Some Bioactivities Screening and Extraction of Essential Oil from *Valerianaofficinalis*L. (Kantbalu)

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Abstract

One of the well-known traditional medicinal plants, *Valeriana officinalis* L. (Kantbalu) was chosen for this research work. This study was to examine the determination of nutritional values, cytotoxicity and antimicrobial activities of *Valeriana officinalis* L. (Kantbalu). It has been carried out by AOAC method for determination of nutritional values. According to the results of Brine Shrimp Cytotoxicity Bioassay, all of the tested sample (EtOH and watery extracts) of roots of *Valeriana officinalis* L. (Kantbalu) have cytotoxic effect. The antimicrobial activities of the various crude extracts (PE, EtOAc, EtOH and watery extracts) from roots of *Valeriana officinalis* L. were determined against six strains of microorganisms by agar well diffusion method at Myanma Pharmaceutical Industrial Enterprise, Research Department. The highest antimicrobial activity was observed in EtOH extract whereas PE, EtOAc and watery extract showed the minimum activity. The chemical constituents of essential oil such as trans-calamenene, methylsterate, α-Guaiene, trans-ligustilide, cis-ligustilide, hexadecanoic acid and methyl ester from the roots of Kantbalu were identified by Gas Chromatography- Mass spectrometry method at National Analytical Laboratory, Yangon, Myanmar. The research indicated that root of *Valeriana officinalis* L. has a good source of many constituents and different pharmacological activities.

Keywords: *Valeriana officinalis* L., cytotoxicity, antimicrobial activity, essential oil

Introduction

Valerian root was absolutely first used as a treatment for brain disorder within the late 16thcentury (Sundaresan and Kasthuri, 2018). It is light grayish brown and about the size of a finger joint, bearing many rootlets. The fresh root has no odor, while the dried root smells distinctly unpleasant, due to isovaleric acid. In traditional, Chinese, Myanmar and Japanese medicine *Valeriana officinalis* is used commonly. Valerian has been shown to encourage sleep, improve sleep quality, and reduce blood pressure. The valerian root is sedative, mild anodyne, hypnotic, antispasmodic, carminative and hypotensive. Traditionally, it has been used for hysterical states, excitability, insomnia, hypochondriasis, migraine, cramp intestinal colic and rheumatic pains etc. (Elham and Ali, 2012). Valerian essential oil is composed of various alkaloids, acids, terpenes and flavonoles, many of which contribute directly to the wide range of applications the valerian oil. One of the oldest and more studied benefits of valerian essential oil is its ability to treat the symptoms of insomnia and improve the quality of sleep. Its many active components coordinate an ideal release of hormones and balances the body' s cycles to stimulate restful, thorough, undisturbed sleep. Since dried roots attract rats and cats, it can be used as a bait to lure them away from other areas. Valerian is also a component of many gerbil mixtures, which are widely used to treat sleeping disorders (Shirin, 2013).

Materials and Methods

The dried sample was purchased from Theingi market. The dried pieces were made into powder by using grinding machine and it was used for chemical and biological investigations. The nutritional values such as moisture, fibre, ash, protein and fat were examined at Ministry of Agriculture, Livestock and Irrigation, Small Scale Industries Department, Yangon, Myanmar. The brine shrimp *(Artemia salina*) was used in this study for cytotoxicity bioassay (Ali *et al.,* 2013).

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Brine shrimp cysts (0.5) g was added to the 1L of artificial seawater in separating funnel. This funnel was placed near the lamp. Light is essential for the cysts to hatch. Brine shrimp cysts required to hatch under constant supply of oxygen for 24-hour incubation at room temperature. After 24-hour incubation, hatching of brine shrimp cysts was occurred and the lived shrimps (napulii) were ready for cytotoxicity test. The antimicrobial activity of four crude extracts such as petroleum ether, ethyl acetate, ethanol and water of roots of *Valeriana officinalis* L. was determined against six strains of microorganisms such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *Escherichia coli* by employing agar well diffusion method (Finegold *et al.,* 1976). The tests were screened at Myanma Pharmaceutical Industrial Enterprise, Research Department. The essential oil from the roots of *Valeriana officinalis* L. was extracted by steam distillation method. The oil was extracted with n-hexane in a separating funnel. The n-hexane was evaporated at 60-70C to get the essential oil (Srivastava *et al*., 2003).

Results and Discussion

Determination of Nutritional Values

Nutritional values such as moisture, ash, protein, fibre, fat in the roots of Kantbalu were determined by AOAC method and the results indicated that moisture (12.64 %), ash (6.84 %), fat (7.24 %), fibre (5.18 %), protein (12.22%) and carbohydrate (55.88%) and energy value of (337) kilocalories in 100 g of sample.

Table 1. Nutritional Values of Roots of *Valeriana officinalis* L. (Kantbalu)

No	Nutrients	Contents %
1.	Moisture	12.64
2.	Ash	6.84
3.	Protein	12.22
4.	Fiber	5.18
5.	Fat	7.24
6.	Carbohydrate	55.88
	Energy value	337 (kcal/100g)

Investigation of Cytotoxicity of Watery and Ethanol Extracts

The cytotoxicity of watery and ethanol extracts of root *Valeriana officinalis* L. (Kantbalu) was evaluated by brine shrimp cytotoxicity bioassay. This assay is simple, high through put cytotoxicity test of bioactive chemicals. It is based on the killing ability of test sample on a simple zoological organism- brine shrimp (*Artemia salina).* It is a preliminary toxicity screen for further experiments on mammalian animal models. The cytotoxicity of crude extracts was expressed in terms of mean ± SEM (standard error mean) and LD_{50} (50% Lethality Dose). In this experiment, potassium dichromate ($K_2Cr_2O_7$) and caffeine were used as standard. Potassium Dichromate is generally used as the positive control for this brine shrimp bioassay and caffeine, a natural product and artificial sea water, as negative control. The nauplli were counted against a lighted background after 24-hour initiation of test. From these results, LD_{50} values of watery and ethanol extracts of Kantbalu were less than 1µg/mL and 7 µg/mL. Standard caffeine did not show cytotoxicity until 1000 µg/mL concentration whereas LD_{50} of standard $K_2Cr_2O_7$ was less than 1 µg/mL.

Table 2. Cytotoxicity of Different Doses of EtOH and H_2O Extracts of the Roots of *Valeriana officinalis* L. (Kantbalu)

Extracts	**Percent Survival of Brine Shrimp (Mean ± SEM) at Various Concentration (µg/mL)**				
	1	10	100	1000	LD_{50} (µg/mL)
Watery	43.33±0.33	53.33±0.33	63.33±0.33	86.66±0.33	7
Ethanol	56.67±0.33	73.33±0.33	86.67±0.33	100±0	<1
***$K_2Cr_2O_7$**	63.33±0.33	66.67±0.33	76.67±0.67	100±0	<1
****Caffeine**	0±0	0±0	26.67±0.33	36.67±0.33	>1000

***** = Standard for positive control ** = Standard for negative control

Screening of Antimicrobial Activity of Crude Extracts by Agar Well Diffusion Method

In this present work, antimicrobial activity of four crude extracts (H_2O, EtOH, EtOAc, PE) obtained from root of *Valeriana officinalis* L. were investigated on 6 different strains of microorganisms such as *Bacillus subtilis, Staphylococus aureus, Pseudomonus aeruginosa, Bacillus pumilus, Candida albicans,* and *Escherichia coli* and by agar well diffusion method. The results indicated that EtOH extracts exhibited the highest activity against all tested microorganisms (zone of inhibition ranging 18mm). However, PE, EtOAc and watery extracts of roots sample showed minimum antimicrobial activity of inhibition ranging from 12-15 mm. Therefore, the antimicrobial activity of EtOH extract is almost higher than that of other extracts.

Table 3. Inhibition Zone Diameter of Various Crude Extracts of *Valeriana officinalis* L. (Kantbalu)

Organisms	Inhibition Zone Diameter of Extracts (mm)			
	PE	EtOAc	EtOH	H_2O
Bacillus subtilis	12(+)	12(+)	14(+)	-
Staphylococcus aureus	15(++)	12(+)	17(++)	-
Pseudomonas aeruginosa	-	-	14(+)	12(+)
Bacillus pumilus	12(+)	12(+)	18(++)	-
Candida albicans	14(+)	14(+)	18(++)	-
Escherichia coli	12(+)	13(+)	15(++)	-

Diameter of agar well = 10mm

10mm-14mm = (+)

15mm-19mm = (++)

20mm above = (+++)

No activity = (-)

Detection of Organic Compounds in Essential Oil

Gas chromatographic mass spectrometry (GC-MS) is the single most important tool for identification of unknown organic compounds by matching with reference spectra. According to GC-MS chromatogram, the peak appears at the retention time 12.8 min with 100% relative abundance. At this retention time, the GC-MS spectrum shows the molecular ion peak m/z 202, indicating the molecular weight of a compound to be 202 with the molecular formula $C_{15}H_{22}$. The signal at m/z 202 (R.T.12.848) corresponds M+$[C_{15}H_{22}]^+$., while the peak at m/z 159 corresponds to loss of a propyl group. Therefore, it can be referred that the compound is trans-calamenene. At the retention 21.4 min, the GC-MS spectrum shows the molecular ion peak m/z 298, indicating the molecular weight of a compound to be 298 with the molecular formula $C_{19}H_{38}O_2$. The signal at m/z 298 (R.T.12.42) corresponds M+$[C_{19}H_{38}O_2]^+$., while the peak at m/z 74 corresponds to loss of methyl acetate. Therefore, it can be referred that the compound is methyl stearate. At the retention 9.27 min, the GC-MS spectrum shows the molecular ion peak m/z 204, indicating the molecular weight of a compound to be 204 with the molecular formula $C_{15}H_{24}$. The signal at m/z 204 (R.T.9.274) corresponds M+$[C_{15}H_{24}]^+$., while the peak at m/z 161corresponds to loss of a propyl group. Therefore, it can be referred that the compound is alpha-guaiene. At the retention 14.3 min, the GC-MS spectrum shows the molecular ion peak m/z 190, indicating the molecular weight of a compound to be 190 with the molecular formula $C_{12}H_{14}O_2$. The signal at m/z 190 (R.T.14.306) corresponds M+$[C_{12}H_{14}O_2]^+$., while the peak at m/z 161 corresponds loss of a ethyl group. Therefore, it can be referred that the compound is cis-ligustilide. At the retention 15.26 min, the GC-MS spectrum shows the molecular ion peak m/z 190, indicating the molecular weight of a compound to be 190 with the molecular formula $C_{12}H_{14}O_2$. The signal at m/z 190 (R.T.15.267) corresponds M+$[C_{12}H_{14}O_2]^+$., while the peak at m/z 161 corresponds to loss of a ethyl group. Therefore, it can be referred that the compound is trans-ligustilide. At the retention 17.7 min, the GC-MS spectrum shows the molecular ion peak m/z 270, indicating the molecular weight of a compound to be 270 with the molecular formula $C_{17}H_{34}O_2$. The signal at m/z 270 (R.T.17.714) corresponds M+$[C_{17}H_{34}O_2]^+$., while the peak at m/z 74 corresponds to a loss of ester group. Therefore, it can be referred that the compound is hexadecanoic methylester.

Table 4. Chemical Compositions of Essential Oil

No.	Compound	Retention Time(min)	M.W	Compound from Kantbalu species	Remarks
1.	Alpha-guaiene	9.27	204	Alpha-guaiene	*Valeriana officinalis*
2.	Trans-calamenene	12.848	202	Trans-calamenene	*Valeriana officinalis*
3.	Cis-ligistilide	14.306	190	Cis-ligustilide	*Levisticum officinale*
4.	Trans-ligustilide	15.267	190	Trans-ligustilide	*Levisticum officinale*
5.	Hexadecanoic acid, methylester	17.714	270	Hexadecanoic acid, methylester	*Valeriana italica*
6.	Methyl stearate	21.420	298	Methyl sterate	*Valeriana tuberosa*

Conclusion

The nutritional values determined by AOAC method provided moisture (12.64%), ash (6.84%), fat (7.24%), fiber (5.18%), protein (12.22%) and carbohydrate (55.88%) and energy value of 337 kcal/100g. The cytotoxicity of watery and ethanol extracts was 7µg/mL and less than 1µg/mL respectively. The LD_{50} value of $K_2Cr_2O_7$ was less than 1µg/mL. Caffeine was not cytotoxic to brine shrimp up to the maximum dose of 1000µg/mL. Therefore, watery extract showed lower cytotoxicity effect than the ethanol extract. According to antimicrobial screening, all extracts showed antimicrobial activity. Ethanol extract showed higher antimicrobial activity with the inhibition zone (18 mm) than the watery extract with the inhibition zone (12 mm). The chemical constituents of essential oil from Kantbalu were identified by gas chromatography –mass spectrometry method at Department of Research and Innovation, National Analytical Laboratory, Yangon. The six compounds were found in essential oil of Kantbalu where, trans-calamenene, methyl stearate, Cis-ligustilide, Trans-ligustilide, α-guaniene and hexadecanoic acid methylester. The research indicated that root of *Valeriana officinalis* L. has a good source of many constituents with different pharmacological activities and can be used as a source of alternative medicine.

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