascorbic acid and other antioxidants of the 2.2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) oxidation catalyzed by peroxidase: a new approach for determining total antioxidant status of foods. Anal Biochem 1996;236:255–261.

39. Bhattacharya S, Zaman MK. Pharmacognostical evaluation of *Zanthoxylum nitidum* bark. Int J Pharm Tech Res 2009;1:292–298.

40. Sonibare MA, Olatubosun OV. Pharmacognostic and free radical scavenging evaluation of *Cyathula prostata* (Blume) L. Phcog J 2015;7:107–116.

41. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of Xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J Agric Food Chem 1992;40:945-948.