PHYTOCHEMICAL SCREENING AND EVALUATION OF VARIOUS EXTRACTS OF *CASSIA FISTULA* L. FRUIT PULP FOR ANTIMICROBIAL ACTIVITY

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Abstract

Cassia fistula L., belonging to family Fabaceae, is known for its beautiful inflorescences and medicinal properties of various plant parts. This plant is called Ngu-shwe-war in Myanmar. It is widely cultivated and used in folk medicine. The present study was designed to evaluate the preliminary phytochemical constituents and antimicrobial activity of fruits of *Cassia fistula* 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 literatures (Backer, 1968; Burkill, 1935; Hooker, 1881; Dassanayake, 1981 and Hu-Qi-ming, 2009). The diagnostic characters of dried fruit pulp powder of *Cassia fistula* L. were investigated for standardization of powdered drugs. Then the powdered sample was subjected to phytochemical analysis in order to find out the presence of phytochemical constituents. From this, it is known that it contained alkaloids, glycoside, reducing sugar, saponin, steroid, terpenoids, carbohydrate, tannin, phenolic compound, flavonoid, starch, protein and amino acid. For antimicrobial activity, the fruit pulp powder of *Cassia fistula* L. was extracted with six different solvents to carryout antimicrobial screening in vitro on six different types of microorganisms by paper disc diffusion method. It was found that the watery extract showed most significant antimicrobial activity on *Aspergillus flavous* and *Candida albicans* whereas ethanol extract also gave highest activity on *Escherichia coli*. The phytochemical investigations and antimicrobial activity of fruit of *Cassia fistula* L. prove its importance as a valuable medicinal plant.

Keywords: *Cassia fistula* L., Morphology, Phytochemistry, Antimicrobial activity

Introduction

Medicinal herbs are moving from fringe to mainstream use with a greater number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals. Researchers have aimed at identifying and validating plant-derived substances for the treatment of various diseases. Interestingly it is estimated that more than 25% of the modern medicines are directly or indirectly derived from plants (Danish, 2011). Nature always stands as a golden mark to exemplify the outstanding phenomena of symbiosis. Natural products from plant, animal and minerals have been the basis of the treatment of human disease. 80% of people in developing countries still relays on traditional medicine based largely on species of plants and animals for their primary health care. Medicinal plants are important for pharmacological research and drug development, not only when plant constituents are used directly as therapeutic agents, but also as starting materials for the synthesis of drugs or as models for pharmacologically active compounds. The derivatives of medicinal plants are non-narcotic with little or no side effects (Banjare Paul, 2014).

Cassia fistula L. (family Fabaceae) is known as not only the medicinal plant but also ornamental plant. It is also commonly known as golden or yellow shower because of its characteristics yellow flowers in pendulous raceme and with typical branches. It is distributed in various regions including Asia, South Africa, China, West Indies and Brazil. It has been extensively used in Ayurvedic system of medicine for various ailments (Joshi, 2004).

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According to Hartwell, *Cassia fistula* plants are used in folk remedies for tumors of the abdomen, glands, liver, stomach and throat cancer, carcinomata and impostumes of the uterus. Ayurvedic medicine recognizes the seed as antibilious, aperitif carminative and laxative the root for adenopathy, burning sensations, leprosy, skin diseases, syphilis and tubercular glands the leaves for erysipelas, malaria, rheumatism and ulcers the buds for biliousness constipation, fever, leprosy and skin disease the fruit for abdominal pain, constipation, fever, heart disease and leprosy. Unani use the leaves for inflammation the flowers for a purgative the fruit as anti-inflammatory, antipyretic, abortifacient, demulcent, purgative, refrigerant good for chest complaints eye ailments, flu, heart and liver ailments, and rheumatism. In the West Indies, the pulp and leaves are poulticed onto inflamed viscera, e.g., the liver (Anitha & Miruthula, 2014).

This research aims to provide a comprehensive review on the phytochemical and antimicrobial aspects of fruit of *Cassia fistula* L.

Materials and Methods

Collection of plant materials

In this study, fruit pulp of *Cassia fistula* L. were collected in the month of April-May, 2019 from local area of Banmaw Township. Then fruit pulp were dried and finely powered and used for the study.

Diagnostic characters of the fruit pulp powder

The sensory characters of fruit pulp powder were observed in sight and the microscopical characters of powder sample were examined under the light microscope. Chloral hydrate solution was used as cleaning reagent.

Preliminary phytochemical investigation

For the phytochemical study, fruit pulp powder of *Cassia fistula* L. were used and carried out at the Department of Botany, University of Yangon according to the methods of British Pharmacopoeia (1968) and Trease and Evans (2002).

Antimicrobial activity of *Cassia fistula* L. fruit

Antimicrobial activity of different solvent extracts from *Cassia fistula* L. fruit were carried out on six microorganisms by paper disc diffusion method at the Department of Botany, University of Yangon.

Preparation of the crude extracts

About 5 g of the powder sample was extracted with 20 ml of each solvent (ethanol, methanol, pet-ether, acetone, ethyl acetate and water) respectively. The crude extracts were then filtered. After filtration, the extracts were concentrated to dryness and the residues were transferred to a pre-weighed bottle and were stored in desiccators for further studies.

Test organisms

The test organisms used in this study were *Aspergillus flavous*, *Bacillus subtilis*, *Candida albican*, *Escherichia coli*, *Pseudomonas fluorescens* and *Xanthomonas oryzae*.

Antimicrobial screening

Isolated bacterial strains grown on nutrient agar were inoculated into 50 ml conical flasks containing 10 ml of sterile growth medium. Then, they were incubated at 30°C for 72 hours on a reciprocal shaker at 200 rpm. 0.3 ml of test organisms was added to assay medium, then poured into plates. After solidification, paper discs impregnated with different solvent extracts were applied on the test plates and these plates were incubated for 24-36 hours at 30°C. After incubation

for 24-36 hours, the inhibition zone which appeared around the paper discs indicated the presence of bioactive compounds which inhibit the growth of test organisms. Then, the zones of inhibition diameter including 6 mm paper disc were measured with the aid of a transparent ruler.

Results

Scientific Name : *Cassia fistula* L.
Commons Name : Golden shower, purging cassia
Myanmar Name : Ngu-shwe-war
Family : Fabaceae
Flowering and fruiting Period : March to September

Taxonomic description

A medium-sized deciduous tree, 6-9 meters tall with a straight trunk and spreading branches. Young stem is pale grey in colour and smooth, while the mature stem is dark brown in colour and rough. Leaves: alternate, paripinnately compound, 20-40 cm long, petiolate, long stalked stipulate. Leaflets: 4-5 pairs, opposite, ovate, acute or acuminate, base usually rounded. Inflorescences: axillary, drooping raceme. Flower: yellow, very showy, complete, bisexual, irregular, zygomorphic, hypogynous. Calyx: sepals 5, synsepalous, lanceolate, pubescent, yellowish green. Corolla: petals 5, apopetalous, obovate, unequal, shortly clawed, yellow. Androecium: stamens 10, free, vary in length; filaments long and curved; anther dithecous, introrse, dorsifixed and basifixed. Gynoecium: carpel 1, linear, pubescent, unilocular, many ovules in each locule, marginal placentation; style long; stigma reduced and curved. Fruit: elongated and rounded pod, 30-60 cm long with 60-75 seeds. Seeds are pointed at end and blunt at the other (as shown in Figure.1).

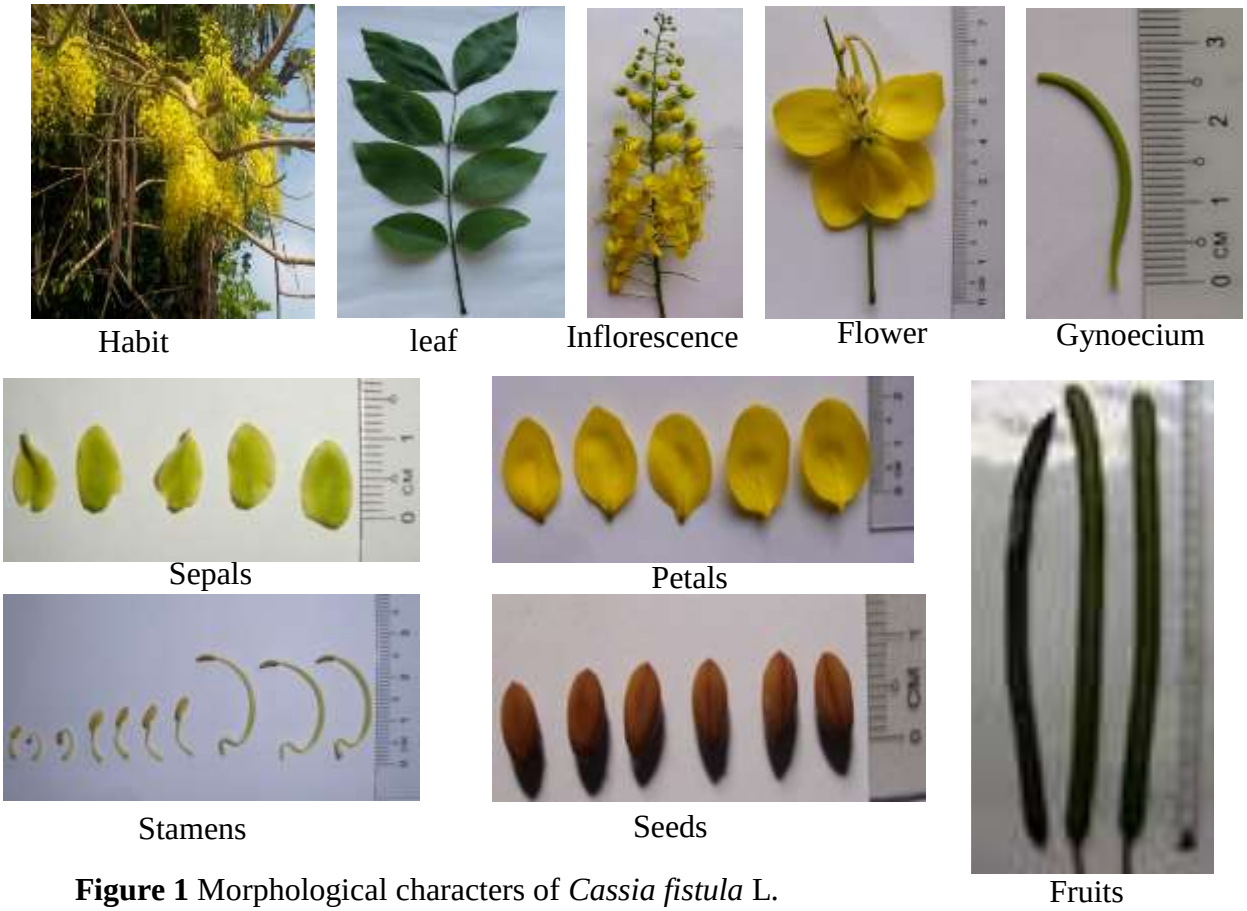


Figure 1 Morphological characters of *Cassia fistula* L.

Diagnostic characters of fruit pulp powder

Sensory characters of fruit pulp powder of *Cassia fistula* L.

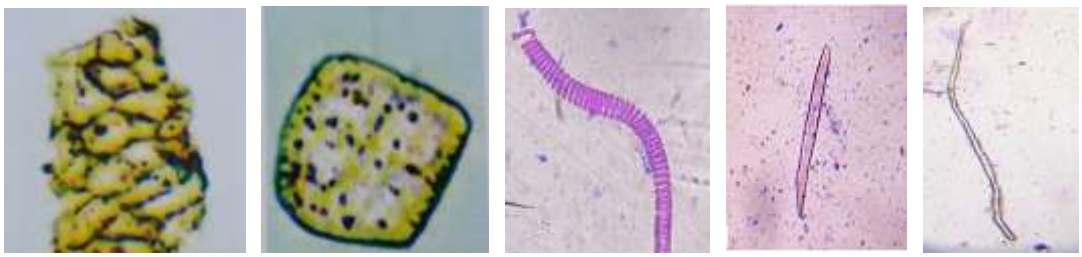
Colour = dark brown

Odour = characteristic

Taste = sweet and mucilaginous

Texture = sticky

Figure 2 Fruit pulp powder



Epidermal cells Stone cells Spiral vessel Treached Fibre

Figure 3 Microscopical characters of fruit pulp powder of *Cassia fistula* L.

Preliminary phytochemical investigation

Preliminary phytochemical test on the fruit pulp of *Cassia fistula* L. was investigated and the presence or absence of phytochemical constituents in this plant were presented in Table 1 and Fig. 4.

According to preliminary phytochemical study, it is found that the fruit pulp of Cassia fistula L. contained alkaloid, glycoside, phenolic compound, flavonoid, steroid, terpenoid, α-amino acid, starch, reducing sugar, saponin, tannin, carbohydrate and protein. These tests were shown in Figure 4.

Figure 4 Preliminary phytochemical test of the fruit pulp of Cassia fistula L.

Table 1 Phytochemical test on the fruit pulp of *Cassia fistula* L.

No	Constituents	Extract	Test Reagent	Observation	Result
1.	Alkaloid	Methanol	1% HCL + Mayer's reagent 1% HCL + Hager reagent 1% HCL + Wagner's reagent.	White ppt. Yellow ppt. Reddish Brown ppt.	+
2.	Glycoside	Methanol	1 ml H_2O + NaOH	Yellow colour	+
3.	Phenolic compound	Methanol	2 ml H_2O + 10% $FeCL_3$	green colour	+
4.	Flavonoid	Methanol	HCL (dil) + Mg coil	Pink colour	+
5.	Steroid	Methanol	$CHCL_3$ + conc. H_2SO_4	Green colour	+
6.	Terpenoid	Methanol	$CHCL_3$ + conc. H_2SO_4	Reddish brown colour	+
7.	α- amino acid	Water	Ninhydrin reagent	Pink spot	+
8.	Starch	Water	I_2 solution	blue-black ppt.	+
9.	Reducing sugar	Water	1 ml H_2O + mixture equal part Fehling's A and B	Brick red ppt.	+
10.	Saponin	Water	Shaken with 2 ml H_2O	Frothing	+
11.	Tannin	Water	5% $FeCL_3$ + dil H_2SO_4	Yellowish-brown ppt.	+
12.	Carbohydrate	Water	1 ml benedict's reagent and boil for few minute	Brick red ppt.	+
13.	Protein	Water	Million's reagent (heated)	White ppt. turned red when heated	+

+ = Present – = Absent ppt = Precipitate

Antimicrobial activity

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

Table 2 Inhibition zone exhibited by different extracts of *Cassia fistula* L. Fruit pulp

No.	Extract	Microorganisms					
		A. flavous	***B. subtilis***	***C. albican***	***E. coli***	***P. fluorescens***	***X. oryzae***
1.	Pet-ether	16 mm	8 mm	12mm	8 mm	8 mm	10 mm
2.	Acetone	14 mm	8 mm	8 mm	14 mm	8 mm	8 mm
3.	Ethyl acetate	12 mm	10 mm	12 mm	12 mm	8 mm	8 mm
4.	Ethanol	10 mm	16 mm	12 mm	18 mm	8 mm	16 mm
5.	Methanol	14 mm	14 mm	16 mm	12 mm	14 mm	14 mm
6.	water	18 mm	14 mm	18 mm	16 mm	12 mm	12 mm
7.	Control	-	-	-	-	-	-

Paper disc = 6 mm - = no activity

In this experiment, acetone extract was found to be highest activity on *Aspergillus flavous* and *Escherichia coli*. Ethyl acetate shows significantly the antimicrobial activity on *Aspergillus flavous, Candida albican* and *Escherichia coli*. Ethanol extract showed the highest activity against *Escherichia coli* (inhibition zone 18 mm). Methanol extract and pet-ether extract are more sensitive against *Candida albican* and *Aspergillus flavous* respectively. Water extract showed highest

activity on *Aspergillus flavous* and *Candida albican* (inhibition zone 18 mm). The antimicrobial test were showed in Figure 5.

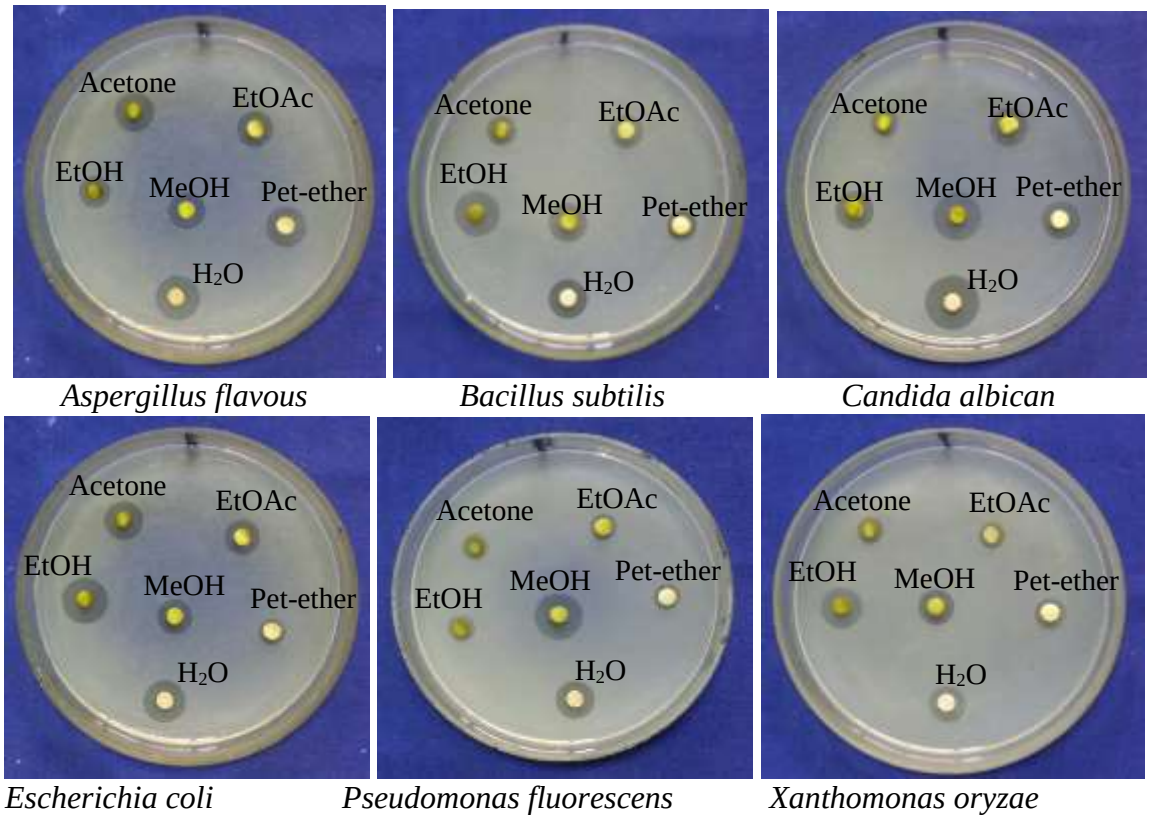


Figure 5 Antimicrobial test of different extracts from fruit pulp of *Cassia fistula* L.

Discussion and Conclusion

Research on *Cassia fistula* L. was made from two aspects such as phytochemical study and antimicrobial study. The specimens were collected from Banmaw Township, Kachin State. It is a medium tree and commonly found in many places as ornamental plant. It belongs to the family Fabaceae. The Myanmar name is Ngu-shwe-wa. This plant has been widely used as traditional medicine. Entire parts of the plant have medicinal values. The roots, leaves, fruits and seeds are the parts of the plant used as medicine.

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).

In the present study, *Cassia fistula* L. is a medium-sized deciduous tree with a straight trunk and spreading branches. The leaves are pinnately compound, petiolate and long stalked stipulate. Leaflets: 4-5 pairs, opposite, ovate. Inflorescences are axillary and drooping raceme. Flower are yellow, very showy, bisexual, zygomorphic, hypogynous. Calyx contained sepals 5, synsepalous. Corolla consisted of petals 5, apopetalous, unequal, shortly clawed. Stamens are 10, free, vary in length. Carpel are 1, marginal placentation; style long; stigma reduced and curved. Fruit are elongated and rounded pod. These morphological characters of *Cassia fistula* L. were in accordance with those described by Backer (1968), Burkill (1935), Hooker (1881), Dassanayake (1981) and Hu-Qi-ming (2009).

In the microscopic characters of fruit pulp powder of *Cassia fistula* L., fragment of parenchyma cells, stone cells, vessels, treachid and fiber were found.

Indian medicinal plants reported that pulp of the pod contains anthraquinone glycosides, pectin and tannin. In compendium of Indian medicinal plants, Rastogi and Mehrotra revealed that fruit pulp of *Cassia fistula* L. Contained proteins and carbohydrates. The research of Anitha and Miruthula showed to be contained flavonoid, glycoside, amino acid, tannin, saponin, anthraquinone, steroid, terpenoid and reducing sugar in the fruit of *Cassia fistula* L.

In this research, the preliminary phytochemical test revealed that alkaloid, glycoside, phenolic compound, flavonoid, steroid, terpenoid, α- amino acid, starch, reducing sugar, saponin, tannin, carbohydrate and protein are present in fruit pulp of *Cassia fistula* L. The result of this study indicated that the leaves of this plant contain some major bioactive compounds needed for organisms. So, this plant proved to be very active.

In the antimicrobial activity, the fruit pulp of *Cassia fistula* L. was extracted with different solvents. The extracts were used to carry out antimicrobial screening on *Aspergillus flavous, Bacillus subtilis, Candida albican, Escherichia coli, Pseudomonas fluorescens and Xanthomonas oryzae*. The result showed that the highest activity (zone of inhibition in diameter is about 18 mm) was demonstrated by the ethanol extract against *Escherichia coli* and water extract against *Aspergillus flavous* and *Candida albican.* According to result, watery extract of fruit of *Cassia fistula* L. is effective in treatment bronchitis disease caused by *Aspergillus flavous* and skin infection, vaginal candidiasis, cardiac infection, sinus irritation, sores and ringworm caused by *Candida albican* may also be affected. Whereas diarrhea, dysentery, abscess, septic wounds, bed sores caused by *Escherichia coli* can be protected by ethanol extract of fruit. Therefore, it is recommended that the different components detected in fruit of this plant should be isolated and tested against the susceptible microorganism 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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References

Anitha J. & S. Miruthula (2014). Anti-inflammatory and Phytochemicals Analysis of *Cassia fistula* L. Fruit Pulp Extracts. "*International Journal of Pharmacology*". 1(3): 207-215.

Backer, C.A. and R.C.B. Van Den Brink, (1965). **Flora of Java**.Vol: II, The Netherlands N.V.P. Noondhoff-Groningen.

Banjare L. and S. Paul. (2014). Phytochemical Screening and Evaluation of Various Extracts of *Lageneria siceraria* for Antioxidant Activity. "*International Journal of Pharmacognosy*" 1(2): 107-12.

Burkill, H. M. (1995). **The useful plants of West Tropical Africa**. Royal botanic garden kewl (u,k)

British Pharmacopeia, (1968). Published Under the Direction of the General Medical Council.

Cruickshank, R. (1975). **Medicinal Microbiology**. Churchill Living Stone Ltd., London.

Danish M., P. Singh, G. Mishra, S. Srivastava, K. K. Jha, R. L. Khosa (2011). Cassia fistula L. – An Important Medicinal Plant: A Review of Its Traditional Uses, Phytochemistry and Pharmacological Properties. "*Journal of Natural Product of Plant Resources*", 1 (1): 101-118

Dassanayake, M.D. and W.D. Claylon, (1981). **Flora of Cyelon**, Vol.III, A. A. Balkema / Rotterdam/Brookfield.

Hooker, J.D. (1881). **Flora British India**. Vol.I. Reeve & Co.,Ltd. London.

Hu-Qi-ming. (2009). **Flora of Hong Kong**, Vol.III, Hong Kong Herbarium, Agriculture, Fisheries Conserval on Department.

Hundley and Chit KoKo. (1987). **List of Trees, Shrubs, Herbs and Principal Climbers, etc**, Government Printing Press, Yangon.

Joshi KP, Chavan D and Patwardhan WB: (2004). Molecular markers in herbal drug technology. *Cwr Sci*; 87: 159-165.

Khare C. P. (2007). *Indian Medicinal Plants,* Springer.128.

Kress and Yin Yin Kyi, Daw. (2003). **A Checklist of the Trees, Shrubs, Herbs and Climbers of Myanmar**, Department of Systematic Biology - Botany, National Museum of Natural History Washington, DC.

Kumar D, Kumar A, Prakash O. (2014). Pharmacognostic Investigation of Clerodendrum phlomidis Linn. f. Root. *The Journal of Tropical Life Science*. 4(2)-96-100.

Marini Bettolo, G. B., M. Nicolet tic and M. Patmia. (1981). Plant Screening by Chemical Chromatographic Procedure Under Field Conditions. *Journal of Chromatogram*.

Rastogi, R. P., B. N. Mehrotra. (2004). *Compendium of Indian Medicinal Plants,* Central Drug Research Institute, Lucknow and National Institute of Science Communication and Information Resources, New Delhi.Vol. 3,140.

Trease, G.E. and W.C. Evans. (2002). **Pharmacognosy**. 15th Ed., Harcourt Publishers Limited. Landon.

World Health Organization. (1990). **Medicinal Plants in Vietnam**. Western Pacific Manila: WHO Regional Office.