SCREENING OF PHYTOCHEMICALS AND SOME BIOLOGICAL ACTIVITIES OF LEAF OF *SENNA SIAMEA* (LAM) MEZALI

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Abstract

The study aimed screening some phytochemical constituents of the leaf of *Senna siamea* (Lam.) Mezali, as well as some biological activities, including antioxidant, antimicrobial, and cytotoxic effects. It was widely used in traditional medicine to treat various complaints. The cytotoxic activity of the ethanol and water extracts was determined by a brine shrimp lethality bioassay. The ethanol extract was more cytotoxic than the water extract. The antioxidant activity of ethanol and water extracts of *Senna siamea* was determined by DPPH assay. These assays found that the ethanol extract (IC_{50} = 39.53 µg/mL) in antioxidant activity was more potent than the water extract (IC_{50} =69.93 µg/mL).The antimicrobial activity of four crude extracts (pet-ether, ethyl acetate, ethanol and water) was determined by the agar well diffusion method. The ethyl acetate extract was found to have higher potent antimicrobial activity than PE, EtOH and watery extracts. The findings suggest that the various extracts possess substantial potential for therapeutic application.

Keywords*: Senna siamea*, antioxidant, cytotoxicity, phytochemical and antimicrobial activities

Introduction

Senna siamea is an angiosperm native to Southeast Asia (Burma, Ceylon, India, Japan, Malaysia, Sri-Lanka and Thailand) and widely distributed in Africa (Cote Ivoire, Eritrea, Ethiopia, Ghana, Kenya, Malaysia, Nigeria, Sierra Leone, Southern Africa, Tanzania, Togo, Uganda and Zambia), in Latin America (Cuba, Chile, Antigua and Barbuda, Lucia, St Vincent and the Grenadines and Trinidad and Tobago), and in Oceania (Australia and Fiji Firstly classified in the Caesalpiniaceae family, then in those of Leguminoseae, *S. siamea* is now classified among the Fabaceae. This plant is a shrub which has a medium-size, 10-12 m tall, occasionally reaching 20 m. The bole is short; the crown dense and rounded at first, later becoming irregular and spreading. The young bark is grey and smooth, and later with longitudinal fissures. The leaves are alternate, 15-30 cm long compound, with 6-14 leaflets each ending in a tiny bristle. The flowers are bright yellow, in large, up to 60 cm long, upright, with pyramid-shaped panicles. The fruits are flat with indehiscent pod, 5-30 cm long, and constricted between the seeds. There are about 20 seeds per pod. The seeds are bean-shaped, greenish-brown, and 8-15 mm long (Hill, 1992). The leaves are the most used parts' the plant especially by African and Asian population in preparation of the herbal remedies. In Burkina Faso, fresh and dried leaves decoction (boiled for 20 min in 1 L of water) is drunk with lemon juice or for body bath throughout the day to treat malaria and liver disorders (Bukar, et al., 2009). The young fruits and leaves are also eaten as vegetables in Thailand. The flowers and young fruits are used as curries (Kiepe, 2001). The root decoction is used against fever, constipation, hypertension, and insomnia. The decoction of the bark is drunk against diabetes. The flowers decoction is drunk or used in body bath against malaria and liver disorders. This decoction is also effective against insomnia and asthma. The seeds are used to charm away intestinal worms and as antidote for snake and scorpion bites (Bukar, *et al*., 2009).

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Botanical Aspect of *Senna siamea* (Lam) MeZaLi

Family	Fabaceae
Botanical name	*Senna siamea* (Lam.)
Myanmar name	Mezali
Part used	Leaf

Materials and Methods

Plant Material

In June 2024, the leaf of *S. siamea* was taken from Hinthada Township in the Ayeyarwaddy Region of Myanmar. The plant was identified at the University of Yangon, Department of Botany. After the sample was cleaned and allowed to dry in the shade for a week, it was chopped into extremely small pieces and processed with an electric grinder to a fine powder. The sample in powder form was kept in an airtight container.

Preparation of Extracts

Each 50 g of the leaf of *S. siamea* dried powder was extracted with ethanol, petroleum ether, ethyl acetate and water by sonication using each solvent (100 mL, 1 h) at 70 ℃. Each filtrate was evaporated under reduced pressure by a rotatory evaporator to yield different solvent extracts.

Preliminary Phytochemical Tests

Preliminary phytochemical tests on the powdered sample were carried out according to the reported methods in order to classify the types of organic constituents present in the samples, such as alkaloids, α-amino acids, carbohydrates, cyanogenic glycosides, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, starch, steroids, tannins and terpenoids, by appropriate reported methods (M-Tin Wa, 1972).

Cytotoxicity Effects by Brine Shrimp Lethality Bioassay

The cytotoxicity of ethanol and aqueous extracts of the leaf of *S. siamea* was assessed using a brine shrimp lethality bioassay, following the methodology outlined by (Olowa et al. 2013). The brine shrimp (*Artemia salina*) was used in this bioassay (Ali *et al.,* 2013). 5 mg of the corresponding crude extract was dissolved in 5 mL of distilled water to create the sample solution. Sample solutions with concentrations of 800, 400, 200, 100 and 50 μg/mL were obtained by serially diluting the stock solution ten times with distilled water. Using a Pasteur pipette, 10 live brine shrimp nauplii were transferred to each chamber, which was then allowed to stand at room temperature for 24 h. Following a 24 h incubation period, the percentage of brine shrimp that survived was counted, and the 50 % lethal dosage ((LC_{50}) was determined. Using the previously described method, the cytotoxic effect of control solutions (($K_2Cr_2O_7$) and caffeine) was also evaluated.

Antioxidant Action by DPPH Free Radical Scavenging Assay

Preparation of DPPH solution

DPPH (4.732 mg) was thoroughly dissolved in 95 % ethanol (100 mL). This solution was freshly prepared in the brown-coloured reagent bottle and stored in the fridge for no longer than 24 h. Each tested sample (20 mg) and 20 mL of ethanol were thoroughly mixed by shaker. The mixture solution was filtered, and the stock solution (100 µg/mL) was obtained. By adding ethanol, DPPH solutions with different concentrations of 100–6.25 µg /mL were prepared from the stock solution.

Determination of antioxidant activity

The effect of Mezali on the DPPH radical was determined by using the method of (Marinova and Batchvarov 2011). The control solution was prepared by mixing 1.5 mL of 120 µM DPPH solution and 1.5 mL of 95 % ethanol using a shaker. The test sample solution was also prepared by mixing thoroughly 1.5 mL of 120 µM DPPH solution (Absorbance = 0.8941 used for control) and 1.5 mL of each sample solution (100-6.25 µg/mL). The mixture solutions were allowed to stand at room temperature for 30 min. Then, the absorbance of these solutions was measured at 517 nm by using UV-7504 spectrophotometer. Absorbance measurements were made in triplicate for each concentration, and then the mean values so obtained were used to calculate the percent inhibition of oxidation by the following equation:

$$\% \text{ RSA} = \frac{A_{DPPH-}(A_{smaple-}A_{blank})}{A_{DPPH}} \times 100$$

where, % RSA = % radical scavenging activity

A_{DPPH} = absorbance of DPPH in EtOH solution

A_{Sample} = absorbance of sample and DPPH solution

A_{blank} = absorbance of sample and EtOH solution

The experimental results were performed in triplicate. The data were recorded as mean ± standard deviation.

Screening of Antimicrobial Activity of the Samples by Agar Well Diffusion Method

The antimicrobial activity of four crude extracts, such as pet-ether, ethyl acetate, ethanol, and water extracts from the leaf of *S. siamea,* was determined against eight strains of microorganisms, such as *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas fluorescens, Agrobacterium tumefaciens, Bacillus pumilus, Micrococcus luteus, and Candida albicans.*by employing the agar well diffusion method (Mukhrizah *et al*, 2011). These tests were screened at the Department of Chemistry, Pathein University.

Preparation of nutrient agar medium

A mixture of meat extract (0.5 g), peptone (0.5 g), sodium chloride (0.25 g) and (1.5) g of agar powder was placed in a sterilized conical flask and100 mL of sterile distilled water was added to obtain nutrient agar medium. The resulting mixture was heated to dissolve the contents. Then, the pH of this solution was adjusted to 7.2 with 0.1 M sodium hydroxide solution. It was sterilized in an autoclave at 121 ℃ for 15 min.

Determination of antimicrobial activity by agar well diffusion method

The agar well diffusion method was used to evaluate the antimicrobial activities of the extracts against bacteria and fungi. Different extracts (1 mg each) were dissolved in 1 mL of their respective solvent. After inoculation, plates were dried for 15 min. A hole with a diameter of 8 mm was punched aseptically with a sterile cork or a tip and a volume 0.1 mL of extract solution at the desired concentration was introduced into the well. Then, agar plates were incubated under suitable conditions depending upon the microorganisms. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested. The plates were incubated for 24 h to allow the extract to diffuse through the agar media to form zones of inhibition. The extent of antimicrobial activity was measured by the diameter of the inhibition zone.

Results and Discussion

Phytoconstituents in Mezali

The phytochemical screening of the leaf *S. siamea* was preliminarily carried out by the appropriate methods, and the results are shown in Table 1. The phytochemical tests revealed the presence of the secondary metabolites such as alkaloids, α-amino acids, carbohydrates, glycosides, saponins, phenolic compounds, flavonoids, tannins, steroids and terpenoids, but cyanogenic glycosides, starch, and reducing sugars were absent in the leaf of *S. siamea*.

Table 1. Results of Preliminary Phytochemical Tests of the leaf of *S. siamea*

No.	Test	Extracts	Test Reagent	Observation	Remark
1	Alkaloids	1% HCl	(i)Wagner's	reddish brown ppt	+
			(ii) Dragendorffs’	orange ppt	+
			(iii) Mayer's	white ppt	+
			(iv) Sodium picrate	yellow ppt	+
2	α-amino acids	H_2O	Ninhydrin	violet spot	+
3	Carbohydrates	H_2O	10 % α-naphthol & conc:H_2SO_4	red ring	+
4	Cyanogenic glycosides	H_2O	Sodium picrate solution	no brick red colour	-
5	Flavonoids	EtOH	Mg ribbon & conc: HCl	pink colour	+
6	Glycosides	H_2O	10 % lead acetate	white ppt	+
7	Phenolic compounds	EtOH	1 % $FeCl_3$	green colour	+
8	Reducing Sugars	H_2O	Fehling's solution A & B	no brick red ppt	-
9	Saponins	H_2O	distilled water	frothing	+
10	Starch	H_2O	iodine solution	no blue colour	-
11	Steroids	PE	acetic anhydride & conc:H_2SO_4	green colour	+
12	Tannins	EtOH	1 % gelatin	white ppt	+
13	Terpenoids	$CHCl_3$	acetic anhydride & conc:H_2SO_4	pink colour	+

(+) = presence (-) = absence (ppt) = precipitation

Cytotoxicity Effect of Crude Extracts of the leaf of *S. siamea*

Most toxicity studies that use the brine shrimp assay determine the toxicity by counting the survived nauplii after 24 h of exposure to the test. The cytotoxicity activity of ethanol and watery extracts of the leaf of *S. siamea* was evaluated by the brine shrimp lethality bioassay. The cytotoxic effect was expressed as LC_{50} values (50 % Lethality Concentration). Standard Potassium dichromate ($K_2Cr_2O_7$) and caffeine were chosen because ($K_2Cr_2O_7$) is a well-known toxic agent in this assay and caffeine is a natural product. The results are shown in Table 2. The ethanol extracts exhibited more cytotoxic effect than water extract. From the results, the LC_{50} values of ethanol and watery extracts (399 µg/mL and 570 µg/mL) respectively. For the standard $K_2Cr_2O_7$ was 89.98 µg/mL and caffeine was >1000 µg/mL. The resulting LC_{50} values were compared with the Deciga-Campos criteria. The ethanol extracts showed medium toxicity due to LC_{50} values between 500 and 1000 µg/mL.

Table 2. Cytotoxicity of Ethanol and Watery Crude Extracts of the leaf of *S. siamea*

Test Sample	% Mortality of Brine Shrimp Concentration ± SD in Different Concentration of Sample (µg/mL)					IC 50 (µg/mL)
		100	200	400	800	
(water extract)	6..67 ± 0.58	16.67 ± 1.58	33.34 ± 1.00	40.00 ± 0.58	63.34 ± 0.57	570
(ethanol extract)	23.34 ± 0.57	36.67 ± 1.53	43.34 ± 1.52	56.67 ± 0.00	66.67 ± 0.57	299
* patassium dichromate	36.67 ± 0.53	53.34 ± 0.58	72.00 ± 1.00	85.47 ± 1.15	100.00 ± 0.00	89.98
** Caffeine	0.00 ± 0.00	0.00 ± 0.00	6.67 ± 1.53	16.67 ± 0.58	23.34 ± 1.53	>1000

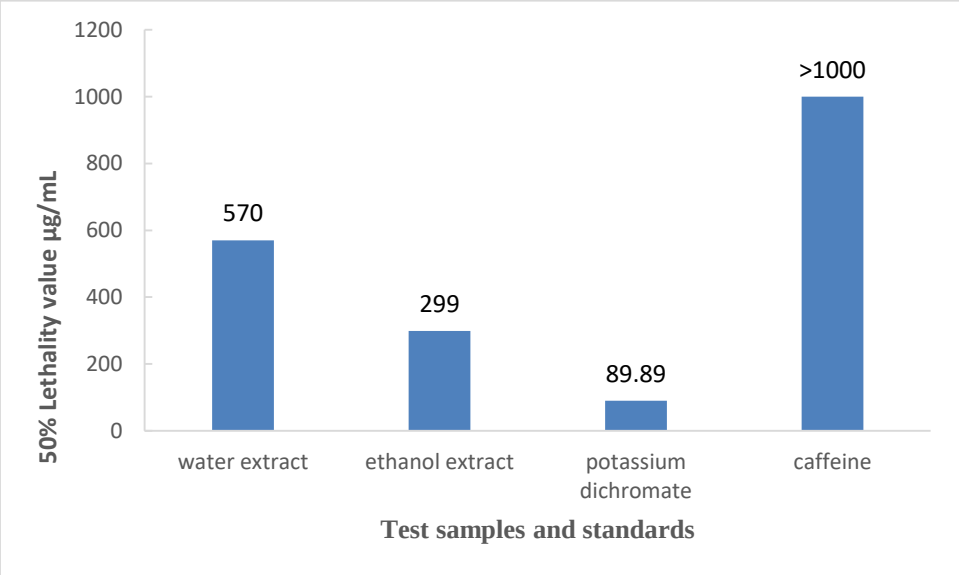

Figure 1. Brine shrimp lethality of crude extracts of the leaf of *S. siamea*

In vitro Antioxidant Assay

The free radicals scavenging capacity of the extract was determined using the stable free radical containing DPPH. It can accept an electron or hydrogen radical. The odd electron in it makes the solution appear deep violet in colour. The absorption vanishes when DPPH accepts an electron, resulting in delocalization. The DPPH radical scavenging ability is supposed to be due to its hydrogen donating property (Soares *et al*., 1997). In the DPPH free radical scavenging assay, 6.25-100 µg/mL of water and ethanol extracts of the *S. siamea* were used. A decrease in absorbance exhibits an increase in radical scavenging activity. The radical scavenging activities of two crude extracts were expressed in terms of % RSA and IC_{50} (50 % inhibitory concentration). The IC_{50} values of ethanol extracts (39.53 µg/mL) showed higher radical scavenging activity than water extract compared to ascorbic acid (1.15 µg/mL). The results are shown in Table 2 and Figure 2.

Table 3 Average % Radical Scavenging Activity and IC_{50} Values of Crude Extracts of the leaf of S. siamea

Extracts	% RSA ± SD at different concentration (µg/mL)					IC_{50} (µg/mL)
	6.25	12.5	25	50	100	
Water extract	14.06 ±0.016	23.27 ±0.013	39.63 ±0.010	45.53 ±0.017	61.57 ±0.003	63.93
Ethanol extract	19.63 ±0.004	30.66 ±0.005	41.33 ±0.006	56.24 ±0.012	70.30 ±0.010	39.53

Table 4 % RSA (Radical Scavenging Activity) of Standard Ascorbic Acid

Sample	% RSA ± SD at different concentration (µg/mL)					IC50 (µg/mL)
	0.625	12.5	25	5	10	
Ascorbic acid	40.68 ± 0.041	50.85 ± 0.041	61.51 ± 0.005	78.30 ± 0.020	88.11 ± 0.001	1.15

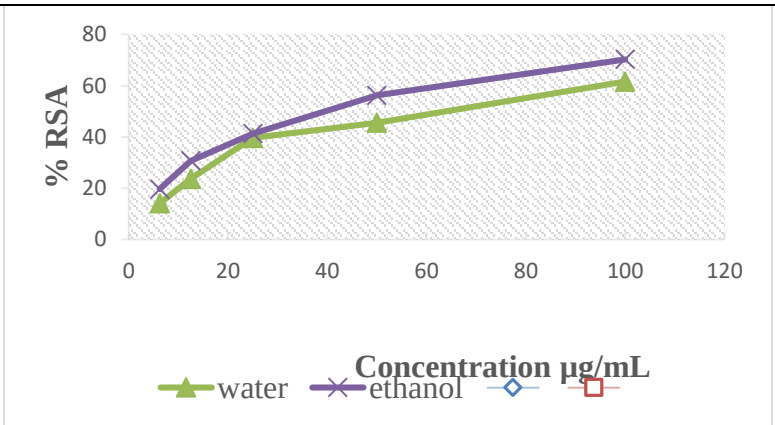

Figure 2. DPPH radical scavenging activities of crude extracts of the leaf of *S. siamea* at different concentrations

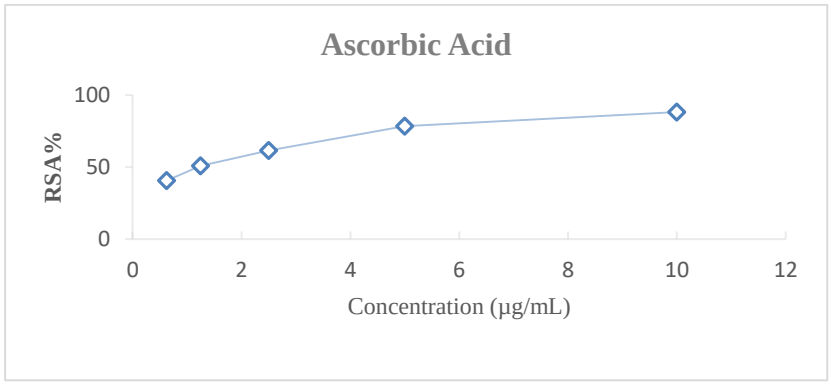

Figure 3. DPPH free radical scavenging activities of standard ascorbic acid

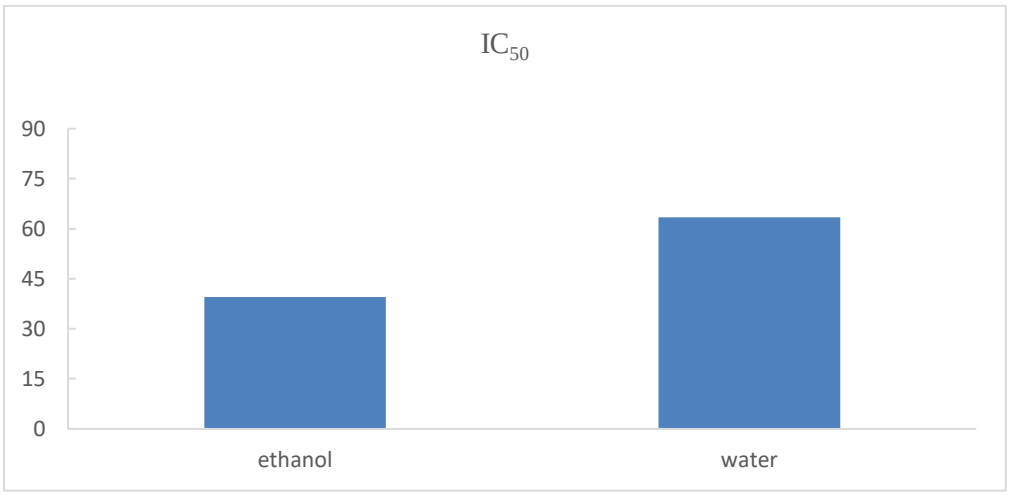

Figure 4. Comparison of percent inhibition and IC_{50} value of crude extracts of the leaf of *S. siamea*

Antimicrobial Activity

The antimicrobial activity was determined by measuring the diameter of the zone of inhibition and recording it. According to the method described by (Selvamohan et al., 2012), in this investigation, four extracts (PE, EtOH, EtOAc and water) of the leaf of *S. siamea* were tested on bacteria (*B. subtilis, E. coli, P. fluorescens, A. tumefaciens, B. pumilus, M.luteus and S. aureus*) and the *C. albicans* fungus by the agar well diffusion method. The measurable zone diameter, including the well diameter, shows the degree of completeness of the antimicrobial activity. In this experiment, the well diameter was set as 8 mm. The ethyl acetate extract demonstrated the higher antimicrobial activity than the other extracts. These extracts showed a higher inhibition zone diameter than standard chloramphenicol and nystatin.

Table 5 Inhibition Zone Diameter of the Leaf of *S. siamea* Against Eight Different Microorganisms

No	Type of Microorganisms	Inhibition Zone Diameter mm)				
		Water (mm)	EtOH (mm)	EtOAc (mm)	PE (mm)	STD (mm)
1	*Agrobacterium tumefaciens*	17	15	29	16	27
2	*Bacillus pumilus*	18	15	28	16	27
3	*Bacillus subtilis*	18	15	30	16	27
4	*Candida albicans*	17	15	29	17	27
5	*Escherichia coli*	17	15	28	16	27
6	*Micrococcus luteus*	18	15	29	16	28
7	*Pseudomonas fluorescens*	18	15	29	17	28
8	*Staphylococcus aureus*	18	15	28	17	28

Agar well diameter = 8 mm

10 mm-14 mm = low activity

15 mm- 20 mm = medium activity

20 mm above = very high activity

*STD for bacterial = chloramphenicol

for fungus = Nystatin

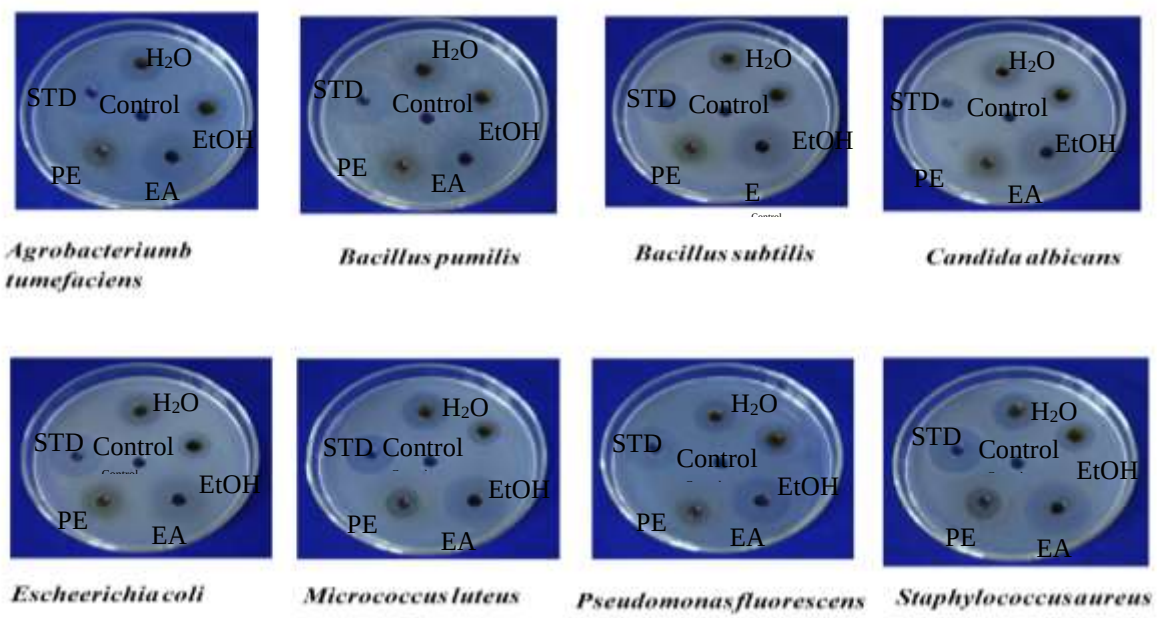

Figure 5. Screening of antimicrobial activities of the leaf of *S. siamea*

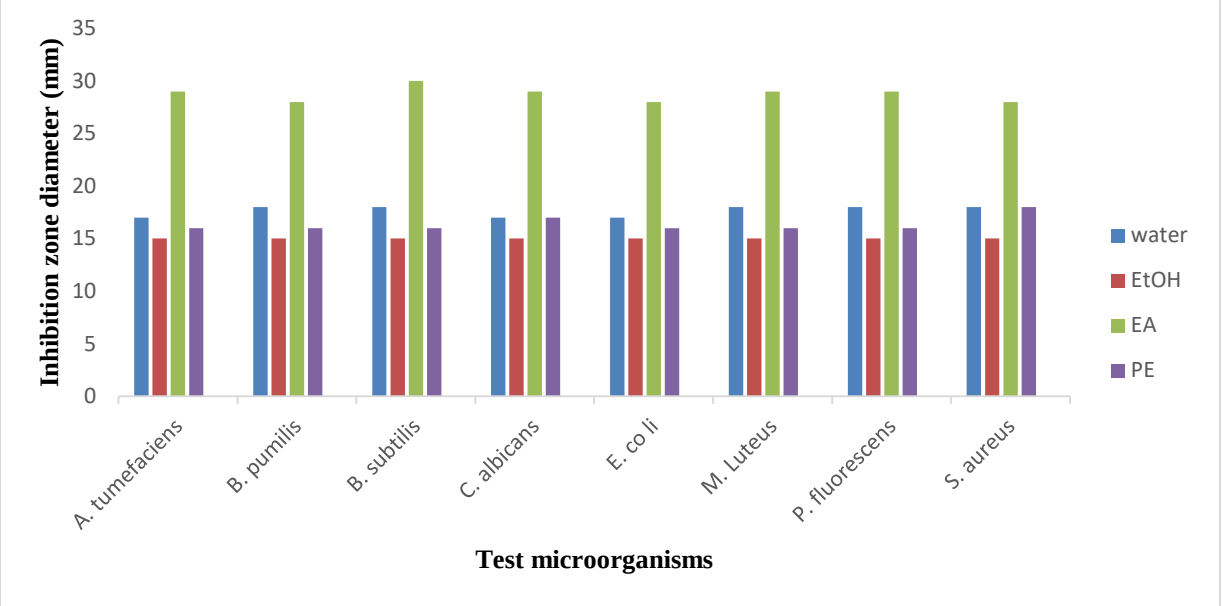

Figure 6. Inhibition Zone diameters for crude extracts of the leaf of *S. siamea*

Conclusion

In this research work, the following inferences could be deduced. The preliminary phytochemical analysis revealed the presence of alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, steroids, tannins and terpenoids. Moreover, cyanogenic glycosides, reducing sugar and starch were absent. The screening of antioxidant activity of ethanol extract (IC_{50} = 39.53 µg/mL) is higher than that of watery extract (IC_{50} = 69.93 µg/mL). The ethyl acetate extract of the leaf of *S. siamea* had the highest antimicrobial activity (inhibition zone diameter 28-30 mm) compared to the pet-ether, watery and ethanol extracts because it contained flavonoids, phenols and tannin. According to the results, the ethanol extract exhibited more cytotoxicity than the water extract that may be used in traditional medicine formulations to treat many kinds of disease.

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