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Research Article

Physico-chemical and primary phytochemical screening of *pisolithus arrhizus* collected from Western Ghats region of Karnataka

S Nataraja[1], R Meghalatha[1*], Ashok Chittaragi[2] and M Krishnappa[2]

[*1]Research Scholar, Dept of Botany, Sahyadri Science College (Autonomous) Shimoga, Karnataka, India
[1]Associate professor, Dept of Botany and Seed Technology, Sahyadri Science College (Autonomous) Shimoga, Karnataka, India
[2]Research Scholar, Dept of P. G. Studies and Research in Applied Botany, Mycology Lab, Bio-Science Comlpex, Jnana Sahyadri, Kuvempu University, Shankaraghatta-577451, Shimoga, Karnataka, India
[2]Professor, Dept of P. G. Studies and Research in Applied Botany, Jnana Sahyadri, Kuvempu University, Shankaraghatta-577451, Shimoga, Karnataka, India

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ABSTRACT

The present study was aimed to investigate the physico-chemical and primary phytochemical screening of *P. arrhizus* fruiting bodies. The physico-chemical parameters of *P. arrhizus* 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 *P. arrhizus* were prepared using different solvents like chloroform, ethyl acetate, methanol and aqueous solvents. The phytochemical screening of the fruiting bodies extracts was performed. The presence carbohydrates (Molisch's test) and proteins (Biuret test) in ethyl acetate and methanol where as absent in chloroform and aqueous were indicated by the test conducted. 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 physico-chemical analysis. These studies provided referential information in regard to its identification parameters assumed significantly in the way of acceptability.

Keywords: *Pisolithus arrhizus*, Wild mushrooms, Primary tests, Physico-chemical parameters, Western Ghats.

INTRODUCTION

Fungi are ubiquitous [1], exceptionally diverse group of heterotrophic organisms and play principal role in the forest ecosystems [2]. They are important eukaryotes and possess more diverse array of reproductive strategies than most of the other organisms [3, 4]. 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 [5]. 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 [6]. Fungi are a major component of forest soils and serve as indicators of stress and disturbance resulting due to various forest management practices [7]. Although identification of relevant indicators in nature has been a difficult task, these can be very useful tools in conservation strategies [8]. 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 [9]. Several

***Corresponding Author Address: Mrs. R. Meghalatha,** Research Scholar, Dept of Botany, Sahyadri Science College (Autonomous) Shimoga, Karnataka, India; E-mail: meghabotany@gmail.com*

studies of research indicate that mushrooms have been used as a bio indicator to determine the heavy metal pollutions [10, 11]. 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 [12]. 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 [13]. The present study aims to provide information of physico-chemical and preliminary phytochemical screening of *Pisolithus arrhizus* collected from Western Ghats region of Karnataka.

EXPERIMENTAL SECTION

Mushroom material: The *Pisolithus arrhizus* was collected from Western Ghats regions of Sagar (T), Shimoga (D), Karnataka. They were harvested fresh during rainy season in the month of June to August 2011, The *P. arrhizus* 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 [14]. It was identified as *P. arrhizus* with the help of faculty of Department of P. G. Studies and Research in Applied Botany, Kuvempu University, Jnana Sahyadri, Shankaraghatta-577451, Shimoga (Dist) Karnataka.

Extraction of mushroom materials: The extracts were prepared according to the methodology of Indian Pharmacopoeia [15]. The powdered materials were subjected Soxhlet extraction by using various solvents namely chloroform (0.25 mg), ethyl acetate (0.70 mg), methanol (4.2 gm) and aqueous (3.0 gm). Each extraction was carried out for 48 hours at suitable temperature. The yield of each extracts were recorded (Graph-1) and preserved at 4° C for further experiments.

Physico-chemical parameters: Physico-chemical parameters of *Pisolithus arrhizus* 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) [16].

***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 [17, 18].

***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 [17, 18].

***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 [19, 20].

***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 [21].

***Determination of acid insoluble ash*-**One gram of ash was weighed and 10 ml of concentrated H_2SO_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 [21].

Primary phytochemical studies: Primary phytochemical test of various extracts of roots powder of *Pisolithus arrhizus* were performed for phytochemical analysis of carbohydrates, polysaccharides, proteins, lipids and oils [22, 23].

RESULTS AND DISCUSSION

Physico-chemical parameters: The result of physicochemical of *P. arrhizus* mushroom ash was given in the Table-1. 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 phytochemical studies: Primary phytochemical screening of the extracts obtained from *P. arrhizus* revealed the presence of carbohydrates (Molisch's test) in ethyl acetate and methanol extracts, where as absent in chloroform and aqueous solvent extracts. Proteins (Ninhydrin's test**)** completely absent, in the same (Biuret test) also present in ethyl acetate and methanol extracts, here also absent in chloroform and aqueous solvents extracts. Remaining test like, polysaccharides, lipids and oils, completely absent in all four 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 [24]. 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 *P. arrhizus* the results obtained confirmed therapeutic potency of some mushroom used in traditional medicine [25].

CONCLUSION

The present study on phytochemical and physico-chemical investigation of *Pisolithus arrhizus* fruiting bodies will be providing useful information in regard to the presence of active phytoconstituents. 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.

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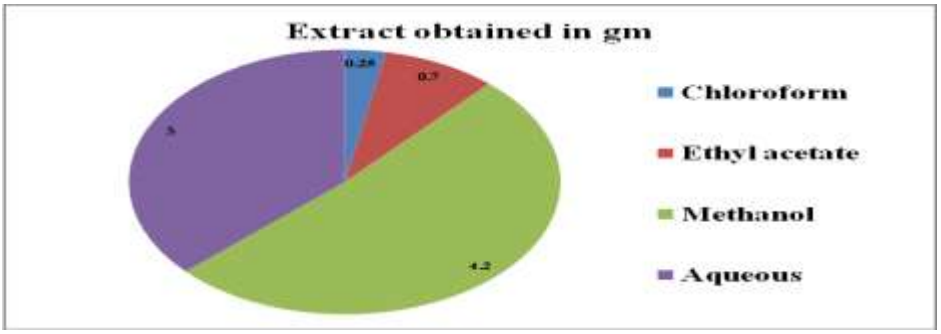

Graph 1: Showing yield of *Pisolithus arrhizus* in different solvents

Table 1: Physico-chemical parameters of *Pisolithus arrhizus*

Parameters	Average values in % age
Total ash content	9.5
Acid-insoluble ash	5.2
Water soluble ash	14.28
pH of 5 % w/v solution of aqueous extract	16.48
Foreign matter	14.0
Moisture content	15.4
Alcohol soluble extractive	60

Table 2: Primary phytochemical screening of different extracts of *Pisolithus arrhizus*

Tests	Extracts			
	Chloroform	Ethyl acetate	Methanol	Aqueous
Test for Carbohydrates				
Molisch's Test	-	+	+	-
Test for Polysaccharides				
	-	-	-	-
Test for Proteins				
Biuret test	-	+	+	-
Ninhydrin's test	-	-	-	-
Test for Lipids				
	-	-	-	-
Test for Oils				
	-	-	-	-

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

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