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ANTI-INFLAMMATORY AND AMYLASE INHIBITORY PROPERTIES OF INDIAN EDIBLE MACROFUNGI *Dacryopinax spathularia* (Schwein) AND *Schizophyllum commune* (Fries)

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ABSTRACT
The present study deals with the two edible macrofungi *Viz*. *D. spathularia* and *S. commune,* which are subjected to phytochemical screening and their extracts have been tested for their anti-inflammatory and amylase-inhibitory impact. Results showed that *D. spathularia* extract shows 14.01% inhibition of Hyaluronidase enzyme at 100 microgram concentration, whereas *S. commune* extract shows 15.76% hyaluronidase inhibition at the same cocentration. In addition to these medicinal properties, extracts from both macrofungi were tested to have amylase inhibition properties, which is of great importance in the field of alternative medicines for Diabetes melitus. At 1000 microgram concentration the *D. spathularia* extract is found to have 38.24% inhibition of amylase, whereas *S. commune* extract is found to have 48.19% amylase inhibition activity.

INTRODUCTION

Inflammation is a kind of primary physiologic immune defence mechanism of the body that occurs in response to infection, introduction of foreign noxious agents into the body, burn, allergens or other stimuli and chronic disease (Kumar *et al*., 2004; Sati *et al*., 2011). The inflammatory response is the result of a complex set of interactions between the cells and the soluble factors that may occur in any tissue in response to trauma, infections, or postischaemic, toxic, or autoimmune injury (Nathan, 2002). During inflammation complement system, fibrinolytic system and hyaluronidase enzyme are activated in plasma , therefore the hyaluronidase enzyme activity can be considered as an indicative parameter of inflammation (Suleyman *et al*, 2004). Besides, Inflammation is also one of the indications of oxidative stress in the body (Ceriello and Motz, 2004). The pro- inflammatory cytokines stimulates the production of free radicals like Reactive oxygen species (ROS) and reactive nitrogen species (NOS) through the activation of protein-kinases signalling in chronically inflamed tissue (Tiwari, 2004; Federico *et al*., 2007). Overaccumulation of Free radicals in the body (oxidative stress) is inextricably connected with inflammation in various physiologic phenomena as well as diseases such as, cardiovascular disease and atherosclerosis (Cottone et al., 2008; Uno and Nichols, 2010), pulmonary disease (Jelic *et al*., 2008), rheumatoid arthritis (Stamp *et al*., 2012), cancers (Reuter *et al*., 2010), metabolic syndrome (Assumpcao *et al*., 2008), obesity and diabetes (Wasser and Weiss, 1999) etc. Diabetes is a group of metabolic diseases characterized by increased blood Glucose level (hyperglycemia) due to defects in insulin secretion. To treat the Type 2 diabetes one therapeutic approach is to decrease the postprandial hyperglycemia. The modern days treatment of diabetes involves several medicines such as biguanides, sulfonylureas, and thiozolidinediones, which may have undesired effects associated with their uses (Fowler, 2007). Therefore natural dietary sources supplemented with antidiabetic properties are getting more importance in the treatment of diabetes as they are free from side effects and less expensive when compared to synthetic hypoglycemic agents. There are several edible macrofungal species which shows the antidiabetic impact by inhibiting the pancreatic alpha-amylase enzyme (Bello *et al*., 2017). The inhibition of this enzyme results into delayed carbohydrate digestion and prolonged overall carbohydrate digestion time, which result into the reduction in glucose absorption rate and consequently the rise in postprandial plasma glucose level will get slowed down (Prasanth *et al*., 2001). Several indigenous medicinal plants and macrofungi have a significant potential to inhibit the á-amylase enzyme activity (Yamac *et al*., 2008). Therefore the *in-vitro* assay of amylase inhibition by the extracts of the two experimental macrofungal species may infer their antidiabetic activities.

Edible Mushrooms or macrofungi are widely used for their high nutritional value, as well as they have been highly

appreciated for their medicinal and therapeutic properties (Karaman *et al*., 2012). Many workers have demonstrated that numerous edible macrofungi have remarkable bioactivities like antioxidant, anti-inflammatory, antiviral, antitumor, anti-diabetic and immunoregulatory effects (Fan *et al*., 2006). *Dacryopinax spathularia* (Schwein) and *Schizophyllum commune* (Fries) are the two potent macrofungi belonging to group Basidiomycota and has been used traditionally for the treatment of various diseases and disorders such as antiviral, antitumour, antibacterial, immunomodulating, anti-inflammatory , anti diabetic, nephroprotective, hepatoprotective activities (Mitko *et al*., 2008; Adebayo *et al*., 2012). Kumar *et al*. (2018) has reported the antioxidant impact of both macrofungal extracts in detail against hydroxyl radical, super oxide anion radical, their free radical scavenging activity and the total antioxidant capacity (TAC), but their use as anti-inflammatory and anti-diabetic agent has not been worked out. Therefore the present study was undertaken for confirmatory evaluation of the mycochemical composition and to estimate the anti-inflammatory activity and amylase-inhibition activity of the two edible macrofungi *Dacryopinax spathularia* and *Schizophyllum commune*.

MATERIALS AND METHODS

Collection of Macrofungi

Fresh fruiting bodies of *D. spathularia* and *S. commune* were collected from different sites of three National Parks (Orang National Park, Kaziranga National Park and Manas National Park) of Assam, which were identified in laboratory of Department of Botany, Gauhati University, Guwahati, Assam and were carried to Department of Zoology, Ranchi University, Ranchi for further analysis.

Extract preparation

The fresh fungi have been washed, disinfected by treating with $HgCl_2$ and then subjected to repeated washing. The fungi have been dried in shade under room temperature for six to seven days, powdered and then sieved. Then the powdered samples have been subjected to extraction by sox let using distilled water for aqueous extract and ethanol for ethanolic extract. The extracts thus obtained, have been filtered, concentrated and dried using rotary flash evaporator maintained at 45ºC for proper dehydration. Percentage yield of each extract has been calculated and the dried extract has been stored in air tight containers at room temperature for further estimation (Dandapat and Sinha, 2015).

Table 1: Qualitative Mycochemical analysis of macrofungal extracts

Compounds	*D. spathularia*	*S. commune*
Carbohydrates	+	+
Glycosides	+	+
Proteins	+	+
Free amino acids	-	-
Alkaloids	+	+
Steroids	+	+
Triterpenoids	+	+
Steroids	+	+
Flavonoids	+	+
Tannins	+	+
Phenolics	+	+
Saponins	+	+

Mycochemical screening

Qualitative mycochemical screening of fungal extracts has been done following the method of Sofowara, (2008) , Dandapat *et al*. (2013) and previously published standards (Harborne, 1984).

Anti-inflammatory assay

The anti-inflammatory activity of the ehanolic extracts of *D. Spathularia* and *S. Commune* has been estimated by measuring the hyaluronidase inhibition activity following Ling *et al*. (2003). 10, 50 and 100 microgram/ml concentrations of both sample extracts were assayed for hyaluronidase inhibition acivity using Indomethacin as reference standard.

Amylase-inhibition assay

The amylase inhibition activity of the ehanolic extracts of *D. Spathularia* and *S. Commune* has been assayed by the chromogenic DNSA (3,5- dinitrosalicylic acid) method (Miller, 1959; Sudha *et al*., 2011). The amylase inhibition activity was estimated by measuring the absorbance of test samples at 540 nm, Acarbose was used as the reference standard.

RESULTS AND DISCUSSION

Mycochemical analysis and Anti-inflammatory activity

The preliminary mycochemical screening confirms the presence of Phenolics, Alkaloids, Flavonoids, Tannins, Saponins and other nutritional components in both the macrofungal extracs. The qualitative analysis of different compounds in the two experimental species of macrofungi is shown in (Table 1).Procida *et al*. (1971) has reported hyaluronidase activity in blood increases during inflammation and decrease inflammation and decrease in inflammation parallels a decrease in hyaluronidase activity. Wang and Marcone (2011) and Badalyan (2012) reported that Edible mushrooms produce a vast diversity of bioactive compounds such as polysaccharides, proteoglucans, phenolic compounds, steroids, and lectins which have a wide range of therapeutic effects and can act as immune-modulatory, anticarcinogenic, antiviral, antioxidant, and anti-inflammatory agents. According to the above results, the present study shows that both the macrofungal extracts have significant hyaluronidase inhibition capacity (Fig. 2) which may be considered as a reference of anti-inflammatory impact of the two experimental macrofungal species.

Amylase-inhibition activity

Result of amylase inhibitory activity has been shown in Fig. 2, which reveals that the D. Spathularia extract shows 38.24% and the S. Commune extract shows 48.19% amylase inhibition activity at 1000 microgram/ml concentration. The retardation of starch digestion by inhibition of enzymes such as a-amylase plays a key role in the control of diabetes. Tarling *et al*. (2008) reported that the inhibitors of pancreatic a-amylase delay carbohydrate digestion causing a reduction in the rate of glucose absorption and lowering the post-prandial serum glucose levels. The present work reveals that the extracts of both edible macrofungi studied have significant amylase-

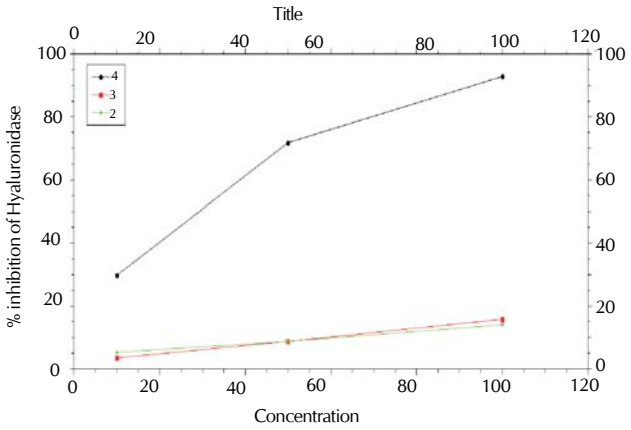

Figure 1: Graph showing % Inhibition of Hyaluronidase by 2 (*D. spathularia*), 3 (*S. commune*) and 4 (Indomethacin)

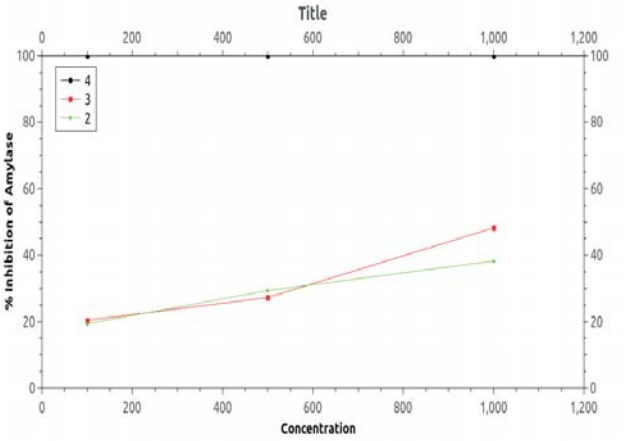

Figure 2: Graph showing % Inhibition of amylase by 2 (*D. spathularia*), 3 (*S. commune*) and 4 (Acarbose)

inhibitory capacities (Fig. 3). Oyedemi *et al*. (2017) has reported that the flavonoid content is responsible for the inhibition of amylase activity in some anti-diabetic medicinal plants . Flavonoids have the ability to form quinone with the 4-oxo-pyrane structure of the amylase enzyme thereby inhibiting its activity (Kim *et al*., 2000). The present work reveals that the two edible macrofungi *Viz*. *D. spathularia* and *S. commune* possess significant flavonoid content which may be responsible for their remarkable amylase inhibitory impact and therefore can be used as alternative medicinal source or dietary constituent for the diabetic patients.

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