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

Kyaw Thu and Phyu Phyu Myint

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 (11.08 and 11.75%), moisture (26.0 and 8.59%), fat (2, 4 and1.81%), and fiber (14.08 and 10.81%) 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 (IC_{50}=161.7 µg/ml) > SN (IC_{50}= 183.7 µg/ml) by MTT assay. The antioxidant activity of DS ethanol extract (IC_{50} = 3.62 µg/ml) was found to be most potent and followed by DS methanol extract (IC_{50}= 3.75 µg/ml), DS aqueous extract (IC_{50} = 4.48 µg/ml), SN methanol extract (IC_{50} = 5.81 µg/ml), SN aqueous extract (IC_{50} = 7.24 µg/ml), and SN ethanol extract (IC_{50} =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.

1. Introduction
In Myanmar, a high proportion of the rural and urban population rely on herbal medicinal plants. According to Myanmar traditional medical belief there are 96 diseases which afflict humankind using fresh or dried roots, stems, barks, leaves, buds and flowers of medicinal plant and the hair, fat, bones and organs of certain insects, reptiles and mammals. Myanmar indigenous medicine is able to heal and cure all 96 maladies. Indigenous medicines are administered as powder, mixtures, decoctions, infusions, percolates, pastes, extracts, preserves, pills or tablets [1]. 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-nwe. The plant contains flavonoids, glycosides, steroids, alkaloids, cuscutalin, cuscutin, and amarvelinodoroside, neritaloside, strospeside, tyramine, ursolic acid, β-sitosterol glucoside, gitoxeginin, methyl cinnamate, dihydroajugapitin, ferulic acid [2]. Traditionally, it is used in treatment of protracted fever, diaphoretic, and as demulcent and as purgative. Various studies on *Cuscuta reflexa* showed that the plant has anticonvulsant [3], antimicrobial [4], antioxidant [5], anti-steroidal [6], anti-inflmmatory [7], antitumour [8], diuretic [9], anti-HIV [10], hypotensive, and hypoglycaemic activity [11]. *Taraxacum officinale* F.H. Wigg. (Compositae) 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 *T. officinale* are responsible for anti-diabetes actions [12]. This herbaceous plant is traditionally used to treat hepatic disease, diabetes mellitus, rheumatoid arthritis, cancer and jaundice [13]. It exerts anti-hyperglycemic, anti-oxidant, anti-inflammatory, anti-allergic and anti-coagulant activities [14, 15].

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 recommended 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.

2. Materials and Methods

Preparation of the samples

The plant materials were collected from Taunggyi Township, Myanmar in December, 2018. The samples were identified the specimen*s* 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 concertation (0.625, 1.25, 2.5, 5 and 10 µg/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.

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 [16]. 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% CO_2 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 $2\frac{1}{2}$ 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.

3. 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 (11.08 and 11.75 %), moisture (26.06 and 8.59%), fat (2.4 and 1.81%), and fiber (14.08 and 10.81%). 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 (w/w)	
		SN	DS
1	Moisture	26.06	8.59
2	Ash		
3	Fat	2.4	1.81
4	Crude Fiber	14.08	10.81
5	Proteins	11.08	11.75
6	Carbohydrate		

EDXRF elemental analysis show 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)	90.0	3.65
2	Calcium (Ca)	9.36	3.64
3	Sulphur (S)	0.0978	-
4	Iron (Fe)	0.134	82.8
5	Phosphours (P)	0.114	0.431
6	Chlorine (Cl)	0.0375	0.806
7	Sodium (Na)	-	3.13
8	Aluminum (Al)	-	1.23

The percent oxidative inhibition values of crude extracts measured at different concentration and the results are summarized in Table 3 and Figure 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, IC_{50} (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 IC_{50} showed the higher the free radical scavenging activity.

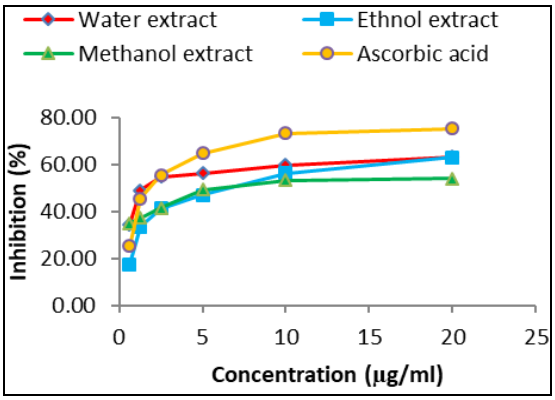

Fig 1: Plot of % oxidative inhibition Vs concentrations (µg/ml) of SN extracts

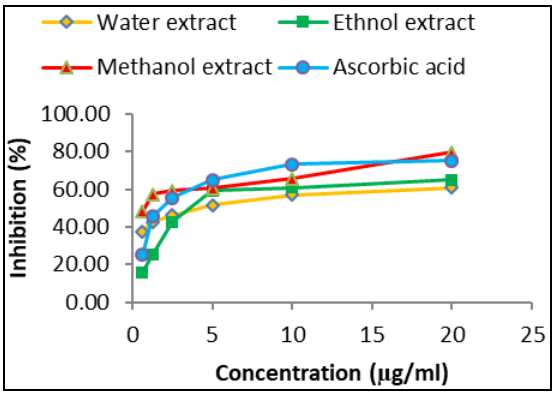

Fig 2: Plot of % oxidative inhibition Vs concentrations (µg/ml) of DS extracts

The antioxidant activity of DS ethanol extract (IC_{50} = 3.62 µg/ml) was found to be most potent and followed by DS methanol extract (IC_{50}= 3.75 µg/ml), DS aqueous extract (IC_{50} = 4.48 µg/ml), SN methanol extract (IC_{50} = 5.81 µg/ml), SN aqueous extract (IC_{50} = 7.24 µg/ml), and SN ethanol extract (IC_{50} =12.21 µg/ml) (Figure 3 and 4).

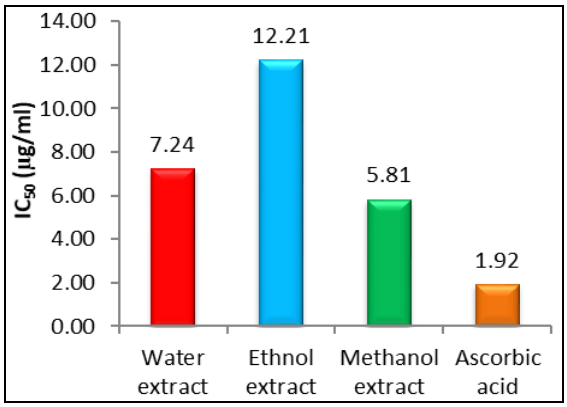

Fig 3: A bar graph of IC_{50} values of SN extracts

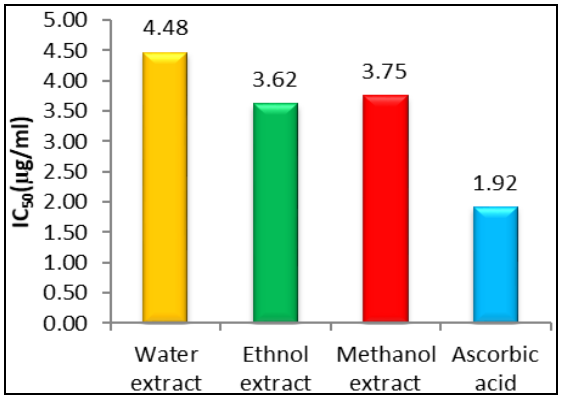

Fig 4: A bar graph of IC_{50} values of DS extracts

The total phenolic content expressed in terms of GAE and yield (%) of SN extract (water, methanol and ethanol) was found to be (257.2±1.79; 284.4±2.88 and 165.4±1.56 mg of GAE/g). For DS sample, total phenol contents are calculated as (154.8±3.91; 201.6.4±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).

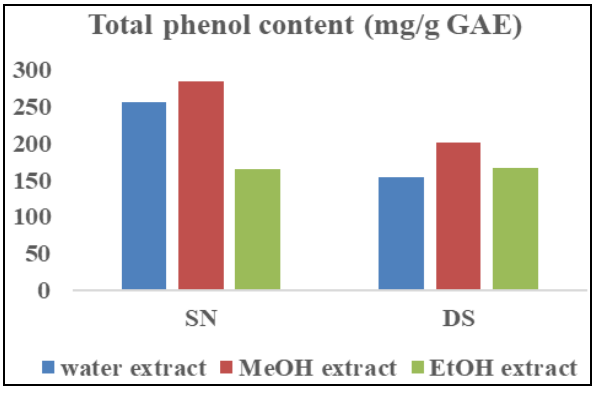

Fig 5: A bar graph of total phenol content

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 ± SD (standard deviation) and IC_{50} (50% Inhibitory Concentration) and the results are shown in Figure 6.

There are many plants extracts have been 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 [17]. 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. IC_{50} values obtained in both cell lines using MTT assay showed that DS was more effective in inducing cytotoxicity in HepG2 cell line, IC_{50} (181.7±2.13µg/ml) and followed by the plant extracts of SN, IC_{50} (183.7±4.01 µg/ml).

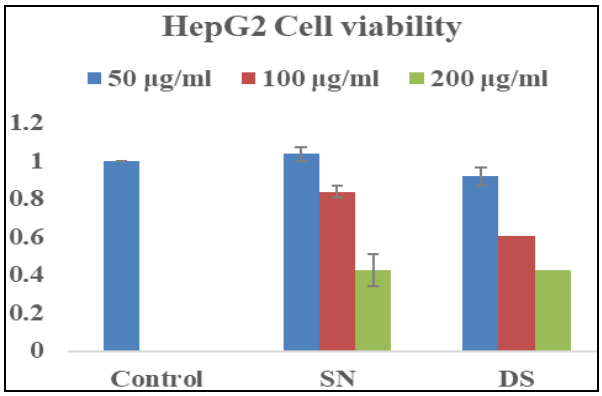

Fig 6: A bar graph of cell viability for 24 h treated by MeOH extracts of SN and DS

4. Conclusion

The results of the study suggest that *Cuscuta reflexa* (Shwe-new; 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.

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