PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITIES OF FLOWERS AND SEEDS OF *GYMNANTHEMUM AMYGDALINUM* WALP.

Khin MyoThant[1], Kyawt Kywat Khine[2], San San Myint[3]

Abstract

In this research, the phytochemical analysis and antimicrobial activities of the flowers and seeds of *Gymnanthemum amygdalinum* Walp. were studied. It belongs to the family Asteraceae. The plants were collected from Kyaing Tong Township, Eastern Shan State. English name is bitter leaf plant and Shan name is Sa paung Lone. These investigations had been carried out by (Harbone, 1973 and Trease and Evans, 2002) in the Pharmaceutical Research Department of Ministry of Industry, Yangon Region. Alkaloid, carbohydrate, glycoside, phenolic compound, α-amino acid, saponin, tannin, flavonoids, steroids, terpenoids, reducing sugar and starch were observed in the flowers of studied plant. Tannin and cyanogenic glycoside did not observed in the seeds of this plant. For antimicrobial activities, the different extracts of flowers and seeds of *G. amygdalinum* were applied on some different microorganisms. The antimicrobial activity was done by using agar well diffusion method. Partially active and active were observed on studied microorganisms. The highest inhibition zones (15 mm- 20 mm) were found in ethanol extract of flowers and petroleum ether extracts of seeds. These results can be used for further pharmaceutical studied.

Keywords: *Preliminary phytochemical analysis and antimicrobial activity.*

Introduction

Gymnanthemum amygdalinum Walp. is a tropical plant belonging to the family Asteraceae and is used widely as vegetable and medicinal plant (Ibrahim *et al*., 2000). It is a shrub of about 2 to 5m with a petiolate leaf of about 6 mm in diameter and elliptic shape. The leaves are green with a characteristic odour and bitter taste. It does not produce seeds and has to be distributed or propagated through cutting. It grows under a range of ecological

[1.] Dr., Associate Professor, Department of Botany, Myeik University
[2.] Dr., Associate Professor, Department of Botany, Yaynanchaung Degree Collegue
[3.] Dr., Lecturer, Department of Botany, Myeik University

zones in Africa and produces a lager mass of forage and it is drought tolerant. It is majorly used for human consumption and has to be washed to remove the bitter taste. Its bitter taste is due to antinutritional factors such as alkaloids, saponins, tannins and glycosides. It stimulates the digestive system as well as reduces fever (Iwu, *et al.*, 1993). This plant contains complex active components that are pharmacologically useful. The roots and the leaves are used in ethno-medicine to treat fever, hiccups, kidney problems and stomach discomfort. The stem and root divested of the bark are used as chew sticks in many West African countries like Cameroon, Ghana and Nigeria (Burkill, 1985). However, extract of bitter leaf had been reported to exert antibiotic action against drug resistant microorganisms and possess antioxidant, anticancer, antiviral, anti-helminthic and anti-inflammatory activities (Akinpelu, 1999; Dahanukar*et al*., 2000). Furthermore, the root provides one of the commonly used chew sticks in Nigeria due to alleged beneficial effect on dental caries (Aregheore*et al*., 1998). The leaves and bark in Ethiopian local medicine are used as purgative, against menstrual pain and wound dressing (Akah and Okafor, 1992; Uhegbu and Ogbuchi, 2004). The present research was aimed to evaluate phytochemical constituents and antimicrobial activities of different solvent extracts of flowers and seeds of these medicinal plants.

Materials and Methods

The flowers and seeds of *Gymnanthemum amygdalinum* were collected from Kyaing Tong Township, Eastern Shan State during the flowering period from January to March in 2017. The identification of species was made by Hooker (1894) and Backer (1963). The samples were dried under shades for three weeks and stored in air tight containers for the phytochemical analysis and antimicrobial activity experiments. For antimicrobial activities, the flowers and seedsof this plantwere extracted by using methanol, ethanol, ethyl acetate, chloroform, pet-ether and water. The solvent extracts were tested six microorganisms (*Bacillus pumalis, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, Candida albicans and Escherichia coli*). Since April 2017, these investigations had

been carried out by (Harbone, 1973 and Trease and Evans, 2002) in the Pharmaceutical Research Department of Ministry of Industry, Yangon Region.

Results

1. Outstanding characters

Small tree, much-branched, spreading, bark grey or brown,smooth stems pubescent with asymmetrical T-shaped hairs;leaves petiolate, elliptic, lanceolatebase rounded, margins coarsely serrate, apex shortly acuminate, apiculate;inflorescent capitulate, numerous in terminal compound corymb form cymes,florets 10-24 per capitulum, sweetly scented, bracteolate, green; flowers bisexual, all tubular, actinomorphic, epigynous, outer series 7-8 florets, inner ones 4-6 florets, creamy corolla 5-lobed, tubular; stamens 5, inserted, anther dithecous; ovary inferior, oblong, shortly pubescent, unilocular, solitary basal ovule, stigma bifid, pappus 37-42, creamy; fruits achenes Fig.(A,B and C).

A. Habit B. inflorescence C. Florets

Figure 1. Morphological characters of *Gymnanthemum amygdalinum*

2. Phytochemical Analysis and Antimicrobial Activities of Flowers and Seeds

The phytochemical analysis of *G. amygdalinum* flowers indicated the presence of alkaloids, carbohydrates, glycoside, phenol, amino acid, saponin, tannin, flavonoids, steroids, terpenoids, reducing sugar and starch. Tannin and cyanogenic glycoside were absent in the seeds. These results were shown in Table 1. Petroleum ether, methanol, ethyl acetate and ethanol and watery extract of flowers and seeds showed an effective antimicrobial activity on six

pathogens. Chloroform extract of flowers did not show activity on *Bacillus subtilis.* Chloroform extract of seeds did not show activity on *Candida albicans.* Ethanol extract of flowers and petroleum ether extract of seeds showed the most effective on studied microbes.Among them ethanol extract of flowers and petroleum ether extract of seeds showed the highest activity on *Escherichia coli.* These results were shown in Table 2 and Figure 2 - 7.

Table1. Phytochemical Constituent of Flowers and Seeds

No.	**Sample**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	Flowers	+	+	+	+	+	+	+	+	+	+	+	+	-
2	Seeds	+	+	+	+	+	+	-	+	+	+	+	+	-

(+) = presence (-) = absence

1= Alkaloid, 2= Carbohydrates, 3= Glycoside, 4= Phenol, 5= α-amino acid, 6=Saponin, 7= Tannin, 8= Flavonoid, 9= Steroid, 10=Terpenoids, 11= Reducing sugar, 12=Starch, 13= Cyanogenic glycoside

Table2 . Antimicrobial activities of different extracts of the flowers and seeds

No.	Sample	**Organisms**																	
		***Bacillus pumalis* (mm)**						***Bacillus subtilis* (mm)**						***Candida albicans* (mm)**					
		P	**C**	**M**	**EA**	**E**	**W**	**P**	**C**	**M**	**EA**	**E**	**W**	**P**	**C**	**M**	**EA**	**E**	**W**
1	Flowers	14	16	17	15	15	12	13	-	12	14	13	12	14	15	17	14	16	13
2	Seeds	14	11	13	13	12	11	15	11	12	11	11	12	14	15	-	13	12	11
No.	Sample	**Organisms**																	
		***Escherichia coli* (mm)**						***Pseudomonas aeruginosa* (mm)**						***Staphylcoccus aureus* (mm)**					
		P	**C**	**M**	**EA**	**E**	**W**	**P**	**C**	**M**	**EA**	**E**	**W**	**P**	**C**	**M**	**EA**	**E**	**W**
1	Flowers	12	12	18	14	20	17	12	14	16	13	17	15	12	11	13	12	14	16
2	Seeds	17	13	13	13	12	11	13	12	12	13	12	12	13	11	12	12	12	12

Agar well – <10 mm (inactive) ,10 mm ~ 14 mm (partially active), 15 mm ~ 19 mm (active)

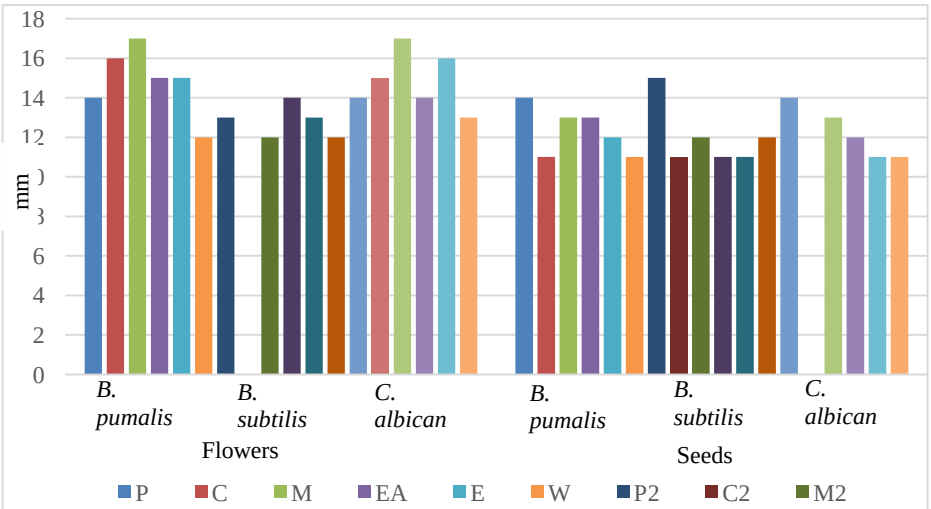


Figure 2. Antimicrobial activities of flowers and seeds of *G.amygdalinum*

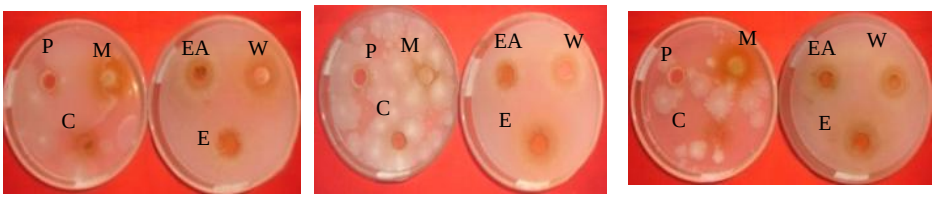


Figure 3. Antimicrobial activities of flowers of *Gymnanthemum amygdalinum*

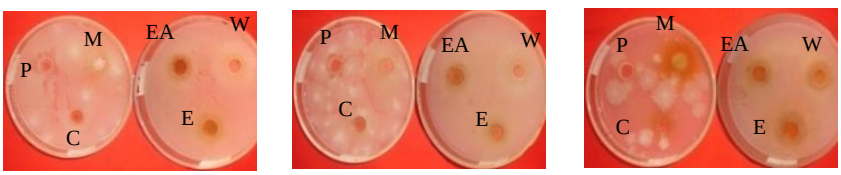


Figure 4. Antimicrobial activities of seeds of *Gymnanthemum amygdalinum*

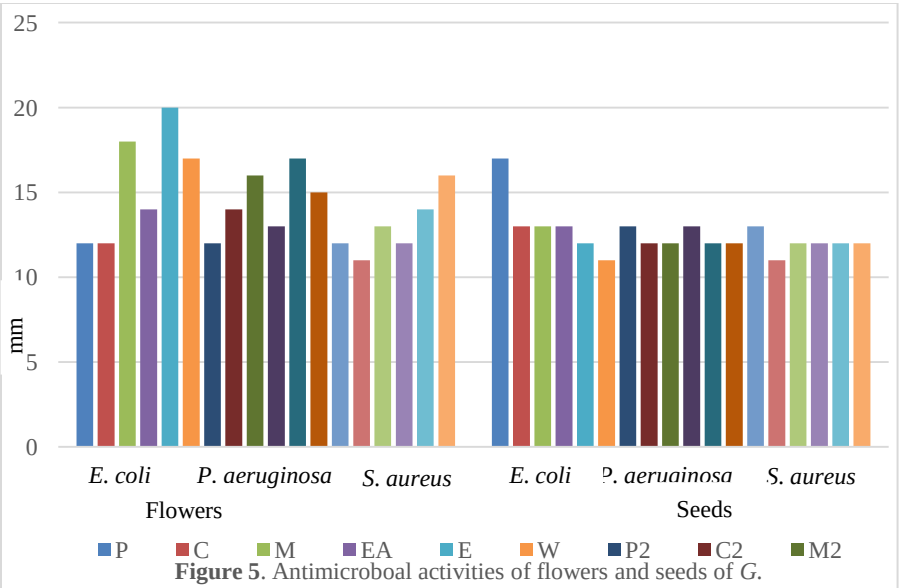
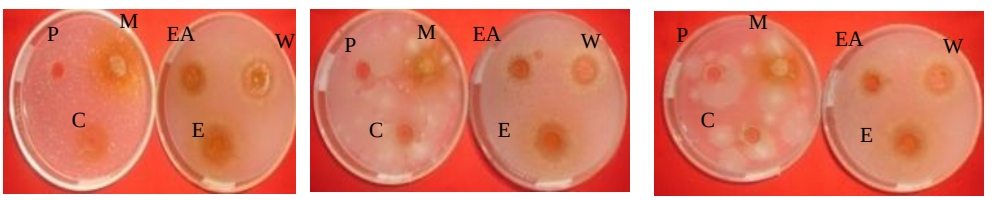
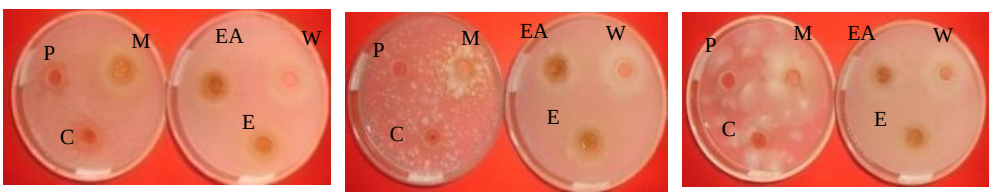


Figure 5. Antimicroboal activities of flowers and seeds of *G. amygdalinum*



Staphylococcus aureus *Pseudomonas aeruginosa* *Staphylococcus aureus*

Figure 6. Antimicrobial activities of flowers of *Gymnanthemum amygdalinum*



Escherichia coli *Pseudomonas aeruginosa* *Staphylococcus aureus*

Figure 7. Antimicrobial activities of seeds of *Gymnanthemum amygdalinum*

P = pet ether, C = chloroform, M= methanol, EA= ethyl acetate, E = ethanol, W= water

Discussion and Conclusion

The phytochemical analysis of the *Gymnanthemum amygdalinum* flowers and seeds showed the presenceof saponin, steroids, terpenoids, and alkaloids. These finding agreed with those mentioned by Adetunji *et al*., 2013. Saponin, flavonoids, alkaloids, and terpenoids were present in the flowers and seeds. These findings agreed with Enyi-Idoh *et al*.,2012. Tannin, alkaloids and saponin were present in flowers and seeds. These findings agreed with Wazis *et al*., 2012. Tannin have accelerate blood clotting, reduce blood pressure, decrease the serum lipid level, produce liver necrosis, and modulate immunoresponses properties (Chung, 1998). Saponin have a favorable effect on cholesterol, can help boost the immune system, have an antioxidant effect, may even support bone strength and act against cancer cells by Sushant (2018) reported that alkaloids are used as a cure for malaria, as an anesthetic, help in curing diabetes, controlling blood sugar levels and treatment of cancer. Jordan (2017) reported that amino acid supplement with improved muscle growth, increase endurance, greater fat burn, reduce fatigue, increase mental focus muscle sparing, improved recovery and reduced muscle soreness. A carbohydrates intensive diet can cause high blood sugar and unwanted weight grain. Terpenoids have antimicrobial, antifungal, antiparasitic, antiviral, anti-allergenic, antispasmodic, antihyperglycemic, anti-inflammatory and immunodulatory properties (Roslin and Anupam 2011). Flavonoids showed antioxidant activity, anticancer, antitumor activity, hepatoprotective activity, anti-inflammatory activity, anti-diabetes activity, antiviral antibacterial and antifungal activity (Xiao, 2016). Reducing sugar intake and weight loss and can create a stable mood and energy levels (Medibank, 2018). Starch can improve insulin sensitivity, very effective at lowering blood sugar levels after meals and help avoid chronic disease (Kris, 2018). According to the data, these plants can be used as drugs. In antimicrobial activities, pet-ether extract was effect on *Staphylococcus aureus* and *Escherichia coli*. These findings agreed with Gashe *et al*., 2017. Chloroform extract showed active on *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans.* These findings agreed with Zenebe *et al*., 2015, Gashe *et al*., 2017.Methanol extract

was active on *Staphylococcus aureus Escherichia coli, and Candida albicans.* These findings agreed with Zenebe *et al*., 2015, Gashe *et al*., 2017. Ethanol extract showed active on *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*. These finding agreed with Adetunji *et al*., 2013, Anibijuwon *et al*., 2012 and Enyi-Idoh *et al*., 2012. *E. coli* infection include diarrhea, abdominal pain and fever. *Pseudomonas aeruginosa* can cause problems ranging from ear infection, bronchitis, respiratory infection and pneumonia (Ann, 2017). *Bacillus* infection are clindamycin and vancomycin and food poisons (Sliman *et al*., 1987). *Candida albicans* can cause skin and vaginal yeast infection (Weng, 2016). Staphylococcus aureus can cause a range of illnesses, from minor skin infections such as pimples, impetigo, boils, cellulitis, folliculitis, carbuncles, scalded skin syndrome and abscesses to life threating diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic, shock syndrome, bacteremia and sepsis (Larry and Charles 2018).In conclusion, this research showed more effective on studied microbes. Many kinds of secondary metabolites were observed in the flowers and seeds of this plant. So this work can help further pharmaceutical study.

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