MORPHOLOGICAL IDENTIFICATION AND PHYTOCHEMICAL INVESTIGATION OF *CASSIA OCCIDENTALIS* L. LEAVES AND ITS ANTIMICROBIAL ACTIVITY

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Abstract

Cassia occidentalis L., belonging to family Fabaceae, is a medicinal plant used as a traditional medicine for the treatment of various diseases. The present study was designed to evaluate the preliminary phytochemical constituents and antimicrobial activity of leaves of *Cassia occidentalis* L. The specimen were collected from Banmaw Township, Kachin State. The morphological characters of this plant have been studied in detail and identified by the available literatures. The dried leaf powder of *Cassia occidentalis* L. was subjected to phytochemical analysis in order to find out the presence of phytochemical constituents. It contained many chemical groups included alkaloids, glycoside, reducing sugar, saponin, steroid, terpenoids, carbohydrate, tannin, phenolic compound, flavonoid, starch, protein and amino acid. For antimicrobial activity, the leaf powder of *Cassia occidentalis* L. was extracted with seven different solvents. The extracts were used to carryout antimicrobial screening in vitro on six different types of microorganisms by agar well diffusion method. It was found that the ethyl acetate extract showed most significant antimicrobial activity on *Pseudomonas aeruginosa*. The phytochemical investigations and antimicrobial properties of leaves of *Cassia occidentalis* L. prove its importance as a valuable medicinal plant.

Keywords : Phytochemical, Antimicrobial, *Cassia occidentalis*

Introduction

Traditional medicine, making use of herbs in different preparations, is greatly relied upon especially by rural dwellers, for the treatment of various ailments. Nowadays, there is growing trend of people moving from synthetic drugs to herbal cure. The plant under investigation is *Cassia occidentalis* L. *Occidentalis* species belongs to the genus *Cassia* and the Family Fabaceae. It is called Stink Weed, Stinking or Negro Coffee (Nuhu, 2008). *Cassia* is one of the largest genera with about 250 species and it is famous genus for its ornamental value and medicinal usage (Cronquist, 1981). *Cassia* plants are widely distributed and well known in Myanmar for their medicinal and ornamental values with brightly-coloured flowers. The potential of the leaf extract of *C. occidentalis* may be related to its antioxidant activity. The extract contains flavonoids which are powerful antioxidant polyphenolic compounds (Nuhu, 2008). The root of *Cassia occidentalis* L. is useful in ringworm and scorpion sting. The leaves are used in cough, asthma, stomachic and fevers. The seeds are also used as a substitute for coffee (Kirtikar and Basu, 1975).

The active principle of many drugs found in plants is phytochemical. The medicinal value of these phytochemicals is because of the presence of chemical substance that produces definite physiological action on the human body. Some of the valuable one include: - Alkaloids, tannins, saponins, glycosides, flavonoids, phosphorus and calcium for cell growth, replacement, body building (Harbone, 1973). *Cassia occidentalis* L. was found to be contain many groups of chemical substance.

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The development of resistance to current antibiotics by disease causing microbes has reinforced research for discovery of new ones. Current trends in drug development process are focused on natural sources, especially source of plant origin due to some proven correlation between the folkloric medicinal uses of some of these plants to biological activity (Kunle, 2009). *C. occidentalis* is an ayurvedic plant with huge medicinal importance. Leaves of this plant have ethno medicinal importance like paste of leaves is externally applied on healing wounds, sores, itch, cutaneous diseases, bone fracture, fever, ringworm, skin diseases and throat infection (Burkill, 1995).

It is the intention of this research work to identify the relevant phytochemical compounds of the plant part that produces the antimicrobial effect and to determine the antimicrobial activity of the plant extract to correlate to its medicinal use.

Materials and Methods

Morphological Studies

Collection, Identification and Preparation

In this study, *Cassia occidentalis* L. was collected during flowering period (December to March) from some areas of Banmaw Township. After the collection, all the vegetative and reproductive parts of the fresh specimens were studied, measured in detail and recorded. The relevant data for taxonomic description of the species were also recorded. Based on the resulting data, the plants were identified with the help of literatures (Backer; 1968, Burkill; 1935, Hooker; 1881, Dassanayake (1981) and Hu-Qi-ming (2009).). All the necessities were documented by photographs. Then, the leaves of this plant were thoroughly washed with water, dried in shade and crushed and powdered with a grinding machine. This powder was stored in the airtight container for further study.

Preliminary phytochemical investigation

For the phytochemical study, powdered leaves of *Cassia occidentalis* L. were used to find out the presence or absence of phytochemical constituents. The preliminary phytochemical tests were carried out at the Department of Botany, Banmaw University according to the methods of British Pharmacopoeia (1968), Marini Bettolo *et. al.* (1981) and Trease and Evans (2002).

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

Antimicrobial activity of different solvent extracts from Cassia occidentalis L. leaves were tested on six microorganisms by agar well diffusion method at Central Research and Development Centre (CRDC).

Preparation of the crude extracts

About 5 g of the powdered leaves was extracted with 20 ml of each solvent (ethanol, methanol, pet-ether, chloroform, acetone, ethyl acetate and water) respectively. The powder sample with respectively solvents was placed in water bath for 6 hours. The crude extracts were then filtered. After filtration, the extracts were dried on water bath to obtained concentrated substances.

Test organisms

The test organisms used in this study were *Bacillus subtilis* (JAP – 0225215), *Bacillus pumalis* (IFO – 12102), *Candida albican* (IFO – 1060), *Escherichia coli* (ATCC – 25922), *Pseudomonas aeruginosa* (IFO – 3080) and *Staphylococcus aureus* (ATCC – 12277).

Antimicrobial screening

In this method, nutrient agar was used as culture media. For initial screening, according to the method nutrient agar was prepared described by Cruickshank, 1975. Nutrient agar was boiled and poured into the test tube and plugged with cotton wool and sterilized in an autoclave at 121°C for 15 minutes. Then, the tubes were cooled down to 30 – 35°C and poured into sterilized petri-dishes and 0.1 – 0.2 ml of microbial suspension from nutrient broth were added into the dishes. The agar medium was allowed to solidify for 2 – 3 hours, then 10 mm agar well was made by the help of sterilized agar well cutter. After that, about 0.2 ml of sample dissolved in their respective solvents was introduced into the agar well and incubated at 37°C for 24 hrs. After incubation for 24 hrs, the inhibition zone which appeared around the agar well indicated 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 were prepared with only solvent for the comparison with plant extracts.

Results

Morphological studies

Scientific Name	:	*Cassia occidentalis* L.
Commons Name	:	Coffee Senna
Myanmar Name	:	Kazaw-boke
Family	:	Fabaceae
Subfamily	:	Caesalpinoideae

Flowering and fruiting Period: December to March

Taxonomic description

Annual, herb, stem erect, 1-2 meters long, 0.5-1.5 cm. thickness at its basal region, branching at nodes spirally. Young stem is green in colour and furrowed, while the mature stem is light brown to dark in colour. Branches many, ascending and smooth. The internode is 2-4 cm. long. Leaves: alternate, paripinnately compound, 9-13-20cm. long, petiolate, petiole (rachis) pulvinate, grooved or nearly round, glabrous, 5-12cm. long, showing dark purplish colour in the grooved portion and greenish on the opposite side. Leaflets:3-5 pairs, opposite, unequal, the lower most smallest and ovate, the superior ones longer, 2.5-8 cm. broad, very short stalk, ovate, oblong to ovate, lanceolate, acute or acuminate, base usually rounded and somewhat oblique, glabrous above and pubescent beneath. The leaves possess a very foetid odour. Inflorescences: racemes few –flowered, axillary, and also forming terminal panicle; bracts caduceus. Flower: Yellow, 1 to 2 cm in diameter, complete, bisexual, regular, actinomorphic, hypogynous. Calyx: sepals 5, free, oblong, glabrous, brownish green. Corolla: petals 5, free, obovate, shortly clawed, yellow. Androecium: stamens 10, free, 6-fertile, 4-sterile, inserted; filaments filiform unequal,

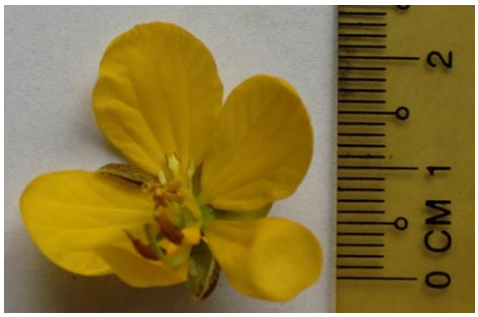
yellow, glabrous; anther dithecous, basifixed, curved, yellow, dehiscent by apical pores. Gynoecium: carpel 1, linear, pubescent, unilocular, many ovules in each locule, marginal placentation; style long filiform, curved, glabrous; stigma capitate. Fruit: flat pods 10-12 cm. long with 10-30 seeds. Areolate seeds are pointed at end and blunt at the other (as shown in Figure.1).

Habit

leaf

Pulvinate petiole

Inflorescence



Flower

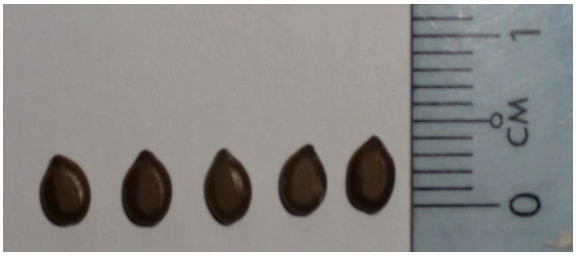
Sepal

Petal

Stamen

ovary

Fruits



Seeds

Figure 1 Morphological characters of *Cassia occidentalis* L.

Preliminary phytochemical investigation

Preliminary phytochemical test on the leaves of *Cassia occidentalis* L. was investigated and the presence or absence of phytochemical constituents in this plant were presented in Table 1 and Figure 2.

Table 1 phytochemical test on the leaves of *Cassia occidentalis* L.

No.	Constituents	Extract	Test Reagent	Observation	Result
1.	Alkaloid	3% HCL	Mayer's reagent Hager reagent Wagner's reagent.	White ppt. Yellow ppt. Reddish Brown ppt.	+
2.	Glycoside	EtOH	1 ml of water and NaOH sol:	Yellow colour	+
3..	Phenolic compound	H_2O	3% ferric chloride sol:	green colour	+
4.	Flavonoid	EtOH	HCL, Mg turning	Pink colour	+
5.	Steroid	EtOH	$CHCL_3$+conc. H_2SO_4	Green colour	+
6.	α- amino acid	H_2O	Dry and sprayed with Ninhydrin reagent	Pink spot	
7.	Terpenoid	EtOH	$CHCL_3$+ conc. H_2SO_4	Pink colour	+
8.	Starch	H_2O	I_2 solution	blue-black ppt.	+
9.	Reducing sugar	H_2O	Benedict Solution	Brick red ppt.	+
10.	Saponin	H_2O	Distilled water	Frothing	+
11.	Tannin	H_2O	5% ferric chloride sol:+ dil H_2SO_4	Yellowish-brown ppt.	+
12.	Carbohydrate	H_2O	Fehling sol: A+B	Red colour ppt.	+
13.	Protein	H_2O	Sodium hydroxide sol: + 3% copper sulphate sol:	Red or violet colour	+

+ = Present – = Absent

According to the present study, it is found that the leaves of *Cassia occidentalis* L. contained alkaloid, flavonoid, steroid, terpenoid, glycoside, carbohydrate, saponin, tannin, resin, polyphenol, protein and starch. These tests were shown in Figure 2.

Figure 2 Preliminary phytochemical test of the leaves of *Cassia occidentalis* L.

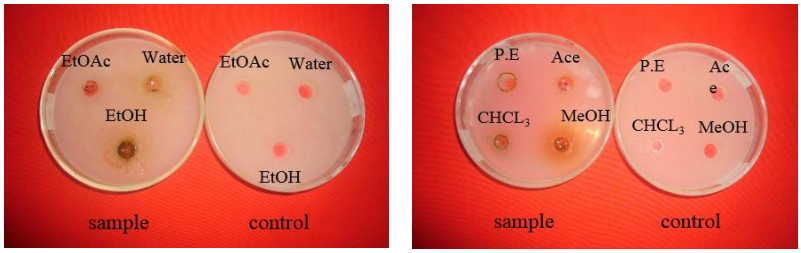
Antimicrobial activity

Screening of antimicrobial activity of leaves of *Cassia occidentalis* L. was carried out by using different solvents namely pet-ether, chloroform, methanol, acetone, ethyl acetate, ethanol and water. The diameter of inhibition zones that appeared were given in Table 2.

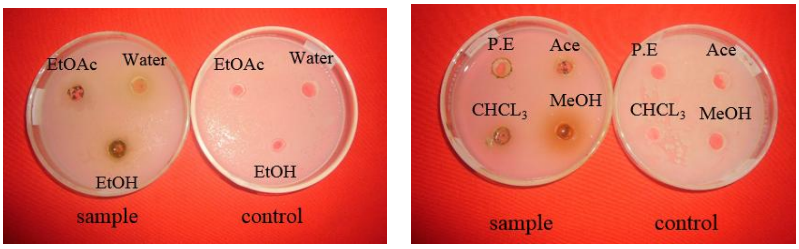
Table 2 Inhibition zone exhibited by different extracts of leaves of *Cassia occidentalis* L. against six microorganisms

No.	Extract	Microorganisms					
		B. subtilis	***S. aureus***	***P. aeruginosa***	***B. pumilus***	***C. albican***	***E. coli***
1.	Pet-ether	-	-	-	-	-	-
2.	Chloroform	-	-	-	14 mm	12 mm	-
3.	Methanol	14 mm	14 mm	13 mm	12 mm	13 mm	14 mm
4.	Acetone	14 mm	15 mm	15 mm	14 mm	14 mm	12 mm
5.	Ethyl acetate	-	-	30 mm	-	-	14 mm
6.	Ethanol	14 mm	13 mm	-	-	11 mm	13 mm
7.	water	12 mm	-	-	-	11 mm	-

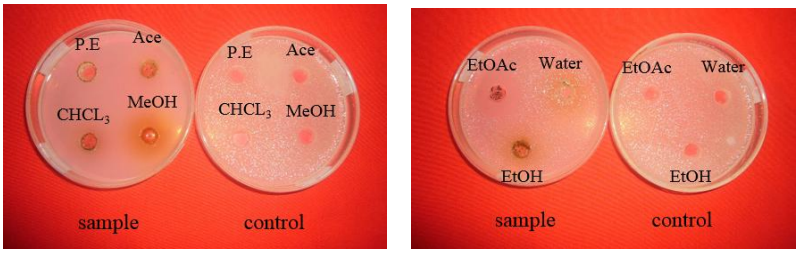
Agar well = 10 mm - = No activity



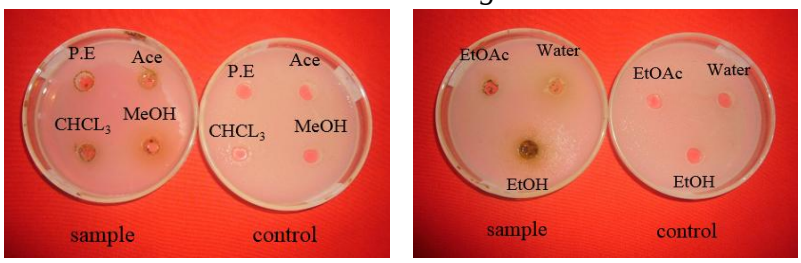
Bacillus subtilis



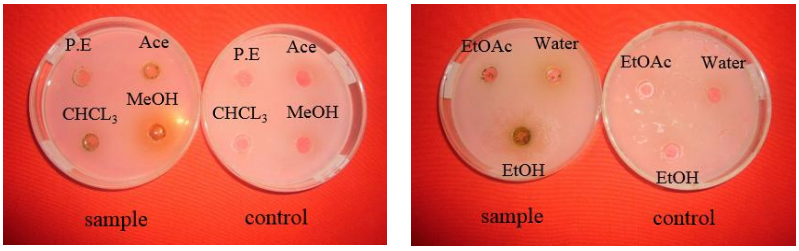
Staphylococcus aureus



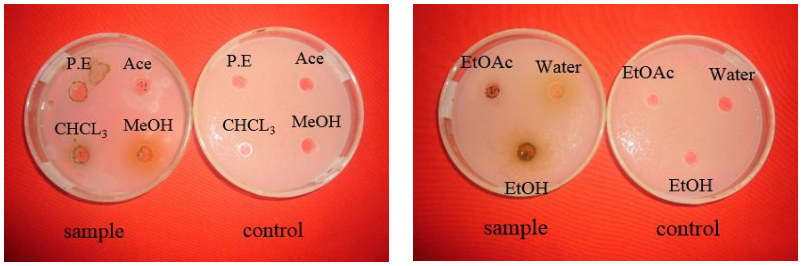
Pseudomonas aeruginosa



Bacillus pumalis



Candida albicans



Escherichia coli

Figure 3 Antimicrobial test of different solvent extracts from leaves of *Cassia occidentalis* L.

In this experiment, pet-ether extract was found to be insensitive to all tested organisms. All other extracts did not show significantly the antimicrobial activity on tested organisms except that ethyl acetate extract showed the highest activity especially more sensitive against *P. aeruginosa* (inhibition zone 30 mm). Controlled experiment containing only solvent did not show inhibitory activities on any of the organisms as shown in Figure 3.

Discussion and Conclusion

Research on *Cassia occidentalis* L. was made from two aspects such as phytochemical study and antimicrobial study about diseases caused by microorganisms. The specimens were collected from Banmaw Township, Kachin State. It is an erect herb and commonly found in road sides. It belongs to the family Fabaceae. The common name is Kazaw Boke. This plant has been widely used as traditional medicine. Entire parts of the plant have medicinal values. The roots, leaves and seeds are the parts of the plant used.

Crude drugs are usually obtained from wild sources and are mostly collected by illiterate and unskilled people unaware of their botanical information, authentication and standardization parameters. This usually affects the safety of the final product. For safe and efficacious herbal medicine production, appropriate control of starting material is extremely crucial (Kumar, 2014). *Cassia occidentalis* is a plant with potentially limitless uses and is of importance to properly establish a partial monograph for its correct identification. The morphological characters of *Cassia occidentalis* mentioned in result were in accordance with those described by Backer (1968), Burkill (1935), Hooker (1881), Dassanayake (1981) and Hu-Qi-ming (2009).

The preliminary phytochemical test revealed that Alkaloid, flavonoid, steroid, terpenoid, glycoside, carbohydrate, saponin, tannin, resin, polyphenol, protein and starch are present in leaves of *Cassia occidentalis*. The result of this study indicated that the leaves of this plant contain some major bioactive compounds needed for organisms. So, this plant proved very active.

In the antimicrobial activity, the leaves of *Cassia occidentalis* were extracted with different solvents. The extracts were used to carry out antimicrobial screening on *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albican* and *E. coli*. The result showed that pet-ether extracts are not effective against all of the test organisms and methanol and acetone extract are effective against all of the test organisms. The highest activity (zone of inhibition in diameter is about 30 mm) was demonstrated by the ethyl acetate extract against *Pseudomonas aeruginosa*. Curickshank (1975) stated that *Pseudomonas aeruginosa* causes urinary tract infection, respiratory system infection, bone and joint infection, chronic lung, eye infection, burn infection. Therefore, it is recommended that the different components detected in leaves of this plant should be isolated and tested against the susceptible microorganism (*Pseudomonas aeruginosa*) in order to arrive at the most potent structure. Further in-depth research has to be carried out to use the phytochemicals in pharmaceutical industry as a substitute for medicine.

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