Investigation of Phytoconstituents and Antimicrobial Activity of V*erbena officinalis* Linn

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Abstract

As the interest of uses from the various part of Verbena officinalis Linn.*, preliminarily phytochemical investigation and antimicrobial property was performed. This study revealed the prominent phytochemicals such as phenolic compounds, flavonoids, steroids and terpenoids were presented in the seed, leaves and stem of V.officinalis. All plant samples contain the triterpenoid compound, ursolic acid by checking thin layer chromatographic (TLC) method. Antimicrobial activity of plant was screened by agar well diffusion method on the different six microorganisms. The ethyl acetate extract of V officinalis can mostly inhibit the growth of Pseudomonas aeruginosa that compare to other five microorganisms namely: Bacillus pumalis, Bacillus subtilis, Escheichia coli, Staphylococcus aureus* and *C. albicans.*

Keywords: *Verbena officinalis, agar well diffusion method, Pseudomonas aeruginosa*

1. Introduction

Most of researches in Myanmar especially in University level concerning with Organic Chemistry are finding of Phytochemicals and determination of pharmacological activities from medicinal plants and herbs. The preliminary phytochemical investigation has to be done as a first step in the survey of medicinal plants to standardize. The medicinal plants and herbs are important, useful for every person in Myanmar as well as various parts of world.

According to currently interest, the plant selected in this research is *Verbena officinalis* Linn., a well-known plant of the family Verbenaceae, which is a large family of perennial herbaceous plants. The Myanmar name of the plant is Saung-taw-ku and also called Say-saung-taw-ku. It was found that Verbenaceae consists of about 30 genera and some 1,100 species. The genus *Verbena comprises* about 200-250 species worldwide. *Verbena officinalis* is distributed throughout the plains of subcontinent, Pakistan and India and up to 7,000 ft. on Himalaya from Kashmir eastwards. The plant is fairly common near water in waste lands and cultivated fields in northern and western regions of the world, between 500 to 2000 m. It is also found in North Africa, as well as in China and Japan [1] throughout Europe and has been introduced to many other parts of the world such as North America and South Africa [2]. In Myanmar, the plants were widely grown by roadsides and sunny pastures of hilly regions especially Southern Shan State.

Figure 1. (a) Naturally wide distribution (b) plant (c) leaves (d) flower of *Verbena officinalis* Linn.

1.1. Pharmacological activities and Chemical constituents of *Verbena officinalis* Linn.

Verbena officinalis Linn. has been used in the traditional Austrian medicine internally (as tea or liqueur) for the treatment of infections and fever. The medical use of common vervain is usually as an herbal tea and it is used in folk medicine for the treatment of inflammatory disorders, skin burns, abrasions and gastric diseases [3]. In addition, the plant has diuretic, expectorant properties, antidepressant, anticonvulsant effect as well as it is used for the treatment of jaundice, cough, cold and digestive problems. The previous researches pointed out that the various extracts of this plant have shown antifugal, antibacterial, antioxidant, analgesic, anti-rheumatic and nerve growth factor-potentiating activities [4].

By the reviewing of the previous papers, the main constituents of *Verbena officinalis* are iridoid glycosides, namely verbenalin, hastatoside, aucubin, apigenin and a flovone compound: luteolin. The aerial part of *V. officinalis* yields an essential oil such as citral, geraniol, limonene and verbenone as main constituents by steam distillation. The pet-ether and chloroform extract of *V. officinalis* gives β-sitosterol, ursolic acid, olenolic acid and minor triterpenoid derivatives of ursolic acid and olenolic acid. The MeOH extract gives two iridoidglycosides (verbenalin and hastatoside), a phenylpropanoidglycoside, verbascoside and β-sitosterol-D-glucoside [3, 4]. Other constituents include the flavone derivative artemetin, phenylpropane glycosides verbascoside and eukovoside and the triterpenes, lupeol [5]. As a consequence of presenting vast majority of phytoconstituents, *Verbena officinalis* Linn. (Say-saung-taw-ku) which grown in Southern Shan state, Myanmar was interested to study the promising phytoconstituents.

2. Materials and Methods

2.1. Plant Materials

The whole plant of *V. officinalis* was collected from compound of Panglong University, from June to September on 2016.The plant was identified by an authorized Botanist at the Department of Botany, Panglong University. The screening of phytochemicals by standard method and thin layer chromatography (TLC) from the plant sample were carried out at Department of Chemistry, University of Yangon. The antimicrobial activity of samples was carried out at Department of Foods and Pharmaceuticals Research, Insein Township, Yangon at December, 2018.

2.1.1. Sampling and Preparation of plant extract

The plant sample was washed properly with running tap water for several times and then it was separated into different parts (seeds, leaves and stem) and air dried under the shade. After one week, dried mass was grounded into coarsely powder. The powders obtained were kept in small air-tight plastic bottles with proper labeling for further use.

The powdered samples; seed, leaves and stem were extracted in distilled water and methanol separately. After one day, soaking with solvents and then filtered. The filtrate was used for phytochemical screening and TLC screening.

2.2. Phytochemical Determination of Plant Extracts

2.2.1. Standard method

Phytochemical evaluates for various phytoconstituents of the extracts were undertaken using standard qualitative methods [6, 7]. The extracts were screened with the presence of biologically active compounds like alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic steroids, terpenoids, tannins, etc.

2.2.2. Phytochemical Screening of Plant Extracts by TLC method

The phytochemical exploration from plant sources is a fundamental work of all researches which were related to medicinal plant chemistry. The phytochemical constituents of petroleum-ether (60-80°C), ethyl acetate and methanol extracts were analyzed by thin layer chromatographic method using detecting reagents as described in figure 3.1. In this work, TLC pre-coated plates (GF_{254}) were used. Before using these ready-made plates were dried in an oven at 110°C to remove moisture. And then the desired size were cut with sharp knife to load the extract and sample loaded with capillary tube and run with ethyl acetate:methanol (9:1) solvent system [8]. The chromatograms were tested by spraying with 10% H_2SO_4 solution and heated to 120° C for 5 minutes and visualized under UV lamp with the wavelength of 254 and 365 nm. The R_f (retention factor) values of resulting spots were calculated and compared the literature data. The second part of research was to find the property against some pathogens. The antimicrobial activity was investigated with agar well diffusion method by using two gram negative bacteria (*Escheichia coli, Pseudomonus aeruginosa*) and three gram positive bacteria (*Bacillus pumalis, Bacillus subtilis, Staphylococcus aureus*) and one fungus (*Candida albicans*).

3. Results and Discussion

3.1 Phytochemical Investigation of Seed, Leaves and Stem of *Verbena officinalis*

The preliminary phytochemical investigation was carried out by using standard methods, which have been used by former researcher as usual.

Table. 3.1. Phytochemical Investigation of Various parts of *Verbena officinalis*

Compound type	Seed	Leaves	Stem
Alkaloids	-	-	+
α-amino acids	-	+	-
Carbohydrates	+	+	+
Flavonoids	+	+	+
Glycosides	-	-	+
Phenolic compounds	+	+	+
Saponnins	+	+	+
Steroids	+	+	+
Terpenoids	+	+	+
Tannins	+	+	+

+ = presence, - = absence

These tests showed the presence of promising phytochemicals such as carbohydrates, flavonoids, phenolic compounds steroids, terpenoids and tannins, the seed, leaves and stem of *V. officinalia*. In detail keeping watch the table 3.1, alkaloids and glycosides were present only in the stem sample, likewise, α-amino acid only in leaves of *V. officinalia*, though these types of compound may be active ingredients for most of medicinal plants. Moreover, the phenolic compounds were present in the leaves and stem rather than seeds due to a slowly reaction with 3% ferric chloric solution. By reviewing the former article [3], terpenoid compounds containing plants in Verbenaceae family possess pharmacological effect namely anti-tumor, anti-inflammatory, antidiarrheal activity and *etc*.

3.2 Thin Layer Chromatographic checking of Major Phytoconstituents

The TLC result confirmed the presence of steroids, terpenoids, essential oils and phenolic compounds in

plant samples. Terpenoids were major constituent in three parts: seed, leaves and stem of *V. officinalis* which was pointed out in thin layer chromatogram. The R_f values of the resulting plate (0.51) were indicated with the similar R_f of triterpenoid type, ursolic acid according to literature [8]. In addition, it was visualized under UV_{254} and UV_{365} lamp into light brown and fluorescent spot (Figure 2) respectively. By doing this experiment, the developed fluorescent spots on TLC plate indicated that terpenoids may contain in the seed, leaves and stem of *V. officinalis*.

R_f = 0.51

ethyl acetate : methanol
(9:1)

Figure 2. TLC behavior of (1) crude ursolic acid, Ethyl acetate extract of (2) seeds, (3) leaves and (4) stem of *V. officinalis* by checking under UV_{365} lamp

3.3 Antimicrobial Activity of *Verbena officinalis*

In this screening, there was no response of all extracts such as petroleum ether, ethyl acetate, ethanol and water extract on *Bacillus pumalis*, *Escheichia coli* and *Staphylococcus aureus.* The pet-ether extract of seed sample has activity on *Bacillus subtilis* (12 mm in diameter), 11 mm in leaves and 13 mm in stem. Similarly, in the pet-ether extract of leaves sample has little activity with a diameter of 11 mm on *C. albicans*. In contrast, ethyl acetate extract of leaves showed broad spectrum at *Pseudomonus aeruginosa* (20 mm in dia.). According to the antimicrobial activity, it was found that the highest activity at *Pseudomonus aeruginosa* indicated to use as a drug for some diseases such as urinary tract infection, respiratory system infection, dermatitis and joint infection etc.

Table. 3.2. Antimicrobial activities of seed, leaves and stem of *Verbena officinalis*

Microorganisms	Inhibition zone diameter of Plant Sample (mm)											
	Seed				Leaves				Stem			
	1	2	3	4	1	2	3	4	1	2	3	4
B. subtilis	12	-	-	-	11	-	-	-	13	-	-	-
P. aeruginosa	-	-	-	-	-	20	-	-	-	-	-	-
C. albicans	-	-	-	-	11	-	-	-	-	-	-	-

1 = petroleum, 2 = ethyl acetate, 3 = ethanol and 4 = water extract of *V. officinalis*
- = not observed in broad spectrum

4. Conclusion

The current study pointed out that scientific exploration of phytochemical constituents on the seeds, leaves and stem of *V. officinalia* (Say-saung-taw-ku in Myanmar name) was firstly carried out in the research field of University level. According to the results, there are many valuable phytoconstituents such as carbohydrate, flavonoids, phenolic compounds, saponins, steroids, triterpenoids and tannin in the ethyl acetate and alcoholic extracts of plant parts. By screening of the antimicrobial activity, ursolic acid (triterpenoid) containing ethyl acetate fractions of *V. officinalia* (leaves) was consistent with that fraction of sample supported broad spectrum on *Pseudomonus aeruginosa*. In this way, the selected plant parts can be utilized as great potential antimicrobial drug that assist to traditionally uses.

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