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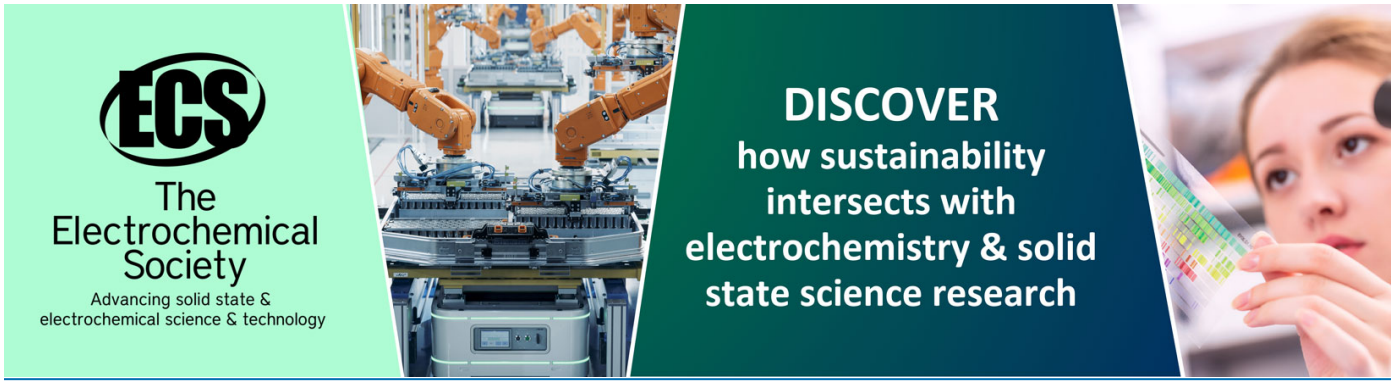

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Development of Antioxidant Film Based on Blends of *Stenochlaena palustris* Flour and Poly(Vinyl Alcohol)

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Abstract. The aim of this work is to develop antioxidant films based on blends of *Stenochlaena palustris* flour and poly(vinyl alcohol) (PVA). Four different PVA types were tested. All films produced were analyzed for their antioxidant activity by diphenyl-picrylhydrazyl radical scavenging (DPPH) method. This study found that S. palustris flour-88% HD PVA blends film contains the highest antioxidant activity. The efficiency study of the film shows the EC_{50}, T_{EC50} and Antioxidant Efficiency value of 20 mg/ml, 55 min and 0.91×10^{-3} respectively. The use of low concentration PVA and addition of glycerol made the film more flexible with greater elongation at break but low tensile strength. The formation of hydrogen bonds between the blend components was confirmed by the FTIR spectra analysis. The thermogravimetric analysis shows the thermal degradation of the film produced.

1. Introduction

In recent years, antioxidants have gained a lot of importance because of their potential as prophylactic and therapeutic agents in many diseases. Antioxidants are substances which counteract free radicals and prevent the damage caused by them [1]. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. Antioxidants which have been used in diet or as formulated agents have some common problems in their efficacy as therapeutic agents which are related to their physicochemical and biopharmaceutical properties. Many of the antioxidants have poor oral bioavailability, due to low solubility, permeability, stability and/or drug biotransformation before they reach systemic circulation [1]. Development of antioxidant films based on blends of *Stenochlaena palustris* (Burm) flour and poly(vinyl alcohol) (PVA) will somehow eliminate the used through oral route. This antioxidant films can be used mainly in cosmetic applications and a potential material in production of biodegradable packaging or plastic.

S. palustris a scrambling fern, is distributed in a large part of the tropical areas from southern and northern India through Malaysia to Polynesia and Australia [2]. The red young fronds are used as spinach and are said to be particularly beneficial when eaten at breakfast-time during the fasting month. The juice is used to cure fevers, the long rhizomes to bind baskets and fish traps, and a very durable rope can be made from the stems [3].

2. Experimental Section

2.1. Materials
Poly(vinyl alcohol) (99-100% HD, 95% HD, 88% HD, & 75% HD) were purchased from Acros Organics. 2,2-diphenilpicrylhydrazyl free radical (DPPH) was purchased from Merck. Kaempferol, myricetin, quercetin dihydrate, and rutin hydrate were purchased from Sigma-aldrich Co. Glycerol and methanol were purchased from HmBG. S. palustris were collected from open area at Wang Tepus, Jitra, Kedah. Fresh S. palustris leaveswere dried in the oven at 60˚C for 24 h. The dried samples then ground and the resulting flour were sieved at 45 μm to produce fine flour.

2.2. Sample Preparation
S. palustris flour/PVA blended films were made by the casting technique. The two solutions were first prepared separately. The *S. palustris* flour solution was prepared with 4 g of *S. palustris* flour/100 g solution. The *S. palustris* flour and distilled water were mixed for 2 hours with a magnetic stirrer. The *S. palustris* flour dispersion was then heated to 75˚C and glycerol (20 g glycerol/100 g polymers) was added. The temperature was increased to 85˚C for 60 min with constant magnetic stirring [4]. The PVA solution was prepared with PVA (4 g/100 g solution) and distilled water. The PVA was stirred for at least 4 h at room temperature, and then heated at 85 to 95˚C depending of the PVA type, until the PVA was completely dissolved [4]. Films were produced by varying the PVA content from 10 to 100% (w/w) of the dry matter. Blends of *S. palustris* flour and PVA solutions were prepared by mixing these two solutions. The blends were heated at 85˚C for 1 h with constant stirring but avoid frothing to increase the interaction between the components [4]. Water was added to maintain the volume. The film forming solutions were cast onto a glass plate with thickness of about 0.5 - 1 mm, after which air bubbles were removed by flaming and each solution was dried for 24 h at 55 ˚C to form the desired films. Complete drying was avoided as some moisture was required for films to remain flexible and not to crack. The films were finally removed (by peeling) from the glass.

2.3. Antioxidant Assay
Antioxidant properties of the films were carried out by using DPPH method as described in [5] with slight modification. An aliquot of 22 μL of the sample was added to 200 μL of DPPH (60 μM) in a 96-well flat-bottom microplate. The absorbance was recorded in a VersaMax microplate reader at 517 nm after 30 min incubation time at 37˚C [5]. The radical scavenging activity was calculated by the following equation:

$$\text{DPPH activity}(\%) = \left[1 - \frac{\text{Absorbance of sample at 517nm}}{\text{Absorbance of DPPH at 517nm}}\right] \text{x}100 \quad (1)$$

2.4. Efficiency Study
The efficiency of the antioxidant activity was carried out only on blended film of 88% HDPVA with *S. palustris* flour at 55% PVA concentration as this film show the best antioxidant activities. An aliquot of 22 μL of the sample at various concentration (10 – 100 mg/mL) was added to 200 μL of DPPH (60 μM) in a 96-well flat-bottom microplate. The absorbance was recorded in a VersaMax microplate reader at 517 nm after 30 min incubation time until plateau at 37˚C [6]. The remaining level of DPPH (% $DPPH_{REM}$) in the reaction medium was calculated using the following relation:

$$\%\ \text{of remaining DPPH} = 100.\left(A_{s517nm(t=30)}/A_{c517nm}\right) \quad (2)$$

where $A_{s\ 517\ nm}$ is the absorbance of the sample measured at t = 30 min and $A_{c\ 517\ nm}$ is the absorbance of the control. The percentage of remaining DPPH against the standard concentration was then plotted to

obtain the amount of antioxidant necessary to decrease the initial DPPH concentration by 50 %. The time needed to reach the steady state to EC_{50} concentration (T_{EC50}) was calculated graphically. Taking into account that both, EC_{50} and T_{EC50}, affect the antiradical capacity, a new parameter: antioxidant efficiency (AE) [7], which combines these two factors, was defined:

$$AE = 1/EC_{50}T_{EC_{50}} \quad (3)$$

2.5. Film Characterization

For Fourier transform infrared (FTIR) spectroscopy analysis, the infrared spectra were recorded between 4000 and 600 cm^{-1} at 4 cm^{-1} of resolution with a Fourier transform IR (FT-IR) spectrometer RX1 (Perkin Elmer). The films were dried at 100 ˚C for 48 h and ground into powder before the infrared (IR) measurement. Thermogravimetric analysis was carried our using a Perkin Elmer (TGA7) under nitrogen atmosphere. Sample (about 10 mg) were heated from room temperature to 500 ˚C at a heating rate of 10 ˚C/min. The tensile strength (σ_b) and elongation at break (ε_b) of the films in both dry and wet states were measured using a universal testing machine Haunsfield (H10KS), at a tensile rate of 10 mm/min. The wet films were measured immediately after immersed in water for 30 min. The σ_b and ε_b values are the averages of three measurements.

3. Results and discussion

3.1. PVA Selection

Four different types of PVA were tested in the formulation of blended films with equal proportions of *S. palustris* flour-PVA. All films were homogeneous and easily peelable from the support except for 75% HD PVA. The characteristics of the film produced were simplified in Table 1. Even though it had been dried for 72 h, the *S. palustris* flour-75% HD PVA film was unable to peel smoothly. This may due to the low compatibility between this partially hydrolyzed and low molecular weight PVA and the *S. palustris* flour. Several factors associated with PVA may explain the effects of molecular weight and concentration.

At a high molecular weight polymer with a high degree of hydrolysis, the solution viscosity increases significantly and hence reduced rate of solvent evaporation [8]. When using low molecular weight PVA, the viscosity of solution was lower thus increased the rate of solvent evaporation. This conditions cause less formation of hydrogen bond between the PVA chains and water molecules. Low molecular weight PVA comparatively has low ability of film producing.

Table 1 Characteristics of film produced

PVA Types	Film produced
99-100% HD	Easily peeled at high concentration and at low concentration
95% HD	Easily peeled at high concentration and at low concentration
88% HD	Easily peeled at high concentration and at low concentration
75% HD	Not peeled at high and low concentration, tend to rupture when separate from support

3.2. Antioxidant Activity

The antioxidant activity of the *S. palustris* flour-PVA films were carried out after 1 h of incubation time at 37 ˚C with three different concentration of the film (25, 50 & 100 mg/mL) and five different PVA concentration (10, 32.5, 55, 77.5 & 100%). Percentage of antioxidant activity was calculated and presented as graph percentage of antioxidant activity versus PVA concentration. Fig 1, 2 and 3, shows the antioxidant activity of *S. palustris* flour-99-100% HD PVA blends film, *S. palustris* flour-95% HD

PVA blends film & *S. palustris* flour-88% HD PVA blends film respectively. Antioxidant activities were lowest at 100% PVA concentration for all type of PVA. Comparing all three types of PVA, 88% HD PVA shows highest antioxidant content for all concentration used. While 95% HD PVA shows lowest antioxidant content compare to the other two types of PVA, might be due to the higher molecular weight of 95% HD PVA.

In the higher molecular weight PVA, in which the polymer component was much more concentrated and viscous, there were very high tendency of the antioxidant from the *S. palustris* flour react more on the concentrated PVA. Therefore, the amount of hydrogen or electron that will react to DPPH much more lower resulting low antioxidant activity. The highest antioxidant activities were recorded from film developed from 88% HD PVA at 55% PVA concentration.

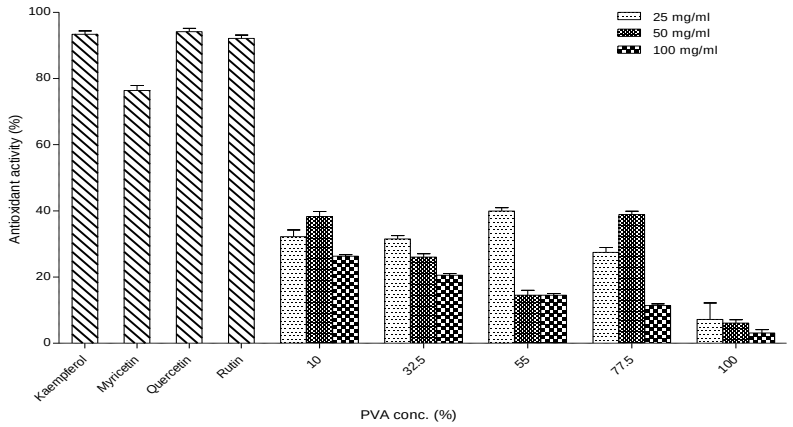

Fig. 1 Antioxidant activity of *S. palustris* flour- 99-100% HD PVA blends film at different concentrations of the PVA and different dilution rate of the film. Values are the means ± SEM (n = 4).

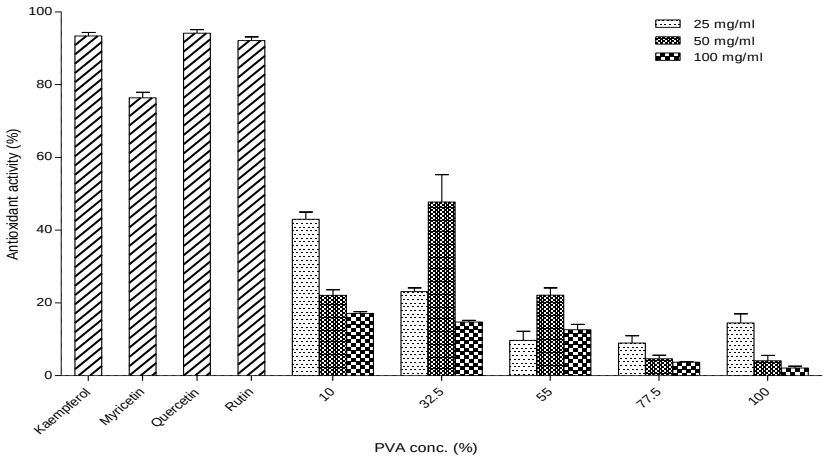

Fig. 2 Antioxidant activity of *S. palustris* flour- 95% HD PVA blends film at different concentrations of the PVA and different dilution rate of the film. Values are the means ± SEM (n = 4).

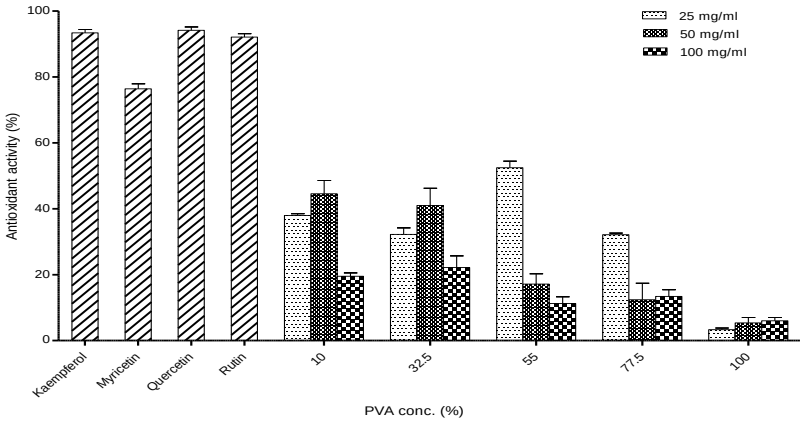

Fig. 3 Antioxidant activity of *S. palustris* flour- 88% HD PVA blends film at different concentrations of the PVA and different dilution rate of the film. Values are the means ± SEM (n = 4).

3.3. Efficiency Study

The efficiency of the antioxidant activity was conducted on blends film of 88% HD PVA with *S. palustris* flour at 55% PVA concentration of varies concentration (10 - 100 mg/mL) (Fig. 4). Reacting DPPH solution with each concentration of films sample for every 30 min incubation time until plateau at 37 ˚C facilitates the extraction of antioxidant compounds from the sample thereby increasing the measured antioxidant activity of the sample. The results showed that the absorbance decreased as the radical was scavenged by antiradicals, through donation of hydrogen, to give the reduced form DPPH-H. Basically, the antioxidant activities of the blends films were increased with concentration up to 20 mg/mL and further increased up to 100 mg/mL resulted in significant decrease of antioxidant activity. This might be explained by the fact that at higher concentrations, films act as oxygen carrying agent and serves as pro-oxidant in the oxidation of lipid [9].

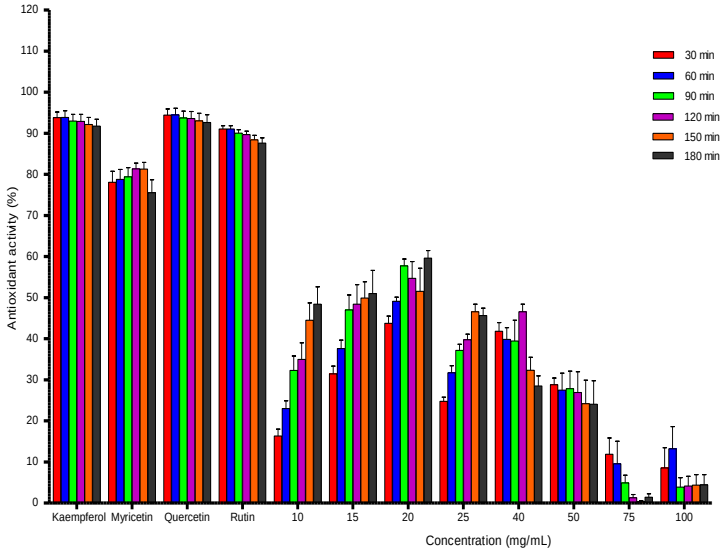

Fig. 4 Antioxidant activity of various concentration of *S. palustris*-88% HD PVA blends films. Values show means ± SEM (n = 4).

Fig. 5 illustrates antioxidant behavior of *S. palustris*-PVA blend film, the values of % DPPH remaining as a function of time are presented at a concentration of sample in the reaction mixture varies from 10 to 100 mg/mL. In the presence of film solutions a rapid initial decrease of DPPH

content was followed by slow subsequent disappearance of DPPH. There were significant differences between the slopes after the end of the initial fast step. These differences were related to the role of secondary slow reactions (dimerization or disproportionation) of the radicals formed.

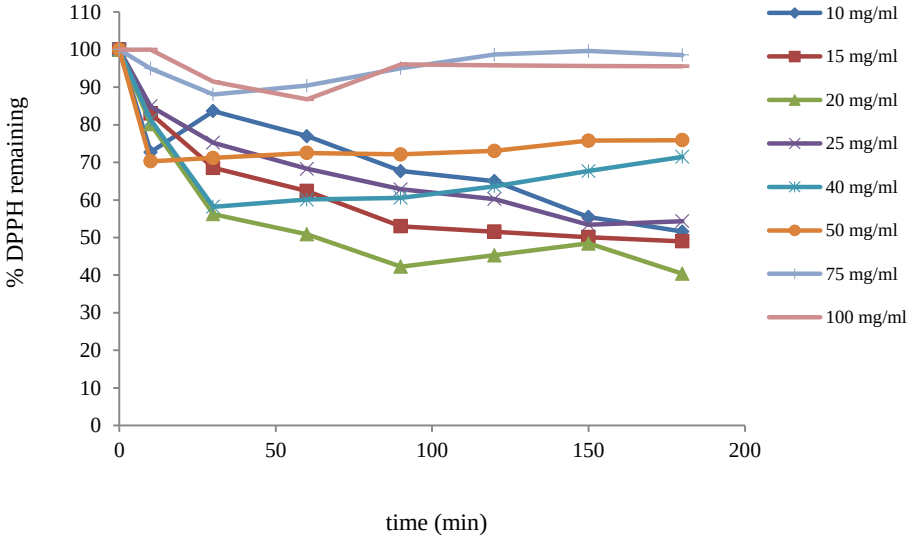

Fig. 5 Antioxidant behaviour of different film concentration

Antioxidants in blends film may not necessarily freely available to react with DPPH, hence they react at different rates and the reaction will often not go to completion in a reasonable assay time. Therefore, the sample size that can lower the initial absorbance of DPPH solution by 50% has been chosen as the endpoint for measuring the antioxidant activity [10]. This sample size was called EC_{50}. The lower EC_{50}, the higher the antioxidant activity of a compound is [11]. Fig. 6 shows the EC_{50} value was 20 mg/mL.

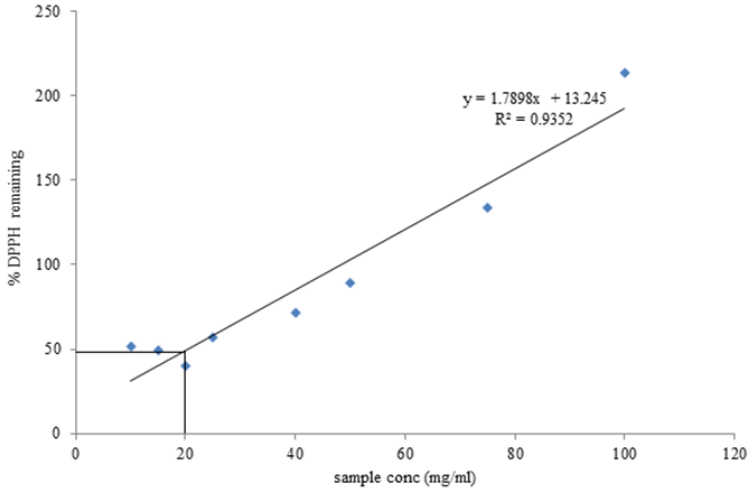

Fig. 6 Determination of EC_{50}

T_{EC50} was the time needed to reach a steady state at the concentration corresponding to EC_{50}. This parameter was obtained by plotting the times at steady state against the concentration for each antioxidant compound [7], as illustrated in Fig. 7. Based on T_{EC50} values determined, the kinetic behavior of the antioxidant compound can be classified as follows: < 5 min (intermediate); 5 – 100 min (rapid) and > 100 min (slow). The T_{EC50} value of *S. palustris*/PVA blend film was about 55 min, therefore it was classified as rapid.

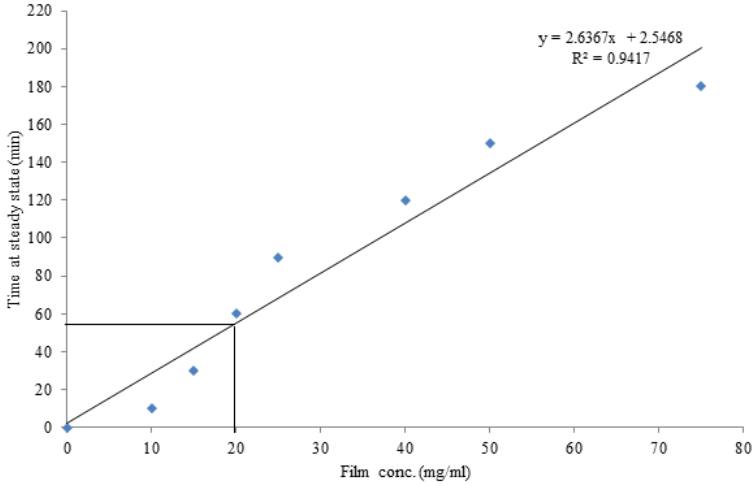

Fig. 7 Determination of the time needed to reach the steady state to EC_{50} concentration.

DPPH method permits to evaluate not only the electron or hydrogen atom donating properties of antioxidants, but also the rate of their reaction towards the free radicals. The AE, was calculated in order to easily characterize the behaviour of a substance as antioxidant. It was found that AE value of the studied film was 0.91 x 10^{-3}. The AE values were classified as follows: AE $\leq 1 \times 10^{-3}$ = low; $1 \times 10^{-3} < AE \leq 5 \times 10^{-3}$ = medium; $5 \times 10^{-3} < AE \leq 10 \times 10^{-3}$ = high and AE > 10×10^{-3} = very high [12]. Therefore, the AE of *S. palustris*/PVA blend film was suggested as low rate of reaction. AE was more adequate parameter than widely used EC_{50}, which is not completely discriminatory to select the antioxidant compound.

3.4. Film Characterization

The main IR bands observed in *S. palustris* flour-PVA 88% HD (55% PVA concentration) film spectra were then investigated. The *S. palustris* flour-88% HD PVA blended film spectrum displayed the typical profile of a polysaccharide in the 1600 to 600 cm^{-1} range (characteristic peaks attributed to COC bond stretching), due to starch content of *S. palustris* flour [4]. No strong peak was observed indicate that low amount of anhydroglucose ring presence in *S. palustris* flour. The band at 3899.73 cm^{-1} was due to hydrogen-bonded hydroxyl group (O – H) [13].

Assessing the thermogravimetric data of a polymer was means to asses the phase transformation (degradation) of the polymer. When studying the phase transformation of a polymer, one of the most important point to determine was the glass transition temperature; where the polymer structure turns rubbery upon heating and glassy upon cooling. The thermal degradation of blends film is known to take place in various steps such as; a small weight loss of about 1-7 % below 100 ˚C is assigned to the release of moisture from the sample. The sample show one stage degradation within the range of 105 - 300 ˚C. The thermal stability also depends on molecular weight and crystallinity of the polymer. Generally, lower the molecular weight or lower the cristallinity, the easier the degradation of the polymer.

Fig. 8 shows the variations of tensile strength and elongation at break of the film. The values of tensile strength and elongation at break after performed the experiment at triplet were 0.0977 ± 0.0144 MPa and 266 ± 19.95% using 0.5500 ± 0.3606 N load at break. Considering this result, it shows that film produced were actually low in elasticity due to effect of low concentration of PVA and plasticization. The use of glycerol as plasticizer caused a reduction in the resistance (decrease in TS) and an increase in the flexibility (increase in EB) of the films [14]. This behaviour, typical of the plasticization phenomenon, is a consequence of the increase in molecular mobility with the addition of

plasticizer and may be due to the absorbed moisture in the protein films causing protein chains to slip over one another.

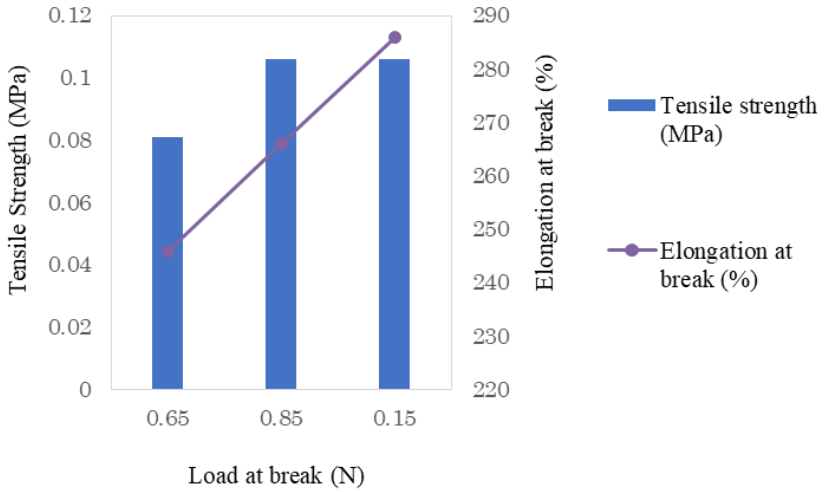

Fig. 8 The tensile strength and elongation at break of *S. palustris*-88% HD PVA (55 % PVA concentration) blends film for different load at break.

4. Conclusions

S. palustris flour-PVA blend film had been successfully developed by using three types of PVA; 88% HD, 95% HD, and 99-100% HD, however unsuccessfully by using 75% HD PVA. The highest antioxidant activity was show by the film formulated with 88% HD PVA at 55% concentration. The efficiency study conducted shows that 20 mg/mL of the film concentration gives the highest antioxidant activity. The EC_{50} value also shows the concentration of 20 mg/mL was needed to reduce radical concentration by 50%. Antioxidant efficiency (AE) of the film, which combines with the T_{EC50} value of time at steady state need to achieve EC_{50} gives the value of 0.91×10^{-3}. The use of glycerol plasticizer gives high elongation at break but low tensile strength of films produced. FTIR analysis gives identical polysaccharide profile and presence of hydrogen bonded hydroxyl group (O – H). The TGA analysis shows the thermal degradation of polymer. The glass transition temperature of blends film was at 305 ˚C and the mass residue was 4.48%.

Acknowledgements

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