Screening of phytochemicals and Antibacterial activities of various extracts of Sadao din (*Glinus oppositifolius*)

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Abstract

Sadao din (*Glinus oppositifolius*) pertained to the Molluginaceae family is an herb and widely distributed almost all over Thailand. These plants had been used in local medicine for the treatment of many ailments such as diarrhea, fever and malaria**.** In this work, the aerial parts of the plant *G. oppositifolius* was prepared into powder. The powder was extracted with various solvents viz water, ethanol, ethyl acetate and chloroform. Then, the extracts were evaporated for analysis of phytochemicals, total phenolic content (TPC) and antibacterial activities. The phytochemical screening showed that alkaloids and flavonoids were presented in all of the extracts, whereas saponins and tannins were found in aqueous and ethanol extracts. The amount of TPC varied in different extract, moreover, ethanol extract showed the maximum TPC of 142.22±12.51 mg of GAE/g. Furthermore, the extracts were tested the *in vitro* antibacterial activity against seven bacteria namely *Bacillus cereus, B. subtilis, Enterobecter aerogenes, Escherichia coli, Pseudomonas aeruginosaj, Staphylococcus aureus* and *S. epidermidis* using disc diffusion technique. The result showed that *B. cereus* and *B. subtilis* were inhibited at the concentration of 2 mg/disc of ethyl acetate extract. The study provided a scientific basis for using of the plant extracts in the retaining of microbial-induced aliments.

Keywords: *Antibacterial activity, Bacillus subtilis, Ethyl acetate, Glinus oppositifolius, Phytochemicals, Total phenolic content.*

1. Introduction

The increasing of bacterial infections is due to the bacteria resistant to many antibacterial agents (Akroum, 2017). The antibacterial agents comprise a large and diverse group of drugs used to treat bacterial infections. The increasing of the usage of antibacterial agents has resulted in the evolution of bacteria to resist the drugs. Searching of newer antibiotics to treat bacterial infection has been the choice to overcome the resistance of the pathogens during treatment several antibacterial agents. (Kalidindi et al., 2015). The medicinal plants seem to be rich in secondary metabolites that used in folk medicine to treat many ailments (Radhia et al., 2018). Various bioactive substances, such as alkaloids, flavonoids, glycosides, saponins and tannins are contained in the plant. Many reports have reported that these phytochemical compounds possess antifungal, antibacterial, antidiarrhoeal, antiviral, anti-inflammatory and antidiabetic activities (Martin-Puzon & Rivera, 2015).

Extraction is the process that bioactive substance may be obtained from the plant. Phytochemicals and bioactivity of the extract is not only affected by the extraction but also by the solvents. Many solvents such as water, ethanol, ethyl acetate and chloroform, have been used for extraction of bioactive compound, due to the variety of the compounds consisted in plant differing solubility properties in different solvents (Truong et al., 2019).

The *Glinus oppositifolius*, the local name is Sadao din, is included in the family of Molluginaceae. This plant is widely spread throughout all regions of Thailand. The shoot or whole plant of *Glinus oppositifolius* is eaten as a vegetable. Moreover, there are reports that tannins, phlabotannins, alkaloids, saponins, flavonoids, steroids, terpenoids, cardiace glycosides glinosides A and B were found in this plant (Chhanda, Muslim, & Rahman, 2014; Traore et al., 2000). Many studies revealed for biological activity from the extract of the *G. oppositifolius* such as, antihyperglycemic (Hoque et al., 2011), antioxidant (Martin-Puzon & Rivera, 2015), antidiarrhoeal (Pattanayak et al., 2012) and antithelmintic (Pattanayak et al., 2011) activities. Furthermore, this plant has been used for treatment of dyspepsia, improve digestion, skin diseases,

stimulate the action of liver, promote the menstrual discharge in women and used as blood (Pattanayak et al., 2011). However, previous reports have showed that phytochemicals and bioactivities of the same genus or species of plant could be different when the sampling was done form different areas (Tiwari & Cummins, 2013).

2. Objectives

This study aimed to evaluate the phytochemicals and examine of antibacterial activities of the various extracts obtained from *Glinus oppositifolius* against seven common pathogens and food spoilage microorganisms.

3. Materials and Methods

3.1 Plant sample

The aerial parts of the plant were washed 3-4 times with running tap water and cut into small pieces. The plant material was dried at 50℃ in a hot air oven for 2 days, and grinded to powder. Then the powder was extracted with different solvents (water, ethanol, ethyl acetate and chloroform). *G. oppositifolius* extract was prepared by brewing 40 g of dried sample in distilled water of 500 ml for 30 min twice and separately with ethanol, ethyl acetate and chloroform using a soxhlet tool for 4 hours. The extract was separated using filter paper, and then concentrated by evaporator. All extracts were stored at 4 °C until further used. The yield of extract, which calculated by weight of dried extract divided by weight of dried plant sample and multiply with 100, was used as an indicator of the various solvents.

3.2 Screening of phytochemicals

The extract was detected in the presence of alkaloids (Mayer's reagent and Wagner's reagent), saponins (Froth test) and flavonoids (Shinoda test) according to Yadav, and Agarwala (2011). Antraquinone glycosides (Borntrager's test), cardiac glycosides (Keller killiani test), tannins (ferric chloride test) and triterpenoids (Salkowski test) in the extract were detected according to De, Dey, and Ghosh (2010).

3.3 Determination of total phenolic content

Total phenolic content (TPC) in the extract was determined by a method explained by Prior, Wu, and Schaich (2005). In brief, 100 µl of 2.5 µg/ml extract were mixed with 2 ml of 2% Na_2CO_3 and incubated for 2 minutes at room temperature. After incubation, 100 µl of Folin-Ciocalteu reagent mixed with methanol in the ratio 1:1 (v/v) were added to the mixture and incubated for 30 minutes at room temperature. The absorbance at 750 nm was determined using spectrophotometer. The concentration of TPC calculated as mg gallic acid equivalents (GAE) per g extract using gallic acid as a standard.

3.4 Antibacterial activities

3.4.1 Bacterial strains

Antibacterial activities were tested against bacteria strains namely *Bacillus subtilis* TISTR 008, *B. cereus* TISTR 687, *Enterobacter aerogenes* TISTR 1540, *Escherichia coli* TISTR 780, *Pseudomonas aeruginosa* TISTR 781, *Staphylococcus aureus* TISTR 1466 and *S. epidermidis* TISTR 518 that obtained from the Thailand Institute of Scientific and Technological Research.

3.4.2 Agar disc diffusion

The antibacterial activity was evaluated by agar disc diffusion method (Rabe & Staden, 1997). The strains were cultured in a Mueller-Hinton broth (MHB) for 18 hour at 37°C. Then, 100 µl of the bacteria at the concentration of 10^8 CFU/ml were spread on the agar plate containing Mueller-Hinton agar (HMA) medium. Sterilized filter paper discs (6 mm in diameters was dropped with 20 µl of the extract, which was dissolved in dimethyl sulfoxide at various concentrations of 0.1, 0.2 and 0.3 g/ml. The final concentration of the extract correlating to 2, 4, and 6 mg/disc was achieved, respectively. After that, the discs was set on the HMA plate. Tetracycling (30 µg) and dimethyl sulfoxide were used as a positive and negative control,

respectively. All of the plates were done in triplicate, and incubated for 24 hour at 37°C. Antibacterial activity was determined after incubation and reported in the mean of the zone of inhibition (mm).

3.4.3 Minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC)

Evaluation of MIC against bacteria (10^8 CFU/ml) was using broth dilution method in MHB. The lowest concentration of the extract with no growth of bacteria after incubation (37°C, 24 h) was achieved as MIC value. The MBC evaluation was carried out by subculturing 100 µl for each tube from MIC assay with no visible bacterial growth to new MHA plate followed by incubation (37°C, 24 h) again and was carried out in triplicate. The lowest concentration of the extract that killing the bacteria was defined as the MBC value.

4. Results and Discussion

4.1 The yield of extraction

Yields of difference extraction are revealed in Table 1. Aqueous extract contains the highest yield (17.43% w/w) followed by ethanol (11.76% w/w), chloroform (3.21% w/w) and ethyl acetate (2.26% w/w). This result revealed that the variety in the yields of extraction was attributed to the polar difference of composition contained in the plants (Javaprakasha, Singh, & Sakariah, 2001). Therefore, it could be reported that the secondary metabolites in *G. oppositifolius* was more extracted by polar solvent (water and ethanol) than extracted by semi-polar (ethyl acetate) and non-polar (chloroform) solvents.

Table1 Yield of the extracts of *G. oppositifolius*

Solvents	Yield (%, w/w)
Water	17.43
Ethanol	11.76
Ethyl acetate	2.26
Chloroform	3.21

4.2 Screening of phytochemicals

The screening of phytochemicals was carried out with water, ethanol, ethyl acetate and chloroform of the extract. The results of the screening represented that all of the extracts consists of alkaloids and flavonoids (Table 2), as tannins and saponins were presented in only the aqueous and ethanol extract. The results represented that the aqueous and ethanol extract were rich in alkaloids, flavonoids, saponins and tannins that corresponding to other studies, who reported that the extract of *G. oppositifolius* contains alkaloids, flavonoids, saponins, and tannins (Chhanda, Muslim, T., & Rahman, 2013; Ramaseshan et al., 2016; Hoque et al., 2011).

Table 2 Screening of Phytochemicals of the extract of *G. oppositifolius*

Phytochemicals	**Tests**	**Solvents**			
		Water	**Ethanol**	**Ethyl acetate**	**Chloroform**
Alkaloids	Mayer's test	+	+	+	+
	Wagner's test	+	+	+	+
Antraquinone glycosides	Borntrager's test	-	-	-	-
Cardiac glycosides	Keller killiani test	-	-	-	-
Flavonoids	Shinoda's test	+	+	+	+
Saponins	Froth test	+	+	-	-
Tannins	Ferric chloride test	+	+	-	-
Triterpenoids	Salkowski's test	-	-	-	-

(+) presence of the phytochemical, (-) absence of the phytochemical

4.3 Total phenolic contents

The amount of phenolic contents varied in different extracts, the highest of total phenolic contents of 142.22±12.51 mg of GAE/g was found in ethanol extract, followed by ethyl acetate (117.25±0.39 mg of GAE/g), chloroform (116.41±2.36 mg of GAE/g) and aqueous (111±0.33 mg of GAE/g) extract, respectively. This resulted maybe owing to the amount of TPC depend on the using of solvent that corresponding to Usman, Bakar & Mahamed (2016), Victor & Grace (2013) and Shalavadi et al. (2019). They found that TPC was more soluble in polar organic solvents (ethanol) than in aqueous solvent (water), semi-polar solvent (ethyl acetate) and non-polar solvent (chloroform).

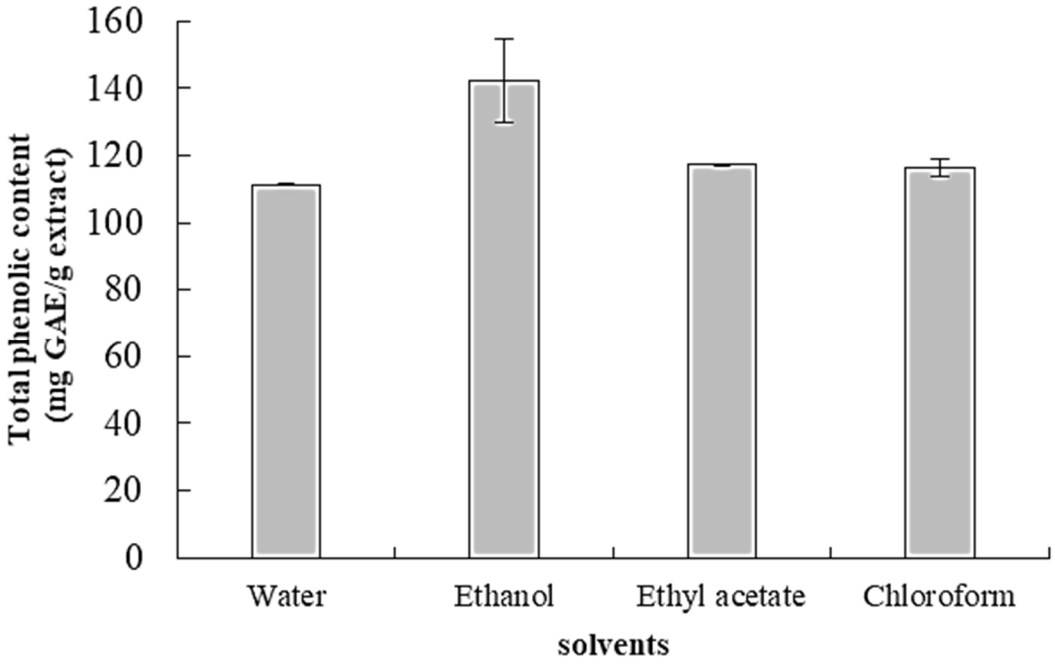

Figure 1 Total phenolic contents of *G. oppositifolius*

4.4 Antibacterial activities

The antibacterial activities of the extracts of *G. oppositifolius* were tested against seven strains as described above (Table 3). The results represented that all of tested bacteria was not inhibited by aqueous extract, while only *B. subtilis* and *B. cereus* were inhibited by ethanol and ethyl acetate. Moreover, *B. cereus* was less sensitive to ethyl acetate extract than *B. subtilis* with lower diameter of the zone of inhibition. The highest antibacterial activity was achieved against *B. subtilis* at 2 mg/disc (7.25 mm), while the control (tetracycline 30 µg) was 21 mm. Although, the best total phenolic contents was obtained from the ethanol extract, the best antibacterial activity was achieved from ethyl acetate extract. This result might be thanks to the low amount of phytochemicals in ethanol extract comparing to the ethyl acetate extract. For this reason, the ethyl acetate extract was chosen for MIC and MBC determination.

The MIC values of ethyl acetate extract were 5 and 10 mg/ml for *B. subtilis* and *B. cereus*, respectively (Table 4). The results showed that MBC of ethyl acetate extract were more than 20 mg for both of *B. subtilis* and *B. cereus*. This result might be due to ethyl acetate extract consisting of alkaloids and flavonoids, which possess antibacterial activities (Afolabi, Akinmoladun, & Dan-Ologe, 2007; Shan et al. 2007; Ogunkunle & Tonia, 2006; Okwu, 2004).

5. Conclusion

In the present investigation, the results from the phytochemical screening of the aerial parts extract (aqueous, ethanol, ethyl acetate and chloroform) of Sadao din (*G. oppositifolius*) revealed the potentials for developing antimicrobial substances from secondary metabolites such as alkaloids and flavonoids. The presence of secondary metabolites in Sadao din makes the plant advantageous for the repairing of aliments.

Table 3 Antibacterial activities of *G. oppositifolius*

Solvents	Stains	Zone of inhibition (mean ± SD)		
		2 mg/disc	4 mg/disc	6 mg/disc
Water	*B. cereus*	-	-	-
	B. subtilis	-	-	-
	E. aerogenes	-	-	-
	E. coli	-	-	-
	P. aeruginosa	-	-	-
	S. aureus	-	-	-
	S. epidermidis	-	-	-
Ethanol	*B. cereus*	-	7.87±0.44	9.12±0.59
	B. subtilis	-	7.67±0.45	9.56±0.98
	E. aerogenes	-	-	-
	E. coli	-	-	-
	P. aeruginosa	-	-	-
	S. aureus	-	-	-
	S. epidermidis	-	-	-
Ethyl acetate	*B. cereus*	6.83±0.71	8.03±0.67	9.68±0.91
	B. subtilis	7.25±0.57	8.46±0.57	9.84±0.69
	E. aerogenes	-	-	-
	E. coli	-	-	-
	P. aeruginosa	-	-	-
	S. aureus	-	-	-
	S. epidermidis	-	-	-
Chloroform	*B. cereus*	-	-	-
	B. subtilis	-	10.28±2.17	12.47±1.64
	E. aerogenes	-	-	-
	E. coli	-	-	-
	P. aeruginosa	-	-	-
	S. aureus	-	-	-
	S. epidermidis	-	-	-

- : not detected; Values are calculated as means ± standard deviation from 3 replicates.

Table 4 MIC and MBC of *G. oppositifolius*

Microorganisms	Concentrations (mg/ml)	
	MIC	MBC
Bacillus subtilis	5	>20
Bacillus cereus	10	>20

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