ABSTRACT

The present study was aimed to comparative primary phytochemical and physico-chemical properties of wild mushrooms fruiting bodies. The physico-chemical parameters of *Phellinus linteus* and *Phellinus wahlbergii* 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 *Ph. linteus* and *Ph. wahlbergii* were prepared using different solvents like petroleum ether, chloroform, methanol and aqueous solvents. The phytochemical screening of *Ph. linteus* and *Ph. wahlbergii* fruiting bodies extracts was performed the presence carbohydrates (Molisch’s test) and proteins (Biuret test and Ninhydrin’s test) in chloroform, methanol and aqueous solvents where as absent in petroleum ether, where as absent in petroleum ether were indicated by the test conducted. The both species are having same phytochemical constituents, in the same time absent for polysaccharides, lipids and oil tests all the different solvent extracts. The *Ph. linteus* mushroom was found to contain highest percentage of alcohol soluble extractive (71.5 %), followed by moisture (22.9 %), foreign matter (18.7 %), where as the *Ph. Wahlbergii* mushroom was found to contain highest percentage of acid soluble ash content (45 %), followed by moisture content (27.57 %), alcohol soluble extractive (19.4 %), water soluble ash (14-15 %), pH of 5 % w/v solution of aqueous extract (14.3 %) for the physico-chemical analysis. These studies provided
referential information in regard to its identification parameters assumed significantly in the way of acceptability.

**Keywords:** *Phellinus* spp, Wild mushrooms, Primary tests, Physico-chemical parameters, Western Ghats.

**INTRODUCTION**

Mushroom is a fleshy, spore bearing fruiting body, a fungus, typically produced above ground on soil or on its food source. Mushroom is most often applied to fungi (Basidiomycota, Agaricomycetes, order Boletales and family Boletaceae) that have stem (stipe), a cap (Pileus) and gills (Lamellae) on the other side of the cap. Mushrooms can be found in the forest around the country [1]. Mushrooms such as the Pleurotus species are known to be among the largest fungi or saprophytic eukaryotes composed of hyphae filament that thrives very well in damp or moist condition [2].

Fungi are ubiquitous [3], exceptionally diverse group of heterotrophic organisms and play principal role in the forest ecosystems [4]. They are important eukaryotes and possess more diverse array of reproductive strategies than most of the other organisms [5, 6]. 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 [7]. 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 [8].

Fungi are a major component of forest soils and serve as indicators of stress and disturbance resulting due to various forest management practices [9]. Although identification of relevant indicators in nature has been a difficult task, these can be very useful tools in conservation strategies [10].

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 [11]. Several studies of research indicate that mushrooms have been used as a bio indicator to determine the heavy metal pollutions [12, 13].
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 [14]. 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 lifespan of trees does not allow for rapid adaptation to environmental changes, forests are particularly sensitive to climate change [15].

The present study aims to provide information of comparative primary phyto and physico-chemical properties of *Phellinus linteus* and *Phellinus wahlbergii*.

**MATERIALS AND METHODS**

**Collection of Mushrooms**

The *Phellinus linteus* and *Phellinus wahlbergii* was collected from Kigga village of Sringeri (T), Chickmagalur (D), Karnataka. They were harvested fresh during rainy season in the month of August and September 2012, The *Ph. linteus* and *Ph. wahlbergii* of mushroom was picked from the litter and decaying wood surface, with help of forceps and then they were cleaned and air dried in an oven at 40˚ C for 48 h. dried mushroom samples were powdered mechanically for further use. Identification was done by comparing their morphological, anatomical and physiological characteristics with the help of standard literatures [16]. The specimen has been deposited at the herbarium of Dept. of Botany, Sahyadri Science College (Autonomous) Shimoga, Karnataka, India.

**Preparation of mushroom extracts**

The dried and powdered by grinder (Bajaj Electrical Limited-Twister Mixer) mushroom material (200 g) was extracted successively with 2000 ml pet ether following chloroform and methanol and aqueous with a Soxhlet extractor for 48 h at temperature not exceeding the boiling point of the solvent [17]. The extract was filtered with Whatman filter paper no.1 and the filter was concentrated in a vacuum at 40ºC using a rotary evaporator [18]. The yield of *Phellinus linteus* and *Phellinus wahlbergii* extracts respectively obtained from petroleum ether was (0.40 and 1.50 gm), followed by chloroform (0.80 and 0.90 gm), methanol (1.6 and 2.0 gm) and aqueous (1.0 and 1.80 gm) Table-1. Each extract was transferred to glass vials and kept at 4˚C before use.
Physico-chemical parameters

Physico-chemical parameters of *Phellinus linteus* and *Phellinus wahlbergii* powder were determined by the following methodology.

**Determination of foreign matter**-One gram 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**-One gram 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) (5 g in 100 ml of water) powder was kept on shaker for 5 h with 140 rpm and filtered. The filtrate was analyzed for the pH using pH meter (Elico, India) [19].

**Determination of water soluble extractive**-Five grams of powder was weighed and added into a 100 ml conical flask. 25 ml of distilled water was added into it and kept on a rotator shaker (140 rpm) 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 [20, 21].

**Determination of alcohol soluble extractive**-Five grams of powder was weighed and added into a 100 ml conical flask. 25 ml of absolute alcohol was added into it and kept on a rotator shaker (140 rpm) 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 [20, 21].

**Determination of total ash content**-The clean and dry crucible (silica) was weighed and its weight was noted. 10 g 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 [22, 23].
Determination of water soluble ash-One g of ash was weighed and 10 ml of distilled water was added into it. The mixture was kept on a shaker with 140 rpm 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 [24].

Determination of acid insoluble ash-One gram of ash was weighed and 10 ml of concentrated H$_2$SO$_4$ was added into it. The mixture was kept on a shaker with 140 rpm 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 [24].

Primary phytochemical studies
Primary phytochemical test of various extracts of roots powder of Phellinus linteus and Phellinus wahlbergii were performed for phytochemical analysis of carbohydrates, polysaccharides, proteins, lipids and oils [25, 26].

RESULTS AND DISCUSSION

Physico-chemical parameters
The physico-chemical parameters of Phellinus linteus and Phellinus wahlbergii 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 Ph. linteus and Ph.wahlbergii were prepared using different solvents like petroleum ether, chloroform, methanol and aqueous solvents.

The Ph. linteus mushroom was found to contain highest percentage of alcohol soluble extractive (71.5 %), followed by moisture content (22.9 %), foreign matter (18.7 %), water soluble ash (14 %) and moderate percentage of pH of 5 % w/v solution of aqueous extract (11.3 %) followed by total ash content (11.2 %) and acid-insoluble ash (7.8 %) (Piechart-1), where as the Ph. wahlbergii mushroom was found to contain highest percentage of acid soluble ash content (45 %), followed by moisture content (27.57 %), alcohol soluble extractive (19.4 %), water soluble ash (15 %), pH of 5 % w/v solution of aqueous extract (14.3 %), and least percentage of total ash content (3.2 %), foreign matter (2.8 %) (Graph-1), for the physico-chemical analysis.
The physico-chemical parameters of *Phellinus linteus* and *Phellinus wahlbergii* both species are having nearest percentage in water soluble ash (14 and 15 %). The *Phellinus linteus* contain highly percentage of the all parameters when compare to the *Phellinus wahlbergii* (Piechart-1 and Graph-1).

**Table 1: Showing yield of mushrooms extracts from different solvents**

<table>
<thead>
<tr>
<th>Mushroom</th>
<th><em>Phellinus linteus</em></th>
<th><em>Phellinus wahlbergii</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvents</td>
<td>Extract obtained in gm</td>
<td></td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>1.50</td>
<td>0.40</td>
</tr>
<tr>
<td>Chloroform</td>
<td>0.90</td>
<td>0.80</td>
</tr>
<tr>
<td>Methanol</td>
<td>2.0</td>
<td>1.6</td>
</tr>
<tr>
<td>Aqueous</td>
<td>1.80</td>
<td>1.0</td>
</tr>
</tbody>
</table>

![Piechart 1: Shows physico-chemical parameters of Phellinus linteus values (%)](image1)

![Graph 1: Shows physico-chemical parameters of Phellinus wahlbergii](image2)
Table 2: Primary phytochemical screening of different solvent extracts of *Phellinus linteus* and *Phellinus wahlbergii*

<table>
<thead>
<tr>
<th>Tests</th>
<th>Extracts</th>
<th>Petroleum ether</th>
<th>Chloroform</th>
<th>Methanol</th>
<th>Aqueous</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Phellinus linteus</em></td>
<td>Test for Carbohydrates</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Molisch’s Test</td>
<td>Test for Polysaccharides</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Test for Proteins</td>
<td>Biuret test</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ninhydrin’s test</td>
<td>Test for Lipids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Phellinus wahlbergii</em></td>
<td>Test for Carbohydrates</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Molisch’s Test</td>
<td>Test for Polysaccharides</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Test for Proteins</td>
<td>Biuret test</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ninhydrin’s test</td>
<td>Test for Lipids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Test for Oils</td>
<td>Note: ‘+’ = Present, ‘-’ = Absent</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Primary phytochemical studies

The phytochemical screening of *Ph. linteus* and *Ph. wahlbergii* fruiting bodies extracts was performed the presence carbohydrates (Molisch’s test) and proteins (Biuret test and Ninhydrin’s test) in chloroform, methanol and aqueous solvents where as absent in petroleum ether, where as absent in petroleum ether were indicated by the test conducted. The both species are having same phytochemical constituents, in the same time absent for polysaccharides, lipids and oil tests all the different solvent extracts (Table-2). The current environmental issues of global warming and climate change would adversely affect the regeneration and growth pattern of the delicate fungi [27].

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 extracts of Ph. linteus and Ph. wahlbergii the results obtained confirmed therapeutic potency of some mushroom used in traditional medicine [28].

CONCLUSION
The present study on phytochemical and physico-chemical investigation of Ph. linteus and Ph. wahlbergii fruiting bodies will be providing useful information in regard to the presence of active phytoconstituents. Further study will be required in the way of biological activity and pharmacological properties of the active compound.

ACKNOWLEDGMENT
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