Preliminary Phytochemical Test and Antimicrobial Activities of Leaves of *Cassia alata* L. (Thinbaw-mezali)

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Abstract

The medicinal plant of *Cassia alata* L. belongs to the family Caesalpiniaceae, commonly known as Pwesay-mezali, Thinbaw-mezali or Pwegaing in Myanmar. In this paper, preliminary tests on phytochemical constituents and antimicrobial activities of leaves of *C. alata* L. have been carried out. Preliminary phytochemical examination on leaves determined the presence or absence of chemical constituents. This examination showed the presence of alkaloid, glycoside, reducing sugar, saponin, phenolic compound, flavonoid, α-amino acid, carbohydrate, starch, tannin and acid or base compound except the cyanogenic glycoside. In addition, *in vitro* screening of antimicrobial activity, the leaves extracts of *C. alata* L. were prepared by various solvents such as pet-ether, chloroform, methanol, acetone, ethyl acetate, ethanol and water. The crude extracts were tested on six pathogenic microorganisms by using agar-well diffusion methods. According to this experiment in leaves, methanol, ethanol and acetone extracts showed moderately against on six pathogenic microorganisms. Moreover ethyl acetate extract showed moderately against on *Bacillus pumalis*, *Bacillus subtilis*, *Escherichia coli* and *Candida albicans* but no effective antimicrobial activity on *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Watery extracts of leaves showed against only on the *Staphylococcus aureus*. While pet-ether (60 - 80)ºC and chloroform extract showed no effective antimicrobial activity on six pathogenic microorganisms.

Keywords: Phytochemicals, Antimicrobial activity.

Introduction

The medicinal plant of *Cassia alata* L. belongs to the family Caesalpiniaceae. This plant is commonly known as Pwesay mezali, Thinbaw-mezali or Pwegaing in Myanmar and Ring worm senna, craw craw plant in English (Hundley and Chit Ko Ko, 1961, San Khin, 1970 and Kress, 2003).

Cassia *alata* L. is native to tropical South America and widely distributed in the tropical and warm temperate regions of the world (Kitikar & Basu, 1935 and Lawrence, 1969). This plant is cosmopolitan in the tropic; found in lower Bengal, Western Peninsula, Burma and Malacca (Watt, 1972). It grows wild in wet places but is also cultivated in various parts of Myanmar for its medicinal purposes (Myat Myat Ohn Khin, 1970).

Caesalpiniacae family includes 160 genera and the genus *Cassia* comprise 580 species (Wealth of India, 1950; http://www.jpsr.pharmainfo.in and https:// www.britannica.com). The genus *Cassia* includes 450-480 species (Lawrence, 1969).

Plants produce numerous natural products which have therapeutic benefits as traditional medicines for curing diseases. Drugs derived from natural products are usually secondary metabolites such as alkaloids, terpenoids, phenols, resins and tannins etc. These secondary metabolites are produced by a certain types of plant only (Buzarbarua, 2003).

The leaves and stem of *Cassia alata* L. have antiseptic and laxative properties, constipation, oedema, hepatitis and icterus in a tea like infusion. The leaves are regarded as an excellent medicine for ringworm. The leaves are also used in other skin diseases, purgative properties, herpes and snake bite. The decoction of leaves are drunk by women to hasten delivery of children. The leaves and flowers are used as relieving dyspnoeal oppression, promoting expectoration and tonic. The wood decoction is used as purgative (Kitikar & Basu, 1935; Prajapati, 2003).

Cassia alata L. is used as indigenous medicine locally in many countries. It is also used to urinary disorders, flatulence, dyspepsia, anorexia, laxative and purgative, digest, ringworm, scabies and other skin diseases in Myanmar (Myat Myat Ohn Khin, 1970 and Ashin Nagathein, 1976).

Thus, the aims of this research are to promote the Myanmar traditional medicine, to examine the medicinal plant of *Cassia alata* L. scientifically and to know its medicinal values. The main objectives are to investigate the preliminary phytochemical constituents and to examine the antimicrobial activities from different solvent extracts from leaves of this plant.

Materials and Methods

Sample collection and preparation

The specimens of *Cassia alata* L. were collected from Thaketa Township, Yangon Division during the flowering period of October - January, 2018. After the collection, the morphological characters of the specimens were identified by the available literatures such as Hooker, 1885, Kitikar & Basu, 1935; Hundley & Chit Ko Ko, 1961; Backer, 1963; Lawrence, 1969; U San Khin, 1970 and Kress, 2003.

Then, the samples were washed and then dried at room temperature until it reaches constant weight. After that, the samples were ground into powder and stored in air tight containers.

Preliminary phytochemical test from powdered leaves of *Cassia alata* L.

Preliminary phytochemical test was carried out to determine the presence or absence of phytoconstituents from the powdered leaves of *Cassia alata* L. These tests were on alkaloid, glycoside, reducing sugar, saponin, cyanogenic glycoside, phenolic compound, flavonoid, α- amino acid, carbohydrate, starch, tannin and acid or base compounds. The powdered leaves were tested qualitatively according to general chemical test books (British Pharmacopeia, 1965; Tin Wa, 1972 and Trease and Evans, 2002). The results were shown in Table (2).

In vitro antimicrobial activity of different solvent extracts from leaves of *Cassia alata* L.

For the determination of antimicrobial activity of the leaves extract from *Cassia alata* L. *in vitro*, agar-well diffusion method was used because of its simplicity, speed of performance, economy and reproducibility. The solvent extracts were tested against six pathogenic microorganisms by using agar-well diffusion method by (Cruickshank, 1970) at the Pharmaceutical and Food Research Department (PFRD). The extent of antimicrobial activity was measured from the diameter zone of inhibition. The microorganisms include *Bacillus pumalis*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*.

Table 1. Name of the test organisms and their code number

No.	Name of test organisms	Code number	Occurrence (Disease)
1	*Bacillus pumalis*	IFO-12102	Eye infection, soft tissue infection
2	*Bacillus subtilis*	JAP-0225215	Pathogenic group, anthrax in man and animals
3	*Escherichia coli*	ATCC- 25922	Urinary tract infections, peritonitis abscess, septic, wound and bed sores, diaorrhoea and dysentery.
4	*Pseudomonas aeruginosa*	IFO- 3080	Urinary tract infections, respiratory system infection, burn surgical wounds infection
5	*Staphylococcus aureus*	ATCC-12277	Food poisoning, boils, wound sepsis, muscle cramping, na vomiting, headache, carbuncle, staphylococcal pneumonia
6	*Candida albicans*	IFO- 1060	Fungal spp. pathogenic, skin infection, vaginal candidiasis ringworm

Antimicrobial screening

The antimicrobial activity of different solvent extracts from leaves of *Cassia alata* L. **was** performed by agar-well diffusion method. In this method, nutrient agar was used as culture media. For initial screening, nutrient agar was prepared according to the method described by (Cruickshank, 1970).

Nutrient agar was boiled and 20 - 25 ml of the medium was poured into each test tube and plugged with cotton wool and sterilized at 121ºC for 15 minutes in an autoclave. Then, the tubes were cooled down to 30 - 35ºC and poured into sterilized petridishes and 0.1 - 0.2 ml of test organism was also added into the dishes. The agar was allowed to set for 2 - 3 hours. And then, 10mm plate agar-well was made with the help of sterilized agar-well culture. After that, about 0.2 ml of sample was introduced into the agar-well and incubated at 37ºC for 24 hours. The inhibition zone appeared around the agar-well, indicating the presence of antimicrobial activity. Then, the zones of inhibition diameter including 10 mm agar-well were measured with the aid of a transparent ruler.

At the same time, the controlled experiments using solvent only were prepared for the comparison with samples. The antimicrobial activities of different solvent extracts from leaves were measured from the diameter zone of inhibition as in Table (3) and Figure (9).

Results

Morphological characters of *Cassia alata* L.

Scientific Name - *Cassia alata* L.
Myanmar Name - Pwesay-mezali, Thinbaw-mezali or Pwegaing
English Name - Ring worm senna, craw craw plant
Family - Caesalpiniaceae

The plant is perennial shrub, stem woody, erect with downy branches. Leaves are alternate, pinnately compound, paripinnate, deciduous, triangular stipule present; leaflets subsessile, brodly rounded, minutely mucronulate at the tip, margin entire, oblique at the base, glabrous. Inflorescences are racemose, terminal spike, erect, peduncle long. Flowers are yellow in colour, short pedicel, bracteates; yellow, conspicuous, caducous, ebracteolate, bisexual, complete, zygomorphic, hypogynous, ovary superior; sepals are 5, aposepalous, petaloid, ovate, quincunical aestivation, inferior; petals are 5, apopetalous, petaloid, ovate with claw, imbricate, inferior; stamen are $7+3^{st}$, apostemonous, unequal, 3 posterior stamen staminode, and remaining stamen fertile, anther dithecous, basifixed, terminal dehiscence, inferior; carpels 1, monocaperally, unilocular, marginal placentation, style short, stigma terminal, hairy, ovary superior. Fruits are pod with angle, dehiscent. Seeds are quandrangular in shape, flattened, green in young and mature in black colour and endospermic.

Morphological characters of *Cassia alata* L.


Fig. (1) Habit of the plant


Fig. (2) Leaves


Fig. (3) Inflorescences


Fig. (4) Flower


Fig. (5) L.S of flower

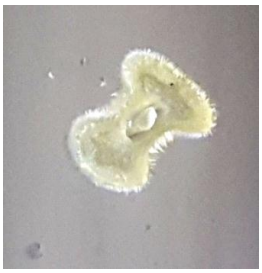


Fig. (6) T.S of ovary


Fig. (7) Fruits


Fig. (8) Seeds

Preliminary phytochemical test from powdered leaves of *Cassia alata* L.

The results showed the presence of alkaloid, glycoside, reducing sugar, saponin, phenolic compound, flavonoid, α-amino acid, carbohydrate, starch, tannin and acid or base except cyanogenic glycoside in leaves. These results were shown in Table (2).

Table (2) Preliminary phytochemical test from powdered leaves of *Cassia alata* L.

No.	Tests	Test reagents	Observation	Results
1	Alkaloids	1.Mayers's 2.Dragendroff's	white ppts. orange ppts.	+ +
2	Glycoside	10% Lead acetate	white ppts.	+
3	Reducing Sugar	1.Benedict's solution 2.Fehling solution	brick red ppts. brick red ppts	+ +
4	Saponin	Distilled water	frothing	+
5	Cyanogenic glycoside	Sodium picrate paper	no colouration	-
6	Phenolic compounds	Ferric chloride solution	brown colour	+
7	Flavonoid	Benzene + $FeCl_3$	yellow colour	+
8	α-amino acid	Ninhydrin reagent	violet colour	+
9	Carbohydrate	10% α-napthol + H_2SO_4 (conc:)	pink ring	+
10	Starch	Potassium iodide solution	blue black	+
11	Tannin	Ferric chloride solution	brown ppts.	+
12	Acid or basic	Bromocresol green	green/red colour	Neutral

+ = present, - = absent

Antimicrobial activity of six different solvent extracts from leaves of *Cassia alata* L.

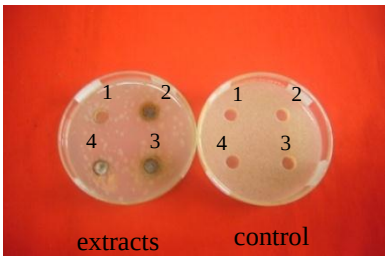
The results in the screening of the antimicrobial activity of six different solvent extracts from leaves of *Cassia alata* L. were as shown in Table (3).

Table 3. Antimicrobial activity of six different solvent extracts from leaves of *Cassia alata* L.

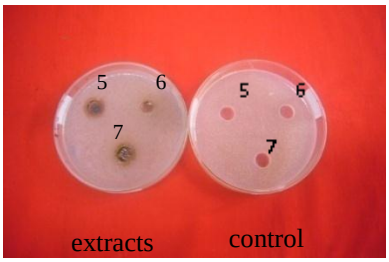
Sample	Solvents	*Bacillus pumalis*	*Bacillus subtilis*	*E.coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Candida albicans*
	$CHCl_3$	-	-	-	-	-	-
	Pet-ether	-	-	-	-	-	-
Leaves	MeOH	15mm	14mm	15mm	15mm	15mm	16mm
	Acetone	14mm	13mm	13mm	15mm	15mm	15mm
	EtOAc	16mm	15mm	15mm	-	-	15mm
	EtOH	15mm	14mm	15mm	15mm	15mm	14mm
	H_2O	-	-	-	-	15mm	-

Agar well- 10mm

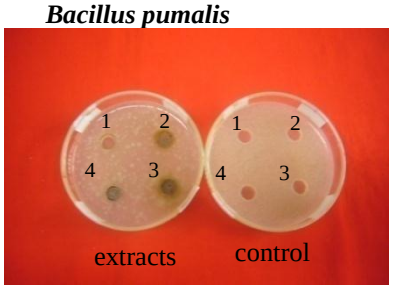
According to this experiment in leaves, methanol, ethanol and acetone extracts showed moderately against on six pathogenic microorganisms. Moreover ethyl acetate extract showed moderately against on *Bacillus pumalis*, *Bacillus subtilis*, *Escherichia coli* and *Candida albicans* but no effective antimicrobial activity on *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Watery extracts of leaves showed against only on the *Staphylococcus aureus*. Pet-ether (60 - 80)ºC and chloroform extract showed no effective antimicrobial activity on six microorganisms. These results were shown in Figure (9).



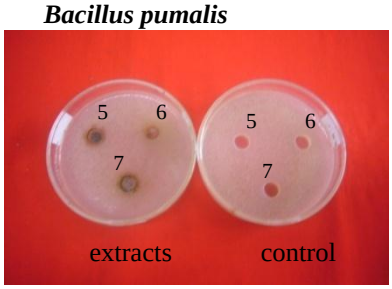
Bacillus pumalis



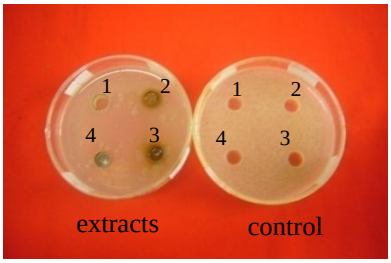
Bacillus pumalis



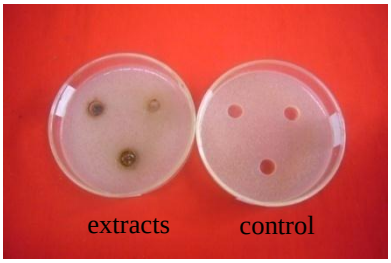
Bacillus subtilis



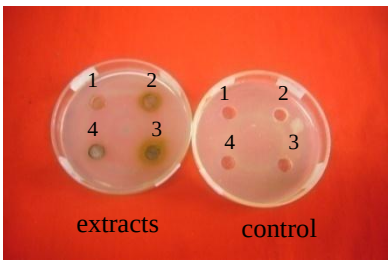
Bacillus subtilis



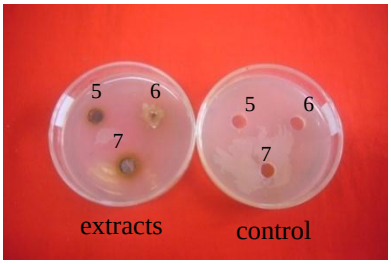
E. coli



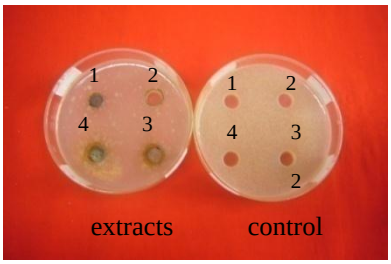
E. coli



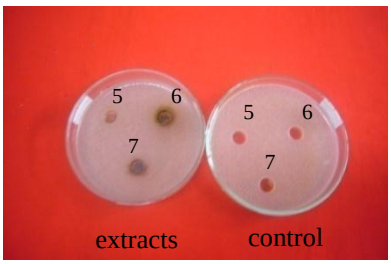
Pseudomonas aeruginosa



Pseudomonas aeruginosa

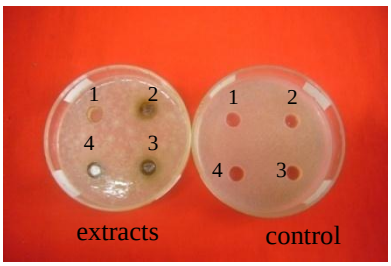


Staphylococcus aureus

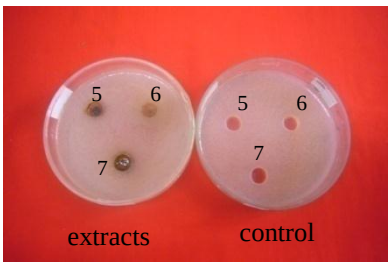


Staphylococcus aureus

1. Petroleum-ether 2. Acetone 3. Methanol 4. Chloroform
5. Ethyl acetate 6. Water 7. Ethanol



Candida albicans



Candida albicans

1. Petroleum-ether 2. Acetone 3. Methanol 4. Chloroform
5. Ethyl acetate 6. Water 7. Ethanol

Fig. (9) Antimicrobial activities from leaves of *Cassia alata* L.

Discussion and Conclusion

In this research, preliminary phytochemical test and antimicrobial activities of leaves of *Cassia alata* L. have been described.

The plant is shrub, finely downy branches. Leaves are alternate, pinnately compound, paripinnate, subsessile. Inflorescences are terminal and axillary spike; flowers are yellow, conspicuous bract. Sepals are petaloid, stamen unequal, fruits are pod. These characters are in agreement with those given by Hooker, 1885; Kirtikar & Basu, 1935 and Prajapati, 2003.

In the investigation of preliminary phytochemical test revealed that the presence of alkaloids, glycosides, reducing sugar, saponin, phenolic compounds, flavonoids, α-amino acid, carbohydrate, tannin and the absence of cyanogenic

glycoside and organic acid or base in leaves. These results were in agreement with those given by Doughari and Okafor, 2007.

In the study of antimicrobial activity, the various solvent extracts of leaves of *Cassia alata* L. **were** obtained by using pet-ether (60 - 80)ºC, chloroform, methanol, acetone, ethyl acetate, ethanol, and water were investigated. These various solvent extracts were tested by agar-well diffusion method. The results showed methanol, ethanol and acetone extracts showed moderately against on six pathogenic microorganisms. Moreover, ethyl acetate extract showed moderately against on *Bacillus pumalis*, *Bacillus subtilis*, *Escherichia coli* and *Candida albicans*, but no effective antimicrobial activity on *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Watery extracts of leaves showed against only on the *Staphylococcus aureus*. While pet-ether (60 - 80)ºC and chloroform extract showed no effective antimicrobial activity on six microorganisms.

According to the result, absence of cyanogenic glycosides indicates that the leaves of *Cassia alata* L. were not toxic for humans. In addition, the bioactive compounds of flavonoid and phenolic compounds have been reported to be used by plant for protection against bacterial, fungal pesticidal infection and are responsible for antimicrobial activity.

Therefore, the medicinal plant of *Cassia alata* L. has valuable medicinal properties for human used as antibiotic drugs in traditional medicine.

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