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Optimization of extraction conditions and assessment of antioxidant, α-glucosidase inhibitory and antimicrobial activities of *Xanthium strumarium* L. fruits

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ABSTRACT

In this study, *Xanthium strumarium* L. fruit was extracted and the product obtained was analyzed for its antioxidant, antimicrobial and antidiabetic activities. The effects of solvent (methanol), extraction time and solid/solvent ratios on total phenolic content (TPC), 1,1-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radical scavenging activity and the ferric reducing antioxidant power (FRAP) were investigated. The results indicated that optimal methanol concentration, extraction time and solid to solvent ratio were 60%, 30 min and 1:5, respectively. Corresponding TPC, DPPH and FRAP values were 12.1 mg of GAE/g of sample, 72.5% and 92.6 μM of Fe (II)/g of sample, respectively. Methanol concentration and solid to solvent ratio were found to have significant effects on TPC, DPPH and FRAP values. On the other hand, the fruit extracts demonstrated antibacterial activity against *Staphyloccocus aureus* and *Streptococcus agalactiae*. When tested for antidiabetic potential, the extract exhibited strong α-glucosidase inhibitory effect with IC_{50} value of 15.25 μg/ml. Electrochemical measurements were found to be helpful for determining the content as well as redox properties of the extracted compounds. The results could be of significant importance for industrial applications of the extract in novel plant-derived drugs or functional foods.

1. Introduction

Xanthium strumarium L. (Family: Compositae) is a weed used as traditional medicine in many parts of the world (Ferrer et al., 2014). The plant and its roots, fruits, seeds and leaves possess pharmacological properties, which have been exploited to treat various diseases (Jawad et al., 1988; Kamboj and Saluja, 2010; Kishore et al., 1982; Lee et al., 2002). In general, phytochemicals isolated from *X. strumarium* L. were reported to have high antioxidant, antimicrobial activity and especially very potent hypoglycemic effects (Nguyen et al., 2012; Scherer and Godoy, 2014). Extracts from the plant containing terpenes, d-limonene and d-carveol exhibit strong antifungal activity against both pathogenic and non-pathogenic fungi (Bisht and Singh, 1978). Similarly, the extract demonstrated strong inhibitory effects against selected strains of gram-positive and gram-negative bacteria and the activity was ascribed to the presence of xanthol in its composition (Jawad et al., 1988). On the other hand, *X. strumarium* L. contains high amount of phenolic compounds and flavonoids, which have significant antioxidant activities and free radical scavenging activities (Kamboj and Saluja, 2010; Rad et al., 2013; Sadiq et al., 2017). It has been suggested that antioxidant activity of the extract could play vital role in traditional uses of the plant (Kamboj and Saluja, 2010). Antihyperglycemic effects of *X. strumarium* L. extract have been reported both in vitro and in vivo investigations (Favier et al., 2005; Kupiecki et al., 1974; Nguyen et al., 2012). The effects were attributed to caffeic acids, phenolics and possibly terpenoids present in its fruit. Nguyen et al. (2012) showed that among 38 medicinal plants screened for their α-glucosidase inhibitory activity, *X. strumarium* L being one of the plants displayed the high potency. The extract ability to inhibit α-glucosidase was also reported by Hwang et al. (2016). Recently, there have been growing interests for plant extracts in food, pharmaceutical and cosmetic industries due to concerns of the negative effects of synthetic ingredients on human health and environment (Cavalcanti et al., 2013). Plant-derived traditional medicines were used in primary health care for about 65% of world's population (Cragg et al., 2009) and more than half of currently available drugs are natural compounds or their derivatives (Sánchez-Lamar et al., 2016). Moreover, as the excessive and repeated use of antibiotics results in the development of antibiotic resistant microbial

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strains, the plant-derived extracts may be important sources of new therapeutic agents (Sharifi-Rad et al., 2015). To apply natural products in food or pharmaceutical products, their bioactive compounds need to be separated or concentrated from the raw materials (Cavalcanti et al., 2013). However, to set up an efficient extraction process, there is clear need to understand key factors and their effects on the yield and bioactivity of the extracted compounds. Extraction efficiency is the function of major process conditions such as solvent type, temperature, solvent to feed ratio, contact time, particle size, etc. (Sánchez-Lamar et al., 2016). There are no universal extraction conditions, but optimized process variables need to be developed for particular products. Optimization of a process could be conducted by either empirical or statistical methods (Liyana-Pathirana and Shahidi, 2005). One-factor-at-a-time empirical approach is usually time-consuming and may not account for the interactions between different factors. Response surface methodology (RSM) is a useful statistical tool which can reduce the number of experiments and produce statistically acceptable results (Silva et al., 2007). RSM has been successfully applied to optimize the extraction of phenolic compounds and other active components from various plant materials (Liyana-Pathirana and Shahidi, 2005; Majeed et al., 2016; Silva et al., 2007).

The applications of plant-derived phytochemicals could be greatly facilitated if their redox behaviors and potential correlations with bioactivities were elucidated. Redox behaviors of natural compounds can be readily characterized by electrochemical methods. These approaches are highly preferred due to its simplicity, sensitivity, reduced reagent consumption and assay time (Blasco et al., 2007; Oliveira-Neto et al., 2016). Various studies demonstrated that oxidation potentials of many antioxidants correlated to their antioxidant efficiency (Huang et al., 2004). In addition, antioxidant activity of various extracts such as green pepper, teas, coffee have been evaluated via electroanalysis (Chatterjee et al., 2007; Kilmartin and Hsu, 2003).

The objectives of this study were to optimize the extraction of active compounds from *X. strumarium* L fruits using response surface methodology. Antioxidant, antibacterial activity and α-glucosidase inhibition of the extracts were determined. Correlations between biological activities and redox properties of the extract were also investigated.

2. Materials and methods

2.1. Chemicals and reagents

Gallic acid, α-glucosidase, 2,2-diphenyl-1-picrylhydrazyl, ferric reducing antioxidant power and Folin-Ciocalteu phenol reagents were acquired from Sigma-Aldrich (USA). Mueller Hinton agar was procured from Hi-Media (India). All other chemicals and reagents were of analytical grade and acquired from CT Laboratory Co., LTD (Thailand).

2.2. Sample preparation

X. strumarium L. fruit samples were obtained from a local supplier. The plant samples were identified by Tu Bao Ngan, Vietnam Academy of Sciences and Technology (Vietnam). A specimen voucher (VNMN.TBN.2017.001) was deposited in the Herbarium of National Museum of Nature (Vietnam). The fruits were thoroughly rinsed with sterilized distilled water to remove all dirt from the surfaces. The samples were dried in a hot air oven at 60 °C and then ground into fine powder by a grinder (Philips Co. Ltd., China). The obtained samples were stored at − 20 °C in zip lock bag until further use.

2.3. Optimization of sample extraction

The extraction of active compounds from *X. strumarium* L. fruit was subjected to ultra-sonication at a frequency of 24 kHz. The extraction conditions were optimized by Response surface methodology (RSM) using Design-Expert® software (Minneapolis, MN, USA). The independent variables X_1, X_2 and X_3 were methanol concentration (X_1: 60%, 80% and 100%), time (X_2: 10, 30 and 50 min) and solid to solvent ratio (X_3: 1:5.0, 1:12.5 and 1:20.0 g/ml), respectively. The variables were coded at three levels − 1, 0 and 1 using the Box-Behnken design. Total phenolic content, DPPH radical scavenging activity and FRAP values were determined as the response variables. The optimal extract of fruits were filtered and concentrated by means of a rotary evaporator (Büchi rotavapor R-144, Switzerland) followed by lyophilization for 24 h in a freeze dryer (Scanvac Cool Safe 55-4, Denmark). The freeze-dried extract powders were stored at 4 °C until further use for antimicrobial and antidiabetic tests. The effects of methanol (X_1), time (X_2) and solid-to-solvent ratio (X_3) on the extraction of bioactive compounds from *X. strumarium* L were analyzed using a quadratic polynomial regression model as shown:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j \quad (1)$$

where, Y was the response variable, β_0 was constant, β_i, β_{ii} and β_{ij} were the linear, quadratic and interactive coefficients. X_i and X_j were the independent variables.

2.4. Analyses

2.4.1. Estimation of TPC, DPPH radical scavenging and FRAP

Folin-Ciocalteu reagent was used to determine the total phenolic compounds (TPC) of fruit extracts as described by Alhakmani et al. (2013). Gallic acid was used as a reference standard to develop calibration curve. TPC was expressed as mg of GAE/g of crude sample. The antioxidant activity of the extracts was determined by DPPH radical scavenging activity according to Scherer and Godoy (2014) with slight modifications. The FRAP analysis of the extract was based on procedures reported by Benzie and Strain (1996). Iron (II) sulfate solution was used to prepare the standard curve and the results were expressed as μM Fe (II)/g crude sample.

2.4.2. Phytochemical analysis

The crude plant material was evaluated for the presence of steroids, alkaloids, flavonoids and phenols, saponins, tannins, glycosides, triterpenoids, carbohydrates, coumarin, quinone and terpenoids following the standard methods as described by Doughari et al. (2012) and Farooq et al. (2014).

2.4.3. Gas chromatography mass spectrometer (GC-MS) analysis of fruit extract

The qualitative analysis of *X. strumarium* L. fruit extract was carried out by using gas chromatography system coupled with mass spectrometer (GC-MS) (QP2010, Shimadzu, Japan). The separation of compounds was carried out by using DB-5 fused silica capillary column (60 m × 0.32 mm ID × 0.25 μm film thickness). Helium was used as carrier gas with a 2.0 ml/min flow rate and the injector temperature was maintained at 280 °C (splitless). The oven temperature was programmed with an initial temperature of 60 °C followed by a temperature increase of 10 °C/min up to 200 °C and hold for 5 min. The temperature was further gradually increased (5 °C/min) to 280 °C and subjected to final hold for 25 min. All data were acquired by collecting the full-scan mass spectra within the scan range 45–500 amu. Compounds were recognized using comparison of their mass spectra with the NIST and Wiley GC-MS library.

2.4.4. In vitro evaluation of biological activities of X. strumarium L. fruit extracts

2.4.4.1. Antibacterial activity. The antibacterial activity was tested following the procedure of Ramakrishnan et al. (2011) with some modifications. Mueller Hinton Agar plates were inoculated with 18 h old broth cultures (adjusted to 0.5 McFarland standard) of

Staphylococcus aureus and *Streptococcus agalactiae*. Wells (8 mm dia. and about two cm a part) were made on each of the plates using sterile cork borer. The stock solutions of fruit extracts were prepared at different concentrations (50–200 μg/ml). The sample without plant extract was used as the negative control whereas tetracycline was used as positive control. The plates were incubated at 37 °C for 24 h and diameters of the inhibition zone (IZ in mm) were measured.

2.4.4.2. Antidiabetic activity. The antidiabetic activity was estimated by α-glucosidase inhibition assay, following the method described by Kim et al. (2008). The IC_{50} value was defined as the concentration of α-glucosidase inhibitor that inhibited 50% of α-glucosidase activity. Antidiabetic activity was evaluated by non-linear regression (dose-response analysis).

2.5. Electrochemical measurement

Electrochemical measurements were carried out at 25 °C in using a portable potentiostat (DY2000, Digi-Ivy, USA). Screen-printed carbon electrodes with working electrode diameter of 3.0 mm (Quasense Co. Ltd., Thailand) were used in this study. The fruit extract was dissolved in 0.1 M KCl (pH 7) at different concentrations (10–400 μg/ml). The cyclic voltammetry was recorded in the potential ranges from − 0.2 to 1.2 V at scan rate of 50 mV s^{-1}.

2.6. Statistical analysis

The data were determined in triplicate and average results were presented. The analysis of variance (ANOVA) was conducted and the effect and regression coefficients of terms in Eq. (1) were determined. $P < 0.05$ was regarded as significant. IC_{50} values were estimated by implementing non-linear regression, log (inhibitor concentration) vs. normalized response model with a variable slope (Sadiq et al., 2015) using GraphPad Prism® version 6.01 (San Diego, US).

3. Results and discussion

3.1. Optimization of ultrasound-assisted extraction of bioactive compounds from X. strumarium L. fruits

The effects of extraction conditions on TPC, DPPH inhibition and FRAP values are presented in Table 1. The results indicated that the optimal extraction was at methanol concentration of 60%, extraction time of 30 min and solid/solvent ratio of 1:5. Corresponding TPC, DPPH inhibition and FRAP values were 12.1 mg GAE/g of sample, 72.5% and 92.6 μM of Fe (II)/g sample, respectively.

3.1.1. Effect of process variables on total phenolic content

Total phenolic contents obtained were from 3.31 to 12.4 mg of GAE/g (Table 1). Analysis of variance (ANOVA) (Table S1-Supplementary information) was performed on the experimental data and the coefficients of model were evaluated to estimate the influence of process parameters on TPC of the extract. The results showed that, effect of methanol concentration was highly significant for extracting TPC from *X. strumarium* L. fruits.

The relationship between TPC and main parameters of the extraction process is given as below:

$$\begin{aligned}\mathbf{TPC\ (mg\ GAE/gram)} = {} & \mathbf{9.41 - 3.33 \times_1 + 0.81 \times_2 - 0.77 \times_3} \\ & \mathbf{- 0.50 \times_1 \times_2 + 0.43 \times_1 \times_3 - 0.85BC} \\ & \mathbf{- 2.02 \times_1^2 - 0.047 \times_2^2 + 0.024 \times_3^2} \end{aligned} \quad (2)$$

The R^2 value of the model was estimated to be 0.961 (Table S1) and the lack of fit for TPC was not significant ($P > 0.05$) so the model can be used to predict the responses.

Changes in TPC in response to variations in methanol concentration, extraction time and solid to solvent ratio are presented in 3D response surface plot (Fig. 1). According to Tan et al. (2013), the extraction of phenolic compounds from plant material was directly related to the compatibility of the phenolic compounds to the solvent and thus, when the compounds were well matched in polarity with the solvent, they could be easily extracted. A combination of alcohol with water was more effective in extracting phenolic compounds than alcohol alone (Prasad et al., 2011). When methanol concentration decreased from 100% to 60%, increase in the phenolic content from 3.31 to 12.40 mg GAE/gram of raw extract was observed. These results were similar to those of Mussatto et al. (2011), who reported that TPC of spent coffee grounds increased with decreasing methanol concentration and increasing solid/solvent ratio. Aqueous mixture of solvents are more efficient in the extraction of phenolic compounds than the mono-solvent systems (Spigno et al., 2007). The increase in extraction time had slightly positive effect on TPC.

3.1.2. Effect of process variables on DPPH radical scavenging activity

Under studied conditions, the antioxidant activities based on DPPH inhibition ranged from 56.00% to 93.68% (Table 1). The ANOVA analysis (Table S2) showed that dominant factors affecting antioxidant activity of the extracts were methanol and solid/solvent ratio.

Table 1
Experimental TPC, DPPH and FRAP values of *Xanthium strumarium* L. fruit extract.

Independent variables				Response variables[1]		
Experiments	Methanol (%)	Time (min)	Solid/solvent ratio (g/ml)	TPC (mg GAE/g of sample)	DPPH inhibition (%)	FRAP (μM Fe (II)/ g sample)
1	60	10	1 :12.5	9.25 ± 0.10	71.38 ± 3.23	54.70 ± 0.23
2	100	50	1 :12.5	4.46 ± 0.04	91.67 ± 1.19	37.40 ± 2.48
3	80	50	1 :20.0	8.65 ± 0.21	89.03 ± 0.28	45.90 ± 1.21
4	80	30	1 :12.5	10.80 ± 0.23	89.40 ± 0.92	58.20 ± 0.09
5	100	30	1 :5.00	4.92 ± 0.14	93.68 ± 0.56	46.20 ± 4.03
6	80	30	1 :12.5	8.74 ± 0.20	91.48 ± 0.59	50.50 ± 0.65
7	80	10	1 :5.00	8.46 ± 0.08	92.65 ± 0.73	58.30 ± 1.40
8	80	10	1 :20.0	9.27 ± 0.53	87.75 ± 0.10	53.80 ± 2.83
9	60	30	1 :20.0	9.08 ± 0.22	73.16 ± 1.02	51.20 ± 1.12
10	100	10	1 :12.5	3.31 ± 0.11	91.30 ± 0.56	29.90 ± 0.28
11	80	50	1 :5.00	11.20 ± 0.03	91.67 ± 0.38	72.30 ± 12.0
12	80	30	1 :12.5	8.66 ± 0.04	90.80 ± 0.36	64.20 ± 7.86
13	100	30	1 :20.0	3.58 ± 0.08	90.44 ± 0.84	28.90 ± 0.75
14	60	50	1 :12.5	12.40 ± 0.02	56.00 ± 6.01	55.50 ± 0.77
15	60	30	1 :5.00	12.10 ± 0.04	72.50 ± 2.19	92.60 ± 9.90

[1] All values are presented as mean ± SD (standard deviation) of three individual determinations. TPC = total phenolic content; DPPH = 1,1-diphenyl-1-picryl-hydrazyl-hydrate; FRAP = ferric reducing antioxidant power.

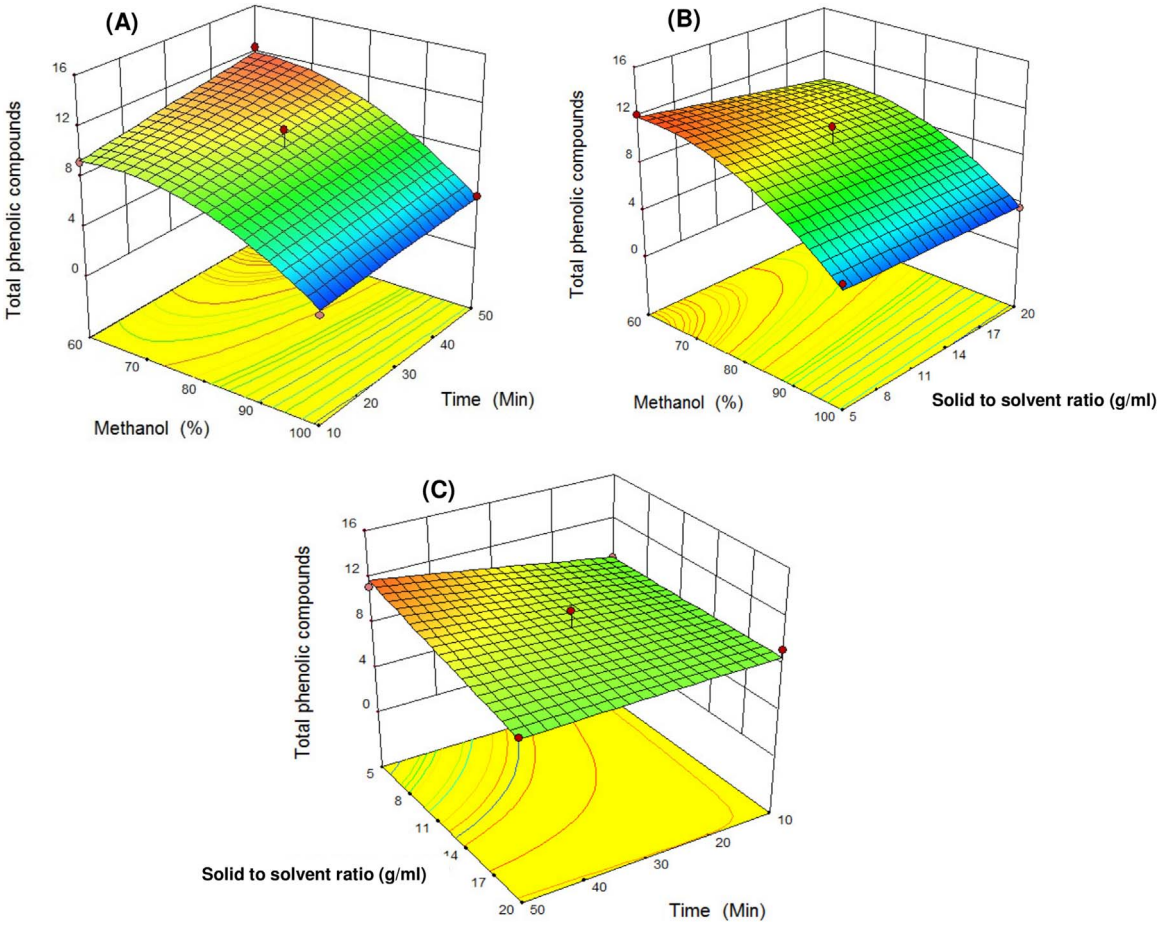

Fig. 1. Response surface plots showing the interactive effects of methanol (X1), time (X2), and solid to solvent ratio (X3) on the TPC (total phenolic content).

Extraction time was not a significant parameter. Increase in methanol concentration (60–100%) and reduction in ratio of solid to liquid (20.0–5.0 g/ml) resulted in enhanced DPPH inhibitory activity of the extract (Fig. 2). Eq. (3) described the relationship between antioxidant activity and key extraction factors:

$$\textbf{Antioxidant activity (DPPH)} = 36.23 + \mathbf{5.96}\,X_1 - \mathbf{0.83}\,X_2 - 33.18\,X_3 + \mathbf{1.57}\,X_1X_2 - \mathbf{4.20}\,X_1X_3 + \mathbf{0.33}\,X_2X_3 - \mathbf{5.27}\,X_1^2 + \mathbf{0.076}\,X_2^2 + 20.83X_3^2 \quad (3)$$

For DPPH scavenging activity, the lack of fit was not significant ($p > 0.05$) and the obtained R^2 value of the model was 0.997.

3.1.3. Effect of process variables on FRAP antioxidant activity

The FRAP values for different extraction treatments were found to range from 28.9 to 92.6 μM of Fe (II)/gram of crude plant material (Table 1). Relationship between FRAP values of *X. strumarium* L. fruit extracts and experimental variables was provided in Fig. 3. It was noted that FRAP antioxidant activity was mainly affected by concentrations of methanol and solid to solvent ratios (Table S3). The extraction of bioactive compounds from plant materials was considerably affected by solid/solvent ratio. A low ratio is conducive to oxidation of phenolics, and more liquids may contain more dissolved oxygen, which exacerbates oxidation process with extended extraction time (Bey et al., 2013). The effects of extraction conditions on FRAP values were described by Eq. (4):

$$\textbf{Antioxidant activity (FRAP)} = 57.61 - 13.96\,X_1 + \mathbf{1.81}\,X_2 - 11.19\,X_3 + \mathbf{1.70}\,X_1X_2 + \mathbf{6.04}\,X_1X_3 - \mathbf{5.47}\,X_2X_3 - \mathbf{8.05}\,X_1^2 - \mathbf{5.19}\,X_2^2 + \mathbf{5.16}X_3^2 \quad (4)$$

3.2. Phytochemical analysis

Analysis of phytochemicals in *X. strumarium* L. fruit extracts indicated the presence of alkaloids, flavonoids, triterpenoids, terpenoids, tannins, saponins, quinone, coumarin, carbohydrates, glycoside and phenol (Table 2).

3.3. GC-MS analysis of fruit extract

Gas chromatography–mass spectrometry (GC–MS) analysis of *X. strumarium* L. fruit extract was carried out to identify organic compounds and overall, 15 metabolites were identified (Table 3). The major phytoconstituents were 2-methoxy-4-methyl-10H-acridin-9-one (16.55%), 4-cyclopropylmethylbenzonitrile (14.65%), methyl linoleate (8.28%), 1-butyl (dimethyl) silyloxypropane (7.44%), decanoic acid (7.04%), 3-hydroxymethyl-2-trimethylsilyloxypentane (6.85%) and ethyl linoleate (4.02%). 2-methoxy-4-methyl-10H-acridin-9-one (16.55%) is a derivative of acridone which are well known for anticancer, antimicrobial, antifungal, antimalarial, antiviral and anti-inflammatory potentials (Parikh et al., 2011; Sepulveda et al., 2013). Decanoic acid (7.04%) and its derivatives hexadecanoic acid methyl ester (0.92%) and octadecanoic acid, methyl ester (0.62%) were reported to associate with antibacterial and antifungal activities (Abubakar and Majinda, 2016; Chandrasekaran et al., 2011). Lin et al. (2014), analyzed ethanolic extract of *Xanthium strumarium* L. fruits by

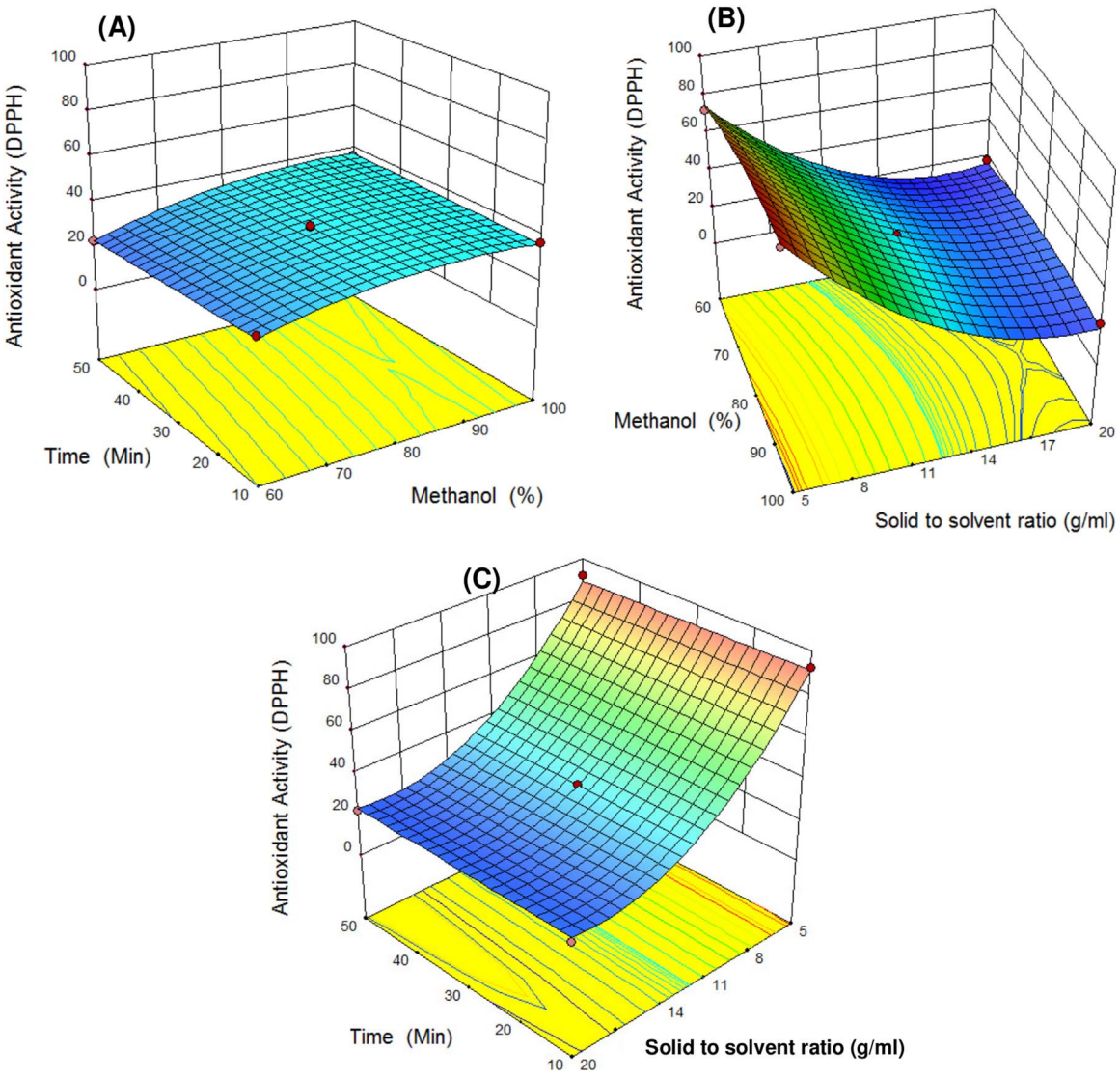

Fig. 2. Response surface plots showing the interactive effects of methanol (X1), time (X2), and solid to solvent ratio (X3) on the antioxidant capacity using DPPH (1,1-diphenyl-1-picryl-hydrazyl-hydrate) inhibition assay.

liquid chromatography-mass spectrometry (LC-MS) and identified the following phenolic compounds; 1-O-caffeoylquinic acid, 3-O-caffeoylquinic acid, chlorogenic acid, 4-O-caffeoylquinic acid, 1,3-O-dicaffeoylquinic acid, 1,4-O-dicaffeoylquinic acid, 1,5-O-dicaffeoylquinic acid, 4,5-O-dicaffeoylquinic acid, 1,3,5-O-tricaffeoylquinic acid and 3,4,5-O-tricaffeoylquinic acid.

3.4. Evaluation of biological properties of *X. strumarium* L. fruit extract

3.4.1. Antibacterial activity

The fruit extracts were tested for antimicrobial properties against *Staphyloccocus aureus* and *Streptococcus agalactiae* (Table 4). It was observed that the increasing concentration of the extract resulted in increased inhibition zone against selected pathogens. According to Rad et al. (2013), methanol extract of *X. strumarium* L. fruit exhibited significant inhibitory activity against the growth of all tested bacterial pathogens and most effective results were reported against *Staphylococcus aureus*.

3.4.2. In vitro evaluation of antidiabetic activity

The in-vitro α-glucosidase inhibitory activity of the *X. strumarium* L. fruit extracts was shown in Table 5. The highest and lowest inhibition values were 95.02% and 37.67% at 400 µg/ml and 10 µg/ml, respectively and inhibition increased with extract concentration (Fig. 4). The IC_{50} and Hillslope values of fruit extract for α-glucosidase inhibition were 15.25 µg/ml (12.90–17.61) and 0.036 µg/ml (0.025–0.046), respectively at 95% confidence interval. The IC_{50} value of *X. strumarium* fruit extract was found to be lower than acarbose (positive control) for α-glucosidase inhibition, which was in accordance with the previous studies (Hyun et al., 2016; Nguyen et al., 2012).

Glycosidase is a hydrolytic enzyme that plays a vital role in digestion of carbohydrates and biosynthesis of glycoproteins. Inhibitors of α-glucosidase may potentially reduce the progression of diabetes by decreasing the digestion and absorption of carbohydrates. According to Nguyen et al. (2012), the fruits of *X. strumarium* showed various biological activities such as antibacterial, antitumour, anticancer, anti-inflammatory, and hypoglycemic activity. However the IC_{50} value of fruit extract for α-glucosidase inhibition was lower in the current study than the previous reports (Nguyen et al., 2012). Natural polyphenols have been reported to inhibit the activity of carbohydrate hydrolyzing enzymes, such as α-amylase and α-glucosidase, because of their ability to bind with proteins (Hyun et al., 2016). The results illustrated that methanol extract of *X. strumarium* fruit efficiently inhibited α-glucosidase enzymes in vitro and showed capability of reducing diabetic

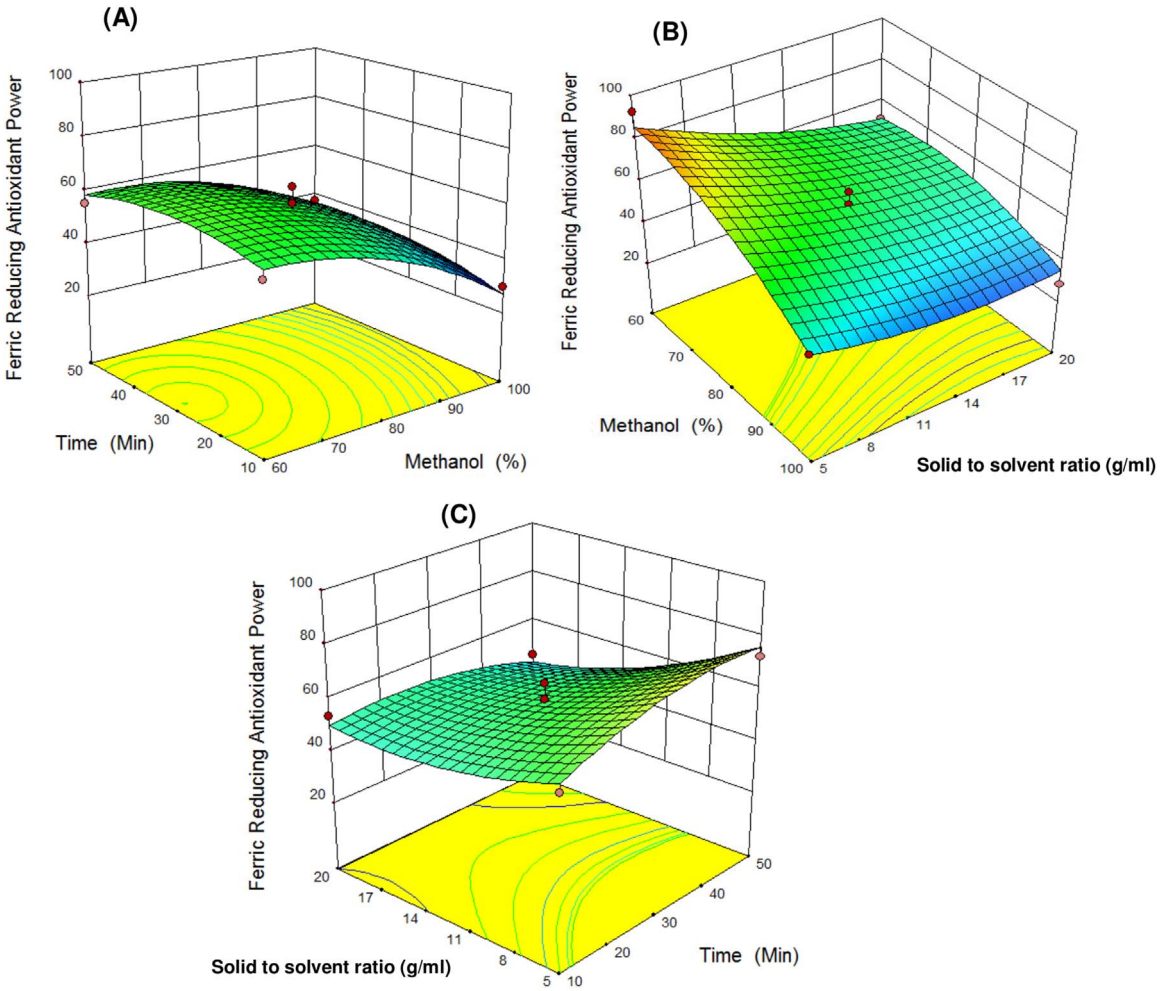

Fig. 3. Response surface plots showing the interactive effects of methanol (X1), time (X2), and solid to solvent ratio (X3) on the antioxidant capacity using FRAP (ferric reducing antioxidant power) assay.

Table 2
Phytochemicals present in *X. strumarium* fruit extract.

Compounds	Fruit extract
Alkaloids	+
Flavonoids	+
Triterpenoids	+
Terpenoids	+
Tannins	+
Saponins	+
Quinones	+
Coumarins	+
Carbohydrates	+
Glycosides	+
Steroids	–
Phenolics	+

Where (+) means present and (−) means absent.

effects.

3.5. Electrochemical behavior of X. strumarium L. fruit extract and correlation with biological activities

The electrochemical behavior of *X. strumarium* L. fruit extract is demonstrated in Fig. 5. In general, the extracts exhibited irreversible oxidation peak around 0.25–0.35 V. Oxidation potential is connected to antioxidant activity of the phenolic compounds and lower the oxidation potential of phenolic compound, the higher is electron donor ability and antioxidation activity (Gil and Couto, 2013). The antioxidant activity of compounds obtained was as high as that of strong antioxidants such as myricetin, quercetin, catechin and epicatechin (E_p: 0.30–0.33 V). Hence, *X. strumarium* L. fruit could be a potential source of highly potent antioxidants. The results also showed that peak oxidation currents (I_p) correlated with concentrations of the extract in the solution (Fig. 5 inset). Therefore, electrochemical measurements were further attempted to determine the content of active compounds and their activities in the extracted products. The correlation analysis showed that TPC exhibited strong relationship with I_p (R^2 = 0.980) whilst α-glucosidase inhibitory activity did not seem to correlate with the measured I_p values (R^2 = 0.655) (Fig. 6).

The trend was explainable as α-glucosidase inhibitory reaction in this case may be mainly based on the bindings of extracted compounds with proteins (Griffiths and Moseley, 1980) while TPC values were estimated from the oxidation of phenolic compounds which is similar to the electron donation-acceptance process on electrode surface. The results demonstrated that electrochemical measurement could be used to quickly evaluate the content of the active phenolic compounds in *X. strumarium* L. fruit extract.

4. Conclusion

The extraction of *X. strumarium* fruit was optimized using response surface methodology. The effects of key process parameters including, solid/solvent ratio and extraction time were evaluated and optimal conditions were presented. The content of extracted phenolic

Table 3
Major compounds in *Xanthium strumarium* L. fruit extract identified by GC-MS analyses.

Peak	Retention time (min)	Area (%)	Compound name	MW	Molecular formula
1	18.22	1.05	2-Aminobutanoic acid	103	$C_4H_9NO_2$
2	20.32	7.44	1-Butyl (dimethyl) silyloxypropane	174	$C_9H_{22}OSi$
3	22.08	0.58	2-Propenoic acid, 2-methyl-, 2-(dimethylamino) ethyl ester	157	$C_8\ H_{15}\ N\ O_2$
4	22.73	2.28	2,4,5,6,7-Pentamethoxyheptanoic acid, methyl ester	294	$C_{13}H_{26}O_7$
5	23.85	6.85	3-Hydroxymethyl-2-trimethylsilyloxypentane	190	$C_9H_{22}O_2Si$
6	28.94	14.65	4-Cyclopropylmethylbenzonitrile	157	$C_{11}H_{11}N$
7	30.61	7.04	Decanoic acid	172	$C_{10}\ H_{20}\ O_2$
8	30.91	4.61	6-Desoxy-l-gulitol	166	$C_6H_{14}O_5$
9	35.20	0.92	Hexadecanoic acid, methyl ester	270	$C_{17}\ H_{34}\ O_2$
10	38.42	8.28	Methyl linoleate	294	$C_{19}\ H_{34}\ O_2$
11	38.87	0.62	Octadecanoic acid, methyl ester	298	$C_{19}\ H_{38}\ O_2$
12	39.64	4.02	Ethyl linoleate	308	$C_{20}\ H_{36}\ O_2$
13	41.56	16.55	2-Methoxy-4-methyl-10H-acridin-9-one	239	$C_{15}H_{13}NO_2$
14	46.54	1.13	Carbamic acid, 2-(dimethylamino)ethyl ester	132	$C_5H_{12}N_2O_2$
15	52.72	1.37	3-n-Butylthiophene-1,1-dioxide	172	$C_8H_{12}O_2S$

Table 4
Antimicrobial activity of *X. strumarium* L. fruit extracts.

Concentration (μg/ml)	Diameter of inhibition zone (mm)	
	Streptococcus agalactiae	***Staphylococcus aureus***
50	11.9 ± 0.1	13.1 ± 1.4
75	11.8 ± 0.6	14.2 ± 0.9
100	11.9 ± 0.4	14.8 ± 1.2
125	12.9 ± 0.3	14.7 ± 0.4
150	13.1 ± 0.3	16.3 ± 1.8
200	17.1 ± 0.4	18.7 ± 3.3
Tetracycline (30 μg)	18.6 ± 1.8	21.8 ± 0.7

Results were expressed as mean ± S.D (standard deviation).

Table 5
α-glucosidase inhibitory activity of *X. strumarium* fruit extract.

Sample	Concentration (μg/ml)	Inhibition (%)
Xanthium strumarium L. fruit extracts	10.0	37.67 ± 0.01
	12.5	54.69 ± 0.04
	25.0	72.04 ± 0.02
	50.0	82.71 ± 0.02
	100.0	87.95 ± 0.02
	200.0	92.29 ± 0.01
	400.0	95.02 ± 0.01

Results expressed as mean ± SD (standard deviation).

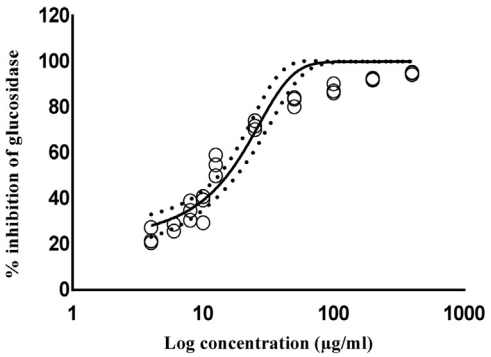

Fig. 4. Estimated α-glucosidase inhibition of *X. strumarium* extract at different concentrations, using non-linear regression. Open circles represent observations, the solid lines represent the estimated mean curves, and the dotted lines represent the 95% confidence intervals of the mean estimates.

compounds and their properties were mainly influenced by methanol concentration and solid/solvent ratios. The extract was found to have significant antimicrobial and antioxidant activity under studied

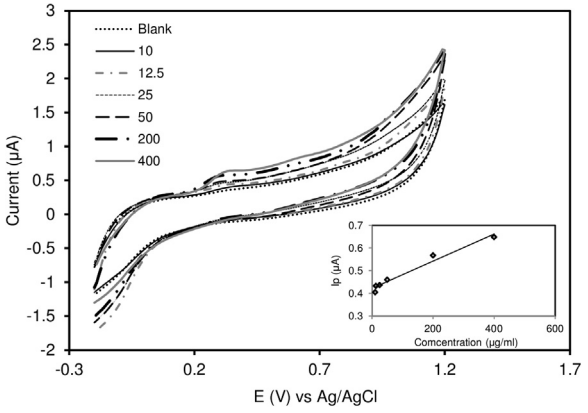

Fig. 5. Cyclic voltammograms of the extracts at different concentrations in 0.1 M KCl (pH 7.00). Scan rate: 50 mVs-1 and voltage range: − 0.2 to 1.2 V.

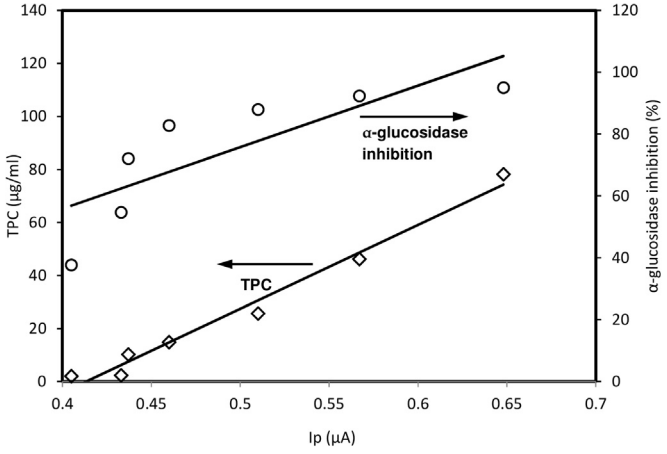

Fig. 6. Correlation between peak oxidation current, total phenolic content (TPC) and α-glucosidase inhibition.

conditions. In addition, *X. strumarium* L. extract exhibited very strong α-glucosidase inhibitory effect with IC_{50} value of 15.25 μg/ml. Electrochemical behavior of the extract was characterized and oxidation peak current could be a viable method to determine the properties and content of active compounds present in the extract. The findings could be helpful for industrial extraction and applications of *X. strumarium* fruit in potential pharmaceutical products.

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Conflict of interest

Authors declare no conflict of interest.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bcab.2018.02.004.

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