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ACTIVE COMPOUND, ANTIOXIDANT, ANTIPROLIFERATIVE AND EFFECT ON STZ INDUCED ZEBRAFISH OF VARIOUS CRUDE EXTRACTS FROM *Boletus qriseipurpureus*

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ABSTRACT

Boletus qriseipurpureus (gelam mushroom) is a mushroom used by locals in Tok Bali, Kelantan, Malaysia to treat diabetes, cervical cancer and breast cancer. The active compounds in *B. qriseipurpureus* remain unidentified. Therefore, in this study, we investigated the potential medicinal properties of *B. qriseipurpureus* extracts (hot water, cold water and methanol extracts) by conducting preliminary phytochemical screening, *in vitro* antioxidant, *in vitro* antiproliferative and *in vivo* antidiabetic test. Biochemical assays were performed to detect the presence of alkaloids, anthraquinones, flavonoids, reducing sugars, saponins, steroids and tannins in *B. qriseipurpureus* extracts. 1,1-diphenyl-2-picrylhydrazyl (DPPH) was used to evaluate the antioxidant capacity of *B. qriseipurpureus* extracts, while MTT cell proliferation assay (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) was used to investigate the extracts antiproliferative activity. The effects of *B. qriseipurpureus* extracts on streptozotocin (STZ) induced zebrafish were examined at the concentration of 45 mg/mL, 90 mg/mL and 135 mg/mL. The effect of extracts were measured by the regenerative growth rate of the amputated caudal fin for fourteen days post transection. Screening of the mushroom extracts for active compounds revealed the presence of alkaloids, flavonoids, saponins and tannins in all test extracts. Reducing sugars and anthraquinones are only detected in hot water and cold water of *B. qriseipurpureus* extracts. The half maximal inhibitory concentration (IC_{50}) of DPPH by hot water, cold water and methanol extract of *B. qriseipurpureus* are 1.79 mg/mL, 1.97 mg/mL and 3.98 mg/mL respectively. The MTT assay indicated that all extracts exhibited significant antiproliferative effects on MCF-7 cell line after 72 hours with IC_{50} of 7.7 mg/mL for hot water extract, 8.2 mg/mL for cold water extract and 16.1 mg/mL for methanol extract but do not display any significant cytotoxic effect. The STZ induced zebrafish treated with 135 mg/mL of hot water *B. qriseipurpureus* extract for 14 days showed the highest regeneration growth rate of caudal fin (5.04 ± 0.43%) compared to fish treated with metformin (5.72 ± 0.64 %). In this study, we showed the potential of hot water *B. qriseipurpureus* extracts as a potent therapeutic agent for diabetes and as an alternative natural source of antioxidant.

Key words: *Boletus qriseipurpureus*, active compound, antioxidant, antiproliferative, antidiabetic, zebrafish

INTRODUCTION

In recent years, mushrooms have attracted researches attentions for their functional properties in medical application (Sun *et al.,* 2011). Some mushrooms have functional roles in antinflammatory, antitumor, anticancer, antibacterial, antioxidant, antiviral, antiallergic, hypoglycemic and haematological properties (Barros *et al.,* 2007a; Barros *et al.,* 2007b; Chen *et al.,* 2009; Dore *et al.,* 2007; Faccin *et al.,* 2007; Garcia *et al.,* 2010; Guillamon *et al.,* 2010; Yoshino *et al.,* 2008).

B. qriseipurpureus is a species of wild edible mushroom found along the coast from Bachok, Kelantan to Kuala Terengganu. This mushroom is locally known as "Cendawan (malay word for mushroom) Gelam" as it grows in the sandy area with gelam trees (*Melaleuca cajuput Powell*). It is used by the locals to treat diabetes, cervical cancer

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and breast cancer. Methanol extract of *B. qriseipurpureus* was shown to be rich with natural antioxidant metabolites such as 2,4,6-trimethylacetophenone imine, glutamyl tryptophan, azatadine and lithocholic acid glycine conjugate (Yuswan *et al.,* 2015). We recently conducted a study on *B. qriseipurpureus* extracts (hot water, cold water and methanol) to determine the effect of extracts on diabetes related enzymes (alpha-amylase and alpha-glucosidase) (Muniandy *et al.,* 2015). The study shows that hot water extract possessed the highest inhibition activity of α-amylase and α-glucosidase in a concentration dependent manner with the IC_{50} value 87 mg/mL and 89 mg/mL respectively. The study also suggested that hot water extract of *B. qriseipurpureus* contains bioactive compound that can inhibit α-amylase and α-glucosidase enzyme activity.

Since diabetes has been related to antioxidant, and antioxidant has been correlated with antiproliferation (Fatimah *et al.,* 2012; Wing *et al.,* 2007), here we determined the active compound, antioxidant, antiproliferative and antidiabetic properties of *B. qriseipurpureus* extracts. To investigate the antidiabetic properties of these extracts *in vivo*, tail regeneration assay of streptozotocin (STZ), a diabetogenic drug which damage insulin-producing beta cells of the pancreas (Rossini *et al.,* 1977) induced zebrafish was utilised as described by Robert *et al.* (2013).

MATERIALS AND METHODS

Sample Preparation and Extraction

Wild *B. qriseipurpureus* were forage from the rural area of Tok Bali, Bachok, Kelantan. Extracts of *B. qriseipurpureus* were prepared according to Ahmad *et al.* (2014) with slight modification. All mushrooms used in the extraction were freeze-dried immediately after harvest. For the cold water (28 ± 2ºC) and methanol extraction, 50 g of blend mushroom sample was soaked in 200 mL of cold water and methanol, respectively. The mushrooms were left for 36 hours at room temperature (28 ± 2ºC) with continuous shaking at 200 rpm. For hot water extraction, a total of 50 g of powdered sample was immersed in 200 mL of hot water (95 ± 5ºC) and left for 4 hours at room temperature with continuous shaking at 200 rpm. The hot water and cold water extracts were filtered using filter paper (Whatman No1). The filtrates were then freeze-dried under vacuum *condition* at -50ºC for five days. The methanol was evaporated from the methanol extract using rotary evaporation technique at 50ºC with a speed of 90 rpm until completely dry. All extracts were stored at 4ºC in a clean sterile container for further use.

Mycochemical Screening

Alkaloids

Alkaloids detection was performed according to the method described by Kasolo *et al.* (2010). Extract (0.01 g) was added to 3 drops of 2% hydrochloric acid. 3 drops Dragendorff reagent was added to the solution, precipitating the alkaloids.

Anthraquinones

Anthraquinones detection was performed according to the method described by Ayoola *et al.* (2008). Extract (0.1 g) was added to 2 mL of 10% hydrochloric acid and heated for 2 minutes. The solution was filtered while warm. The liquid filtrate was then added with CH_3Cl_3 in a ratio of 1:1 and shaken. Then, a few drops of 10% ammonia were added. The presence of pink or red colour on the bottom of the test tube indicates the presence of anthraquinones.

Flavonoids

Detection of flavonoids was conducted using Shinoda test as described by Sabri *et al.* (2012). Extract (0.1 g) was added into a solution of 5 mL of distilled water and 1 ml of 10% H_2SO_4. About 0.5 g of magnesium was added into the solution. The solution turns red in presence of flavonoids.

Reducing sugars

Detection of reducing sugars was carried out using Fehling test as described by Sabri *et al.* (2012). Extract (0.1 g) was added into 1 mL of distilled water and 2 mL of mixed reagent Fehling A and B (what is the ratio of Fehling A to B/vol: vol). The mixture was then incubated at 60ºC. Brick red precipitation formed at the bottom of the test tube indicates the presence of reducing sugars.

Saponins

Saponins detection was done according to the method described by Adebayo *et al.* (2012). Extract (0.1 g) was mixed in 5 mL of water, and boiled for 2 minutes. Undissolved extract were left to precipitate at the bottom of the tube. The supernatant was used for the next step of the test. For the foaming test, 1 mL of the supernatant was mixed with 4 mL of distilled water and shaken. The formation of bubbles which last for 15 minutes indicates the presence of saponins. For the emulsion test, 2 drops of olive oil was added to the liquid solution of foaming test and mixed. The emulsion formation indicates the presence of saponins.

Steroids

Steroids detection was conducted using Salkowski test as described by Sharma *et al.* (2013). Extract (0.05 g) was added to 0.5 mL of distilled

water. 2 mL of chloroform was then added to the mixture, and mix by swirling the tube. 2 ml of 10% H_2SO_4 was added slowly to the mixture. Formation of red colour in the chloroform layer and the layer of yellowish green colour indicates the presence of steroids.

Tannins

Tannins detection was performed using ferric chloride test as described by Ayoola *et al.* (2008). Extract (0.05 g) was added to 2 mL of distilled water, boiled, and then filtered. Four drops of 0.1% $FeCl_3$ was then added to the filtrate. The change of colour to blue-black, blue-green or green indicates the presence of tannins.

Antioxidant Assay with DPPH

DPPH assay was performed according to the method proposed by Ahmad *et al.* (2014). Different concentrations of the crude extract (1 mL) in distilled water were added to 4 mL of 0.004% DPPH in methanol solution. The mixtures were incubated at room temperature, in the dark for 30 minutes before reading the absorbance against a blank at 517 nm. Inhibition of free radical by DPPH in percent (I%) was calculated using the following way:

$$\text{Scavenging effect (\%)} = (A_{Blank} - A_{Sample} / A_{Blank}) \times 100$$

Where A_{Blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{Sample} is the absorbance of the test compound. The value of 50% inhibition (IC_{50}) was calculated from the graph plotted inhibition percentage against extract concentrations. Tests were carried out in triplicate.

Antiproliferative Assay

Antiproliferative assay was conducted as described by Shao *et al.* (2013) with slight modification to determine the antiproliferative effects of *B. qriseipurpureus* extracts on African green monkey's kidney epithelial cell line (Vero) and breast cancer cell lines (MCF-7) using the MTT assay.

All cells were grown in RPMI 1640 medium and maintained in 37°C incubator with 5% CO_2. To measure cell viability, 1×10^5 cells/mL of cells were seeded in a 96-well plate and incubated for 24 hours at 37ºC. Cells were then treated with the extracts and incubated for 24, 48 or 72 hours. After incubation, 20 µL MTT solution (4 mg/mL) was added in each well and incubated in 37°C with 5% CO_2 saturation for 4 hours. After the incubation, the medium was removed, and the insoluble formazan product was dissolved in 100 µL of dimethyl sulfoxide. Absorbance was measured at 570 nm using a microplate reader. MTT assay was repeated on 48 hours and 72 hours. Percentage of inhibition was calculated as,

$$\text{Inhibition Percentage (\%)} = 100 - ((A_{570} \text{ of treated cells} / A_{570} \text{ of control cells}) \times 100)$$

The value of 50% inhibition (IC_{50}) was calculated from graph which shows the inhibition percentage of viable cells against extract concentrations. Tests were carried out in triplicate.

Tail regeneration assay

Zebrafish for this study has been obtained from Prof. Alexander Chong Shu-Chien, Universiti Sains Malaysia. *In vivo* activity of various crude extracts from *B. qriseipurpureus* on STZ induced zebrafish was conducted according to Robert *et al.* (2013). All procedures are performed following the guidelines described in "Principles of Laboratory Animal Care" (National Institutes of Health publication no. 85-23, revised 1985) and the approved Rosalind Franklin University Institutional Animal Care and Use Committee animal protocol 08-19.

A total of 105 zebrafish was divided into two main groups which are normal (without STZ treatment; 4 groups of 5 fish each) and induced STZ (STZ treatment; 5 groups of 5 fish each). The STZ induced group were injected with 0.35 mg/g of 0.3% streptozotocin on day 1, 3, 5, 12 and 19. At this point the zebrafish are considered to have been in a prolonged state of hyperglycemia and exhibit the diabetic complications (Robert *et al.,* 2013). 0.35 mg/g of 0.09% sodium chloride was used as a mock treatment. On day 21, the caudal fin of all fish was amputated in a straight line proximal to the first lepidotrichia branching point. Routine examination on the regenerative growth of caudal fin was done at 24 and 72 hour following transection. On day 24 (72 hours after transection), 5 µL test extracts were force feed to the fish for 14 days once daily and caudal fin regenerative growth was checked and recorded on day 1, 3, 5, 7 and 14. Metformin (30 mg/mL) was used as a positive control drug.

Caudal fin regeneration growth rate was calculated as the percentage of differences of fin areas (mm^2) grow over the number of days.

RESULTS AND DISCUSSION

Mycochemical in *B. qriseipurpureus* extracts

The active ingredient screen of the different mushroom extracts methods revealed the presence of alkaloids, flavonoids, saponins and tanins in all available forms (hot water, cold water and methanol

extract). Reducing sugars and anthraquinones however are only present in aqueous extracts (hot water and cold water) as shown in Table 1. The presence of alkaloids, saponins and tannins in extracts is an indication that this species has pharmacological importance (Adebayo *et al.,* 2012). According to Wink *et al.* (1998) alkaloids have medicinal properties such as antihypertensitivity (indole alkaloid), antimalaria (quinine), antiarrhythmia (quinidine) and anticancer (vincristine). Anthraquinones and its derivatives have antitumor properties as it can inhibit cell division, induce apoptosis and inhibit metastases (Huang *et al.,* 2007). In addition, anthraquinones can also act as antifungal agent. According to Yao *et al.* (2004), most flavonoids have been shown to have antioxidant activity, free radical scavenging activity and anticancer activity. Flavanoids also capable of preventing coronary heart disease and some flavonoids demonstrates the ability of antivirus HIV (Human Immunodeficiency Virus). Extensive research has been done and proven that saponins can stimulate the immune system, reduce cholesterol levels, has anticancer, antioxidant, antiviral and antifungal activity and causes hypoglycemia (Francis *et al.,* 2002). In traditional medicine, especially in Japan and China, plant extracts containing tannins has been used to treat diarrhea, abdominal pain and duodenal tumor (Bruyne *et al.,* 1999). Therefore, the present compounds in *B. qriseipurpureus* extracts indicate the medicinal properties of this species that need to be discovered.

Antioxidant properties of *B. qriseipurpureus* extracts

Fig. 2 shows the scavenging effect of DPPH against different concentrations of the extracts from *B. qriseipurpureus*. Hot water extract, cold water extracts and methanol extract each recorded DPPH scavenging effect of 82.28 ± 0.03, 75.55 ± 0.01 and 60.67 ± 0.09 respectively at 10 mg/mL. The DPPH scavenging effect of the control antioxidant compound, ascorbic acid in this experiment is 95.04 ± 0.02%. Our results show that there is a correlation between the concentrations of the extracts with DPPH scavenging effect. Value of DPPH scavenging effect increased in parallel with

Table 1. Active compounds of the crude extracts from *B. qriseipurpureus*

Phytochemicals	AL	AN	FL	RS	SA	ST	TA
Hot Water Extract	+	+	+	+	+	–	+
Cold Water Extract	+	+	+	+	+	–	+
Methanol Extract	+	–	+	–	+	–	+

+, present; –, absent.
AL= Alkaloids, AN= Anthraquinones, FL= Flavanoids, RS= Reducing Sugars, SA= Saponins, ST= Steroids, TA= Tannins

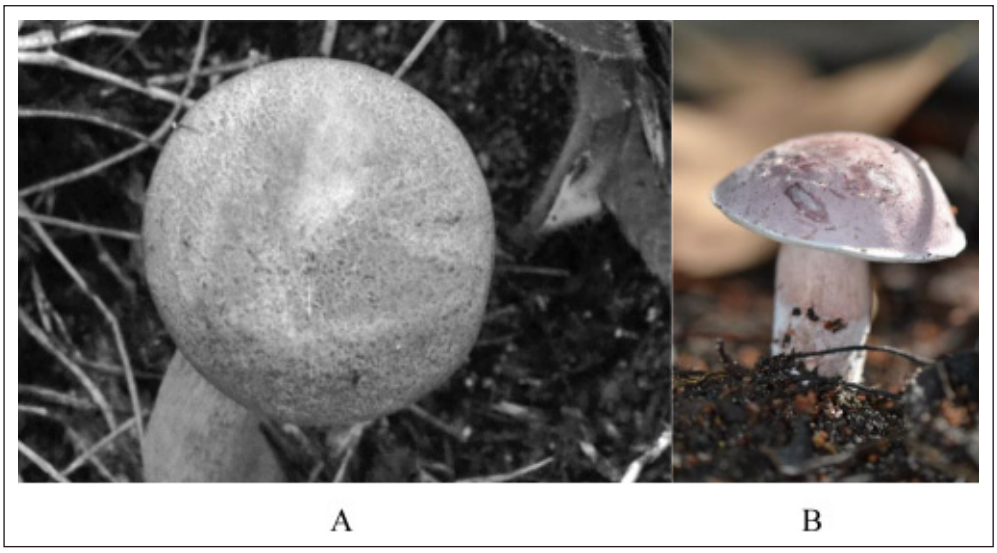

Fig. 1. *Boletus qriseipurpureus.*
A: Top View. B: Side View.

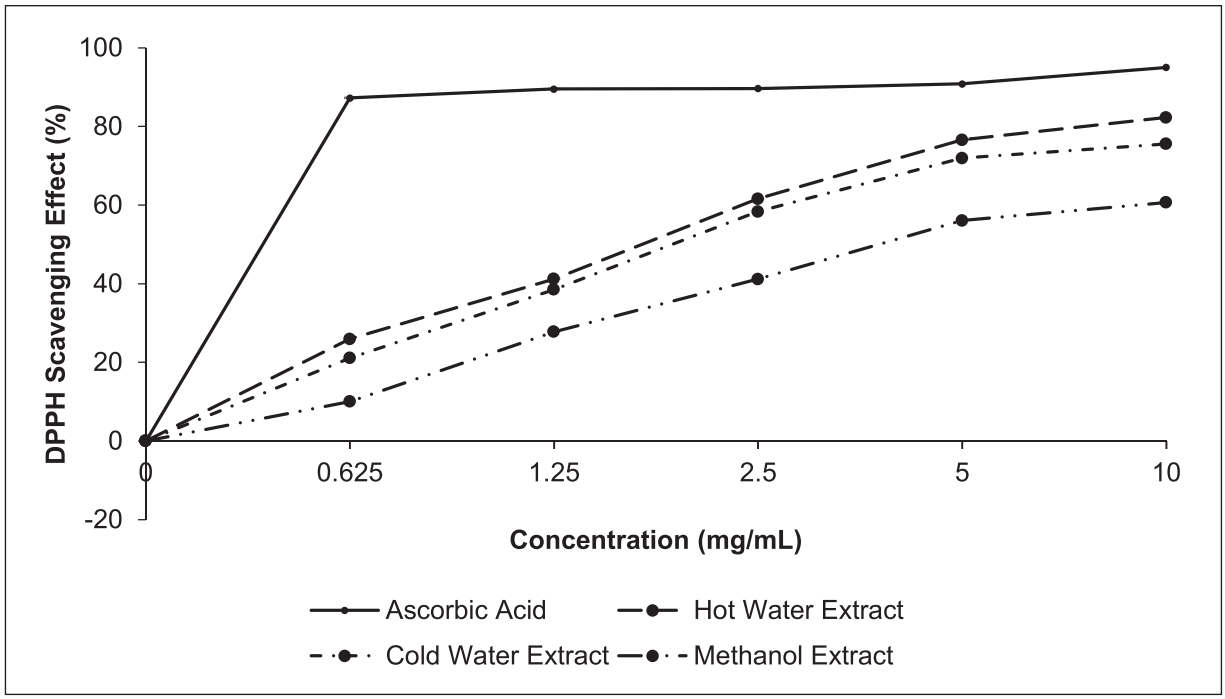

Fig. 2. DPPH scavenging effect of the crude extracts from *B. qriseipurpureus.*

increasing extract concentration, suggesting that all of the extracts in this study have antioxidant activity.

IC_{50} is the minimum concentration of substrate required to inhibit 50% of DPPH free radicals. By determining the IC_{50} value, a sample can be said to have either high or low antioxidant activity. The concentration of the extracts from *B. qriseipurpureus* required to inhibit 50% of DPPH is shown in Table 2. Hot water, cold water and methanol extract from *B. qriseipurpureus* each recorded IC_{50} of 1.79 mg/mL, 1.97 mg/mL, and 3.98 mg/mL respectively. Overall, the hot water extract and cold water extract showed good antioxidant potency compared to the methanol extract of *B. qriseipurpureus*.

Previous studies on fruiting bodies of *B. edulis* extracts showed a lower IC_{50} values for hot water extract (0.57 mg/mL) followed by ethanol extract (0.62 mg/mL), cold water extract (0.66 mg/mL) and methanol extract (0.73 mg/mL) (Emanuel & Sultana 2013). This shows that extracts of *B. edulis* mushrooms have better antioxidant potency than *B. qriseipurpureus.*

Table 2. IC_{50} of DPPH scavenging effect by the crude extracts from *B. qriseipurpureus*

Test sample	IC_{50} of DPPH scavenging effect (mg/mL)
Ascorbic Acid	0.34
Hot Water Extract	1.79
Cold Water Extract	1.97
Methanol Extract	3.98

Antiproliferative activity of *B. qriseipurpureus* extracts treated cell lines

In vitro antiproliferative assay of hot water, cold water and methanol extracts from *B. qriseipurpureus* had shown that growth inhibition correlates with increasing concentration of *B. qriseipurpureus* extracts. Fig. 3 and Fig. 4 showed the growth reduction of breast cancer cell line (MCF-7) and Vero cell line against different concentrations of *B. qriseipurpureus* extracts at 72 hours after treating. The figs. show that inhibition percentage of MCF-7 cell line is higher compared to Vero cell line. For example, at the concentration of 100 mg/mL, hot water extract from *B. qriseipurpureus* showed inhibition percentage of 60.45% ± 0.02 and 96.42% ± 0.04 on Vero and MCF-7 cell lines respectively. This proves the antiproliferative nature of the extract against MCF-7 cell line but the inhibition takes place after 72 hours of treatment.

IC_{50} in antiproliferative assay is the minimum concentration of substrate required to inhibit 50% of cell viability. By determining the IC_{50} value, a sample can be said to have either high or low anti-proliferative activity. The concentrations of the extracts from *B. qriseipurpureus* required to inhibit 50% Vero cells and MCF-7 cells viability are shown in Table 3 and Table 4 respectively. Hot water

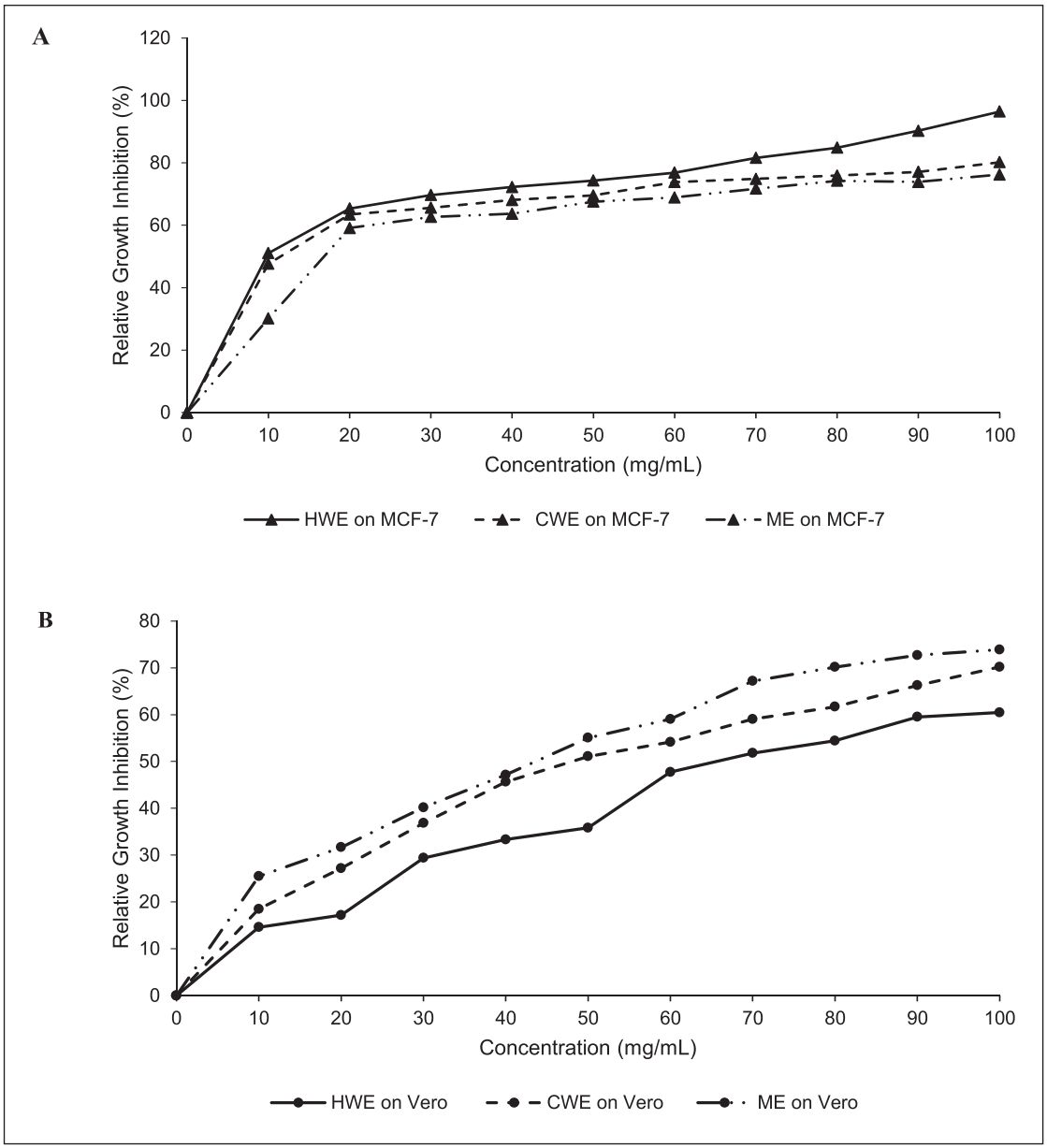

Fig. 3. Inhibition percentage of the crude extracts from *B. qriseipurpureus* on MCF-7 cell line (A) and Vero cell line (B) after 72 hours.

extract from *B. qriseipurpureus* recorded the lowest antiproliferative activity on Vero cell line (IC_{50}, 65.63 mg/mL) and highest antiproliferative activity on MCF-7 cell line (IC_{50}, 9.79 mg/mL) compared to all extracts tested.

A recent study that was conducted using six species of *Boletus* (*B. aereus*, *B. reticulatus*, *B. purpureus*, *B. rhodoxanthus*, *B. atanas* and wild *Boletus* species) shows that these species contain metabolite compounds especially secondary metabolites such as phenolic acids (Sandrina *et al.,* 2011) which provides the ability for antioxidant and anticancer activity. Through the antiproliferative test it is assumed that *B. qriseipurpureus* also contains phenolic acids that leads to anticancer activity towards MCF-7 cell line.

Regeneration of *B. qriseipurpureus* extracts treated STZ induced fish tail

According to Robert *et al.* (2013), the diabetic zebrafish shows decrease in body weight and delayed caudal fin regeneration growth. Observation by weight of zebrafish taken in this study is also similar to that recorded by Robert *et al.* (2013). Table 5 and Table 6 shows the weight of each group of normal and STZ induced zebrafish during the induction of diabetic (normal group was injected with sodium chloride) and after treatment of 14 days.

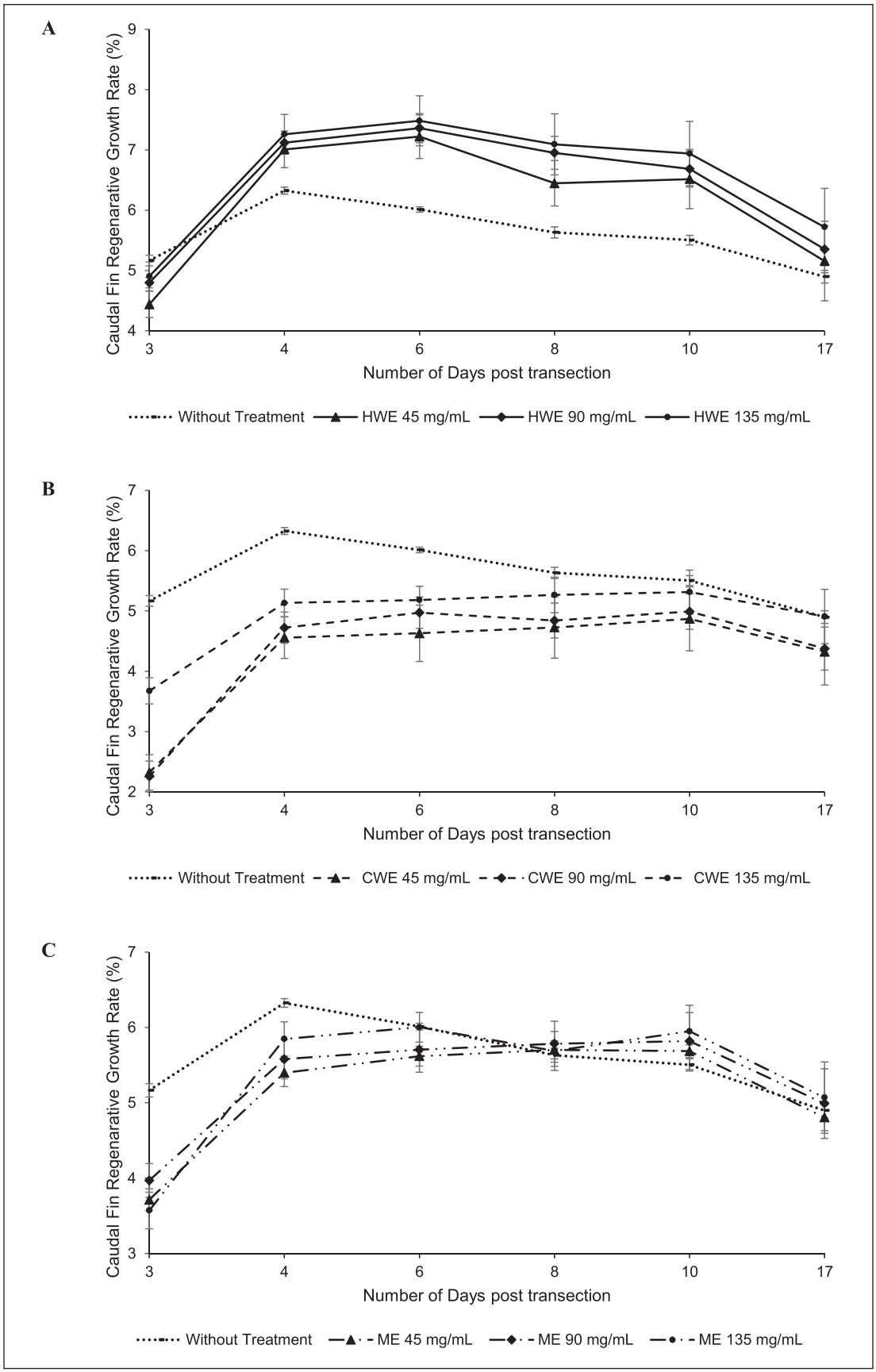

Fig. 4. Percentage of caudal fin regeneration growth rate of the normal fish feed with hot water extract (A), cold water extract (B) and methanol extract (C) from *B. qriseipurpureus* after transection.

Percentage growth rate of the caudal fin regeneration were calculated as, ((Area of new grown fin (mm^2) – Area of new grown fin on day 3 (mm^2))/Total of area of new grown fin (mm^2)*100)/ Number of days. ($p<0.05$).

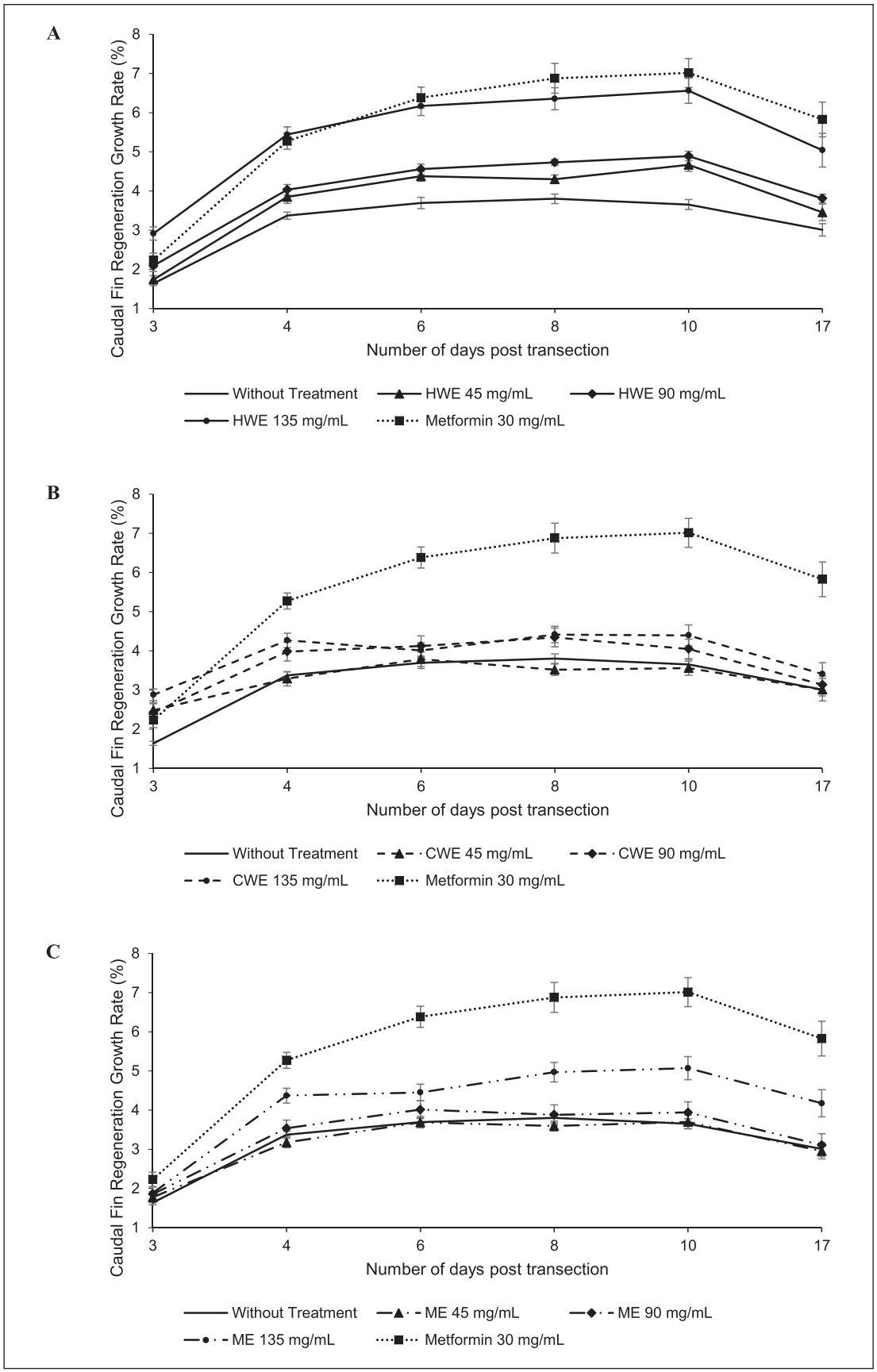

Fig. 5. Percentage of caudal fin regeneration growth rate of the STZ induced fish feed with hot water extract (A), cold water extract (B) and methanol extract (C) from *B. qriseipurpureus* after transection.

Percentage growth rate of the caudal fin regeneration were calculated as, ((Area of new grown fin (mm^2) – Area of new grown fin on day 3 (mm^2))/Total of area of new grown fin (mm^2)*100)/ Number of days. ($p<0.05$).

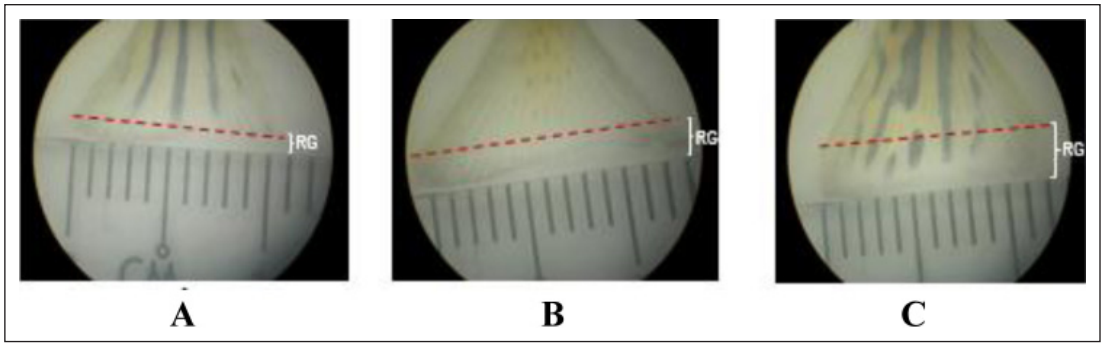

Fig. 6. Image of regenarative caudal fin growth from STZ induced zebrafish after 14 days post-transection. A. No treatment; B. With treatment of 135 mg/mL hot water extract from *Boletus qriseipurpureus*; C. With control treatment of 30 mg/mL metformin.
The red dotted line represents the amputation plane and RG demarks the regenerative growth.

Table 3. IC_{50} of Inhibition on Vero cell line by the crude extracts from *B. qriseipurpureus*

Test sample	IC_{50} on 24 Hours	IC_{50} on 48 Hours	IC_{50} on 72 Hours
Tamoxifen	–	96.7 µg/mL	35.0 µg/mL
Hot Water Extract	–	–	65.63 mg/mL
Cold Water Extract	–	–	48.04 mg/mL
Methanol Extract	–	–	43.62 mg/mL

Table 4. IC_{50} of Inhibition on MCF-7 cell line by the crude extracts from *B. qriseipurpureus*

Test sample	IC_{50} on 24 Hours	IC_{50} on 48 Hours	IC_{50} on 72 Hours
Tamoxifen	88.2 µg/mL	61.6 µg/mL	24.6 µg/mL
Hot Water Extract	–	–	9.79 mg/mL
Cold Water Extract	–	–	11.48 mg/mL
Methanol Extract	–	–	16.85 mg/mL

Table 5. Weight of normal fish during the injection (with sodium chloride) phase and after treatment for 14 days**.** Each value represents the mean value ± SD of n=5 fish in each group

Group	Weight of Fish (g)					
	I 1	I 2	I 3	I 4	I 5	T 14
Without Treatment	0.354 ± 0.087	0.355 ± 0.065	0.356 ± 0.078	0.362 ± 0.098	0.367 ± 0.078	0.377 ± 0.143
HWE 45 mg/mL	0.354 ± 0.123	0.354 ± 0.054	0.356 ± 0.083	0.362 ± 0.102	0.366 ± 0.087	0.377 ± 0.092
HWE 90 mg/mL	0.355 ± 0.096	0.356 ± 0.065	0.357 ± 0.093	0.362 ± 0.89	0.367 ± 0.098	0.375 ± 0.083
HWE 135 mg/mL	0.358 ± 0.064	0.359 ± 0.086	0.361 ± 0.091	0.363 ± 0.123	0.368 ± 0.108	0.371 ± 0.073
CWE 45 mg/mL	0.352 ± 0.071	0.353 ± 0.045	0.353 ± 0.102	0.358 ± 0.142	0.364 ± 0.087	0.372 ± 0.063
CWE 90 mg/mL	0.356 ± 0.081	0.357 ± 0.089	0.357 ± 0.083	0.362 ± 0.078	0.367 ± 0.76	0.375 ± 0.083
CWE 135 mg/mL	0.358 ± 0.101	0.360 ± 0.084	0.361 ± 0.115	0.364 ± 0.088	0.370 ± 0.091	0.381 ± 0.052
ME 45 mg/mL	0.354 ± 0.042	0.355 ± 0.082	0.356 ± 0.076	0.364 ± 0.098	0.369 ± 0.103	0.376 ± 0.139
ME 90 mg/mL	0.352 ± 0.034	0.353 ± 0.073	0.354 ± 0.093	0.361 ± 0.065	0.365 ± 0.054	0.373 ± 0.062
ME 135 mg/mL	0.352 ± 0.053	0.352 ± 0.054	0.353 ± 0.134	0.359 ± 0.076	0.364 ± 0.065	0.376 ± 0.87

HWE= Hot Water Extract, CWE= Cold Water Extract, ME= Methanol Extract, I= Injection, T 14= Treatment Day 14.

Table 6. Weight of STZ induced fish during the diabetic induction phase and after treatment for 14 days. Each value represents the mean value ± SD of n=5 fish in each group

Group	Weight of Fish (g)					
	I 1	I 2	I 3	I 3	I 4	T 14
Without Treatment	0.356 ± 0.058	0.356 ± 0.087	0.355 ± 0.098	0.353 ± 0.088	0.347 ± 0.089	0.344 ± 0.092
HWE 45 mg/mL	0.355 ± 0.062	0.355 ± 0.078	0.353 ± 0.132	0.348 ± 0.079	0.343 ± 0.087	0.349 ± 0.089
HWE 90 mg/mL	0.353 ± 0.072	0.353 ± 0.036	0.352 ± 0.092	0.347 ± 0.087	0.341 ± 0.103	0.349 ± 0.134
HWE 135 mg/mL	0.356 ± 0.056	0.355 ± 0.087	0.353 ± 0.045	0.349 ± 0.065	0.342 ± 0.089	0.352 ± 0.156
CWE 45 mg/mL	0.354 ± 0.053	0.354 ± 0.071	0.353 ± 0.043	0.348 ± 0.076	0.342 ± 0.124	0.343 ± 0.078
CWE 90 mg/mL	0.351 ± 0.065	0.351 ± 0.054	0.349 ± 0.098	0.344 ± 0.049	0.339 ± 0.078	0.342 ± 0.054
CWE 135 mg/mL	0.357 ± 0.078	0.356 ± 0.076	0.355 ± 0.067	0.352 ± 0.067	0.347 ± 0.132	0.350 ± 0.043
ME 45 mg/mL	0.353 ± 0.054	0.353 ± 0.039	0.351 ± 0.034	0.346 ± 0.087	0.340 ± 0.076	0.341 ± 0.0887
ME 90 mg/mL	0.358 ± 0.065	0.358 ± 0.087	0.356 ± 0.065	0.350 ± 0.087	0.344 ± 0.056	0.349 ± 0.0106
ME 135 mg/mL	0.359 ± 0.076	0.358 ± 0.054	0.356 ± 0.067	0.350 ± 0.085	0.345 ± 0.089	0.351 ± 0.1555
Metformin 30 mg/mL	0.354 ± 0.094	0.353 ± 0.076	0.352 ± 0.073	0.348 ± 0.076	0.341 ± 0.107	0.353 ± 0.167

HWE= Hot Water Extract, CWE= Cold Water Extract, ME= Methanol Extract, I= Injection, T 14= Treatment Day 14.

STZ induced zebrafish showed weight loss during the phase of diabetic induction, while the normal fish increased in weight. However, increased in body weight in STZ induced group was seen after the treatment with the *B. qriseipurpureus* extract.

Caudal fin regeneration growth rate of the normal fish and STZ induced fish feed with crude extracts from *B. qriseipurpureus* is shown in Fig. 4. Continuous caudal fin growth was observed in both groups. However growth rate was seen to decrease after the 6th day (normal fish) and 10th day (STZ induced fish) of post transection. Normal fish feed with hot water extract recorded higher caudal fin growth rate compared to fish feed with other extracts. Among the hot water extract, the highest concentration (135 mg/mL) showed the highest caudal fin growth rate in normal fish. Similar trend was seen in STZ induced fish fish; fish feed with 135 mg/mL hot water extract showed highest caudal fin growth rate comparable to the fish fed with the positive control drug (metformin). The results suggest that hot water extract from *B. qriseipurpureus* may cause hypoglycemic within the fish and the activity increases with the increase in the extracts concentration.

CONCLUSION

Our findings show that, the hot water extract of *B. qriseipurpureus* has the maximum antiproliferative activity, DPPH scavenging activity and antidiabetic activity. Antioxidant activity and cytotoxicity effect has positive linear relationship (Wing *et al.,* 2007) and could delay the development of complications in diabetic mellitus (Fatimah *et al.,* 2012). This evidence supports the oral statements of the local people that claimed gelam mushroom could be used as a remedy for diabetes. The present study was restricted to the preliminary screening of active compounds, antioxidation, antiproliferative and antidiabetic test of the selected extracts. Further characterization and identification of compounds within the hot water extract of *B. qriseipurpureus* need to be done before this extract can be used as an alternative remedies to defeat cancer and diabetes.

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