boiling water bath for 10 minutes after 1 minute of cooling in the ice-water bath. Aliquots in the test tubes were then left to cool to room temperature prior to the addition of 0.1 mL of 3% L-cysteine (Sigma Aldrich) solution and the solutions were allowed to stand for 30mins to ensure thorough mixing as reported by Lim et al. [9]. Absorbance reading of all the samples was measured in a 96-well plate at 396 nm and 427 nm by using a BioTek Epoch microplate spectrophotometer (Vermont, USA) [19]. Calibration curve ranging from 0.002-0.02% (w/v) of a commercial fucoidan (F_{ysk}) was used to determine the content of fucoidan in the sample extracts. This spectrophotometric analysis was also used in order to calculate the purity of fucoidan based on its content and the initial concentration of sample used (0.02%). n =3 replication was conducted to ensure more accurate results.

Statistical analysis

All analyses were conducted in triplicates (n=3) and collected data were expressed in terms of mean and standard deviation. The statistical analysis was performed using both t-test and one-way ANOVA followed by Duncan's multiple range test (DMRT) using SPSS software for Windows Version 22. Difference in values of mean was considered significant when $p < 0.05$.

Results and Discussion

Yield of fucoidan extract

Techniques of extraction greatly influenced the yield of fucoidan extract. Different mechanical and chemical processes such as the solvent extraction and steam distillation are normally used for the extraction of some bio-compounds from plant sources. The selection of methods differs according to the nature of soluble polysaccharides to be extracted (fucoidan) to obtain its maximum yield. Based on the study that has been conducted, it showed that the acid extraction (fucoidan extracted by HCl) of all parameters gave lower yield in terms of its dry weight percentage (% w/w) compared to that of the alkaline extraction (Figure 1). The alkaline extraction of fucoidan at 65 °C for 3 hours yielded statistically the highest amount of fucoidan, 3.81±0.73% compared to other extraction parameters. In contrast, the least yield of fucoidan extracted by HCl at 85 °C for 5 hours was 0.13±0.05%. Similar result was reported by Ale that the alkaline extraction yielded a significant higher amount of fucoidan compared to acidic extraction [20].

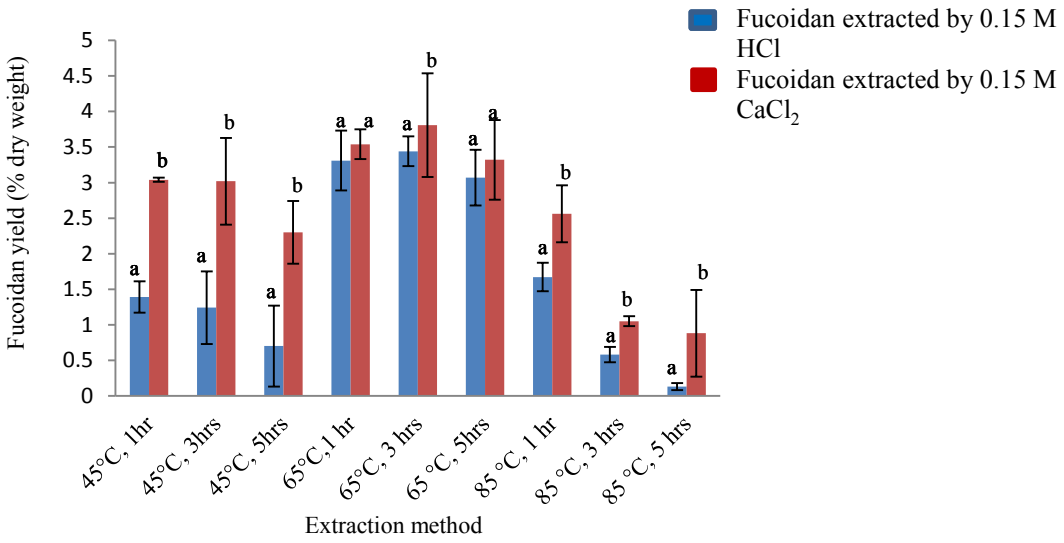

Figure 1. Graph of fucoidan yield (dry weight percentage) against the extraction method. [a-b] Different letters on fucoidan yield indicate a statistically significant difference ($p< 0.05$)

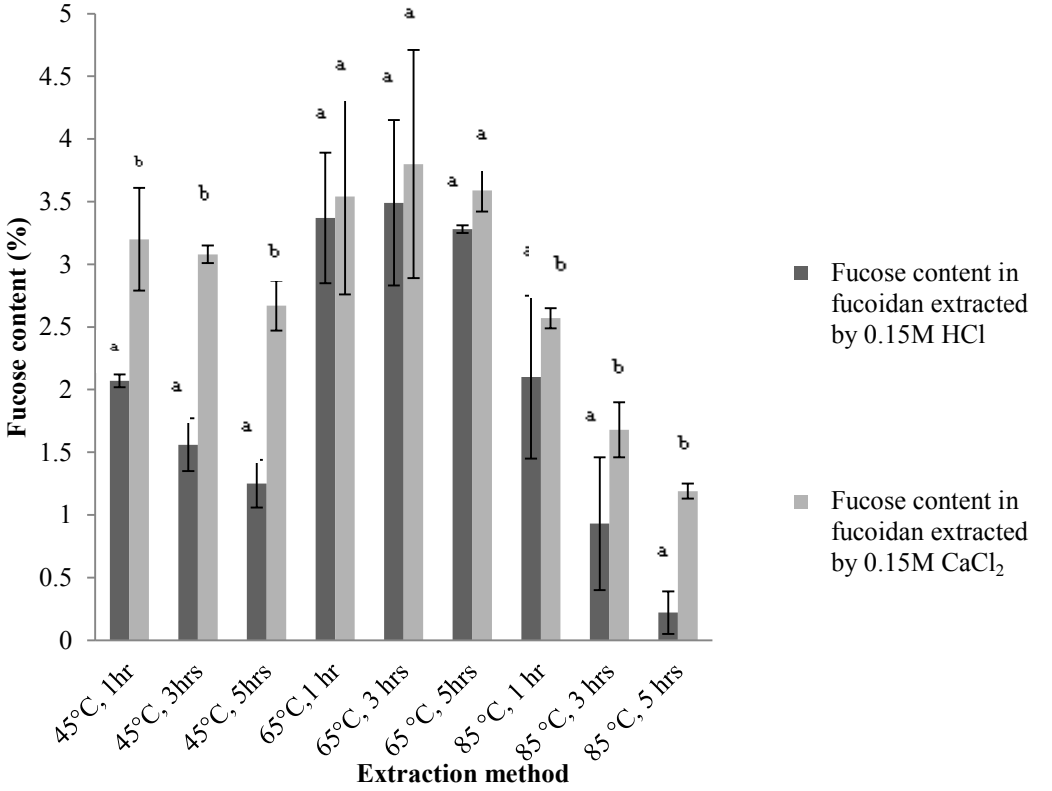

Figure 2. Graph of fucose content (%) against the extraction method. [a-b] Different letters on fucoidan yield indicate a statistically significant difference ($p < 0.05$)

Fucoidan purity

The quality of the extracted fucoidan is normally determined by its purity. The colour of fucoidan also affects the quality of fucoidan in terms of its purity [27]. Purity of the fucoidan extract can be determined visually as lighter colour of fucoidan indicates that it tends to have higher purity, thus higher quality. On the contrary, darker colour of fucoidan extract shows that the extract has a low purity. However, the purity of fucoidan can be measured more accurately by using the colorimetric method as described [19].

In this study, fucoidan obtained from both extraction methods were compared in terms of their purity by using colorimetric method, followed with *t*-test analysis. Based on the results shown in Table 1 below, fucoidan obtained from alkaline extraction (using $CaCl_2$) had a significantly higher purity ($p< 0.05$) than the fucoidan obtained from acidic extraction. This might be due to the existence of other soluble polysaccharides especially alginate that contained in the extract from acidic extraction. During the extraction of fucoidan by HCl, simultaneous extraction of alginate occurred, causing the alginate to be present as the alginic acid. Presence of alginic acid in the fucoidan extract significantly affected the purity of fucoidan when tested. On the contrary, the presence of alginic acid in the fucoidan extracted by alkaline solvent, $CaCl_2$ was determined to be in the form of alginate (insoluble).