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INVESTIGATION OF PHYTOCHEMICAL CONSTITUENTS AND ANTIMICROBIAL ACTIVITIES OF FLOWERS OF *Sesbania grandiflora* (Linn) (Pauk pan phyu)

Moh Moh Myint Kyaw,
Assistant Lecturer, Department of Engineering Chemistry,
University of Technology (Yatanarpon Cyber City), Pyin Oo Lwin, Myanmar
E-mail: mmmkkyaw@gmail.com

Abstract: *The phytochemical screening and antimicrobial activities of traditional medicinal vegetable plant* ***Sesbania grandiflora*** *(Pauk pan phyu) were done. The aim is to be searched and collected some indigenous medicinal plants. To investigate the phytochemical constituents and study the antimicrobial activities of the flowers of* ***Sesbania grandiflora*** *(Linn.) Phytochemical studies have shown that flowers contain alkaloid, flavonoid, glycoside, phenolic compound, reducing sugar, saponin and tannin, which are responsible for its various pharmacological properties. Moreover, the antimicrobial activity of* ***Sesbania grandiflora*** *(Pauk pan phyu) could be determined with various solvent by Agar well diffusion method on six selected microorganisms such as* ***Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumalis, Candida albicans*** *and* ***E.coli*** *. The EtOH extract of flowers responds medium activity on* ***Staphylococcus aureus*** *and* ***Candida albicans****. The EtOAc extract of leaves and flowers (Pauk pan phyu) show high activity on all selected* microorganisms.

1. INTRODUCTION:

From the earliest times, herbs have been prized for their pain relieving and healing abilities. A majority of the world's population in developing countries still relies on herbal medicine to meet its health needs.

Also in Myanmar, there are many thousands of medicinal plants. Most of people used the traditional medicinal plants for the treatment of diseases and to relief pain. The study of traditional indigenous medicinal plants and their usage in therapy play a very important role.

Medicinal plants can be important sources of unknown chemical substances with potential therapeutic effects. Besides, the World Health Organization has estimated that over 75% of the world's population still relies on plant-derived medicines, usually obtained from traditional healers, for basic health-care needs.In Myanmar, there are many kinds of flowers of vegetables plants that have medicinal properties. Flower vegetables are flowers of traditional vegetables plants that have flowers and have been used for cooking since ancient time. It was believed that consumption of these flower vegetables can cure illness and diseases. They also help people who suffer from diarrhea, which indicates the anti-microbial activity of these vegetables (Somanpan, 1990, Vichirasup, 1995, Boonyaprapatsara, 1996 and 2000). The flower vegetables are composed of various chemical compounds that can be grouped as flavonoid, anthraquinone and glycoside. Flavonoid is excepted to be the main component that plays a major role in microbial inhibition.

Sesbania grandiflora belonging to family Leguminosae (Hindi, Agati, Hadga) found in various regions of India, Srilanka and South East Asia.

Sesbania grandiflora (Fabaceae), commonly known as Agati is a widely available, fast growing plant, generally popular for its animal fodder use. Traditionally, the plant has been used for the treatment of headache, in fever, as a tonic, in catarrh, as an astringent etc. The flowers have been reported to have antimicrobial activity. It shows hypolipemic, anti-ulcer and anti-inflammatory properties as well. The flowers of ***Sesbania grandiflora*** are edible and are often used to supplement meals (Gutteridge and Shelton, 1998).

2. LITERATURE REVIEW:

Botanical Description

Figure 1 Flowers of ***Sesbania grandiflora*** (Linn.)

Family	:	Fabaceae
Botanical name	:	***Sesbania grandiflora*** (Linn.)
Common name	:	Butterfly tree
Myanmar name	:	Pauk pan phyu
Part Uses	:	Flowers

Sesbania grandiflora (Linn.)

Sesbania grandiflora is a small. loosely branching tree that grows up to 8-15 m tall and 25-30 cm in diameter; stems tomentose, unarmed; roots normally heavily nodulated with large nodules; the tree can develop floating roots. Flower clusters hanging at leaf base have 2-5 large or giant flowers; pink, red or white, pealike, 5-10 cm in length, curved, about 3 cm wide before opening. Pods long and narrow, hanging down 30-50 cm by 8 mm; septate, wide, flat, with swollen margins and about 15-40 pale-coloured seeds; seed is beanlike, elliptical, red brown, 6-8 in a pod, 3.5 mm, each weighting 1 g. The generic name is derived from an Arab word for one of the species, S. sesban. The specific epithet means large-flowered in Latin.*Sesbania grandiflora* originated either in India or Southeast Asia and grows primarily in hot and humid tropical areas of the world.

Phytoconstituents in the Selected Plant

Alkaloid

Group of basic organic substances of plant origin, are containing at least one nitrogen atom in a ring structure in the molecule. Many have important physiological actions and are used in medicine. e. g, cocaine, nicotine, quinine, morphine.

Alkaloids are a group of naturally occurring chemical compounds that contain mostly basic nitrogen atoms. This group also includes some related compounds with neutral and even weakly acidic properties. Many alkaloids are toxic to other organisms. They often have pharmacological effects and are used as medications as recreational drugs, or in entheogenic rituals. Examples are the local anesthetic and stimulant cocaine, the psychedelic psilocin; the stimulant caffeine, nicotine, the analgesic morphine, the antibacterial berberine, the anticancer compound vincristine, the antihypertension agent reserpine, the cholinomimeric galatamine, the spasmolysis agent atropine, the vasodilator vincamine, the anti-arhythmia compound quinidine, the anti-asthma therapeutic ephedrine and the antimalarial drug quinine. Although alkaloids act on a diversity of metabolic systems in humans and other animals, they almost uniformly invoke a bitter taste.

Steroid

Steroids are derived lipids which include sterols, the bile acids, certain hormones and glucosides and vitamin D. Among the synthetic steroids of therapeutic value are a large number of anti-inflammatory agents, anabolic agents, and oral contraceptives.

Tannin

Tannins are derivatives of gallic with the approximate formula $C_{76}H_{52}O_{46}$. Tannins are contained in galls and in bark, wood and leaves and or fruits of tannins plants. They are used in tanning in leather and they also serve as mordants in dyeing cotton fabric.

Tannins are divided into two classes. The first comprises the tannins formed by a polyhydric alcohol such as glucose, in which the hydroxyl groups are partially or completely esterified by gallic acid or related compounds.Class of complex origin compounds of vegetable origin. Consists of mixtures of derivatives of poly-hydroxy benzonic acids; tannic acid is an example. In medicine, tannins are used as astringents.

Terpene

Terpenes are natural products that are structurally related to isoprene, which has the molecular formula C_5H_8. Plant terpens have strong antitumor, anticancer and antimicrobial activities. They can be responsible for skin allergies in humans.

Terpenes are class of hydrocarbons occurring in many fragrant essential oils of plants. Colorless liquids, generally with a pleasant smell; include pinene, $C_{10}H_{16}$, the chief ingredient of turpentine, and limonene, $C_{10}H_{16}$, found in the essential oils of oranges and lemons.

Glycoside

Derivatives of glucose in which one hydrogen atom in the molecules is replaced by an organic radical. The term glycoside is applied generally to such compounds of all sugars. Glycoside are widely distributed in plants; examples are the anthocyanin pigments and the cardiac glycosides such as digoxin and auabain, which are used medicinally for their stimulant effect on the heart.

Flavonoid

Many flavonoids are easily recognized as flower pigments in most angiosperm families (flowering plants). However, their occurrence is not restricted to flowers but include all parts of the plant.

Flavonoids, an amazing array of over 6,000 different substances food in virtually all plants, are responsible for many of the plants colour. They provide health benefits against cancer and heart diseases and are used in several industries. The flavonoids are water soluble, polyphenolic compounds possessing 15 carbon atoms; two benzene rings, joined by a linear there carbon chain. One of a group of naturally occurring phenolic compounds many of which are plant pigments. They include the *anthocyanins, flavonols and flavones. Patterns of flavonoid distribution have been used in taxonomic studies of plant species. Flavonids have antioxidant activity. The higher the flavonoids contents, the stronger the antioxidant activity. Some of the activities attributed to flavonoids include anti-allergic, anti-cancer and anti-inflammatory.

Reducing Sugar

Reducing sugar is a monosaccharide or disaccharide sugar that can donate electrons to other molecules and can therefore act as a reducing agent. The possession of a free ketone (-CO-) or aldehyde (-CHO) group enables most monosaccharides and disaccharides to act as reducing sugars.

Saponin

Saponins are found in a number of plants. In the animal kingdom, saponins are found in most sea cucumbers and starfish. Saponins have a bitter nature. They are made of both fat-soluble and water-soluble components. The two constituents combined create foam. Because of these characteristics benefits of saponins, they are often used commercially in cosmetic as well as in beverages. Saponins have strong antibacterial, antiviral and antifungal properties, protecting plants from infection. Foods containing saponins pass on the same benefits to humans.

3. MATERIAL & METHOD:

Sample Collection

The flowers of ***Sesbania grandiflora*** (Linn.) (Pauk pan phyu) were collected from Kanthar market, Magway Region. The collected samples were cut into small pieces and allowed to air-dried in shade at room temperature. Finally, the samples were crushed and blended into powder form and stored in well-stoppered bottle and stored for further experiments.

Phytochemical Screening of *Sesbania grandiflora* (Linn.) by Test Tube Method

Phytochemical screening of leaves and flowers were done according to standard procedures. The following procedures were applied in this work.

Test for Alkaloid

Dried powdered sample (2 g) was boiled with 1%HCl for about 10 minutes, allowed to cool and then filtered. The filtrate was divided into two portions in test tubes. The first portion was then tested with Dragendroff's reagent and the second with Mayer's reagent respectively. The formation of precipitates indicates the presence of alkaloids.

Test for Flavonoid

Dried powdered sample (2 g) was extracted with 95% ethanol (25cm^3) and concentrated alcoholic HCl solution (4 cm^3) was treated with a few pieces of magnesium turning and a few drops of concentrated sulphuric acid. The appearance of pink color indicates the presence of flavonoids.

Test for Glycoside

Dried powdered sample (2g) was boiled with distilled water (100 cm^3) for about 10 minutes, allowed to cool and filtered. The filtrate was treated with a few drops of 10% lead acetate solution. The formation of white precipitates indicates the presence of glycosides.

Test for Phenolic Compound

Dried powdered sample (2 g) was boiled with distilled water and filtered. The filtrate was treated with a few drops of 10% ferric chloride solution. The bluish black color indicates the presence of phenolic groups.

Test for Reducing Sugar

Dried powdered sample (2 g) was boiled with distilled water for about 10 minutes and then filtered. The filtrate was boiled with a few drops of Benedict's solution for about 2 minutes. The formation of brick red precipitate indicates the presence of reducing sugars.

Test for Saponin

Dried powdered sample (2 g) was put into the test tube followed by the addition of distilled water. The mixture was vigorously shaken for a few minutes, allowed to settle for 10 minutes. Formation of stable foams indicates the presence of saponins.

Test for Steroid

Dried powdered sample (2 g) was introduced into a round-bottomed flask followed by the addition of petroleum ether. The mixture was kept on water-bath under reflux for 15 minutes and filtered. The filtrate was treated with acetic anhydride (3 drops), chloroform and the mixture was shaken. Then a few drops of concentrated sulphuric acid were carefully added and shaken. The mixture was left in a dark place for a few minutes. The appearance of greenish blue color indicates the presence of steroids.

Test for Tannin

Dried powdered sample (2 g) was extracted with distilled water and filtered. The filtrate (10 cm^3) was then transferred to a test tube and a drop of 10% ferric chloride solution was slowly introduced into the test tube using a glass tube. If a bluish black color is produced which disappear on addition of a few drops of dilute sulphuric acid solution followed by the formation of yellowish brown colour indicates the presence of tannin.

Test for Terpenoid

Dried powdered sample (2 g) was boiled with ethanol 25 cm^3 for about 10 minutes and filtered. 3 drops of acetic anhydride, 1 cm^3 of chloroform and one drop of concentrated sulphuric acid were added to ethanol extract and recorded the observed color. Pale yellow coloration indicates the presence of terpenoids.

Procedure

Preparation of Agar Medium

Trypticase soy agar 40 g were suspended in 100 cm^3 of distilled water in a sterile conical flask and covered with aluminium foil. Then suspension was mixed thoroughly and heated to completely dissolve the powder on a hot plate stirrer. The trypticase soy agar solution was sterilized in an autoclave at 121°C for 15 minutes. The temperature of agar solution was reduced to 50°C on a constant temperature bath. Trypticase soy agar was then poured into the sterile petri-dishes near the flame of spirit burner. The agar medium was allowed to solidify and sealed tightly in a polyethene plastic bag. The medium was stored in a refrigerator until it was used. The solidified agar medium was dried in an incubator 42°C before it was used.

Preparation of Agar Slant Medium

Triple sugar iron agar (65 g) was suspended in (1000 cm^3) of distilled water in a sterile conical flask, cover with aluminium foil and mixed thoroughly and heated to completely dissolve the powder on a hot plate stirrer. The triple sugar iron agar solution was transferred into the tubes (4 cm^3 for each) and sterilized by autoclaving at 121°C for 15 minutes. After sterilization, the test tubes were placed in a slant position and allowed to solidify.

Preparation of Broth Medium

Trypticase soy broth (30 g) was suspended in (1000 cm^3) of distilled water in a conical flask, covered with aluminium foil, mixed thoroughly and heated to completely dissolve on a hot plate stirrer. The broth solution was transferred into the test tubes (3 cm^3 in each tube) and sterilized by autoclaving for 15 minutes at 121° C.

Culture of Bacteria

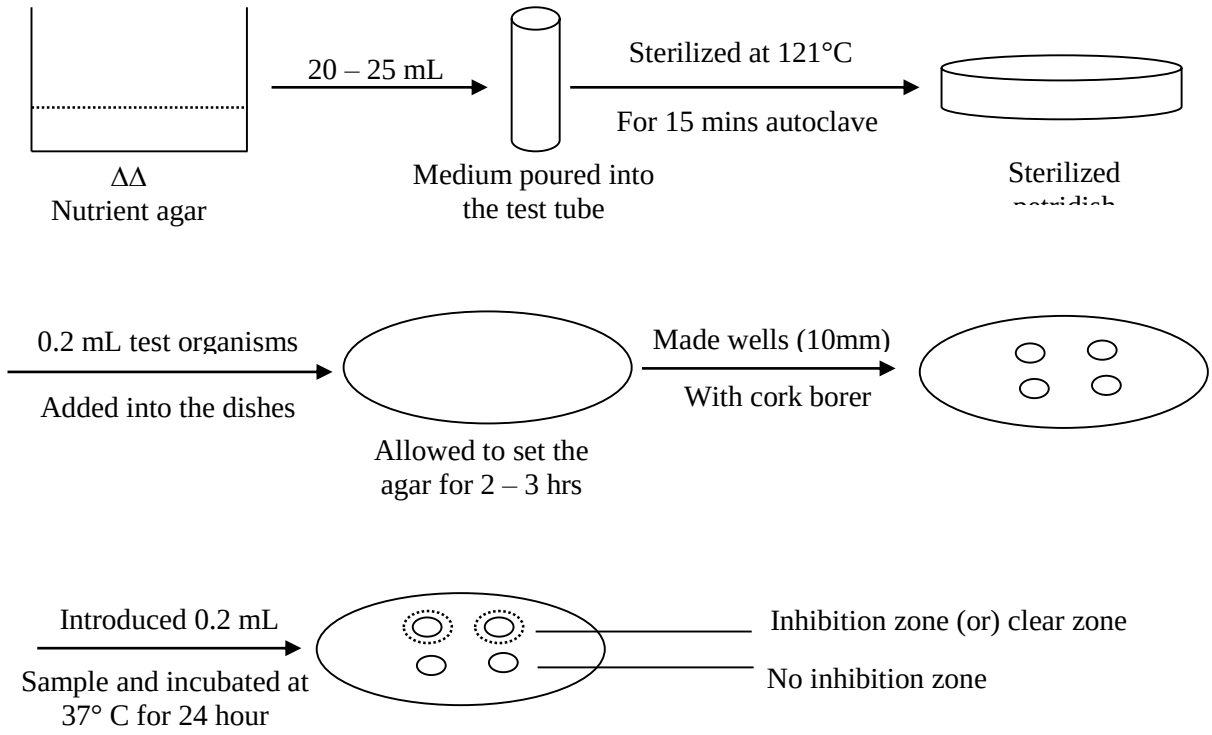
A few colonies of the organism to be tested were inoculated into the triple sugar iron agar and incubated at 37°C for 24 hours in an incubator. A few colonies of the organism from the triple sugar iron agar were introduced into the trypticase soy broth and incubated for 3 hours at 37°C to obtain the bacterial suspension of moderate cloudiness. This contained approximately 10^5 to 10^7 organisms per cm^3.

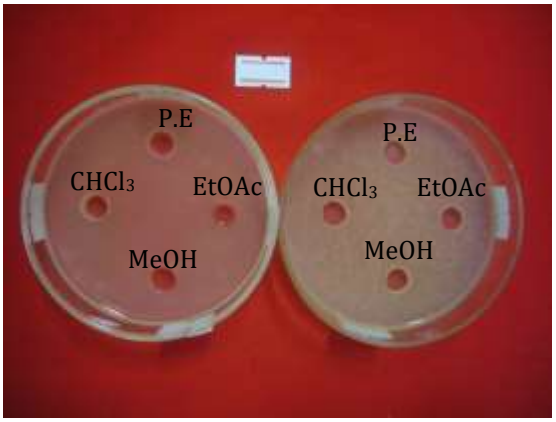
Screening by Agar Well Diffusion Method

The agar well diffusion method was used to test the antimicrobial action of the extracts on 24 hours broth culture of the organisms used.

The extracts of PE, $CHCl_3$, EtOAc, EtOH and MeOH (1g each) were dissolved in 1cm^3 of their respective solvents. 0.2 ml each of the bacterial suspension from broth culture was spread evenly onto the surface of the nutrient agar plates. Immediately after hardening of the agar, well were made with a 10 mm sterile cork-borer. After removing

the agar, the wells were filled with 0.2 ml each of the drug extract to be tested. The plates were incubated at 37°C for 18-24 hours. The diameters of the inhibition zone including 10 mm wells were measured and recorded in mm. The results are shown in Table (2) and Figure (2).

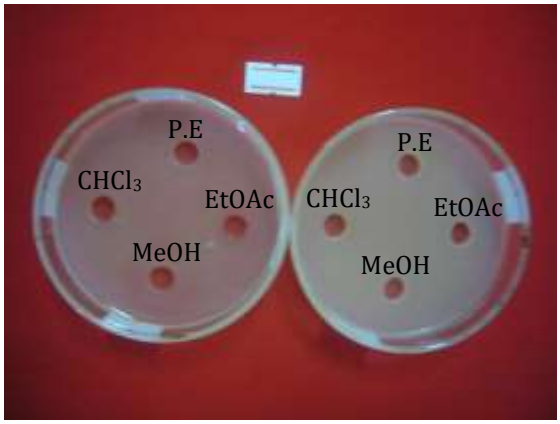




(a) Bacillus subtilis

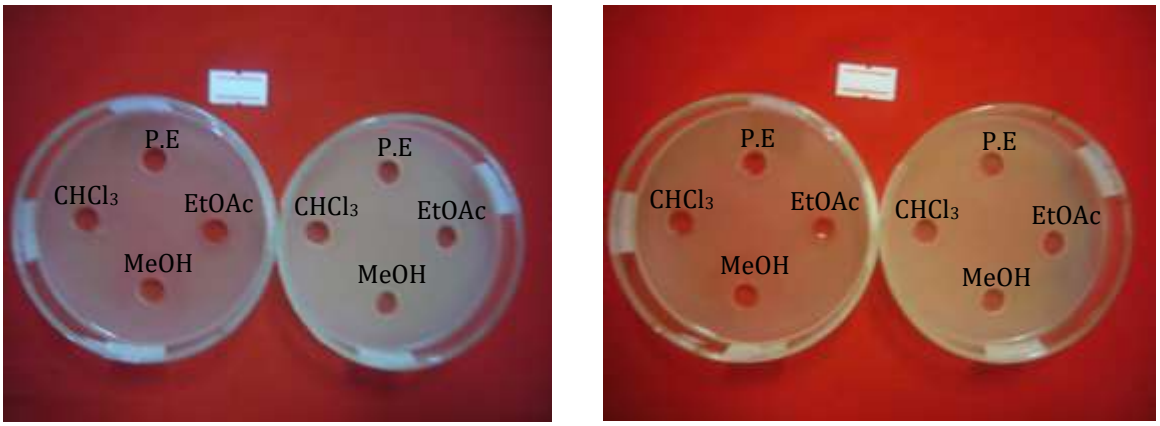
(b) *Staphylococcus aureus*

(c) *Pseudomonas aeruginosa*



(d) *Bacillus pumalis*

Figure 2. Flow diagram for screening of antimicrobial activity by agar well diffusion method



(e) *Candida albicans* **(f) *E.coli***

Figure 3. Antimicrobial Activities of Various Solvent Extract of Flowers of ***Sesbania grandiflora*** (Linn).

4. RESULT & DISCUSSION:

Phytochemical Investigation of Leaves and Flowers Samples by Test Tube Method

In this research, after preparation flowers samples (Pauk pan phyu) ,the identification for the types of compounds present in the were done by using phytochemical test tube method. In accordance with this test, variety of constituents contain in these samples are tabulated in Table (1).

Table.1 Results of Phytochemical Tests on the *Sesbania grandiflora* (Linn)

No	Tests	Solvent Extract	Test Reagents	Observation	Sample
					Flowers
1	Alkaloid	1% HCl	Dragendroff's reagent	yellow brown ppt	+
			Wagner's reagent	greenish colour	+
2	Flavonoid	95% EtOH	Mg ribbon, conc: HCl	pink colour	+
3	Terpene	95% EtOH	acetic anhydride, conc: H_2SO_4, $CHCl_3$	pale green colour	–
4	Steroid	Pet-ether	acetic anhydride, conc: H_2SO_4, $CHCl_3$	greenish colour	–
5	Saponin	Distilled water	Distilled water	frothing	+
6	Phenolic compound	Distilled water	10% $FeCL_3$	brown ppt	+
7	Glycoside	Distilled water	10% lead acetate	cream ppt	+
8	Tannin	Distilled water	10% $FeCl_3$, dil: H_2SO_4	brown colour	+
9	Reducing sugar	Distilled water	Benedict's solution	green colour	+

(+) = presence (–) = absence

According to this table, flowers contain alkaloid, flavonoid, saponin, phenolic compound, glycoside, tannin and reducing sugar respectively.

Antimicrobial Activities of the flowers of *Sesbania grandiflora* (Linn.)

The antimicrobial activity of flowers (Pauk pan phyu) were tested with various solvent extract by using Agar well diffusion method in DCPT (Development Center for Pharmaceutical Technology) Insein, Yangon. The applying microorganisms are ***Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumalis, Candida albicans*** and ***E.coli*** species.The resultant data are tabulated in table (2).The length of the diameters show the degree of antimicrobial activity. The larger the inhibition zone diameter, the higher the antimicrobial activity.

Table (2) Results of Antimicrobial Activities of the ***Sesbania grandiflora*** (Linn)

Sample	Solvents	Organisms					
		I	**II**	**III**	**IV**	**V**	**VI**
	n-hexane	-	-	-	-	-	-

Paun pan phyu (Flower)	$CHCl_3$	12 mm (+)	13 mm (+)	13 mm (+)	13 mm (+)	13 mm (+)	12 mm (+)
	EtOAc	24 mm (+++)	32 mm (+++)	25 mm (+++)	28 mm (+++)	29 mm (+++)	30 mm (+++)
	EtOH	14 mm (+)	15 mm (++)	14 mm (+)	14 mm (+)	15 mm (++)	14 mm (+)
Control	Pet-ether	-	-	-	-	-	-
	$CHCl_3$	-	-	-	-	-	-
	MeOH	-	-	-	-	-	-
	n-hexane	-	-	-	-	-	-
	EtOAc	-	-	-	-	-	-
	EtOH	-	-	-	-	-	-
Agar well – 10 mm 10 mm ~ 14 mm (+) 15 mm ~ 19 mm (++) 20 mm above (+++)		* Organisms* (I) ***Bacillus subtilis*** (N.C.T.C- 8236) (II) ***Staphylococcus aureus*** (N.C.P.C-6371) (III) ***Pseudomonas aeruginosa*** (6749) (IV) ***Bacillus pumalis*** (N.C.I.B-8982) (V) ***Candida albicans*** (VI) ***E.coli*** (N.C.I.B-8134)					

According to the results from Table 2, it was found that pet-ether, $CHCl_3$, MeOH, EtOAc, n-hexane and EtOH extracts of ***Sesbania grandiflora*** (Linn) d flowers showed the antimicrobial activity against all strains of microorganisms.

Among them, EtOAc extract of Pauk pan phyu flowers respond high activity on all tested microorganisms. Furthermore, $CHCl_3$ extract of leaves and flowers show low activity on six selected microorganisms.

In addition, the EtOH extract of flowers show medium activity on ***Staphylococcus aureus*** and ***Candida albicans*** and low activity on ***Bacillus subtilis, Pseudomonas aeruginosa, Bacillus pumalis*** and ***E.coli***.n-hexane extract of flowers have no activity on all tested microorganisms.

Therefore, from the observed data, the various solvent extract of ***Sesbania grandiflora*** possessed potent antimicrobial activity except pet-ether and n-hexane extract.

5. CONCLUSION:

The present work is deal with the study of phytochemical constituents and antimicrobial activities of the flowers of ***Sesbania grandiflora*** (Pauk pan phyu). The qualitative phytochemical study revealed the presence of chemical constituents like alkaloid, flavonoid, glycoside, reducing sugar, phenolic compound, steroid, terpene, saponin and tannin.

Furthermore, the screening of antimicrobial activity showed that EtOAc extract flowers have higher activity than the other extracts. The $CHCl_3$ extract of flowers have low activity. Then, the MeOH extract of leaves and EtOH extract of flowers show medium activity on all tested microorganisms.

According to Ayurvedic system of medicine and reputed in the indigenous medicine, the ***Sesbania grandiflora*** (Pauk pan phyu) is claimed to be useful for various ailments and have a wide spectrum of medicinal properties. Thus, the flowers of ***Sesbania grandiflora*** (Pauk pan phyu) are edible and are often used to supplement meals.

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