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[6-1130-P] Functional/Wellness Foods &Nutrition (6th)
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[6-1130-P-04] Investigation of some biological activities of local shallot (*Allium ascalonicum* Linn.) extract from Thailand

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Keywords: shallot extract, prebiotic, antioxidant activity, antibacterial activity, functional food

Shallot (*Allium ascalonicum* Linn.) is a good source of several nutrients and phytochemicals. Shallot-based functional foods have been being developed in our research group. The partial purified shallot extract was prepared according to the processes developed in our group for utilizing as functional food ingredient of many food products. The aims of this present work were then to investigate some biological activities including antibacterial activity and antioxidant activity of shallot extract prepared from local cultivar of Srisaket province, Thailand. The results showed that both crude and partial purified extracts were rich in oligosaccharides and polysaccharides, with degree of polymerization (DP) about 23-283. Interestingly, it was found that purification processes used in this study, based on adsorption method, removed some low molecular weight sugars from shallot extract. ABTS radical scavenging assay was used in antioxidant activity test of the extracts. The crude extract exhibited significantly higher ABTS scavenging activity than the purified extract. The results also revealed that ABTS scavenging activity continuously decreased according to number of purification step. The similar results were found in antibacterial test that shallot extract lost the activity after purification processes. However, crude extract could inhibit growth of pathogenic *Salmonella* Typhimurium and *Staphylococcus aureus* but not for *Escherichia coli* in agar diffusion assay. Moreover, the minimum inhibitory concentration (MIC) values of crude extract on *S.* Typhimurium and *S. aureus* were 114.66 and 163.80 mg/ml, respectively, and only *S.* Typhimurium was disinfected by crude extract with the minimum bactericidal concentration (MBC) value at 147.42 mg/ml. It could be concluded that shallot extract possess high potential to be applied in functional food manufacturing. However, crude extract and purified extract might be suitable for different purposes, including prebiotic, antioxidant and antibacterial uses.

Investigation of some biological activities of local shallot (*Allium ascalonicum* Linn.) extract from Thailand

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ABSTRACT

Shallot (*Allium ascalonicum* Linn.) is a good source of several nutrients and phytochemicals. Shallot-based functional foods have been being developed in our research group. The partial purified shallot extract was prepared according to the processes developed in our group for utilizing as functional food ingredient of many food products. The aims of this present work were then to investigate some biological activities including antibacterial activity and antioxidant activity of shallot extract prepared from local cultivar of Srisaket province, Thailand. The results showed that both crude and partial purified extracts were rich in oligosaccharides and polysaccharides, with degree of polymerization (DP) about 23-283. Interestingly, it was found that purification processes used in this study, based on adsorption method, removed some low molecular weight sugars from shallot extract. ABTS radical scavenging assay was used in antioxidant activity test of the extracts. The crude extract exhibited significantly higher ABTS scavenging activity than the purified extract. The results also revealed that ABTS scavenging activity continuously decreased according to number of purification step. The similar results were found in antibacterial test that shallot extract lost the activity after purification processes. However, crude extract could inhibit growth of pathogenic *Salmonella* Typhimurium and *Staphylococcus aureus* but not for *Escherichia coli* in agar diffusion assay. Moreover, the minimum inhibitory concentration (MIC) values of crude extract on *S.* Typhimurium and *S. aureus* were 114.66 and 163.80 mg/ml, respectively, and only *S.* Typhimurium was disinfected by crude extract with the minimum bactericidal concentration (MBC) value at 147.42 mg/ml. It could be concluded that shallot extract possess high potential to be applied in functional food manufacturing. However, crude extract and purified extract might be suitable for different purposes, including prebiotic, antioxidant and antibacterial uses.

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1. INTRODUCTION

Shallot or red onion (*Allium ascalonicum* L.) is a member of the Alliaceae family, is widely cultivated and consumed in many Asian countries. In Thailand, shallot have been cultivated mainly in Chiang mai, Uttaradit and Srisaket provinces. It constitutes important ingredient in many Asian diets and is known for its medicinal properties apart from its nutritional value. Shallot contains both water-soluble nutrients and oil-soluble substances, with 79.8% moisture, 16.8% carbohydrates, 2.5% proteins, 3.2% dietary fibers, and 7.9% sugars (by fresh weight) (Putnika et al., 2019). It is a good source of sugars (oligosaccharides), minerals (Ca and P), vitamins (A, B6 and C) and various functional phytochemicals (organo-sulfur compounds flavonoids and other phenolic compounds) (Brewer, 2011; Ounjaijean et al., 2018). Consequently, this plant exhibits many biological properties, including antibacterial, antivirus, anti-diabetic, antioxidant, and anti-inflammation activities (Sakaewan et al., 2019). Shallot extract inhibit the expression of genes associated with inflammation, including iNOS, TNF-α, IL-1β and IL-6 (Werawattanachai et al., 2015), inhibit proliferation and growth of tumor cell lines (HeLa and MCF-7) (Hamid-Reza et al., 2011). The extract also possess antimicrobial and antioxidant activities (Mnayer et al., 2014) by the action of two main classes of components, organo-sulfur compounds (allyl trisulfide, allyl-cysteine and diallyl sulfide) and flavonoids (quercetin and kamferal) (Brewer, 2011). Moreover, oligosaccharides containing in shallot are promising to be utilized as prebiotic foods (unpublished data). According to the above functional potentials of shallot for consumers, shallot-based functional foods have been being developed in our research group. The partial purified shallot extract was prepared according to the processes developed in our group for utilizing as functional food ingredient of many food products. This shallot extract will be mainly proposed as the functional ingredient for prebiotic, antimicrobial and antioxidant foods. From our previous results (unpublished data), it was interestingly that partial purified shallot extract exhibited prebiotic property greater than original shallot extract. However, the other biological activities have not been yet studied. The aim of this study was subsequently to investigate some biological activities of crude and partial purified shallot extract, including antibacterial activity and antioxidant activity to evaluate their potential prior applying in functional food manufacturing.

2. MATERIALS AND METHODS

2.1 Shallot and shallot extract preparation

Shallot or red onion or *Hom-daeng* (in Thai) used in this study was a local cultivar cultivated of Srisaket province, Thailand. The extract was prepared by aqueous extraction of fresh and clean shallot. Shallot extract was then further partial purified through a commercial adsorbent. Crude and partial purified extracts were clarified by centrifugation and stored at -20°C during experiment.

2.2 Sugar content analysis

Reducing sugar and total sugar of the extracts were determined by DNS and phenol-sulfuric acid method; respectively. Size of sugar, in term of an average degree of polymerization (DP) was calculated by the ratio between total sugar and reducing sugar content (Wongputtisin et al., 2012). The distribution of individual sugars in shallot extract was investigated by thin layer chromatography (TLC). The aluminum sheet coated by siliga gel (Merck©) was used as stationary phase and mobile phase was a mixture of butanol: ethanol: water (5:3:2). The sugar bands were visualized by dipping in 5% (v/v) H_2SO_4 in methanol and heating at 150°C in hot air oven.

2.3 Antioxidant activity

To generate $ABTS^{\bullet+}$, the protocol according to Re et al. (1999) was used. Five ml of 14 mM ABTS (0.385 g ABTS in 50 ml deionized water) and 5 ml potassium persulfate (0.066 g potassium persulfate in 50 ml deionized water) were mixed together and stand in the dark for 12-16 h before use. To determine scavenging activity of FCSBM extract, 10 µl of extract was added to 990 µl of $ABTS^{\bullet+}$ solution (adjusted the absorbance at 734 nm to 0.700±0.020 before used) and recorded the decreasing of A_{734} every 1 min until stable. The standard antioxidants used in this study were α-tocopherol (Merck®), ascorbic acid (Fisher Chemicals®), butylated hydroxyanisole (BHA, Fluka®). The percent of scavenging activity at 1 min of reaction can be calculated by the formula:

$$\frac{A_{734} \text{ at } 0 \text{ min} - A_{734} \text{ at } 1 \text{ min}}{} \times 100$$

A_{734} at 0 min

2.4 Antibacterial activity

The antibacterial activity of crude and partial purified shallot extract against *Salmonella enterica* serovar Typhimurium TISTR292, *Escherichia coli* and *Staphyrococcus aureus* were studied. The extracts were sterilized using filtration through Sartorius Minisart® syringe filter (0.2 µm). Firstly, gel diffusion assay method was carried out by transferring of 20 µl extract into agar wells which were prior spread with 24 h- old pathogen suspension, subsequently further incubating at 37ºC for 24 h and recording the clear zone around wells.

Minimum inhibitory concentration (MIC) of the extracts against those pathogens was tested. The sterile extract was diluted by nutrient broth (NB) with 2-fold serial dilution (total volume at 5 ml in test tube), then inoculated by approximately 10^8 CFU of pathogen. The tubes were incubated at 37ºC for 24 h The minimum concentration of extract that did not show visible growth was recorded as MIC value. The control treatment diluted by sterile distil water was carried out in parallel. Ten µl of culture broth from those test tubes with no visible growth were spread on *Salmonella – Shigella* agar (SS agar) (Himedia®) and eosin methylene blue agar (EMB agar) (Himedia®) and nutrient agar (NA) and incubated at 37ºC for 24 h for cell enumeration of *S.* Typhimurium, *E. coli* and *S. aureus*, respectively. Minimum concentration of extract with no viable cell was considered as minimum bactericidal concentration (MBC) value.

3. RESULTS AND DISCUSSION

3.1 Shallot extract

Fresh shallot contained approximately 77.1 $\pm$ 0.2% of moisture content (wet basis) and the % yield of shallot extract obtained from electronic juicer was 453 ml/ kg of fresh shallot. This extract was further processed for partial purification of FOS following our unique and specific steps based on adsorption strategy as usual. The extract quality in term of sugar content in both crude and partial purified extracts were analyzed for quality confirmation and shown in Table 1 and Figure 1. Increased cycle of elution through absorbent resulted of decreasing of monosaccharides, while the average DP was increased. FOS was the major group of sugar found in these shallot extracts and also in other *Allium* sp. cultivars. According to our unpublished data, prebiotic property of these partial purified extract was greater than that of crude extract. However, the antioxidant and antibacterial activity of these extracts were subsequently investigated as main objectives of this study.

Table 1. Total sugar, reducing sugar and degree of polymerization of shallot extracts

Extracts	Total sugar (mg/ml)	Reducing sugar (mg/ml)	DP
Crude extract	167.54±2.55^{ab}	7.19±0.04^{i}	23.31^{g}
Partial purified extracts			
1 cycle	191.15±0.70 ^{ab}	3.59±0.06^{h}	53.29^{fg}
2 cycles	190.49±17.62 ^{ab}	2.27±0.02^{g}	84.04^{f}
3 cycles	193.55±2.95^{a}	1.50±0.03^{f}	129.03^{e}
4 cycles	172.38±15.88 ^{ab}	1.13±0.03^{e}	152.10^{de}
5 cycles	166.61±18.45 ^{ab}	0.94±0.02^{d}	177.25^{cd}
6 cycles	158.20±20.29^{b}	0.83±0.01^{c}	189.84^{c}
7 cycles	166.80±23.53 ^{ab}	0.71±0.03^{b}	233.84^{b}
8 cycles	164.64±10.36 ^{ab}	0.58±0.02^{a}	283.87^{a}

Note: different superscript letters mean significant difference at $p<0.05$.

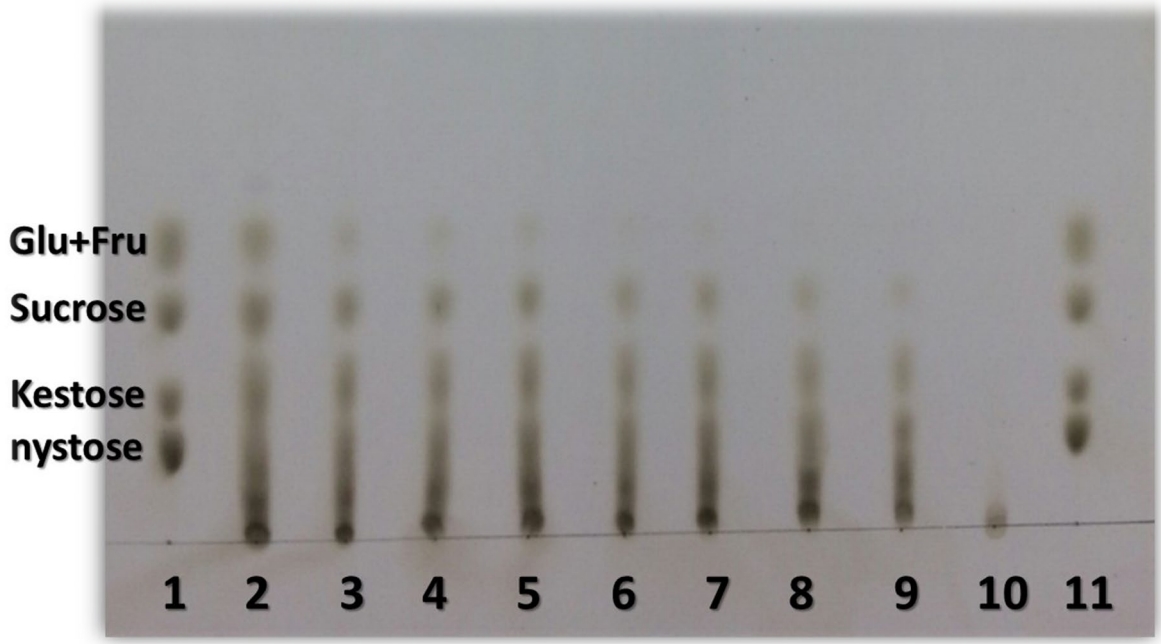

Figure 1. TLC chromatogram of sugar composition in crude and partial purified shallot extracts (Lane 1, 11: Standard sugars; Lane 2: crude extract; Lane 3: purified with 1 cycle; Lane 4: purified with 2 cycles; Lane 5: purified with 3 cycles; Lane 6: purified with 4 cycles; Lane 7: purified with 5 cycles; Lane 8: purified with 6 cycles; Lane 9: purified with 8 cycles; Lane 10: inulin)

3.2 ABTS radical scavenging activity

Several antioxidants were naturally found in *Allium* sp., mainly as flavonoids and even the reducing sugars and amino acids. Antioxidant activity of the extracts were assayed in term of %ABTS radical scavenging activity. The results are shown in Table 2. The scavenging activity of crude extract was 50.66±0.22% and slightly increased ($p<0.05$) about 7% higher than that of crude extract after the first cycle of FOS purification. According to higher concentration of sugar content was obtained after this cycle (Table 1), water moiety might be absorbed on absorbent. Thus, it was possibly that concentration of antioxidants was also increased, even though some were absorbed, resulting of slightly increasing of %scavenging activity. However, further cycles of purification led to continuous decreasing of %scavenging activity. Finally, the activity was lowered about 71% comparing to crude extract after 8 cycles of purification. It was indicated that the crude extract exhibited significantly higher ABTS scavenging activity than the purified extract. During the purification process, it was noticed that color of extract gradually paler along the number of purification steps. Anthrocyanins and flavonols, the dominant flavonoid pigments naturally found in *Allium* sp., especially in red onion, might be also removed from the extract similar to monosaccharides. Their polar molecules can be adsorbed on carbonaceous absorbent via Van de Waals force (Li et al., 2017). The dominant anthrocyanins and flavonols in red onion are cyanidin and quercitin, respectively (Arifin et al., 1999). They play an important role as antioxidant in plants and several health benefits for consumers (Arifin et al., 1999; Pudzianowska et al., 2012; Mnayer et al., 2014).

Table 2. ABTS scavenging activity of crude and purified extracts

Samples	%scavenging activity
Crude extract	50.66±0.22[b]
Partial purified extracts	
1 cycle	54.51±2.15[a]
2 cycles	43.90±0.23[c]
3 cycles	31.42±1.16[d]
4 cycles	23.14±0.87[e]
5 cycles	19.27±0.38[f]
6 cycles	16.53±0.45[g]
7 cycles	14.66±1.09[h]
8 cycles	12.46±0.36[i]

Note: different superscript letters mean significant difference at $p<0.05$.

3.3 Antibacterial activity

The antibacterial activity of shallot extracts against some pathogens were tested. The preliminary results by gel diffusion assay showed that crude extracts could inhibit growth of only *S.* Typhimurium TISTR292 and *S. aureus*, but the purified extracts could not. Unfortunately, it was clear that shallot extract lost its antibacterial activity during our FOS purification processes similar to its anti-oxidation ability. The antibacterial compounds in *A. ascalonicum* include quercitin, diallyl disulfide, trisulfide, tetrasulfide, and so on (Mnayer et al., 2014; Sharift-Rad et al., 2016; Jaisinghani, 2017). Both flavonoids and sulfide compounds can be adsorbed on carbonaceous absorbent. However, the crude shallot extract was further tested for its minimum concentration to inhibit pathogen growth. The MIC experiment resulted consistently to gel diffusion assay (Table 3). Only growth of *E. coli* was not inhibited. *S. aureus* growth was inhibited by only original concentration of extract (163.80 mg/ml), while the minimum concentration of crude extract for inhibition of *S.* Typhimurium was at the ratio 7:3 (114.66 mg/ml). Thus, it was indicated that MIC values of shallot extract for *S.* Typhimurium and *S. aureus* were 114.66 and 163.80 mg/ml, respectively. Minimum bactericidal concentration (MBC) of the shallot extracts could be determined only in case of *S.* Typhimurium and *S. aureus*. They were tested by enumeration the viable cells in the tube with clear broth. Thus, the tubes with dilution from 7:3 – 10:0 and only 10:0 were tested for *S.* Typhimurium and *S. aureus*, respectively. We found that crude extract could not kill *S. aureus*, while the MBC for *S.* Typhimurium was at the ratio 9:1 (147.42 mg/ml).

Table 3. MIC value of crude extract for inhibiting the growth of *Escherichia coli, Salmonella* Typhimurium TISTR292 and *Staphylococcus aureus*

Dilution factor	The growth of bacteria		
	E. coli	*S.* Typhimurium TISTR292	*S. aureus*
10:0	+	-	-
9:1	+	-	+
8:2	++	-	++
7:3	++	-	++
6:4	++	+	++
5:5	++	++	++
4:6	++	++	++
3:7	++	++	++
2:8	++	++	++
1:9	++	++	++
Positive control	++	++	++

"-" = Clear solution, "+" = Medium turbidity and "++" = Very turbidity

4. CONCLUSION

Even though the product from purification process of shallot FOS by using commercial absorbent was efficient for using as prebiotic ingredient (previous unpublished data), but the process markedly affected on antioxidant and antibacterial activity of shallot extract. Both biological activities had been gradually declined during purification process. However, crude shallot extract possessed high potential to be applied in functional food manufacturing as antioxidative and antibacterial agents. Thus, it could be concluded that crude extract and purified extract might be suitable for different purposes, including prebiotic, antioxidant and antibacterial uses

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