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Comparative Study of the Haemagglutination Capabilities of Lectin Extracted from Submerged Cultures of Wild and Mutant Strains of *Schizophyllum commune*

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Abstract

Haemagglutination studies on lectin from both wild (SCW) and mutant strains (SCM1, SCM2, SCM3) of *Schizophyllum commune* using human blood were explored in this study after six days of submerged fermentation. Haemagglutination assay showed that the lectin from all the strains showed slight discrimination in their haemmaglutinating activity against human blood group with strong exhibition of agglutination with blood group O while SCM3 and SCW showed the highest and lowest haemaglutinating activity respectively with a titre score from 4 - 256 HA. Absolute loss of haemagglutination activity was shown by all the four *S. commune* strains tested following exposure to $CuSO_4$ and NH_4SO_4 at 800 mM but optimized by KCl, $MgCl_2$ at 100 mM. Optimal pH for maximal haemagglutination activity was observed at 7.0 for SCW, 6.0 for SCM1 and 8.0 for SCM2 while SCM3 distinctly showed 5.5 and 8.5. Except for SCM3 the thermo stability of haemagglutinating activity was found to improve with the duration of UV irradiation. Inhibitory study in the presence of sugar showed that the partially purified protein from all the strains of *S. commune* in this study was mannose dependent lectin.

Keywords

***Schizophyllum commune*, Haemagglutination, Lectin, Blood Group**

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1. Introduction

Mushrooms have long been appreciated for their flavour and texture; they are also recognized as nutritious food as well as an important source of biologically active compounds of medicinal value [1] [2]. The scientific communities in searching for new therapeutic alternatives have studied many kinds of mushrooms and have found variable therapeutic activities such as anti-carcinogenic, anti-inflammatory, immune-suppressor and antibiotic effects [3] [4]. There are approximately 700 species of higher Basidiomycetes that have been found to possess significant pharmacological activities [5] [6]. Many traditionally used mushrooms from genera, *Auricularia*, *Flammulina*, *Ganoderma*, *Grifola*, *Lentinus*, *Trametes* (*Coriolus*) *and Tremella* have been demonstrated to possess significant medicinal properties [6] [7]. Scientific research in recent years is increasingly confirming the medical efficacy and identifying the biologically active compounds of medicinal mushrooms [8]-[11].

Most edible mushrooms demonstrate medicinal or functional properties when used for a therapeutic intention; the medicinal mushrooms are normally consumed as powdered concentrates or extracts in hot water [12]-[14]. As such these liquid concentrates or dried powdered mushrooms contained in capsules can be considered as dietary supplements (DS) with potential health benefits [15]. Regular intake of these concentrates is believed to enhance the immune response of the human body thereby increasing resistance to disease and, in some cases, causing regression of the disease state [16] [17].

S. commune is an edible mushroom which belongs to the family Schizophyllaceae, the nutritional perspective of the mushroom was also documented in several previous studies [2] [18] [19]. The islanders in Indonesia and Madagascar habitually chew carcophores of this mushroom. *S. commune* was also one of the varieties of mushrooms that were consumed by the Naga tribes in Northern India as food source [20], while the Yoruba people of South Western Nigeria were reported to use *S. commune* to prepare delicious dishes among them [21].

Lectins have drawn increasing attention of some investigators owing to their biological activities including antiproliferative, immunomodulatory, antifungal, antiviral and anti-insect activities [22]. Mushroom lectins have applications in taxonomical, embryological and bacteriological studies, studies of the modification in membrane glycoconjugates and cancer formation, cell sorting, sorting of mutant and tumour cells and isolation of membrane and serum glycoconjugates [23]. Against this backdrop, *Schizophyllum commune* that has long been acknowledged for its medicinal properties is chosen for this present study. It is extremely important because it produces the polysaccharide schizophyllan which shows considerably medicinal properties [7]. However, the present study was carried out to compare the characteristics of lectin from wild type and mutated strains of *S. commune*. These efforts were attempted in a bid to compare the wild type and the mutated strain of *S. commune* as this might have clinical implication in the course of processing *S. commune* for human consumption.

2. Materials and Methods

2.1. Collection of Fungal Strain

S. commune was obtained from deacaying wood of *Mangifera indica*, identified according to the descriptions of Zoberi [24] and Alexopolous *et al*., [2] and authenticated by Professor J. K. Oloke of the Department of Pure and Applied Biology (Microbiology/Biotechnology Unit), Ladoke Akintola, University of Technology, Ogbomoso, Nigeria.

2.2. Sample Preparation and Establishment of Mycelial Cultures

Tissue culture was carried out on fresh carpophores of *S. commune* using the method of Jonathan *et al.*, (25). The mycelial produced were cultured on plates of Potatoe Dextrose Agar (PDA).

2.3. Induction of Mutants of *S. commune*

Mutants of *S. commune* were generated according to the Peak's method as modified by Jonathan *et al.*, (25). Freshly grown plates of wild strain of *S. commune* was exposed to ultraviolet (UV) radiation at 260 nm over a

period of 90 min at 30 min intervals. Each of the mutant obtained at 30, 60 and 90 min were labeled as SCM1, SCM2, SCM3 respectively, while the wild type was tagged SCW.

2.4. Media Formulation and Inoculation

The modified basal medium composition used was according to Ng *et al.*, [22] as follows: *Hibiscus sabdariffa* solution (100 ml), Glucose (6 g), Malt extract (1.6 g), Peptone (2 g), Yeast extract (1.2 g), KH_2PO_4 (0.8 g), $MgSO_4 \cdot 7H_2O$ (0.4 g) and Urea (0.4 g), with pH adjusted to 5.8. The medium was inoculated with about 6 mm agar plugs of each of the wild and mutants strains of *S. commune* of a 5-day-old culture. The batch fermentation set-up was carried out at 28˚C for 6 days with adequate aeration and agitation ensured [25].

2.5. Extraction of Lectin from Submerge Cultures of *Schizophyllum commune*

The broth culture was centrifuged at 1500 rpm for 5 min; pellets were discarded and supernatant collected. Lectin was extracted from the supernatant by 60% ammonium sulphate precipitation performed under low temperature laboratory conditions and then kept overnight. The precipitated solution was centrifuged at 1500 rpm for 5 min; the pellet was dissolved in same buffer solution.

2.6. Assay for Lectin Activity

Agglutination of red blood cells by the crude extract and the various fractions obtained during purification were estimated as described by Bing *et al.*, [26]. A serial two fold dilution of the lectin solution was mixed with 50 µl of a 4% suspension of human erythrocytes in phosphate buffered saline, pH 7.2 at room temperature (the erythrocytes of human blood group A, B, O and AB were fixed with 3% formaldehyde). The plate was left undisturbed for 1 hour at room temperature in order to allow for agglutination of the erythrocytes to take place. The hemagglutination titre of the lectin expressed as the reciprocal of the highest dilution exhibiting visible agglutination unit. The specific activity was the number of hemagglutination per units per mg protein [27].

2.7. Treatment of Blood Samples

Blood samples of known blood groups O, A, B and AB were collected from Olabisi Onabanjo Teaching Hospital, Sagamu. 5 ml of each sample was centrifuged at 1500 × g for 5 min at room temperature. The red blood cells obtained were then washed by centrifugation at 1500 × g for 5 min at room temperature with 0.01 M phosphate buffered saline at pH 7.2. This was repeated twice, 5% hematocrite was mixed with 3% formaldehyde in an EDTA bottle and allowed to stir overnight. The mixture was centrifuged the second time at 1500 × g for 5 min, the red blood cells were then washed again three times with 0.01 M phosphate buffered saline at pH 7.2, after which the cells were collected in stopper bottles and 76 ml of phosphate buffered saline was added to make 4%. Thereafter, it was stored in the refrigerator.

2.8. Hemagglutination Inhibition by Various Carbohydrates

The hemagglutinating inhibition test to investigate inhibition of lectin induced hemagglutination by various carbohydrates were performed in a manner analogous to the hemagglutination test as described by Kuku *et al.*, [28]. Two fold dilutions of sugar samples were prepared in phosphate buffered saline. All the dilutions were mixed with an equal volume (50 µl) of the lectin solution of known hemagglutination units. The mixture was allowed to stand for 1 hour at room temperature and then mixed with 50 µl of a 4% human erythrocyte suspension. The hemagglutination titres obtained were compared with non sugar containing blank. In this study, the sugars used were glucose, galactose, maltose, fructose, sucrose, lactose, raffinose, trehalose and sialic acid. The minimum concentration of the sugar in the final reaction mixture which completely inhibited hemagglutination units of the lectin samples were determined [18].

2.9. Effects of Salts on Hemagglutinating Activity

The hemagglutinating inhibition test to investigate inhibition of lectin induced hemagglutination by various salts were performed in a manner analogous to the hemagglutination test as described by Kuku *et al.*, [28]. Two fold

dilutions of salt samples were prepared in phosphate buffered saline. All the dilutions were mixed with an equal volume (50 µl) of the lectin solution of known hemagglutination units. The mixture was allowed to stand for 1 hour at room temperature and then mixed with 50 µl of a 4% human erythrocyte suspension. The hemagglutination titres obtained were compared with non salt containing blank. The salts used include: Calcium chloride, Iron (III) sulphate, Sodium sulphate, Copper sulphate, Sodium chloride, Iron (III) chloride, Potassium chloride, Magnessium chloride and Potassium di hydrogen phosphate. The minimum concentration of the salt in the final reaction mixture which completely inhibited hemagglutination units of the lectin samples were obtained [27].

2.10. Effects of Temperature on Hemagglutinating Activity

The effect of temperature on the agglutinating activity of the lectin from *S. commune* was determined by carrying out assay at different temperatures according to the method described by Wang *et al*., [18]. The purified lectin was incubated in a water bath for 30 mins at various temperatures: −10, −4, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100˚C, and then cooled to 20˚C. Hemagglutination assay was carried out as previously described.

2.11. Effects of pH on Hemagglutinating Activity

The effect of pH on the activity of the lectin from submerge culture of *S. commune* was determined by incubating the lectin in the following buffers at different pH values of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12. This was done by alternating the pH of 0.15 M NaCl and 0.01 M $NaPO_4$ buffer using concentrated HCL and 1 M NaOH and assaying for hemagglutinating activity. The control values were the agglutination titre of the lectin in phosphate buffered saline, pH 7.2.

3. Results

Table 1 represents the haemagglutination titre of lectin fraction of *S. commune* at which human erythrocyte agglutination occurs. The agglutination efficiencies of the various blood groups ranges from 64 - 1024. However, blood group O showed more agglutination even at a very low concentration of lectin fraction as compared to other blood groups. Thus, blood group O was selected for further study as it exhibited strong agglutination reaction. **Table 2** further indicates the score of haemagglutinating activity elicited by *S. commune* lectin fraction according to haemagglutination titre range. The carbohydrate binding specificity of all the strains of lectin (SCW,

Table 1. Haemmagglutinating activity against different human erythrocyte of *S. commune* lectin fraction.

Blood Group	HA TITRE			
	SCW	SCM1	SCM2	SCM3
O	128	512	512	1024
A	64	128	256	512
B	64	128	128	256
AB	64	256	128	512

Data are haemagglutinating titre (HA). This is defined as the maximum dilution of lectin fraction of *S. commune* at which human erythrocyte agglutination occurs.

Table 2. Scoring of haemagglutination activity elicited by *S. commune* lectin fraction according to haemagglutination titre ranges.

Haemagglutination Score	HA Titre Range
+	4 - 96
++	96 - 256
+++	>256
-	<4

SCM1, SCM2 & SCM3) was determined. Haemagglutinating activity was inhibited by all the sugars tested at specific concentrations. At concentration of 25 mM - 800 mM, inhibitory activity of all the strains of lectin was enhanced by Glucose, Sucrose, Lactose and Maltose. It was also observed that Mannose and Glucosamine inhibited haemagglutinating activity for all the strains of lectin tested at all levels of concentration. However, haemagglutinating activity of SCM3 was strongly enhanced by a wide range of all the sugars tested at all concentration while SCW displayed weak haemagglutinating activity for all the sugars.

In **Table 3**, haemagglutinating activity on 50 µl of erythrocyte was inhibited by Glucosamine and Mannose at 25 mM - 800 mM, Trehalose and Xylose slightly inhibited haemagglutinating activity at 100 mM - 800 mM. At higher concentration of 50 mM - 800 mM, Sialic acid and Fructose inhibited haemagglutinating activity, Raffinose inhibited haemagglutination at 50 mM - 200 mM concentration range and Glucose was found to inhibit at a very high concentration of 800 mM. Lactose, Maltose and Sucrose were found not to be inhibitory to SCW haemagglutination at all concentrations tested.

Table 4 shows haemagglutinating activity of SCM1 with slightly different reactions from that of SCW. At all concentration of 25 mM - 800 mM, Glucosamine, Mannose, Trehalose and Sialic acid inhibited haemagglutinating activity. Xylose and Galactose slightly inhibited haemagglutinating activity at 100 mM - 800 mM and Raffinose and Fructose at 200 mM - 800 mM concentration range. Glucose, Sucrose, Lactose and Maltose exhibited no inhibition for haemagglutination activity at 25 mM - 800 mM.

As shown in **Table 5** and **Table 6** Glucose, Sucrose, Maltose and Lactose showed haemagglutinating activity of SCM2 and SCM3 lectin fractions at a low concentration. However Galactose and Fructose exhibited inhibition for haemagglutination at high concentration of 400 mM & 800 mM. In **Table 5**, Raffinose inhibited haemagglutinating activity at 50 mM - 200 mM concentration range while Xylose exhibited haemagglutinating activity at a very high concentration of 200 mM - 800 mM. Glucosamine, Mannose and Sialic acid inhibited haemagglutinating activity at 50 mM - 800 mM. However, SCM3 as shown in **Table 6**, at concentration of 50 mM - 800 mM, Mannose inhibited haemagglutinating activity and Trehalose at 100 mM - 800 mM inhibited inhibitory activity. And at a higher concentration of 200 mM - 800 mM, sialic acid also inhibited haemagglutinating activity of SCM3.

Haemmaglutinating activity of SCW was inhibited by glucosamine and mannose at 25 - 800 mM, sialic acid

Table 3. Effects of varying concentrations of sugars on haemaglutinating activity of non-UV irradiated *Schizophyllum commune* (SCW) lectin fraction.

Sugars	800	400	200	100	50	25	mmol/L in PBS
Maltose	+	+	+	+	+	++	+
Lactose	+	+	+	+	+	++	+
Sucrose	+	+	+	+	+	+	+
Glucose	-	+	++	+++	+	++	+
Galactose	+++	+++	+	+	+	+	+
Fructose	-	-	-	-	-	+	+
Raffinose	+	++	-	-	-	+	+
Xylose	-	-	-	-	+	+	+
Glucosamine	-	-	-	-	-	-	+
Mannose	-	-	-	-	-	-	+
Trehalose	-	-	-	-	+	+	+
Sialic acid	-	-	-	-	-	+	+

-: No hammaglutination; +: Low hammaglutination; ++: Moderate haemagglutination; +++: High haemmaglutination.

Table 4. Effects of varying concentrations of sugars on haemaglutinating activity of UV irradiated *Schizophyllum commune* (SCM1) lectin fraction.

Sugars	800	400	200	100	50	25	mmol/L in PBS
Maltose	++	+	+	+	+	+	++
Lactose	+	+	+	+	+	+	++
Sucrose	+	+	+	+	+	+	++
Glucose	+	+	+	+	+	+	++
Galactose	-	-	-	-	++	+++	++
Fructose	-	-	-	+	+	+	++
Raffinose	-	-	-	+	++	++	++
Xylose	-	-	-	-	+	+	++
Glucosamine	-	-	-	-	-	-	++
Mannose	-	-	-	-	-	-	++
Trehalose	-	-	-	-	-	-	++
Sialic acid	-	-	-	-	-	-	++

-: No hammaglutination; +: Low hammaglutination; ++: Moderate haemagglutination; +++: High haemmaglutination.

Table 5. Effects of varying concentrations of sugars on haemaglutinating activity of ultra violet light irradiated *Schizophyllum commune* (SCM2) lectin fraction.

Sugars	800	400	200	100	50	25	mmol/L in PBS
Maltose	+	+	+	+	++	++	++
Lactose	+	+	+	+	++	++	++
Sucrose	+	+	+	+	+	++	++
Glucose	+	+	+	++	++	++	++
Galactose	-	-	+	++	++	+	++
Fructose	-	-	+	+	+	++	++
Raffinose	+	++	-	-	-	++	++
Xylose	-	-	-	+	+	++	++
Glucosamine	-	-	-	-	-	++	++
Mannose	-	-	-	-	-	++	++
Trehalose	-	-	-	-	+	+	++
Sialic acid	-	-	-	-	-	+	++

-: No hammaglutination; +: Low hammaglutination; ++: Moderate haemagglutination; +++: High haemmaglutination.

Table 6. Effects of varying concentrations of sugars on haemaglutinating activity of ultra violet light irradiated *Schizophyllum commune* (SCM3) lectin fraction.

Sugars	800	400	200	100	50	25	mmol/L in PBS
Maltose	++	+	+	+++	+++	++	+++
Lactose	+	+	+	+++	+	++	+++
Sucrose	-	+	+	+	+++	+++	+++
Glucose	++	++	++	+++	++	+	+++
Galactose	-	-	+	+	++	+++	+++
Fructose	-	-	+	+	+	++	+++
Raffinose	+	++	+++	+++	+	+	+++
Xylose	-	++	++	+	+	-	+++
Glucosamine	-	-	-	-	+	+	+++
Mannose	-	-	-	-	-	+	+++
Trehalose	-	-	-	-	+	++	+++
Sialic acid	-	-	-	+	+	++	+++

-: No hammaglutination; +: Low hammaglutination; ++: Moderate haemagglutination; +++: High haemmaglutination.

and fructose at 50 - 800 mM, trehalose at 100 - 800 mM, raffinose at 50 - 200 mM concentration range and glucose at 800 mM. Lactose, maltose and sucrose were found not to be inhibitory to SCW haemagglutination at the concentrations tested. Haemmaglutinating activity of SCM1 was inhibited by glucosamine, sialic acid, trehalose and mannose at 25 - 800 mM, xylose and galactose at 100 - 800 mM and raffinose at 200 - 800 mM concentration range. Lactose, maltose and sucrose were found not to be inhibitory to SCM1 haemagglutination at the concentrations tested. Haemmaglutinating activity of SCM2 was inhibited by glucosamine, mannose and sialic acid, trehalose at 100 - 800 mM, xylose at 200 - 800 mM, galactose at 400 - 800 mM and raffinose at 200 - 800 mM concentration range. Lactose, maltose, glucose and sucrose were found not to be inhibitory to SCM2 haemagglutination at the concentrations tested.

Tables 7-10 shows haemagglutinating activity on 50 µl of human erythrocyte at different concentrations of 25 mM - 800 mM of different salts. Haemagglutinating activity was enhanced by all the salts at varying concentration of the salt samples. Inhibition of haemagglutinating activity was observed at very high concentration of lectin fraction at 200 mM - 800 mM. $CaCl_2$, $FeSO_4$, $FeCl_3$, $MgCL_2$, NaCl, KCl, KH_2PO_4 and NaS_2O_8 were found not to be inhibitory to haemagglutinating activity at all the concentrations tested. $FeCl_3$ & $FeSO_4$ slightly inhibited haemagglutinating activity throughout the course of the analysis.

$CuSO_4$ exhibited inhibitory haemagglutinating activity at 100 mM, 200 mM, 400 mM and 800 mM for all the strains of lectin fraction and at 25 mM and 50 mM, haemagglutinating activity was slightly inhibited by $CuSO_4$. **Table 7**, **Table 8** and **Table 9** shows that NH_4SO_4 exhibited inhibitory effect at very high concentration of 400 mM & 800 mM and at lower concentration of 25 mM, 50 mM, NH_4SO_4, $MgCl_2$ and $NaHCO_3$ slightly inhibited haemagglutinating activity.

In **Table 10**, haemagglutinating activity of SCM3 was inhibited by $CuSO_4$ at 100 mM - 800 mM, $NaHCO_3$ at 200 mM - 800 mM concentration range, NH_4SO_4 inhibited inhibitory activity at 800 mM. However, it was observed that all other salts enhanced haemagglutinating activity but $CaCl_2$, $FeSO_4$, $FeCl_3$ slightly inhibited haemagglutinating activity at all the concentration range. **Table 9** shows haemagglutinating activity of SCM2 inhibited by Na_2SO_4 and KH_2PO_4 at 800 mM, with slight haemagglutination occurring at 25 mM - 800 mM by $FeSO_4$, $FeCl_3$. All other salts were found not to inhibit haemagglutinating activity of SCM2 at the concentrations tested.

Table 7. Effects of varying concentrations of salts on haemaglutinating activity of non-ultra violet light irradiated *Schizophyllum commune* (SCW) lectin fraction.

Salt	800	400	200	100	50	25	mmol/L in PBS
$CaCl_2$	+	+	++	+++	+++	+++	+
$FeSO_4$	+	+	+	+	+	+	+
$FeCl_3$	-	+	+	+	+	+	+
Na_2SO_4	-	+	++	+++	+	+	+
$CuSO_4$	-	-	-	-	+	+	+
$MgCl_2$	+++	+++	+++	+	+	+	+
NaCl	+	++	+++	+++	+++	+++	+
KCl	+++	++	+	+	+	+	+
$NaHCO_3$	-	-	-	+	+	+	+
NH_4SO_4	-	-	+	+	+	+	+
KH_2PO_4	+	+	+	+	+	+	+
NaS_2O_8	++	++	++	+	+	+	+

-: No hammaglutination; +: Low hammaglutination; ++: Moderate haemagglutination; +++: High haemmaglutination.

Table 8. Effects of varying concentrations of salts on haemaglutinating activity of ultra violet light irradiated *Schizophyllum commune* (SCM1) lectin fraction.

Salt	800	400	200	100	50	25	mmol/L in PBS
$CaCl_2$	+	+	+	++	+++	++	++
$FeSO_4$	+	+	+	+	+	++	++
$FeCl_3$	+	+	+	+	+	++	++
Na_2SO_4	-	+	++	+++	+	++	++
$CuSO_4$	-	-	-	-	+	++	++
$MgCl_2$	+++	+++	+++	+	+	+	++
NaCl	+	+	++	+++	+++	+++	++
KCl	+++	++	+	+	+	+	++
$NaHCO_3$	-	-	+	+	+	+	++
NH_4SO_4	-	+	+	+	+	+	++
KH_2PO_4	+	+	+	+	+	+	++
NaS_42O_4	++	++	++	+	+	+	++

-: No hammaglutination; +: Low hammaglutination; ++: Moderate haemagglutination; +++: High haemmaglutination.

Table 9. Effects of varying concentrations of salts on haemaglutinating activity of ultra violet light irradiated *Schizophyllum commune* (SCM2) lectin fraction.

Salt	800	400	200	100	50	25	mmol/L in PBS
$CaCl_2$	+	+	+	++	+++	+++	++
$FeSO_4$	+	+	+	+	+	+	++
$FeCl_3$	+	+	+	+	+	+	++
Na_2SO_4	-	+	++	+++	+	+	++
$CuSO_4$	-	-	-	-	+	+	++
$MgCl_2$	+++	+++	+++	+	+	+	++
NaCl	+	+	+	+++	+++	+++	++
KCl	+	+	++	+	+	+	++
$NaHCO_3$	-	-	+	++	++	++	++
NH_4SO_4	-	+	+	++	++	++	++
KH_2PO_4	+	+	+	++	++	++	++
NaS_22O_8	++	++	++	+	+	+	++

-: No hammaglutination; +: Low hammaglutination; ++: Moderate haemagglutination; +++: High haemmaglutination.

Table 10. Effects of varying concentrations of salts on haemaglutinating activity of utltra violet light irradiated *Schizophyllum commune* (SCM3) lectin fraction.

Salt	800	400	200	100	50	25	mmol/L in PBS
$CaCl_2$	+	+	+	++	+++	+++	+++
$FeSO_4$	+	+	+	+	+	+	+++
$FeCl_3$	+	+	+	+	+	+	+++
Na_2SO_4	+	+	++	+++	+	+	+++
$CuSO_4$	-	-	-	-	+	++	+++
$MgCl_2$	+++	+++	+++	+++	++	+	+++
NaCl	+	+	++	+++	+++	+++	+++
KCl	+	+	++	+++	+++	+++	+++
$NaHCO_3$	-	-	-	+	+++	+++	+++
NH_4SO_4	-	+	++	+++	++	+	+++
KH_2PO_4	+	++	++	+++	+++	+++	+++
NaS_2O_8	++	++	++	++	++	+++	+++

-: no hammaglutination; +: low hammaglutination; ++: moderate haemagglutination; +++: high haemmaglutination.

Slight haemagglutination activity of SCM1 was exhibited by $CaCl_2$, $FeSO_4$, $FeCl_3$, $NaHCO_3$, NH_4SO_4 and KH_2PO_4 at 25 mM - 800 mM concentration range. At 400 mM and 800 mM, $NaHCO_3$ inhibited haemagglutinating activity, NH_4SO_4 & Na_2SO_4 inhibited haemagglutinating activity at 800 mM. Other salts were found to enhance haemagglutinating activity of SCM1. Haemmaglutinating activity of SCM3 was inhibited by glucosamine, mannose and trehalose at 100 - 800 mM, sialic acid at 200 - 800 mM, galactose at 400 - 800 mM and xylose at 800 mM. Lactose, maltose, glucose, raffinose and sucrose were found not to be inhibitory to SCM3 haemagglutination at the concentrations tested. Haemmaglutinating activity of SCM2 wild was inhibited by $CuSO_4$ at 100 - 800 mM, Na-citrate at 400 - 800 mM and Na_2SO_4 at 800 mM. Other salts were found not be inhibitory to SCM2 haemagglutination activity at the concentrations tested. Haemmaglutinating activity of SCM1 was inhibited by $CuSO_4$ at 100 - 800 mM, Na-citrate at 400 - 800 mM and Na_2SO_4 as well as NH_4SO_4 at 800 mM each. Other salts were found not be inhibitory to SCM1 haemagglutination activity at the concentrations tested. Haemmaglutinating activity of SCM3 wild was inhibited by $CuSO_4$ at 100 - 800 mM, Na-citrate at 200 - 800 mM and NH_4SO_4 at 800 mM. Other salts were found not be inhibitory to SCM3 haemagglutination activity at the concentrations tested.

The effects of various pH on lectin activity of SCW, SCM1, SCM2 & SCM3 were evaluated in **Figures 1-4**. By incubating the purified lectin at pH below 4, haemagglutinating activity tends towards zero while haemagglutinating activity was observed at pH $\geq$ 5 to be at 90 HU/mg. As the pH concentration increased, Haemagglutinating activity was observed at 100 HU/mg to 200 HU/mg. However, at pH greater than 7, sharp decrease in haemagglutinating activity was observed from 200 HU/mg to zero at pH 12.

Figure 1 shows haemagglutinating activity of SCW to be stable between pH 6 and 7 with maximum haemagglutinating activity recorded at 180 HU/mg and 200 HU/mg. There was sharp decrease in haemagglutinating activity at slightly higher pH than 7 and at pH 12; there was complete loss of haemagglutinating activity. Similar activity was observed for SCM1in **Figure 2**, but complete loss of haemagglutination was observed at pH 11 and 12.

In **Figure 3**, haemagglutinating activity of SCM2 incraesed as pH value increased from 4 to 6 and thereafter wasobserved to remain stable at pH between 6, 7 and 8. At higher pH value of 9 and above, haemagglutinating

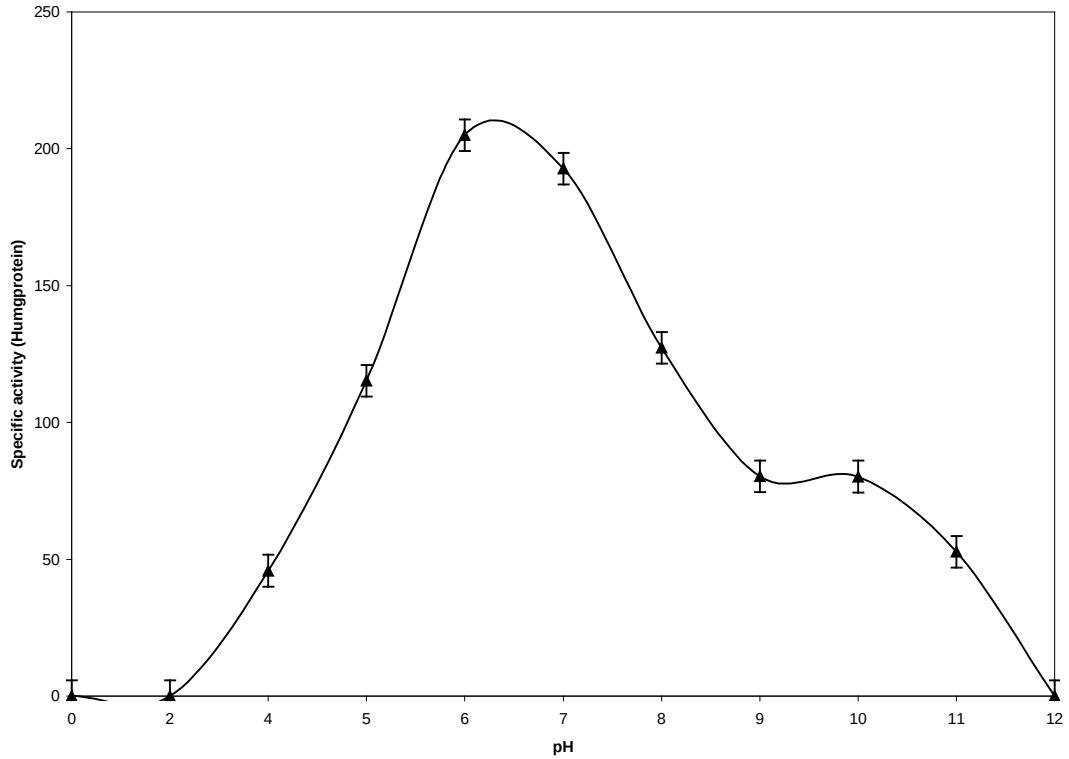

Figure 1. Effect of pH on the haemagglutinating activity of the non-UV irradiated *Schizophyllum commune* lectin extract. Each data point represents mean (SEM) of 3 determinations.

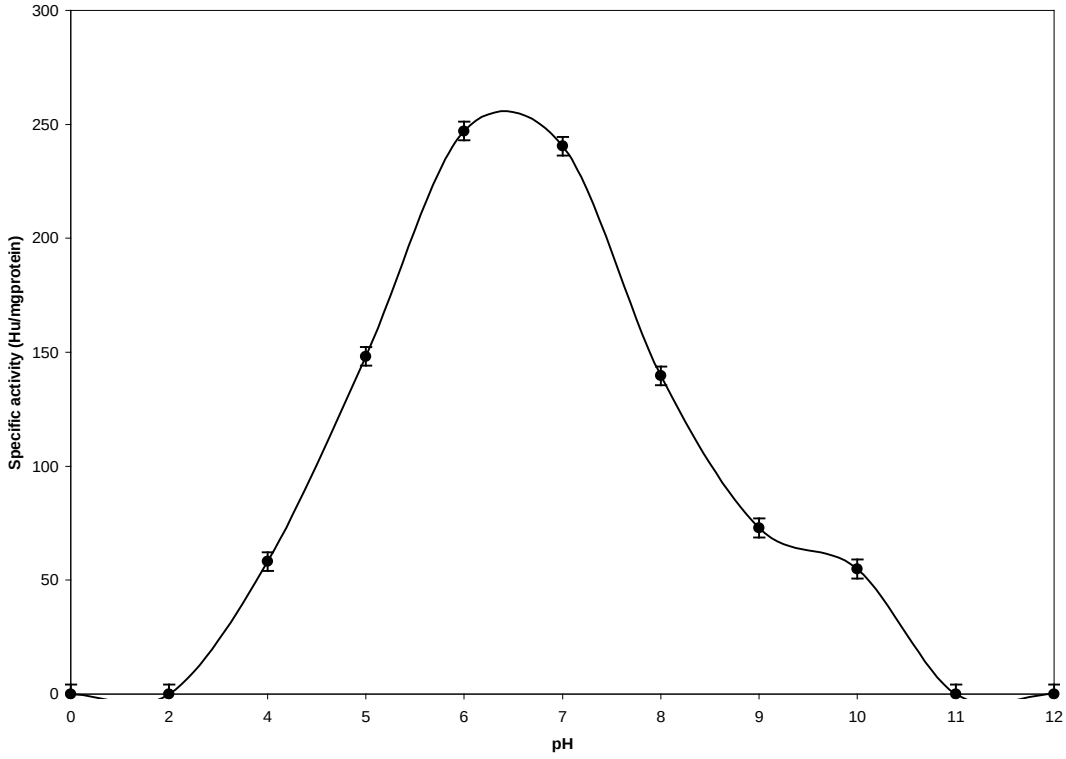

Figure 2. Effect of pH on haemagglutinating activity of *Schizophyllum commune* lectin extract irradiated with UV for 30 min. Each data point represents mean (SEM) of three determinations.

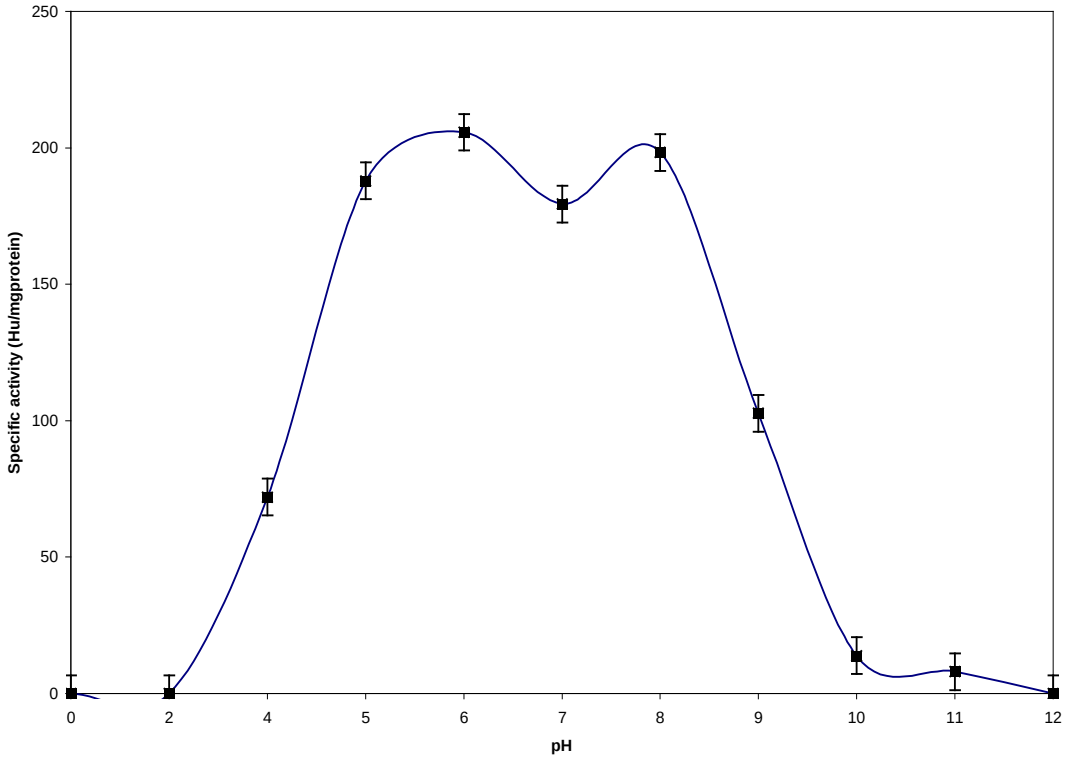

Figure 3. Effect of pH on haemagglutinating activity of *Schizophyllum commune* lectin extract irradiated with UV for 60 min. Each data point represents mean (SEM) of three determinations.

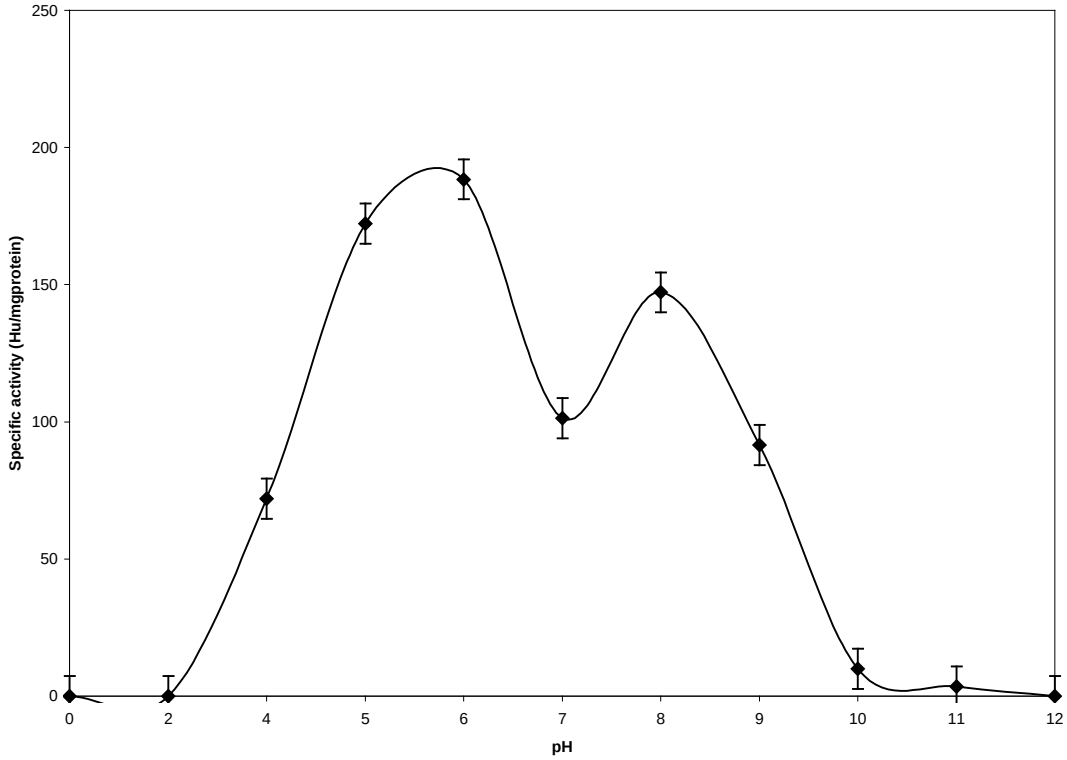

Figure 4. Effect of pH on haemagglutinating activity of *Schizophyllum commune* lectin extract irradiated with UV for 90 min. Each data point represents mean (SEM) of three determinations.

activity gradually decreased from 200 HU/mg to zero and a complete loss of haemagglutinating activity was observed at pH 12.

Haemagglutinating activity of SCM3 as shown in **Figure 4** shows a steady decrease of haemagglutination between pH of 6 - 7 from 200 HU/mg to 100 Hu/mg and between pH 8 to pH 10 from 145 HU/mg to zero and at pH of 12, there was complete loss of haemagglutinating activity. **Figure 5** to **Figure 8** shows the activity of all the strains of lectin tested at various temperature. As shown in **Figure 5**, the protein is in latent form at a temperature of 10˚C. As temperature increased, haemagglutinating activity was observed to increase from 70 HU/mg to 200 HU/mg where it remained stable to a temperature of 50˚C. Gradual decrease in the haemagglutinating activity is observed at much higher temperature of 60˚C and above, however, at 90˚C, there was complete loss of haemagglutination. Similar reaction was observed for SCM1 haemagglutinating activity in **Figure 6** with maximum haemagglutination of the protein occurring between 40˚C and 60˚C. There was sharp decrease in haemagglutinating activity at temperature higher than 60˚C from 198 HU/mg to 10 HU/mg with a complete loss at 90˚C and 100˚C.

As shown in **Figure 7** and **Figure 8**, there was gradual increase in haemagglutinating activity as temperature increased from 10 HU/mg to 200 HU/mg and a sharp decrease from the haemagglutinating activity at temperature of 70˚C and above. However, at 100˚C, there was complete loss in the haemagglutinating activity of both SCM2 and SCM3.

4. Discussion

As shown in this study agglutination potential of lectin from all the strains of *Schizophyllum commune* varied with the concentration of inhibitors. As shown in the result virtually all the lectins from the various strains of *Schizophyllum commune* are Mannose binding. This type of lectin is quite different from the recently reported Lectin [29]. As known they have attracted great interest due to their various biological activities, such as antitumor [30] [31]. Though there was no direct study of the lectin to antitumor activities in the course of this research, however, information from this study could be a useful tool. Lectin activity from this study was noticed

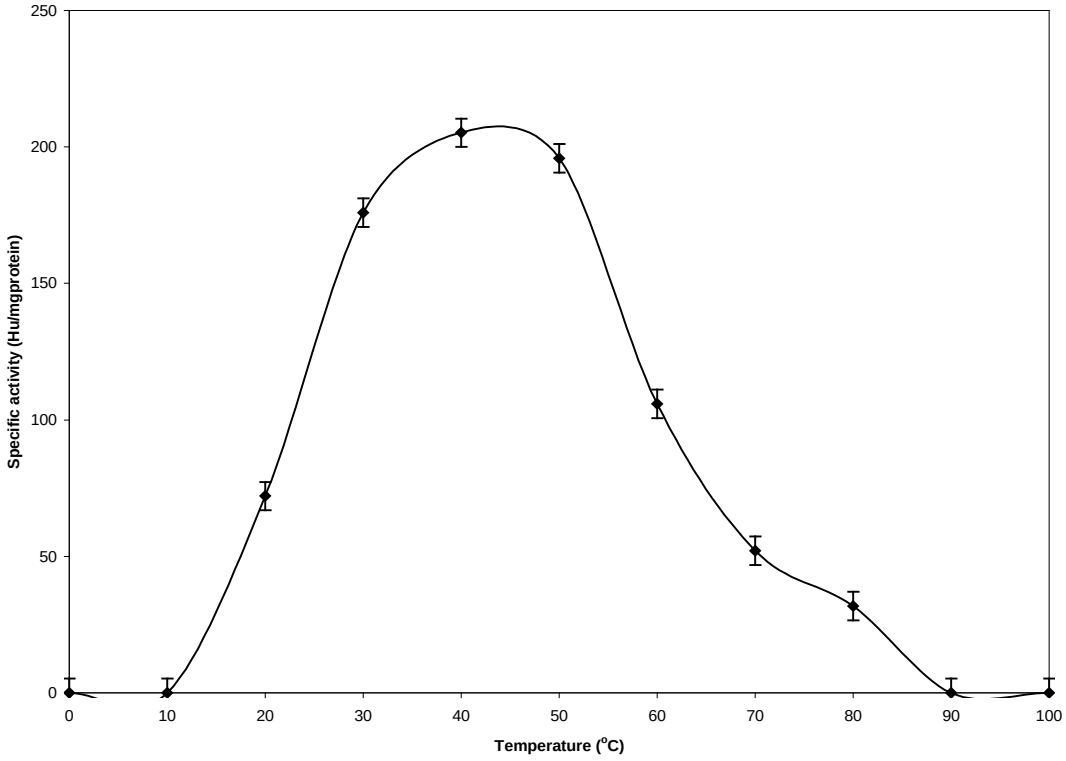

Figure 5. Effect of temperature on the haemagglutinatng activity of non-irradiated *Schizophyllum commune* extract. Each data point represents mean (SEM) of three determinations.

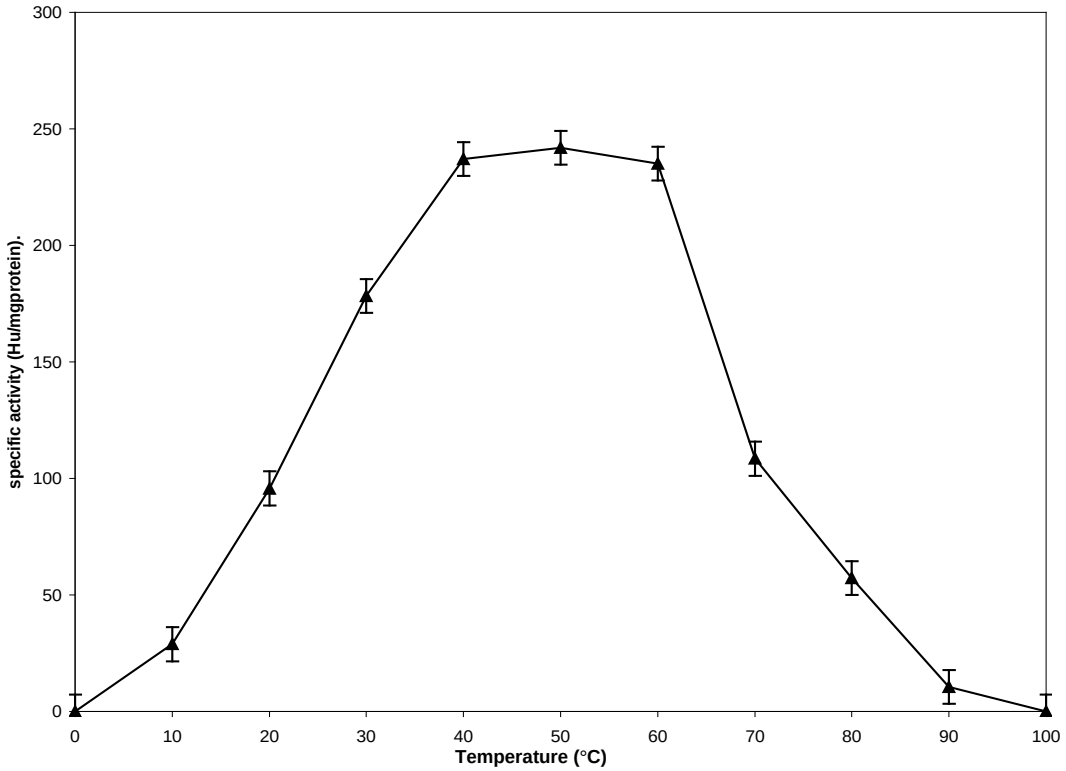

Figure 6. Effect of temperature on the haemagglutinatng activity of UV irradiated *Schizophyllum commune* extract for 30 minutes. Each data point represents mean (SEM) of three determinations.

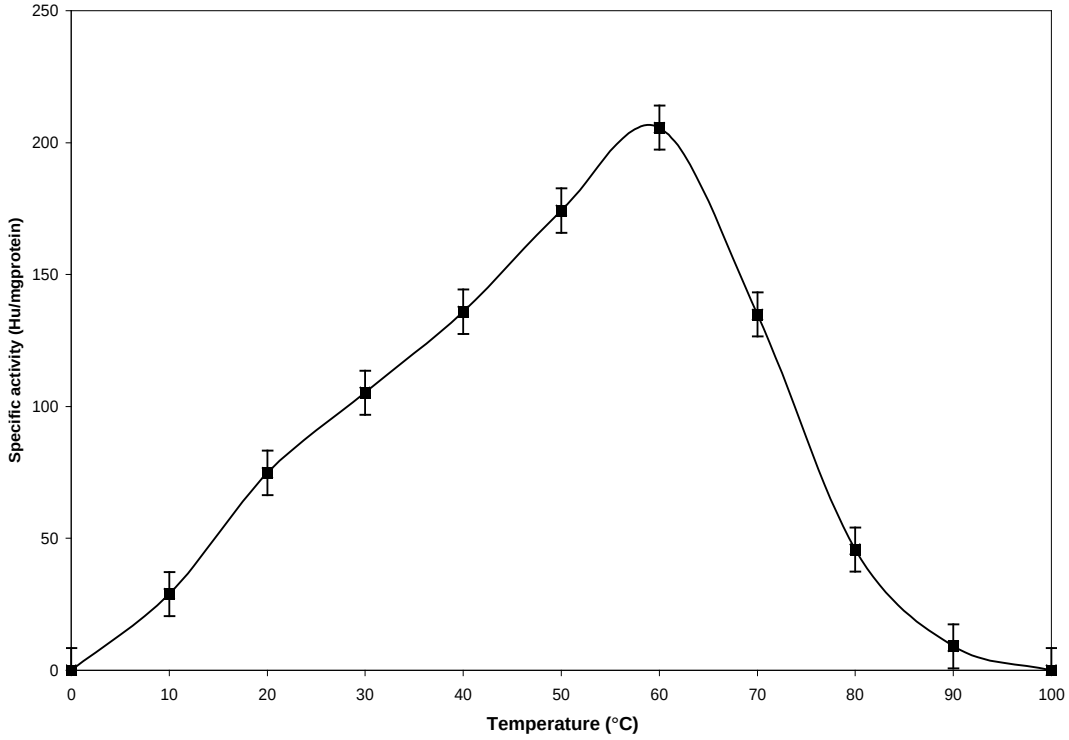

Figure 7. Effect of temperature on the haemagglutinatng activity of UV irradiated *Schizophyllum commune* extract for 60 minutes. Each data point represents mean (SEM) of three determinations.

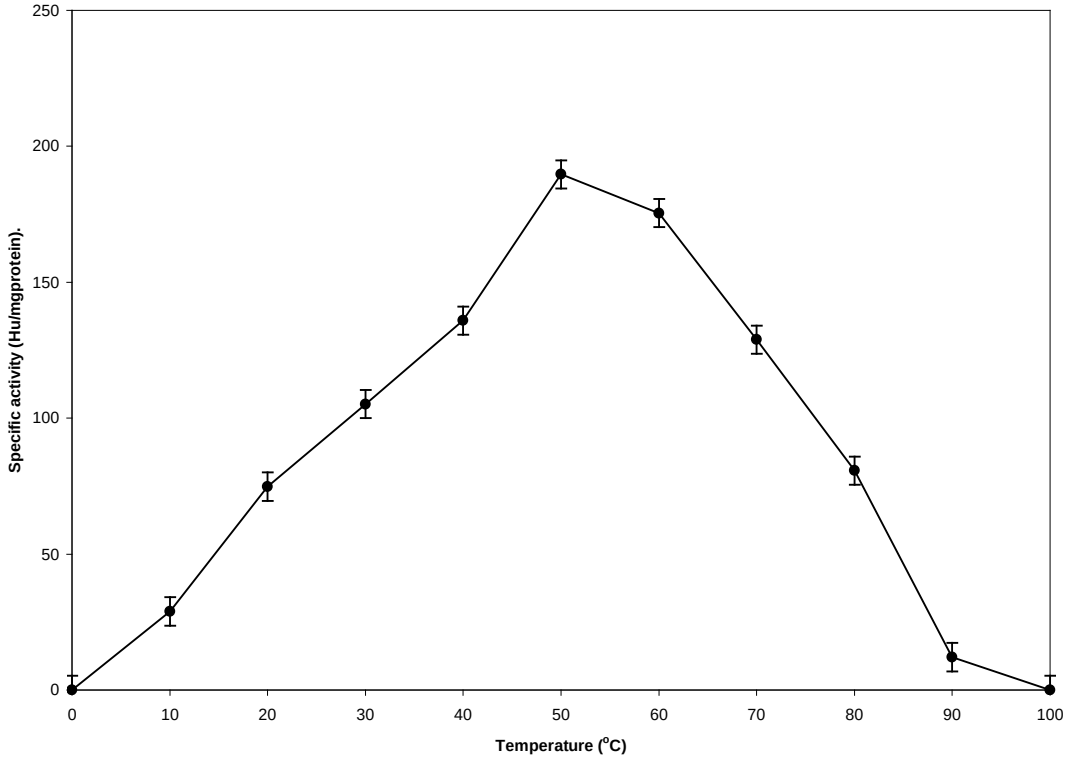

Figure 8. Effect of temperature on haemagglutinating activity of UV irradiated *Schizophyllum commune* extract for 90 minutes.

to have been inhibited by $CuSO_4$ and KH_2PO_4 at all concentrations except from 25 mM and 50 mM. Lectin activity was also inhibited by $CuSO_4$ and KH_2PO_4 at all concentrations. Lectin activity of *Schizophyllum commune* was slightly inhibited by $FeSO_4$ and $FeCl_3$ at all concentrations in contrast to lectin extracted from *Archidendron jiringa* which inhibits lectin activity [32] and *Cissus populnea* which has no effect on lectin activity [33]. Lectin activity was slightly inhibited by $CaCl_2$ at higher concentrations of 200 mM, 400 mM, and 800 mM but did not inhibit the activity of lectin at concentrations of 25 mM, 50 mM, and 100 mM in contrast to lectin extracted from *Manila clam* which is Ca^{2+} depenent [34]. $MgCl_2$ enhanced the lectin activity of *Schizophyllum commune* as the concentration increases. This is similar to the lectin extracted from *Cissus populnea* by Awoyinka and Dada [33].

In this study, hemagglutination activity was inhibited by Glucose, Mannose, Trehalose and Sialic acid even at low concentrations. This is slightly similar to the lectin extracted from *Codiaeum variegatum* [29]. Lectin in this study was found to be heat stable from 20˚C to 30˚C and the heat stability began to decrease at 40˚C and completely lost its activity at 70˚C. In comparison, lectin extracted from *Cissus populnea* was found to be stable from 20˚C to 30˚C [28] [33]. The hemagglutinating activity from the *Pterocladiella capillacea* lectin was affected only by exposure to a temperature at 70˚C [34] [35].

5. Conclusion

In this study hemagglutinating activity of the lectin from both the wild and mutant strains of *Schizophyllum commune* towards human erythrocytes was found to be more selective to blood group O compared to other blood groups. However lectin from *Schizophyllum commune* is exposed to UV irradiation for longer period appeared to possess distinct features compared to lectin from wild and other *Schizophyllum commune* exposed to UV irradiation at a lesser duration. This information may have serious health implication in the consumption of this edible mushroom by human.

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