Abstract
Mushrooms have been used as traditional source of nutritional food and medicinal supplements. *P. tuber-regium* has been well known edible fungi and posses bioactive mycochemicals such as flavonoids, phenols, alkaloids, glycosides, proteins, fatty acids etc. and possess good antioxidant activity. Among estimated biochemicals alkaloid was found in high quantity (28.14 ± 0.32mg/100g) and tannin in low quantity (2.74 ± 0.26mg/100g). The mushrooms extract also posses good antioxidant potentiality. Estimated free radical scavenging cativity was 8.98±1.02%, hydroxy radical scavenging activity was 10.85±0.73% and total antioxidant capacity of the crude extract was 21.50±1.3% at 100µg/100mL concentration of extract.

Key words: Mushroom, mycochemical, medicinal, antioxidant

Introduction.
In recent decades population explosion and its burden is directly associated with burden of communicable and noncommunicable diseases such as diabetes, renal, cardiovascular, respiratory, cancer and diseases associated with microbial infections[1,2]. Generation of oxidative agents or freeradicals (Reactive oxygen and nitrogen species) are very high during pathogenic infections, injuries[3]. It has been found the generation of the oxidative species rapidly increasing due to recent pattern of lifestyle[4]. Free radical at high concentrations damage cellular and subcellular[5] and trig generation of chronic disease and disorders such as Alzheimer’s disease, Parkinson’s disease, multiple sclerosis, ischemic heart disease, chronic renal failure, cancer etc.[6,7]. Traditionally mushrooms have been used as medicine as well as very good source of nutrient [8]. About 700 species of mushrooms have been reported for their significant therapeutic efficacy [9]. Medicinal mushrooms contain various mycochemicals such as tannins, alkaloids, flavonoids, phenolics etc., which associated with remedy of diseases and disorders. *Pleurotus tuber-regium* (Rumph. ex Fr.) Singer, commonly called king oyster mushroom, has been traditionally consumed as medicine and nutraceutical food supplement [10].

The aim of the present study was to evaluate proximate biochemical composition antioxidant activity of *P. tuber-regium*.

Material and Methods
Collection, identification and preparation of extract
Fresh fruiting bodies of *P. tuber-regium* were collected from different sites of Assam and were identified and brought to Department of Zoology, Ranchi University, Ranchi form preparation of extract. Fresh mushrooms were dried in shade, powdered and sieved. 50g of the fine powder was subjected to extraction chamber of soxhlet using distilled water.

Mycochemical analysis
Qualitative analyses of proximate biochemical present in the extract of *P. tuber-regium* were determined following Sofowara [11]. Quantitative estimation of traceable biochemicals was done following Dnadapat et al [12].

Antioxidant activity
Antioxidant activity of *P. tuber-regium* extract was determined on the basis of total antioxidant activity [13,14], freeradical scavenging [15] and hydroxyl radical scavenging activity [16] using standard methods.

Results and Discussion
Mycochemical analysis
The result of qualitative biochemical analyses of *P. tuber-regium* presented in table-1. The result biochemicals such as carbohydrates, glycosides, proteins, tannins, saponins, alkaloids, steroids, and lipids are present in extract of *P. tuber-regium*. 
Previously preliminary biochemical analysis of edible white button mushroom *Agaricus bisporus* was done and presence of biochemical such as saponins, tannins, glycosides, reducing sugar, alkaloid, flavonoid, terpenoid etc. were reported [17].

Table 1: Qualitative biochemical analysis of *P. tuber-regium* extract

<table>
<thead>
<tr>
<th>Mycochemicals</th>
<th>Present(+) or Absent (-)</th>
<th>Inference</th>
<th>Name of Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate</td>
<td>+</td>
<td>Blue coloured solution was observed</td>
<td>Molish’s test</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>Green coloured complex was formed</td>
<td>Anthrone test</td>
</tr>
<tr>
<td>Protein</td>
<td>+</td>
<td>Blue coloured was observed</td>
<td>Bradford’s test</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>+</td>
<td>Orange colour was observed</td>
<td>Dragendroff test</td>
</tr>
<tr>
<td>Steroid</td>
<td>+</td>
<td>Steroid and H₂SO₄ layers separated and sample layer forms cherry red colour and acid layer forms green colour</td>
<td>Salkowskii test</td>
</tr>
<tr>
<td>Triterpene</td>
<td>+</td>
<td>Red colour was formed</td>
<td>Chloroform and Conc. H₂SO₄ test</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>+</td>
<td>Yellow colour formation was observed</td>
<td>NaOH test</td>
</tr>
<tr>
<td>Tannin</td>
<td></td>
<td>Yellow precipitate was observed</td>
<td>FeCl₃ test</td>
</tr>
<tr>
<td>Lipid</td>
<td>+</td>
<td>Original colour of iodine disappeared</td>
<td>Iodine test</td>
</tr>
<tr>
<td>Saponin</td>
<td>+</td>
<td>Clear soap formation was observed</td>
<td>KOH test</td>
</tr>
</tbody>
</table>

Result of qualitative biochemical analysis of traceable mycochemical is presented in figure- 5. Among the mycochemicals alkaloid was found in high quantity (28.14 ± 0.32mg/100g) and tannin in low quantity (2.74 ± 0.26mg/100g). It has been reported other mushroom also contain 64.12 ± 1.2 mg/g phenols, 0.016 ± 0.001 mg/g flavanoid, 0.28 ± 0.04mg/g saponins, 0.1 ± 0.04% alkaloids and 0.014 ± 0.003 % tannins in *Tricholoma nudum* and 6.012 ± 0.91 mg/g phenols, 0.031 ± 0.02 mg/g flavanoids, 0.27 ± 0.008mg/g saponins, 2.0 ± 0.01% alkaloids and 0.014 ± 0.001 % tannins in *Psalliota campestris* and the biochemicals possess therapeutic efficacy [18]. Biochemicals such as phytophenols, flavonoids, tannins, saponins etc. are associated with reduction of reeradicals and decrease the risks of disease and disorders associated with oxidative stress [19-21].

**Antioxidant activity**

Antioxidant activity of *P. tuber-regium* was determined on the basis of free radical scavenging, hydroxyl radical scavenging capacity and total antioxidant activity of the mushroom extract. Results of antioxidant activity of *P. tuber-regium* are presented in figure-6, 7 and 8. BHA (Butylated Hydroxy Anisole) is a synthetic reductin agent and its Freeradical scavenging cativity is quite higher than the extract. However, the extract also shows good freeradical scavenging activity. 100 µg/mL of extract showed highest freeradical scavenging activity (8.98±1.02%) among the tested concentrations of extract. Hydroxy radical scavenging activity of extract was compared with ascorbic acid and found 100µg/mL of extract showed highest (10.85±0.73%) hydroxyradical scavenging activity among the tested extract but the ascorbic acid showed more effective scavenging activity (68.11±2.46%). Total antioxidant capacity of the crude extract of showed very effective result. 100µg/mL extract showed 21.50±1.3% antioxidant activity equivalent to ascorbic acid when compared to same concentration of BHA (65 ± 1.5% antioxidant activity). However 10 µg/mL extract did not show any activity. Antioxidant activity of *Pleurotus florida* and *Calocybe indica* studies and reported hydroxyl radical scavenging of extracts of *P. florida* and *C. indica* 65.41±0.65 % at 1000 µg/ml and 46.99±2.58 % at 1000 µg/ml respectively [22]. It has been reported antioxidant activity of plant and mushroom extracts are concentration dependent and the antioxidant activity depends upon the concentration of bioactive mycochemicals such as alkaloids, tannins, saponins, flavonoids, phenols etc. present in the fruiting body of mushrooms [23]. It has also been reported bioactive chemicals including primary and secondary metabolites of plant and mushroom origine posses reducing power and reduces the reactive oxygen and nitrogen species produce in the humanbody during pathogenic infections, so that they can act as source of good and safe antioxidants [24,25].

On the basis of above results it is concluded that *P. tuber-regium* can be used as fodder and used as potent source of antioxidant.
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Bibliography


