Morphological Characters and Phytochemical Investigation of Leaves of *Hibiscus Cannabinus* L. and its Antimicrobial Activity

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Abstract

The specimens were collected from Banmaw University campus, Banmaw Township in KachinState during the flowering and fruiting period. The collected plants were identified by the literature references to confirm its identity. The present research was carried out to study the morphological characters, qualitative analysis and antimicrobial activity of the leaves of *Hibiscus cannabinus* L. For the qualitative analysis, the powdered leaves was investigated to determine the presence or absence of active chemical constituents according to the methods of Marini Bettalo and Trease and Evans. For the antimicrobial experiment, the six solvent extracts of powdered leaves were tested on six microorganisms by using paper disc diffusion methods. The aim of this study is to find the medicinal plant scientifically which has effective medicinal values. In the result of morphological studies, inflorescences were axillary and solitary cymes and flowers were hypogynous. In qualitative analysis, alkaloids, glycosides, flavonoids, α-amino acids, reducing sugar, carbohydrates, protein, steroids, terpenoids, starch and saponins were found to be present in the leaves of *H. cannabinus* L. Some of these phytochemicals are believed to protect cells from damage that could lead to cancer and help to stop carcinogens from attacking cells. In the results of antimicrobial studies, ethyl acetate and methanolic extracts of leaves showed the highest activity on *E. coli*. Therefore, the leaves of *H. cannabinus* L., a source of natural antimicrobial agents,can be used in medicinal purposes.

Key words: *Hibiscus cannabinus*, Phytochemicals, Antimicrobial activity

Introduction

Kenaf (*Hibiscus cannabinus* L.) is a valuable fiber and medicinal plant from the Malvaceae family. The leaves and seeds have been used in traditional medicine in India and Africa for the treatment of various disease conditions (AyadiRekaya, 2016). Malvaceae or the mallow is a family of flowering plants estimated to contain 244 genera with 4225 known species. The largest genera in terms of number of species include *Hibiscus* (300 species). Representatives occur in all except the coldest parts of the world but are most numerous in the tropics (Christenhusz and Byng, 2016). The specimens were collected from Banmaw University campus, Banmaw Township in Kachin State.

In Myanmar, the family Malvaceae is composed of 16 genera and 75 species (Hundley and Chit Ko, Ko, 1987). The leaves of *Hibiscus cannabinus* L. have medicinal values. Applied externally, the leaves are used as a poultice on pains and bruises. The leaves are purgative. An infusion of the leaves is used in the treatment of coughs. In Ayurvedic medicine, the leaves are used in the treatment of dysentery and bilious, blood and throat disorders. The powdered leaves are applied to Guinea warms in Africa (Website, 1).

In the present study, morphological characters, preliminary phytochemical analysis and antimicrobial studies had been undertaken. As a result, the leaves of *H. cannabinus* L. revealed the present of important active chemical constituents and antimicrobial properties. Thus the leaves of *H. cannabinus* L.,a source of natural antimicrobial agents, can be used in food and medicinal systems.

Therefore, the aim of this study is to find the medicinal plant scientifically which has effective medicinal values and also to investigate the active chemical constituents of the leaves of medicinal plant and to find out the highest activity of leaves extracts on six pathogenic microorganisms.

Materials and methods

Morphological study

The specimens of *Hibiscus cannabinus* L. used in this study were collected in the area of Banmaw University campus, Kachin State. For the identification of their morphological characters, the vegetative and reproductive parts of the plant were collected at their flowering period to fruits and seeds.

Classification and identification were done with the help of available literature cited in Backer (1963), Bailey (1939), Benson (1957), Hooker (1875), Kirtiker and Basu (1935), Rendle (1925), HU Qi-ming (2009) and Kress et.al., (2003).

After the collection, both the vegetative and reproductive parts of the fresh specimen were

measured and recorded for taxonomic description. And then, photographs of the all parts of studied specimens were taken. The collected specimens were properly dried, pressed and mounted on the herbarium sheets. Moreover, these dried specimens were crushed and pounded into powdered form. This powder was stored in the airtight container for another study.

Phytochemical study

For the preliminary phytochemical investigation, the powdered leaves of *Hibiscus cannabinus* L. were carried out to determine the presence or absence of alkaloids, glycosides, phenolic compounds, flavonoids, steroids, α-amino acids, terpenoids, starch, reducing sugar, saponins, tannins, carbohydrates and proteins according to the methods of Marini Bettalo (1981) and Trease and Evans (2002). These experiments were carried out at the Department of Botany, Banmaw University.

Antimicrobial Study

The dried powdered sample of leaves was extracted with acetone, water, ethyl acetate, ethanol, methanol, and pet-ether. The various solvents extracts of leaves were tested on six pathogenic microorganisms such as *Aspergillusflavous, Bacillus subtilis, Candida albicans, Escherichia coli, Pseudomonas fluorescens* and *Xanthomonasoryzae*. These experiments were carried out at the Department of Botany, University of Yangon.

The study of antimicrobial activities was performed by using paper disc diffusion method according to Madigam, (2005).

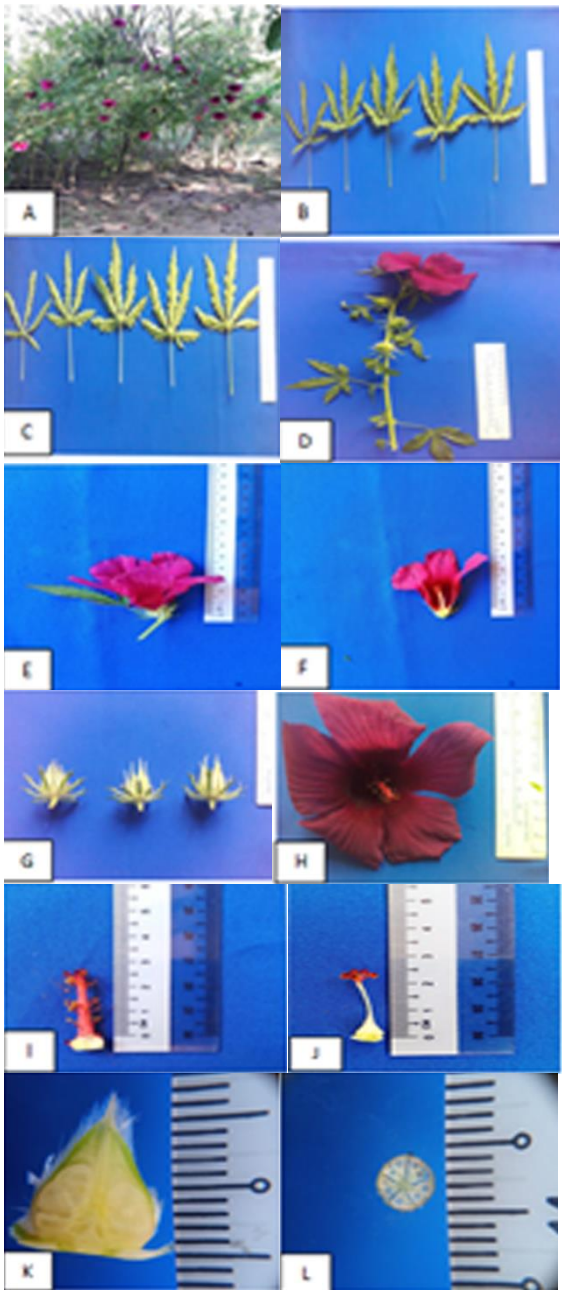
Isolated bacterial strains grown on nutrient agar were inoculated into 50 ml conical flasks containing 10ml of sterile growth medium. Then, they were incubated at 30˚C for 72 hours on a reciprocal shaker at 200 rpm. 0.3 ml of test organisms was added to assay medium, then poured into plates. After solidification, paper discs impregnated with both samples were applied on the test plates and these plates were incubated for 24-36 hours at 30˚C. After for 24-36 hours, clear zones (inhibitory zones) surrounding the test discs indicate the presence of bioactive compounds which inhibit the growth of test organisms.

Results

Scientific Name - *Hibiscus cannabinus* L.
Myanmar Name - Chin-baung-gyi (or)
Chin-baung-kha
Englis Name -Java Jute or Deccan hemp
Family -MalvaceaeFlowering and
Fruiting Period - August to December

Morphological characters of *Hibiscus cannabinus* L.

Perennial shrubs, erect, 1.5-2.0 m high, slightly branched or unbranched, stems woody, solid, provided with small prickles. Leaves alternate, simple, palmatipertite, stipules free lateral, linear, about 5.5 mm long and about 1.0 mm wide, sparely pubescent, the petioles slender, 3.0-10.5 cm long and about 0.2 cm wide, the lamina 3-5 lobed, 7.0-10.0 cm long and 5.5-8.5 cm wide, the margins irregular-serrate, the tips acute, both surfaces glabrous. Inflorescence axillary and solitary cymes, the peduncles absent. Flower campanulate, 4.0 -6.0 cm long and 7.0-8.0 cm in diameter at anthesis, ebracteate, the pedicels cylindrical, about 3.0 mm long and about 1.8 mm wide, sparsely small bristles; epicalyx 7-10, adnate to calyx, linear-lanceolate, 8.0-11.0 mm long and about 1.0 mm wide, the margins entire, and small bristles present, persistent; sepals-5, campanulate, 5-partite, the tubes about 1.0 cm long and about 0.5 cm wide the tips acute, valvate, pubescent, persistent; petals 5, coherent at the base and adnate to the staminal tube, the lobes obovate-spathulate, 5.0-5.3 cm long and 2.0-2.7 cm wide, twisted, the tip slightly crenate, both sides with glabrous; stamens numerous, monadelphous, the stamina tubes entirely antheriferous, adnate to the petals, 1.5-2.0 cm long and 2.0-2.5 cm wide, the filaments filiform, about 2.0 mm long, crimson, dorsifixed, longitudinally dehiscent, extrorse; ovary superior, ovate -oblongoid, about 4.5 mm long and 4.0 mm in diameter, densely pubescent, penta-carpellary, syncarpous, penta-locular, axile placentation, two ovule in each locules in T.S; the styles terminal and slender, about 2.5 cm long, glabrous, the stigmas five-fid, each with capitates stigma. Fruits loculicidal.Seeds numerous, reniform, brown, warted. The resultswere shown in Figure (1).



A. Habit B. Leaves (Upper surfaces) C. Leaves (Lower surfaces)
D. Inflorescence E. Flower F. L. S of Flower
G. Calyx and Epicalyx H. Corolla lobes I. Staminal tube with stamens
J. Gynoecium K. L.S of Ovary L. T. S of Ovary

Figure 1. Morphological characters of *Hibiscus cannabinus* L.

Preliminary phytochemical investigation of leaves of *Hibiscus cannabinus* L.

The preliminary phytochemical investigation was carried out on the powdered leaves. The results in shown in Table (1) and Figure (2).

Table (1) Preliminary phytochemical test of leaves of *Hibiscus cannabinus* L.

No	Constituents	Extract	Test Reagents	Observation	Remark
1.	Alkaloids	2%HCL acid + EtoH	1. Mayer's reagent 2. Hager's reagent 3. Wagner's reagent	White ppt Yellow ppt Reddish brown ppt	+
2.	Glycosides	Ethanol	1 ml of water and sodium hydroxide	Yellow Colour	+
3.	Phenolic compounds	H_2O	3% Ferric chloride solution	Green Colour	+
4.	Flavonoids	Ethanol	Small pieces of Mg, few drops of HCl	Pink Colour	+
5.	Steroids	Pet-ether	Acetic anhydride and Conc: H_2SO_4	Green	+
6.		H_2O, spotted on filter paper		Pink Spot	+
7.	Terpenoids	$CHCl_3$	Acetic anhydride and Conc: H_2SO_4	Pink	+
8.	Starch	H_2O	Iodine Solution	Bluish black ppt	+
9.	Reducing sugar	H_2O	Benedict Solution	Brick red ppt	+
10.	Saponins	H_2O	Distilled Water	Frothing	+
11.	Tannins	H_2O	5%Ferric Chloride slouton and sulphuric acid	No yellowish brown ppt	–
12.	Carbohydrates	H_2O	1 ml of a mixture of equal parts of felling's solution A and B	Brick red ppt	+
13.	Protein	H_2O	NaOH Sol: and 3% $CuSO_4$ Sol:	Red or violet colour	+

(+) Present, (−) Absent

The tests indicated that, alkaloids, glycosides, phenolic compounds, flavonoids, steroids, terpenoids, ∝-amino acids, starch, reducing sugar, saponins, carbohydrates and protein were found to be present and tannin was absence in the leaves of *Hibiscuscannabinus* L.

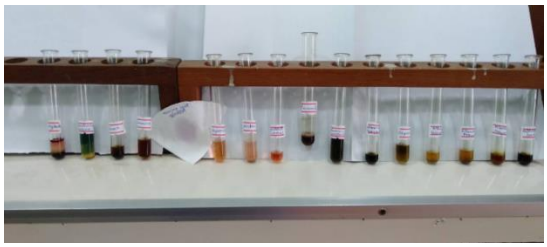


Figure 2. Phytochemical investigation of leaves of *Hibiscus cannabinus* L.

Antimicrobial activity of various solvent extracts of Leaves of *Hibiscus cannabinus* L.

Antimicrobial activity of various solvent extracts such as acetone, water, ethyl acetate, ethanol, methanol and pet-ether extracts were tested on six microorganisms. The results were shown in Table (2) and Figure (3).

Table (2) Antimicrobial activity of various solvent extracts of Leaves of *H. cannabinus* L.

Sample	Test Organisms	Ace	water	EtOAc	EtOH	MeOH	P.E
Leaves	*Aspergillus flavous*	10 mm	10 mm	16 mm	14 mm	14 mm	8 mm
	Bacillus subtilis	8 mm	8 mm	12 mm	12 mm	12 mm	12 mm
	Candida albicans	12 mm	8 mm	8 mm	8 mm	14 mm	16 mm
	Escherichia coli	8 mm	8 mm	18 mm	16 mm	18 mm	10 mm
	Pseudomonas fluorescens	8 mm	8 mm	8 mm	8 mm	8 mm	8 mm
	Xanthomonas oryzae	8 mm	8 mm	12 mm	8 mm	12 mm	10 mm

Paper disc size = 6 mm

In this experiment, ethyl acetate and methanol extracts of leave showed the highest activity especially more sensitive against *E. coli* (18 mm); secondly ethyl acetate, pet-ether and ethanol extracts of leaves showed the activity especially more sensitive agains*A. flavous*, *C. albicans* and *E. coli* (16 mm). Thirdly ethanol and methanol extracts of leaves showed the activity more sensitive against *A. flavous*, *C. albicans* (14 mm). Fourthly acetone, ethyl acetate, ethanol, methanol and pet-ether extracts of leaves showed the activity especially more sensitive against *B. subtilis*, *C. albicans* and *X. oryzae*(12 mm).

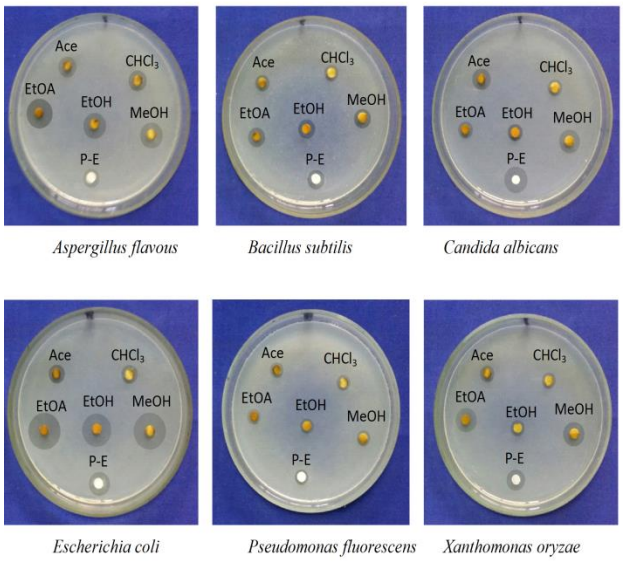


Figure 3 Treatment of various extracts from the leaves of *Hibiscus cannabinus* L.

Discussion

In the present investigation, the morphological studies on both vegetative and reproductive parts of the plants, preliminary phytochemical analysis and antimicrobial activity of the leaves had been undertaken.

As a result of morphological studies, the plant of *H. cannabinus* was perennial shrubs, erect, slightly branched or unbranched. Leaves were alternate, simple, palmatipertite, stipules free lateral, the laminar 3-5 lobed, the margins irregular serrate, the tip acute. Inflorescences were axillary and solitary cymes. Flowers were campanulate, ebracteate, epicalyx 7-10, adnate to calyx, linear-lanceolate, persistent. Sepals were 5, campanulate, 5-partite, the tips acute, pubescent, persistent. Petals wree 5, coherent at the base and adnate to the staminal tube. Stamens were numerous, monadelphous, the staminal tubes entirely antheriferous, adnate to the petals, dorsifixed, extrorse. Ovary was ovate-oblongoid, densely pubescent, penta-carpellary, penta-locular, axile placentation, the stigmas five-fid, superior. Fruits were loculicidal; seeds were numerous, warted. These characters are in aggreement with those mentioned by Backer (1963), Bailey (1939), Benson (1957), Hooker (1875), Kirtiker and Basu(1935), Rendle (1925), HUQ-ming (2009) and Kress et.al., (2003).

The preliminary phytochemical investigation was carried out on the powdered leaves. From these experiments, except the tannin, other twelve groups of compound were found to be present in the leaves of *H. cannabinus* L. According to KailashKaraleet.al., 2014, leaves extract of *H. cannabinus* L. was revealed for the presence of varying amount of alkaloids, saponins, tannins, anthraquinones, cardiac glycosidesand

flavonoids. Some of these phytochemicals are believed to protect cells from damage that could lead to cancer and help to stop carcinogens from attacking cells.

In the results of antimicrobial activity, ethyl acetate and methanol extracts of leaves showed the highest activity especially more sensitive against *E. coli* (18 mm); secondly ethyl acetate, pet-ether and ethanol extracts of leaves showed the activity on *A. flavous*, *C. albicans* and *E. coli* (16 mm). Thirdly ethanol and methanol extracts of leaves showed the activity on *A. flavous* and *C. albicans* (14 mm). Fourthly acetone, ethyl acetate, ethanol, methanol and pet-ether extracts of leaves showed the activity on *B. subtilis*, *C. albicans* and *X. aryzae*(12 mm).

From this finding, it can be inferred that leaves of *H. cannabinus* L. can be effective in the formulation of medicine for the treatment of disease caused by *A. flavous*, *B. subtilis*,*C. albicans*, *E. coli* and *X. oryzae* such as bronchitis, skin infection, vaginal candidiasis alimentary tract infeciton, cholera, diarrhea, vomiting, urinary tract infections and bacteria for leaf blight.

Conclusion

The results of this present study on morphological characters can give a few information on the systematics study on a member of the family Malvaceae. Moreover, the leaves of *H. cannabinus* L. had many active chemical constituents. They are employed for medicinal purposes. Furthermore, the leaves of *H. cannabinus* L., a source of natural antimicrobial agents, can be used in food and medicinal system.

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