

Pharmacological activities of *Cuscuta reflexa* (Shwe-nwe) stem and *Taraxacum officinale* Weber ex F.H. Wigg. (Dai-Si) leaf extracts

Kyaw Thu¹ and Phyu Phyu Myint²

Abstract

Cuscuta reflexa (Shwe-nwe; SN) stem and *Taraxacum officinale* Weber ex F.H. Wigg. (Dai-si; DS) leaf were explored chemically and followed by antioxidant and antiproliferation activities determination. The samples were collected from Taunggyi University Campus, Taunggyi Township, Shan State in 2018. The preliminary phytochemical results showed that the presence of carbohydrate, flavonoids, glycosides, phenolic compound, reducing sugars, saponins, steroids, tannins, terpenoids, and organic acids are present in both samples. The samples (SN and DS) were observed to compose by protein (10.55 and 13.87%), moisture (9.01 and 11.33%), fat (2.53 and 2.70%), fiber (16.50 and 14.13%) and Carbohydrate (35.46 and 53.63 %) were found to be determined by using the AOAC method. In addition, the elements such as K, Cl, Fe, P and S are found as major elements in SN whereas Fe, K, Ca, Na, Si, Al, Mg in DS were found using ED XRF method. The total phenol contents were determined by Folin-Ciocalteu reagent (FCR) method. The total phenolic contents of MeOH and water extracts were 4.12, and 8.26 µg GAE/mg for SN and 2.13 and 1.04 µg GAE/mg for DS respectively. As pharmacological activities, the order of anti-proliferative effect against HepG2 cell line was observed to be methanol extract of DS (IC₅₀=161.7 µg/ml) > SN (IC₅₀=183.7 µg/ml) by MTT assay. The antioxidant activity of DS ethanol extract (IC₅₀ = 3.62 µg/ml) was found to be most potent and followed by DS methanol extract (IC₅₀= 3.75 µg/ml), DS aqueous extract (IC₅₀ = 4.48 µg/ml), SN methanol extract (IC₅₀ = 5.81 µg/ml), SN aqueous extract (IC₅₀ = 7.24 µg/ml), and SN ethanol extract (IC₅₀ =12.21 µg/ml) determined by DPPH radical scavenging assay. Both samples possess significant pharmacological activities and potentially could be applied as herbal medicine.

Keywords: *Cuscuta reflexa* (Shwe-new; SN) stem, *Taraxacum officinale* Weber ex F.H. Wigg. (Dai-si; DS) leaf, Phytochemical constituents, pharmacological activities, herbal medicine

Introduction

Herbal medicine is widely used in Myanmar in traditional healthcare system. Almost 80 % of rural population is dependent on medicinal plants for their primary health care. Now medicinal plants are gaining more importance in pharmaceutical industries for the preparation of new phytomedicines. (Sule *et.al.*, 2010) Herbal industries always use the crude extracts of medicinal plants for their medicine and beautifying constituents. In most cases, they use medicinal plant crude extract without confirming or validating their level of toxicity and active ingredient. (Ayafo *et. al.*, 1982). Herbs and medicinal plants are found in abundance in the country, and serve as highly affordable remedies for diseases. *Cuscuta reflexa* is a leafless yellow twining, parasitic annual plant belonging to Convolvulaceae family and its local name is Shwe-

¹ Kyaw Thu, Dr., Lecturer, Department of Chemistry, Taunggyi University

² Phyu Phyu Myint, Dr., Professor, Department of Chemistry, Loikaw University

new. The plant contains flavonoids, glycosides, steroids, alkaloids, cuscutalin, cuscutin, and amarvelinodoroside, neritaloside, strospeptide, tyramine, ursolic acid, β -sitosterol glucoside, gitoxeginin, methyl cinnamate, dihydroajugapitin, ferulic acid. Traditionally, it is used in treatment of protracted fever, diaphoretic, and as demulcent and as purgative. Various studies on *Cuscuta reflexa* showed that it has anticonvulsant, antimicrobial, antioxidant, anti-steroidal, anti-inflammatory, antitumour, diuretic, anti-HIV, hypotensive, and hypoglycaemic activity. (Mahmood *et.al.*, 1997) *Taraxacum officinale* Weber ex F.H. Wigg is abundant in the warm temperate regions of the northern Hemisphere. It is native to Europe and Asia and its local name is Daisy. Phytosterols, sesquiterpene lactones, flavonoids, and phenolic acids of *Taraxacum officinale* Weber ex F.H. Wigg are responsible for anti-diabetes actions. This herbaceous plant is traditionally used to treat hepatic disease, diabetes mellitus, rheumatoid arthritis, cancer and jaundice. It exerts anti-hyperglycemic, anti-oxidant, anti-inflammatory, anti-allergic and anti-coagulant activities. (Shi *et.al.*, 2008)

Both these two plants contain varieties of natural chemicals that are capable of fighting some infections and healing the human body. This research highlights the current practice and recommends improvement of herbal medicines in the treatment of liver cancer *in vitro* using HepG2 cell lines as well as antioxidant property related to total phenol content.

Materials and Methods

Preparation of the samples

The plant materials were collected from Taunggyi Township, Myanmar in December, 2018. The samples were identified the specimens at Department of Botany, Taunggyi University. The samples were cleaned, carefully dried in shadow and powdered. About 2 g each of ground powder was soaked in 20 ml of different solvent (water, methanol and ethanol) and shaken for 8 h at 180 rpm, 37° C. The soaked substance was filtered throughout Hyundai micro filter paper (110 mm). The solvent was evaporated with rotary evaporator (SB-1200, EYELA, China) at 60 °C. The crude extract was stored in a well-closed container, protected from light and kept in a refrigerator at 4° C.

Determination of nutritional values, phytoconstituents and elementary analysis

The amount of nutrients such as moisture, ash, fat, carbohydrate, fiber, protein, and calories in the samples were determined by recommended analytical methods. Preliminary phytochemical investigation was carried out according to the standard procedures. In dry powder samples for the standardization and quality control were also determined by EDXRF spectrophotometer, at Taunggyi University.

Determination of antioxidant activity

In vitro screening of antioxidant activity the radical scavenging activity of the ethanol, methanol and watery extracts were investigated by using DPPH assay according to the spectrophotometric method. In this experiment, five different concentration (0.625, 1.25, 2.5, 5 and 10 μ g/ml) of each crude extract in ethanol solvent were used. Ascorbic acid was also used as standard and ethanol without crude extract was employed as control. Determination of absorbance was carried out at wavelength 517 nm using UV visible spectrophotometer. Each experiment was done triplicate. (Sharma *et.al.*, 2012)

Determination of total phenol content

The total phenolic content of the extracts was determined by using Folin-Ciocalteu reagent following a slightly modified method of Ainsworth. Gallic acid was used as a reference standard for plotting calibration curve. A volume of 0.5 mL of

the plant extract (100 µg/mL) was mixed with 2 mL of the Folin-Ciocalteu reagent (diluted 1:10 with de-ionized water) and were neutralized with 4 mL of sodium carbonate solution (7.5%, w/v). The reaction mixture was incubated at room temperature for 30 min with intermittent shaking for color development. The absorbance of the resulting blue color was measured at 765 nm using double beam UV-VIS spectrophotometer (UV Analyst-CT 8200). The total phenolic contents were determined from the linear equation of a standard curve prepared with gallic acid. The content of total phenolic compounds expressed as mg/g gallic acid equivalent (GAE) of dry extract.

Determination of antiproliferation activity

The experiment was performed in triplicate. 400 mg of the methanol extract were dissolved in 1 ml of dimethyl sulfoxide (DMSO) and then serially diluted to 200, 100 and 50 µg/ml for *in vitro* cytotoxic assay (anti-proliferation activity).

Cell lines and culture medium

HepG2 cell lines were obtained from American Type Culture Collection (ATCC). HepG2 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) media supplemented with 10 % (v/v) fetal bovine serum and 1% (v/v) of a penicillin/streptomycin solution. Antibiotics (the final concentration to be 100 U/ml penicillin and 100 µg/ml streptomycin) were added to the medium to eliminate potential interference from microbial contamination that may be present on the test material and control samples. Cells were cultured at 37° C and 5% CO₂ humidified atmosphere; complete medium was changed every 2 to 3 days.

Cytotoxic Assay

The cellular toxicity of the methanol extracts of all samples on cultured cells were measured using 3- (4, 5-dimethylthiazol-2-yl) -2, 5- diphenyl tetrazolium bromide (MTT) assay. The cells were grown in 24-well plates at a density of 5×10^4 cells per well. The cells are allowed to grow overnight in a cell culture incubator. The cells were treated with different concentrations of samples (0, 50, 100, and 200 µg/ml) and incubated for 24 h. Later, 50 µl of the MTT solution (5 mg/ml) was added to each well (final concentration of MTT 0.5 mg/ml), and the plate was reincubated for 2½ h. Finally, the medium was removed and 1000 µl of DMSO was added to solubilize the water insoluble formazan crystals which were a metabolite of a tetrazolium salt. The amount of formazan crystal was determined by measuring the absorbance at 595 nm by using a micro plate spectrophotometer (Spectra Max 190). The cell viability curves of the cell line were established based on extract concentration by triplicate experiment after the specified period. (Machana *et. al.*, 2011)

Results and Discussion

The preliminary phytochemical test on the dry powder samples (SN and DS) revealed the presence of alkaloid, glycosides, steroid, α-amino acids, carbohydrates, organic acid, phenolic compounds, and terpenoid. Some nutritional values in SN and DS were found to contain protein (10.55 and 13.87 %), moisture (9.01 and 11.33 %), fat (2.53 and 2.70 %), Ash (7.53 and 5.85 %), Carbohydrate (35.46 and 53.63 %) and fiber (16.50 and 14.13 %). The nutritional values for the samples were found to be good source of fiber and carbohydrate. The results are shown in Table 1.

Table 1 Some Nutritional Values of SN and DS

No.	Parameters	Content (%)	
		SN	DS
1	Moisture	9.01	11.33
2	Ash	7.53	5.85
3	Fat	2.53	2.70
4	Crude Fiber	16.50	14.13
5	Proteins	10.55	13.87
6	Carbohydrate	35.46	53.63

EDXRF elemental analysis shows that SN was found to possess K, Cl, Fe, P, and S as well as DS contained K, Ca, Na, Si, Al, Mg. It can be seen that essential minerals for human health such as calcium and potassium in sample were the most predominant. Ca is key for the health of bones and teeth, but it also affects muscles, hormones and nerve function. The primary functions of potassium in the body include regulating fluid balance and controlling the electrical activity of the heart and other muscles. Potassium supports blood pressure, cardiovascular health, bone strength and muscle strength. According to ED XRF, no toxic element was found in leaf sample. The results were reported in Table 2.

Table 2 Relative Abundance of Some Elements in SN and DS by (EDXRF)

No.	Elements	Relative Abundance (%)	
		SN	DS
1	Potassium (K)	78.86	10.65
2	Calcium (Ca)	19.63	9.46
3	Sulphur (S)	0.0687	-
4	Iron (Fe)	0.164	64.8
5	Phosphours (P)	0.114	0.341
6	Chlorine (Cl)	0.0375	0.689
7	Sodium (Na)	-	3.33
8	Aluminum (Al)	-	1.573

The percent oxidative inhibition values of crude extracts measured at different concentration and the results are summarized in Table 3 and 4. From these experimental results, it was found that as the concentrations increased, the absorbance values decreased i.e. increase in radical scavenging activity of crude extracts usually expressed in term of % inhibition. From the average values of % inhibition, IC₅₀ (50% inhibition concentration) values in µg/mL were calculated by linear regressive excel program.

From these results, it can be clearly seen that both samples possess significant activity (Figure 1 and 2). Among these extracts, since the lower the IC₅₀ showed the higher the free radical scavenging activity.

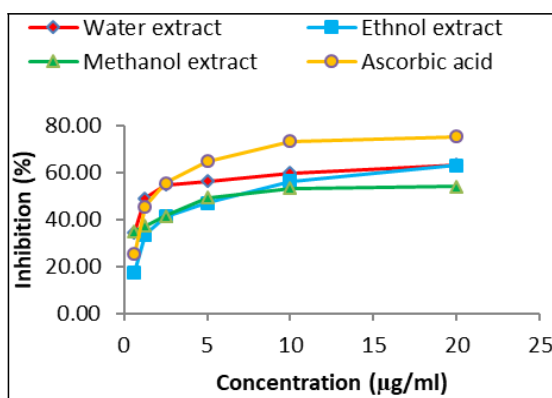


Fig 1 Plot of % oxidative inhibition Vs concentrations ($\mu\text{g/ml}$) of SN extracts

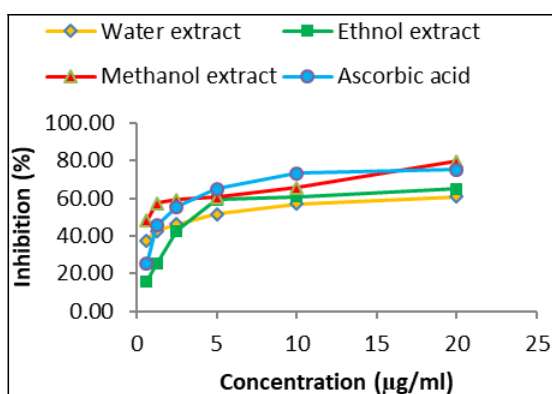


Fig 2 Plot of % oxidative inhibition Vs concentrations ($\mu\text{g/ml}$) of DS extracts

The antioxidant activity of DS ethanol extract ($\text{IC}_{50} = 3.62 \mu\text{g/ml}$) was found to be most potent and followed by DS methanol extract ($\text{IC}_{50} = 3.75 \mu\text{g/ml}$), DS aqueous extract ($\text{IC}_{50} = 4.48 \mu\text{g/ml}$), SN methanol extract ($\text{IC}_{50} = 5.81 \mu\text{g/ml}$), SN aqueous extract ($\text{IC}_{50} = 7.24 \mu\text{g/ml}$), and SN ethanol extract ($\text{IC}_{50} = 12.21 \mu\text{g/ml}$) (Figure 4, Table 4 and Figure 3, Table 3).

Table 3 % Oxidative Inhibition and IC_{50} Values of 95% EtOH, H₂O and MeOH Extracts of SN and Standard Ascorbic Acid

Extract	% inhibition (mean \pm SD) in different concentration ($\mu\text{g/mL}$)						IC_{50} $\mu\text{g/m}$ L
	0.625	1.25	2.5	5	10	20	
EtOH	17.53 ± 0.63	33.38 ± 1.37	41.35 ± 2.47	47.07 ± 3.39	56.18 ± 3.77	63.10 ± 5.39	12.21
H ₂ O	34.55 ± 2.46	49.09 ± 2.68	54.85 ± 1.57	56.36 ± 0.90	59.70 ± 1.79	63.33 ± 3.61	7.24
MeOH	34.91 ± 2.44	37.32 ± 1.30	41.01 ± 3.59	49.39 ± 1.04	53.15 ± 1.33	54.09 ± 2.69	5.81
Ascorbic acid	25.20 ± 1.40	45.58 ± 2.88	55.53 ± 1.13	64.82 ± 3.59	73.32 ± 5.78	75.23 ± 6.81	1.92

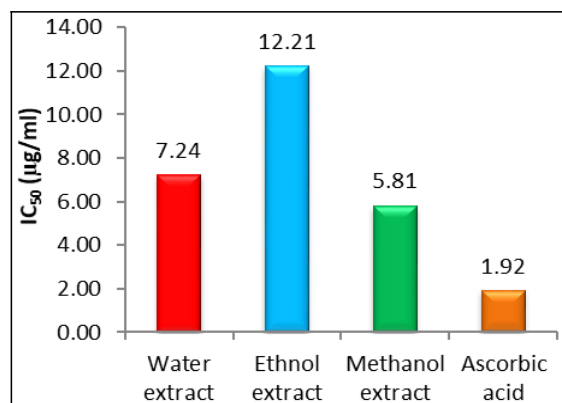


Fig 3 A bar graph of IC₅₀ values of SN extracts

Table 4 % Oxidative Inhibition and IC₅₀ Values of 95% EtOH, H₂O and MeOH Extracts of DS and Standard Ascorbic Acid

Extract	% inhibition (mean ± SD) in different concentration (µg/mL)						IC ₅₀ µg/mL
	0.625	1.25	2.5	5	10	20	
EtOH	15.76 ±1.15	25.00 ±2.31	42.53 ±0.96	59.24 ±2.29	60.87 ±1.54	64.95 ±1.15	3.62
H ₂ O	13.98 ±2.35	17.06 ±1.34	41.00 ±0.84	53.08 ±0.84	57.35 ±0.84	61.61 ±1.01	4.48
MeOH	48.18 ±1.08	57.27 ±3.69	59.09 ±2.17	60.60 ±2.93	65.45 ±1.36	79.69 ±5.93	3.75
Ascorbic acid	25.20 ±1.40	45.58 ±2.88	55.53 ±1.13	64.82 ±3.59	73.32 ±5.78	75.23 ±6.81	1.92

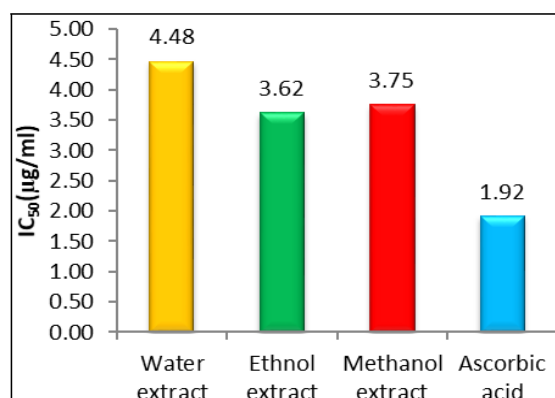


Fig 4 A bar graph of IC₅₀ values of DS extracts

The total phenolic content expressed in terms of GAE and yield (%) of SN extract (water, methanol and ethanol) was found to be $(257.2 \pm 1.79; 284.4 \pm 2.88$ and 165.4 ± 1.56 mg of GAE/g). For DS sample, total phenol contents are calculated as $(154.8 \pm 3.91; 201.64 \pm 4.32$ and 167.6 ± 2.23 mg of GAE/g using the linear equation based on the calibration curve of gallic acid. The results of preliminary phytochemical screening confirmed the presence of various classes of secondary metabolites in the sample including poly phenols (tannins and flavonoids). Plant

polyphenols, produced either from phenylalanine or from its precursor shikmic acid, are important dietary antioxidants because they possess an ideal structural chemistry for free radical scavenging activity (Figure 5).

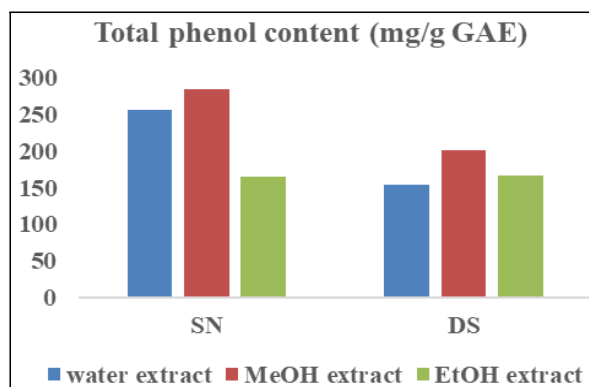


Fig 5 A bar graph of total phenol content

The cytotoxicity (anti-proliferative activity) of methanol extract of SN and DS were evaluated by MTT assay. The cytotoxicity of methanol extract was expressed in term of mean \pm SD (standard deviation) and IC₅₀ (50% Inhibitory Concentration) and the results are shown in Figure 6.

There are many plants extracts used as anticancer agents, even vegetables and fruits may help reduce the risk of cancer in humans. Some tropical plants, namely *Glochidion daltonii*, *Cladogynos orientalis*, *Catimbium speciosum*, *Acorus tatarinowii*, *Amomum villosum* and *Pinus kesiya* were also reported against the human hepatocarcinoma (HepG2) cell line. The cell lines were growth inhibited in a dose- dependent manner after exposure to the plant extracts. The results presented by the optical density (OD) and they indicated that all the different plants methanol extract which mentioned above have a cytotoxic effect in cell lines. IC₅₀ values obtained in both cell lines using MTT assay showed that DS was more effective in inducing cytotoxicity in HepG2 cell line, IC₅₀ (150.8 \pm 2.13 μ g/ml) and followed by the plant extracts of SN, IC₅₀ (169.8 \pm 4.01 μ g/ml).

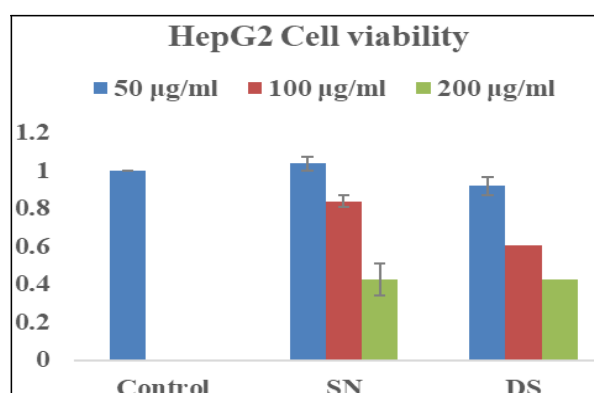


Fig 6 A bar graph of cell viability for 24 h treated by MeOH extracts of SN and DS

Conclusion

The results of the study suggest that *Cuscuta reflexa* (Shwe-nwe; SN) stem and *Taraxacum officinale* Weber ex F.H. Wigg. (Dai-si; DS) leaf are rich in phenolic compounds and have a good antioxidant activity. It can be used as a natural source of antioxidants to prevent the progression of many diseases. The methanolic extract of both samples also produced marked *in-vitro* anti-liver cancer activity

that justifies its use in traditional system of medicine in Myanmar and other Asian countries. However, further detailed investigations are needed to ascertain the mechanisms and constituents behind its anti-liver cancer actions.

Acknowledgements

The authors wish to thank Professor Kim Yeong Shik (College of Pharmacy, Institute of Natural Product Chemistry, Seoul National University, Seoul, Korea) and members of the laboratory for anticancer activity determination. This anticancer determination was also supported by Korea Foundation for Advanced Studies (KFAS).

References

- Ainsworth, E.A and Gillespie, K.M. (2007). "Estimation of Total Phenolic Content and Other Oxidation Substrates in Plant Tissues Using Folin-Ciocalteu Reagent", *Nat Protoc.* **2** (4):875-882.
- Ayafro, J.F, Sondengam, B.L and Ngadyui, B.J. (1982). "Veprisinium salt, A Novel Antibacterium Quaternary Alkaloid From *Vepris Lowsii*." *Planta Med.* **44** (3) 139-142
- Chatterjee, D., Sahu, R.K. Jha., A.K and Dwivedi J. (2011). "Evaluation of Antitumor Activity of *Cuscuta reflexa* Roxb. (Cuscutaceae) Against Ehrlich Ascites Carcinoma in Swiss Albino Mice", *Tropical J Pharm Res*; **10**, 447-454.
- Gilani, A.H and Aftab, K. (1992). "Pharmacological Actions of *Cuscuta reflexa*", *Pharmaceutical Biol.* **30**, 296-302.
- Gupta, M., Mazumder, U.K., Pal, D.K, Battacharya S and Chakrabarty, S. (2003). "Studies on Brain Biogenic Amines in Methanolic Extract of *Cuscuta reflexa* Roxb. and *Corchorus olitorius* Linn. Seed Treated Mice", *Acta Poloniac Pharmaceutica-Drug Res.* **60**, 207-210.
- Gupta, M., Mazumder, U.K., Pal, D.K and Bhattacharya, S. (2003). "Anti-steroidogenic Activity of Methanolic Extract of *Cuscuta reflexa* Roxb. Stem and *Corchorus olitorius* Linn. Seed in Mouse Ovary", *Indian J Exp Biol.* **41**, 641-644.
- Hfaiedh, M., Brahmi, D and Zourgui, L. (2016). "Hepatoprotective effect of *Taraxacum officinale* Leaf Extract on Sodium Dichromate-induced Liver Injury in Rats", *Environ Toxicol.* **3**, 339-349.
- Kalita, D and Saikia J. (2012). "Ethnomedicinal, Antibacterial and Antifungal Potentiality of *Centella asiatica*, *Nerium indicum* and *Cuscuta reflexa* - Widely Used in Tiwa Tribe of Morigaon District of Assam", *India, Int J Phytomed.* **4**, 380-385.
- Katiyar, N.S., Rao, N.V and Gangwar A.K. (2012). "Evaluation of Anti-inflammatory Activity of Stem Extracts of *Cuscuta Reflexa* (Roxb) in Rats", *International J Res Pharm Biomed Sci.* **3**, 1805-1808.
- Machana, S., Weerapreeyakul, N., Barusrux, S., Nonpunya, A., Sripanidkulchai, B and Thitimetharoch, T. (2011). "Cytotoxic and Apoptotic Effects of Six Herbal Plants Against the Human Hepatocarcinoma (HepG2) Cell Line", *Chin Med.* **6**, 39-46.
- Mahmood, N., Piacente, S., Burke, A., Khan, A and Pizza C. (1997). "Constituents of *Cuscuta reflexa* are Anti-HIV Agents", *Antivir Chem Chemother.* **8**, 70-7.
- Petlevski, R., Hadzija, M., Slijepcevic, M., Juretic, D and Petrik, J. (2003). "Glutathione S-transferases and Malondialdehyde in the Liver of NOD Mice on Short-term Treatment with Plant Mixture Extract Phytother" **17**, 311-314.
- Phyu Phyu Myint, May Thu Soe and Htet Htet Hlaing. (2017). "A Study of Phytoconstituents, α -Glucosidase Inhibitory Effect and Antioxidant Activity of *Lagerstroemia Speciosa* L. Leaf and Fruit, *Journal of Pharmacognosy and Phytochemistry*". **6** (4):528-533.
- Sharma, S., Hullatti, K.K, Kumar, S. and Tiwari, B.K. (2012). "Comparative antioxidant activity of *Cuscuta reflexa* and *Cassia Filiformis*", *J Pharm Res.* **5**, 441-443.
- Sharma, S., Hullatti, K.K, Prasanna, S.M., Kuppast, I.J. and Sharma, P. (2009). "Comparative Study of *Cuscuta Reflexa* and *Cassia Riliiformis* for Diuretic Activity", *Phcog Res.* **1**, 327-330.
- Shi, S., Zhao, Y., Zhou, H., Zhang, Y., Jiang, X. and Huang, K. (2008). "Identification of Antioxidants from *Taraxacum mongolicum* by High-performance Liquid Chromatography-diode Array Detection-Radical-Scavenging Detection-Electrospray Ionization Mass Spectrometry and Nuclear Magnetic Resonance Experiments", *J Chromatogr A.* **1209**, 145-152.
- Sule, A., Ahmed, Q.U and Samah, Q.A. (2010). "Screening for Antibacterial Activity of *Andrographis paniculata* Used in Malaysia Folkloric Medicine: A Possible Alternative for the Treatment of Skin Infection" **14**, 445-456
- Wirngo, F.E., Lambert, M.N and Jeppesen, P.B. (2016). "The Physiological Effects of Dandelion (*Taraxacum officinale*) in Type 2 Diabetes", *Rev Diabet Stud.* **13**, 113-131.