

**INTERNATIONAL JOURNAL OF ADVANCES IN  
PHARMACY, BIOLOGY AND CHEMISTRY****Research Article****Physico chemical and Primary Biochemical Studies of  
*Hygrocybe cantharellus* Collected from Western  
Ghats region of Haniya, Shimoga (Dist) Karnataka****Ashok Chittaragi\*, Raja Naika.**Department of Applied Botany, Kuvempu University,  
Jnana Sahyadri, Shankaraghatt, Shimoga, Karnataka, India-577451.**ABSTRACT**

The *Hygrocybe cantharellus* was collected from Western Ghats regions of Haniya, Hosanagar (T), Shimoga (D), Karnataka. They were harvested fresh during rainy season in the month of June to August 2012, for conducted to the physico and primary biochemical studies of *Hygrocybe cantharellus* fruiting bodies. The physicochemical parameters of *H. cantharellus* powder were determined like total ash content, acid-insoluble ash, water soluble ash, pH of 5% w/v solution of aqueous extract, foreign matter, moisture content and alcohol soluble extractive. The extracts of *H. cantharellus* were prepared using different solvents like petroleum ether, chloroform and methanol solvents. The biochemical screening of the fruiting bodies extracts was performed. The presence carbohydrates (Molisch's test) and proteins (Biuret test) in chloroform and methanol where as absent in petroleum ether were indicated by the test conducted. This mushroom was found to contain highest percentage of alcohol soluble extractive (8 %), followed by pH of 5% w/v solution of aqueous extract (18.42%), foreign matter (16.0%), moisture (15.4 %), water soluble ash (12.32%), total ash content 11.3%) and acid soluble ash content (4.5%) for the physicochemical analysis. These studies provided referential information in regard to its identification parameters assumed significantly in the way of acceptability.

**Keywords:** *Hygrocybe cantharellus*, Biochemical analysis, Wild mushrooms, Physicochemical parameters, Western Ghats.

**INTRODUCTION**

Edible mushrooms have been widely utilized as a human food for centuries. These are liked all over the world due to their delicate taste, flavor and health giving properties. Mushrooms also have some medicinal and tonic properties<sup>1</sup>. Mushrooms are also important for the treatment of different diseases in human as is evident from the biochemical analysis of the fruiting bodies of these mushrooms<sup>2</sup>.

Mushrooms are rich source of protein, vitamins, fats, carbohydrates, amino acids and minerals<sup>3</sup>. In mushroom fruiting bodies all essential amino acids, water soluble vitamins and all the essential minerals are present<sup>4</sup>. Mushrooms are also good sources of vitamins like riboflavin, biotin and thiamine<sup>5</sup>. These are low in fat, carbohydrates and salts<sup>6</sup>. Appreciable

amount of dietary fibre is present in their fruiting bodies which are important for the regulation of physiological functions in human beings like regulation of digestive tract<sup>1</sup>. Moreover, mushrooms are low in nucleic acid contents which make them an ideal food for patients suffering from diabetes, obesity and hypertension<sup>7</sup>.

Fungi are ubiquitous<sup>8</sup>, exceptionally diverse group of heterotrophic organisms and play principal role in the forest ecosystems<sup>9</sup>. They are important eukaryotes and possess more diverse array of reproductive strategies than most of the other organisms<sup>10,11</sup>. The divergence in the clusters of fungi and their immense beauty occupy a prime place in the biological world and India has been a cradle for such fungi<sup>12</sup>.

The fungi are an immensely diverse group of organisms, encompassing a huge range of forms in shape, size and colour from microscopic single celled yeasts to large macrofungi, as exemplified by the well-known mushrooms and toadstools<sup>13</sup>. Fungi are a major component of forest soils and serve as indicators of stress and disturbance resulting due to various forest management practices<sup>14</sup>. Although identification of relevant indicators in nature has been a difficult task, these can be very useful tools in conservation strategies<sup>15</sup>.

Today, decline in biodiversity on Earth and practical challenges in describing and enumerating it is rapidly diminishing. So the conservation biologists are relying on environmental characteristics, indicator taxon groups and individual indicator species and higher taxonomic levels for explaining patterns of biodiversity and struggling to preserve the remaining of its natural variability<sup>16</sup>.

Several studies of research indicate that mushrooms have been used as a bio indicator to determine the heavy metal pollutions<sup>17,18</sup>. The environmental factors like, climate change scenario and increasing human impact have become a greater threat to global biodiversity and serious concerns among researchers and the public. Although researchers are constantly on their way for better understanding, less we know about true diversity of life and lack the ability to recognize and to respond intelligently to recent and future environmental changes<sup>19</sup>. Human interference on the earth's climate is becoming more and more obvious. Climate observations reveal the existence of a global warming and global average temperature has increased over the years. Since long life span of trees does not allow for rapid adaptation to environmental changes, forests are particularly sensitive to climate change<sup>20</sup>.

So, keeping in view the importance of mushroom the present study aims to provide information of physicochemical and primary biochemical screening of *H. cantharellus* collected from Western Ghats region Haniya, Shimoga (Dist) of Karnataka.

## EXPERIMENTAL SECTION

### Mushroom material:

The *Hygrocybe cantharellus* was collected from Western Ghats regions of Haniya, Hosanagar (T), Shimoga (D), Karnataka. They were harvested fresh during rainy season in the month of June to August 2012, The *H. cantharellus* of mushroom was picked from the litter and decaying soil surface, with help of forceps and then they were cleaned and kept for shade drying<sup>21</sup>. Identification was done by comparing their morphological, anatomical and physiological characteristics with the help of standard

literatures<sup>22,23</sup>. The voucher specimen (KUABARN-67) has been deposited at the herbarium of mycology laboratory, Department of P. G. Studies and Research in Applied Botany, Bio-Science Complex, Kuvempu University, Jnana Sahyadri, Shankaraghatta-577451, Shimoga district, Karnataka, India.

### Extraction of mushroom materials

The extracts were prepared according to the methodology<sup>24</sup>. The powdered materials were subjected Soxhlet extraction by using various solvents namely petroleum ether (10.8 g), chloroform (11.21g) and methanol (51.16g). Each extraction was carried out for 48 hours at suitable temperature. The yield of each extracts were recorded (Fig-1) and preserved at 4°C for further experiments.

### Physicochemical parameters:

Physicochemical parameters of *Hygrocybe cantharellus* powder were determined by the following methodology.

**Determination of foreign matter**-1g of sample was weighed and foreign matter was carefully separated. The matter differing in colour and texture were considered as foreign. The separated matter was weighed and subtracted from one gram and percentage was calculated.

**Determination of moisture content**-1g of powder was weighed and dried at 80°C for 24 h in hot air oven. After 24 h, the powder was weighed again and the difference in the weight was determined. The percentage of moisture was calculated.

**Determination of pH**-The 5% (w/v) (5g in 100ml of water) powder was kept on shaker for 5 h with 140rpm and filtered. The filtrate was analyzed for the pH using pH meter<sup>25</sup>.

**Determination of water soluble extractive**-5gms of powder was weighed and added into a 100ml conical flask. 25ml of distilled water was added into it and kept on a rotator shaker (140rpm) for 24 h. After 24 h it was filtered and dried in hot air oven set at 80°C for 24 h and weighed again. The difference in the weight was determined and percent of water soluble extractive was calculated<sup>24,26</sup>.

**Determination of alcohol soluble extractive**-5gms of powder was weighed and added into a 100ml conical flask. 25ml of absolute alcohol was added into it and kept on a rotator shaker (140rpm) for 24 h. After 24 h it was filtered and dried in hot air oven set at 80°C

for 24 h and weighed again. The difference in the weight was determined and percent of water soluble extractive was calculated<sup>24,26</sup>.

**Determination of total ash content**-The clean and dry crucible (silica) was weighed and its weight was noted. 10g of powder was weighed in crucible and powder was kept in a muffle furnace and heated up to 300°C for 3-4 h until the whole powder turns into ash. The crucible was cooled and weighed again. The difference in the weight was noted and percent of total ash was calculated<sup>27,28</sup>.

**Determination of water soluble ash**-1g of ash was weighed and 10ml of distilled water was added into it. The mixture was kept on a shaker with 140rpm for 8 h and filtered through ashless filter paper. The ash remained in the paper was kept in a crucible (Silica) and burnt to ash again in a muffle furnace for 3-4 h. The weight of ash obtained was noted and percent of water soluble ash was determined<sup>29</sup>.

**Determination of acid insoluble ash**-1g of ash was weighed and 10ml of concentrated H<sub>2</sub>SO<sub>4</sub> was added into it. The mixture was kept on a shaker with 140rpm for 8 h and filtered through ashless filter paper. The ash remained in the paper was kept in a crucible (Silica) and burnt to ash again in a muffle furnace for 3-4 h. The weight of ash obtained was noted and percent of acid insoluble ash was determined<sup>29</sup>.

#### Primary biochemical studies:

Primary biochemical test of various extracts of *Hygrocybe cantharellus* were performed for biochemical analysis of carbohydrates, polysaccharides, proteins, lipids and oils<sup>30,31</sup>.

## RESULTS AND DISCUSSION

#### Physicochemical parameters:

The result of physicochemical of *H. cantharellus* mushroom ash was given in the Fig-2. The result of ash was shown as fine powder. The percentage of loss on drying was lowest when compared to the percentage of ash content (9.5%). Percentage of ash value was lowest in acid (5.2%) followed by water and alcohol (14.8%). This mushroom was found to contain highest percentage of alcohol soluble

extractive (60%), followed by pH of 5% w/v solution of aqueous extract (16.48%), moisture (15.4%), water soluble ash (14.28%), foreign matter (14.0%), total ash content (9.5%) and acid soluble ash content (5.2%) for the physic-chemical analysis.

#### Primary biochemical studies:

Primary biochemical screening of the extracts obtained from *H. cantharellus* revealed the presence of carbohydrates (Molisch's test) in chloroform and methanol extracts, where as absent in petroleum ether solvent extracts. In case of proteins (Ninhydrin's test) also present all the solvent extracts, in the same time (Biuret test) absent only in petroleum ether extract. Remaining tests like, polysaccharides, lipids and oils, completely absent in all three different solvent extracts (Table-1). The current environmental issues of global warming and climate change would adversely affect the regeneration and growth pattern of the delicate fungi<sup>32</sup>.

The use of natural products including medicinal mushrooms is increasing day by day and the growth of the medicinal mushroom for this reason our investigation, for screening different solvent extract of *H. cantharellus* the results obtained confirmed therapeutic potency of some mushroom used in traditional medicine<sup>33</sup>.

## CONCLUSION

The present study on primary biochemical and physicochemical investigation of *Hygrocybe cantharellus* fruiting bodies will be providing useful information in regard to the presence of active bio-constituents. Further study will be required to bioassay indicated isolation to isolate, identify and characterization the structure of the biologically active compound accountable for pharmacological properties.

## ACKNOWLEDGMENT

Authors are very grateful to Chairman, Department of P. G. Studies and Research in Applied Botany, Bio-Science Complex, Jnana Sahyadri, Shankaraghatta-577451 for laboratory facility and UGC, Government of India, New Delhi for their financial assistant to carry out this work.

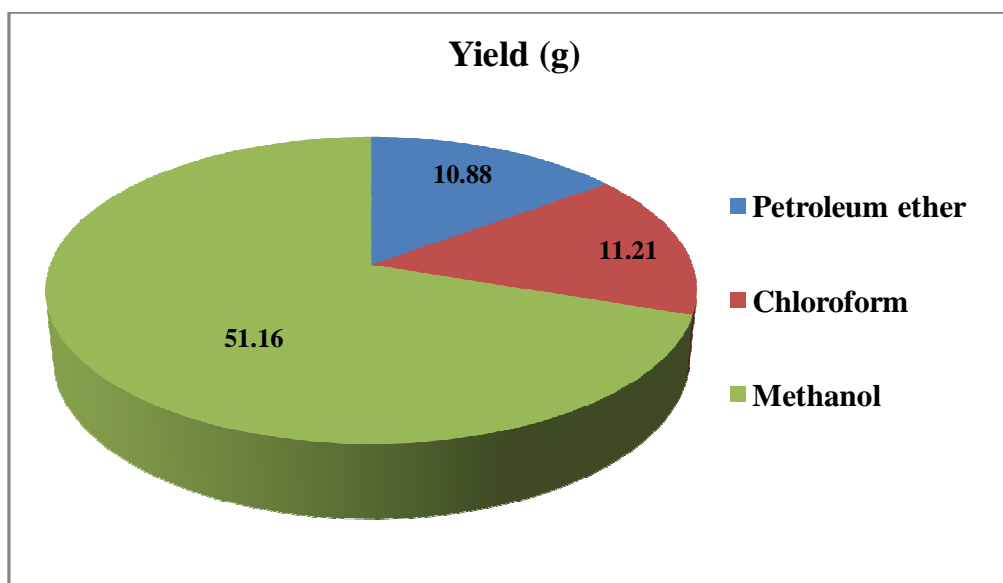


Fig 1  
Showing yield of *Hygrocybe cantharellus* in different solvents

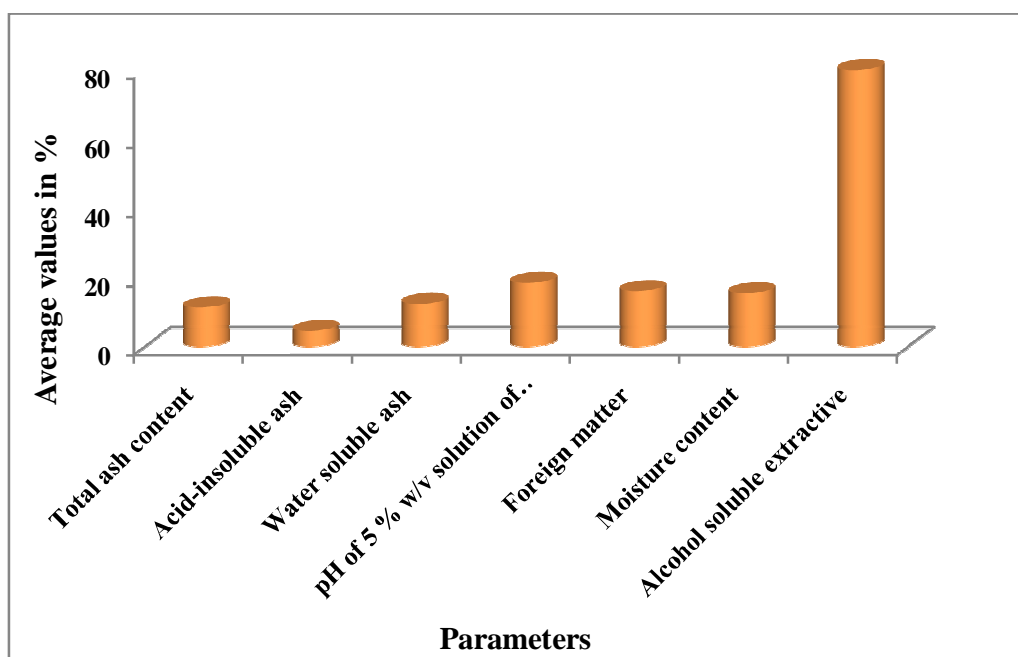


Fig 2  
Shows physicochemical parameters of *Hygrocybe cantharellus*

**Table 1**  
**Primary biochemical properties of different solvent extracts of *Hygrocybe cantharellus***

Sl. No	Tests	Extracts		
		Petroleum ether	Chloroform	Methanol
1	<b>Test for Carbohydrates</b>			
a	Molisch's Test	-	+	+
2	<b>Test for Polysaccharides</b>			
		-	-	-
3	<b>Test for Proteins</b>			
a	Biuret test	-	+	+
b	Ninhydrin's test	+	+	+
4	<b>Test for Lipids</b>			
		-	-	-
5	<b>Test for Oils</b>			
		-	-	-

Note: '+' = Present, '-' = Absent.

## REFERENCES

- Manzi P, Aguzi P, Pizzoferrato L. Nutritional value of mushroom widely consumed in Italy. Food Chemistry, 2001; 73: 321-325.
- Haq IU, Khan MA, Khan SA, Ahmad M. Biochemical analysis of fruiting bodies of *Volvariella volvacea* strain Vvpk, grown on six different substrates. Soil Environ, 2011; 30(2): 146-150.
- Jiskani MM. Energy potential of mushrooms. The DAWN, Economic and Business Review, 2001; pp 4.
- Buigut SK. Mushroom production in sustainable small-scale farming system-opportunities and constraints: a survey of Uasin Gishu district. In: Proceedings of the Horticulture seminar on Sustainable Horticultural Production in the Tropics at Jomo Kenyatta, University of Agriculture & Technology, Juja, Kenya, October 3-6, 2001, Kenya.
- Chang ST, Buswell JA. Mushroom Nutraceuticals. World Journal of Microbiology and Biotechnology, 1996; 12: 473-476.
- Genders R. Mushroom growing for everyone. 3<sup>rd</sup> Ed. Faber and Faber, London. 1990.
- Mushrooms. National Research Centre for Mushroom, Indian Council of Agriculture Research, Chambaghat-173-213, Solan, Himachal Pradesh, India, 2003.
- Schmit *et al.*, Assessment of tree species richness as a surrogate for macrofungal species richness. Biol Cons, 2005; 121: 99-110.
- Newbound *et al.*, Fungi and the urban environment: A review. Landsc Urban Plan, 2010a; 96: 138-145.
- Rudawska M, Leski T. Trace elements in fruiting bodies of ectomycorrhizal fungi growing in Scots pine (*Pinus sylvestris* L.) stands in Poland. Sci Total Environ, 2005; 339: 103-115.
- Osono T. Ecology of ligninolytic fungi associated with leaf litter decomposition. Ecol Res, 2007; 22: 955-974.
- Swapna *et al.*, Diversity of macrofungi in semi-evergreen and moist deciduous forest of Shimoga district-Karnataka, India. J Mycol Plant Pathol, 2008; 38: 21-26.
- Bridge P, Spooner B. Soil fungi: Diversity and detection. Plant and Soil, 2001; 232: 147-154.
- Osono T, Trofymow JA. Microfungal diversity associated with *Kindbergia oregano* in successional forests of British Columbia. Ecol Res, 2012; 27: 35-41.
- Norden *et al.*, Indicators of biodiversity, what do they indicate? – Lessons for conservation of Cryptogams in Oak-rich forest. Biol Conserv, 2007; 135: 369-379.
- Heino J, Soininen J. Are higher taxa adequate surrogates for species-level assemblage patterns and species richness in stream organisms? Biol Conserv, 2007; 137:8-89.
- Cocchi *et al.*, Heavy metals in edible mushrooms in Italy. Food Chem 2006; 98: 277-284.

18. Heino J, Soininen J. Are higher taxa adequate surrogates for species-level assemblage patterns and species richness in stream organisms? *Biol Conserv*, 2007; 137:8-89.
19. Geml *et al.*, Phylogenetic and ecological analyses of soil and sporoma DNA sequences reveal high diversity and strong habitat partitioning in the boreal ectomycorrhizal genus *Russula* (Russulales; Basidiomycota). *New Phytol*, 2010; 187: 494-507.
20. Lindner *et al.*, Climate change impacts, adaptive capacity and vulnerability of European forest ecosystems. *For Ecol Manag*, 2010; 259: 698-709.
21. Chittaragi *et al.*, Antibacterial Potential of *Geastrum triplex* Jungh. Against Plant and Human Pathogens, *Int. J. Pharm Tech Res*, 2013;5(4): 1456-1464.
22. Purkayastha RP, Chandra A. *Manual of Indian Edible Mushrooms*, Today & Tomorrow's Printers and Publishers, New Delhi, India, 1985.
23. Singer R. *The Agaricales in Modern Taxonomy*, Bishen Sing Mahendra Sing Publishers, Dehradun, India, 1986.
24. *Indian Pharmacopoeia*, 2<sup>nd</sup> ed. Government of India, New Delhi. 1966; pp 23.
25. Iqbal *et al.*, Physicochemical Standardization of *Butea Monosperma* (Lam.) Kuntze (Palasha): An Ayurvedic Drug International Journal of Pharmaceutical Quality Assurance, 2010; 2(1): 49-51.
26. Gupta S. *The Ayurvedic system of medicine occurring in Charka, Sushruta*. Neeraj Publishing House, New Delhi, India, Vol-II, 1984.
27. Gupta AK. *Quality standards of Indian medicinal plants*. Indian Council of Medical Research, India Vol-I, 2003.
28. Indrayan *et al.*, Determination of nutritive value and analysis of mineral elements for some medicinally valued plants from Uttaranchal. *Current Sci*, 2005; 89: 1252-1255.
29. Ahmad RV, Sharma RK. Evaluation of drug for standardization. Proceedings of WHO training cum- workshop, Pharmaceutical lab for Indian medicine, Ministry of health and family welfare, Govt. of India, Ghaziabad, 2001.
30. Harbone JB. *Phytochemical Methods: A guide to modern techniques of plant analysis*. Chapman and Hall Ltd, London, 1984.
31. Khandelwal KR. *Practical Pharmacognosy- Techniques and Experiments*. Nirali Prakashan, Pune, 2002.
32. Ashok Chittaragi *et al.*, Comparative antibacterial study of different extract of *Clavaria rosea* Fries against gram negative and gram positive pathogens. *Int J Pharm Sci Res*, 2014; 5(2): 392-396.
33. Chittaragi *et al.*, Nutritive value of few Wild Mushrooms from the Western Ghats of Shivamogga district, Karnataka, India, *Asian J Pharm Clin Res*, 2014; 7(1): 50-53.