PHYTOPHARMACOLOGICAL POTENTIAL OF *DREGEA VOLUBILIS* (GWE-DAUK-NWE) LEAF FOR INHIBITION OF AFLATOXIN PRODUCING FUNGUS AND HEPATOCELLULAR CARCINOMA (HepG2) CELLS

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Abstract

The present work focused on the study of chemical constituents from the leaf of *Dregea volubilis* (L.f.) Benth. ex Hook.f. (Gwe-dauk-nwe) and detoxification activity to aflatoxin producing microorganisms in agricultural products and cytotoxicity to liver cancer cell line (HepG2). The leaf of the plant was collected from Kyunkalay village, Hlegu Township, Yangon Region in July 2018**.** The leaf sample was cleaned, dried and made to powder. Phytochemical investigation of *D. volubilis* leaf was performed and it was found that carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, steroids, tannins, terpenoids, and organic acids were present, however, cyanogenic glycosides and starch were absent. In addition, the elements such as Ca (38.32 %), K (33.72 %), Cl (12.31 %), Al (11.24 %), S (1.95 %), Zn (0.98 %) are found as major elements and small amount of other elements are found as Fe (0.77 %), Mn (0.44 %), Rb (0.11 %), Br (0.08 %), Sr (0.08 %) were also found using ED XRF method. Essential oil of *D. volubilis* leaves was extracted by steam distillation method and the organic components (7-chloro benzofuran, Phenol,2,4-bis(1,1-dimethylethyl), 6,10,14-trimethyl-2-pentadecanone, dibutyl-phthalate, 1-eicosene, phytol and uridine compounds) in it were identified by GC-MS method. The antioxidant activities of watery and ethanol extracts of the leaf sample were determined by DPPH assay method. The IC_{50} value of water and ethanol extracts were found to be 582.12 and > 1000 µg/mL, respectively. *In vitro* detoxification activity of essential oil and extracts (PE,$CHCl_3$, MeOH and H_2O extracts) of *D. volubilis* leaf was tested by aflatoxin producing fungus. Yeast, *Aspergillus flavus* and *Aspergillus niger* were cultured from corn, peanut and chilli using direct culture method. $CHCl_3$, MeOH extracts and essential oil of *D. volubilis* leaf showed detoxification activity on these yeast and fungus microorganisms (inhibition zone diameter 11 mm to 19 mm) as well as PE and H_2O extracts (inhibition zone diameter 11mm to 14 mm). Determination of minimum inhibitory concentration (MIC) of essential oil on two species of organisms, namely *A. niger* and *A. flavus* was carried by potato dextrose agar well diffusion method. The essential oil with different concentrations (1×10^5 to 0.2 µg/mL) showed inhibition zone diameter in the range of 12 to 15 mm. The MIC values for essential oil was found to be 2.5×10^4 µg/mL. (inhibition zone diameter ~ 12 mm). The cytotoxicity of MeOH extract from *D. volubilis* leaf against hepatoma liver cancer cell HepG2 was evaluated by MTT assay. The IC_{50} value of MeOH extract was found to be 168.05 µg/mL for 24 h treated time.

Keywords: *Dregea volubilis* leaf, antioxidant activity, detoxification activity, cytotoxicity, MTT assay

Introduction

Myanmar has a rich tradition of using medicinal plants to treat different diseases. Medicinal plants, also called, herbal medicine, have been discovered and used in traditional medicine practices since prehistoric time. Numerous phytochemicals potential or established biological activity have been identified. Medicinal plants are widely used in non-industrialized societies, mainly because they are readily available and cheaper than modern medicines (Ahn,

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2017). The world health organization estimates that the plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population (Baker, 2005).

Dregea volubilis (Gwe-dauk-nwe) is a plant in the family of Apocynaceae. It is occurring in India to Java and other tropical countries. *Dregea volublis* leaves are ovate, 7.5 to 15 centimeters long, 5 to 10 centimeters wide, rather leathery, rounded or pointed at the base, and pointed at the tip (Sudarsanma, 2015). One popular food in our society is *Drege*a *volubilis* leaves soup with chicken. This bitter taste soup is very popular soup in Myanmar and it is famous for its health benefits as detoxifying remedies especially for alcohol poisoning or alcohol overdose. This leaf soup is also believed to possess numerous medicinal values and benefits to human health and lifestyle. The leaf exhibits some anti-bacterial and antifungal properties that make it a good home remedy to several health issues such as dysentery, diarrhoea, high blood pressure and many others. Therefore, it is important to identify and evaluate the popular ingredient used in relation to efficacy and safety.

Nowadays, in our society, aflatoxins producing microorganisms are regularly found inproperly stored staple communities such as corn, peanut, chili pepper and a variety of species. Chronic exposure increases the risk of developing liver and gall bladder cancer, as aflatoxin metabolites may intercalate into DNA and alkylate the bases through epoxide moiety (Nogueira, *et. al*., 2015). For these and many other reasons, the present investigation was emphasized on the study of phytopharmacological potential from *Dregea volubilis* leaf especially enormous range of biological activities like antioxidant, cytotoxicity and detoxification to aflatoxin producing fungus.

Materials and Methods

The leaf sample of *Dregea volubilis* (Gwe-dauk-nwe) was collected from Kyunkaly Village, Hlegu Township, Yangon Region. After collection, the botanical name of the simple was identified and confirmed as *D. volubilis* leaf at Botany Department, Dagon University. The collected fresh sample was cleaned by washing thoroughly with water and air dried. After drying, the leaf sample was cut into small pieces and ground using grinding machine. And then this powdered sample was kept in the sealed air-tight container to prevent moisture changes and other contamination. It was then used without further purification or refining.

Phytochemical investigation

The dried powdered samples were used to chemical tests for the determination if the presence or absence of the major types of phytochemical constituents using standard procedure (M-Tin Wa, 1972; Marini Bettolo *et al*., 1981; Finar, 1968; Trease and Evans, 1980, Shriner *et al.*, 1980 and Robinson, 1983).

Elemental Analysis of *Dregea volubilis* by ED XRF

In order to determine the heavy toxic metals and micronutrient elements in leaf sample, elemental contents in the leaf of *D. volubilise* were determined by ED XRF method at the Universities' Research Center, Yangon. The major advantage of X-ray spectrometry is that it offers a satisfactory compromise among economy, speed and ease of operation (Ertel, 1991).

Extraction of Essential oil from *Dregea volubilies* Leaf by Steam Distillation Method

Essential oil (0.13 %) was extracted from dried powder sample by steam distillation method. Organic compounds such as 7-chloro benzofuran, Phenol,2,4-bis(1,1-dimethylethyl), 6,10,14-trimethyl-2-pentadecanone, dibutyl-phthalate, 1-eicosene, phytol and uridine compounds could be identified from this extracted essential oil by GC-MS method (James and Martin,1952) at the Department of Research and Innovation, National Analytical Laboratory, Kaba Aye Pagoda Road, Yancon

Determination of Antioxidant Activity

DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical scavenging assay was chosen to assess the antioxidant activity of plant materials. This assay has been widely used of plant materials to evaluate the free radical scavenging effectiveness of various flavonoids and polyphenols in food system (Leea *et al*, 2002). In this experiment, the antioxidant activity was studied on 95 % ethanol and aqueous extract from selected leaf sample by DPPH free radical scavenging assay. DPPH (2 mg) was thoroughly dissolved in 100 mL of 95 % ethanol. This 60 µM DPPH solution was freshly prepared in the brown coloured bottle.

The control solution was prepared by mixing the 1.5 mL of 60 µM DPPH solution and 1.5 mL of 95 % ethanol in brown bottle. Blank solution was prepared by mixing the 1.5 mL of test sample solution with 1.5 mL of 95 % ethanol. Each respective H_2O and ethanol extracts (30 mg) and 30 mL of 95 % ethanol were thoroughly mixed by shaker. The mixture solution was filtered and stock solution was obtained. Desired concentration 1000 µg/mL, 800 µg/mL, 600 µg/ mL, 400 µg/mL, and 200 µg/mL of each solutions were prepared from this stock solution by dilution with appropriate amount of 95 % ethanol.

4 mg of standard Vitamin C was dissolved in 20 mL of 95 % ethanol to get the 200 µg/mL stock solution. Desired concentrations of 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL and 6.25 µg/mL solution were prepared by two-fold serially diluted with ethanol. These bottles were incubated at room temperature and were shaken on shaker for 30 min. After 30 min, the absorbance of these solution was measures at 517 nm by using spectrophotometer (UV-KWF, China). Absorbance measurements were done in triplicate for each solution and the mean values obtained were used to calculate % inhibition of oxidation by the following equation.

$$\% \text{ oxidative inhibition} = \frac{A-(C-B)}{A} \times 100\%$$

% oxidative inhibition = % inhibition of test sample

A= absorbance of the control solution

B= absorbance of the blank solution

C= absorbance of the test sample solution

Then IC_{50} (50 % inhibitory concentration) values were also calculated by linear regressive excel program.

Screening of Detoxification Activity by Potato Dextrose Agar Well Diffusion Method

Detoxification activity of different crude extracts ($CHCl_3$, MeOH, PE, watery extracts and essential oil) of the sample was screened in *in vitro* by Potato Dextrose Agar well Diffusion method (Dorman, 2000). Test Organisms (yeast, *Aspergillus flavus* and *Aspergillus niger*) were

obtained from agricultural products such as corn, peanut and chilli (Figures 1, 2 and 3). This experiment was carried out at Pharmaceutical Research Department (PRD), Ministry of Industry, Yangon.

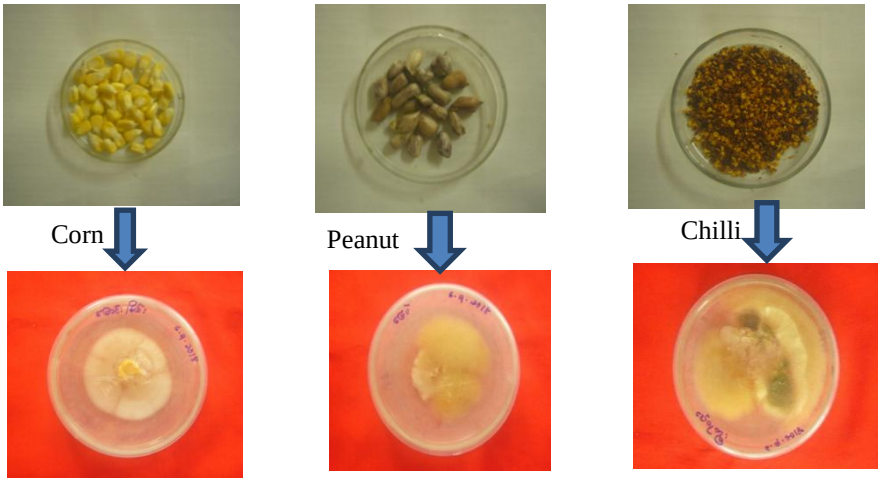


Figure 1 Culture of aflatoxin producing fungi from agricultural products

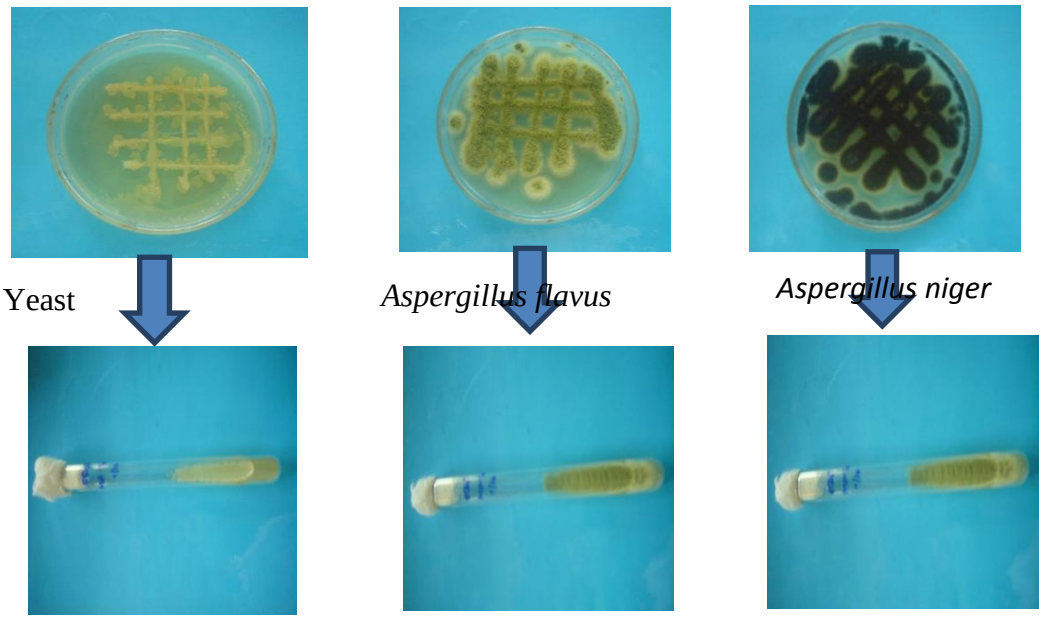


Figure 2 Growth of yeast and fungi from corn, peanut and chilli

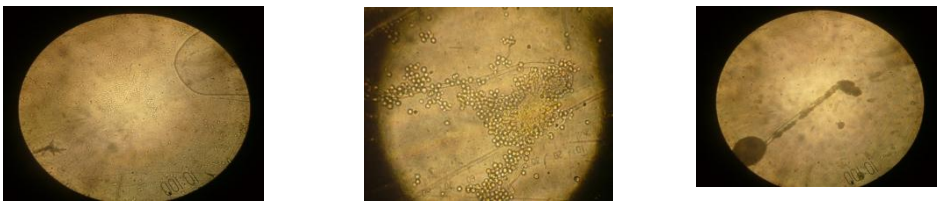


Figure 3 Morphology of Yeast, *Aspergillus flavus* and *Aspergillus niger*

Determination of Minimum Inhibitory Concentration (MIC) of Essential Oil for Detoxification Activity

In order to determine the minimum inhibitory concentration (MIC) of essential oil from leaf which inhibited the microorganisms, the specific concentration of the essential oil prepared in serial dilution in agar plates were used for testing against *A. niger* and *A. flavus* which were obtained from agricultural products such as peanut and chilli. This experiment was carried out at Pharmaceutical Research Department (PRD), Ministry of Industry, Yangon.

Minimum inhibitory concentration (MIC) values of essential oil were determined by potato dextrose agar well diffusion method. 0.2 mL from 100 mg/mL mixture (essential oil dissolved in pet-ether solution) was introduced into the agar well of first Petri dish to obtain the concentration of 1×10^5 µg/mL. By this way, 0.2 mL of each test sample solution was introduced into the agar well of different Petri dishes to obtain the concentrations of 5×10^4, 2.5×10^4, 1.25×10^4, 6.25×10^3, 3.12×10^3, 1.56×10^3, 7.81×10^2, 3.9×10^2 etc., to 0.2 µg/mL. All the Petri dish were incubated at 28 °C for one week. After incubation, the diameters of inhibition zones including 10 mm wells were measured. The amount of sample that showed in inhibition zone diameter was determined as the corresponding MIC value.

Examination of *in vitro* Cytotoxic Activity by MTT Reduction Assay method

The cytotoxicity of methanol extract of the sample was examined by using MTT reduction assay method. HepG2 cells were seeded in a 96 well flat –bottom microliter plate at a density of 1×10^4 cells/ well and allowed to adhere for 24 h at 37 °C in a CO_2 incubator. After 24h the cell were then treated with 0 to 300 µg/mL of methanol extract for 24 h at 37 °C in a CO_2 incubator. Subsequently, 10 µL of MTT solution (5 mg/mL in phosphate buffer solution) were added to each well and incubated for 4 h at 37 °C. The culture medium was discarded, and 100 µL of DMSO solution was added into each well and mixed by gently shaking for 10min. Absorbance (the interesting of the dissolved formazan crystal (purple color) was quantified using the ELISA plate (microplate reader) at 595 nm (Padhya *et al*., 2013). Cell viability was calculated from the mean values of the data from three wells using the equation below and cytotoxic activity was expressed as the IC_{50} (50 % inhibitory concentration) value.

$$(\%)\ \text{Cell viablity} = 100 \times \frac{A_{bs}\,(\text{test sample}) - A_{bs}\,(\text{blank})}{A_{bs}\,(\text{contro}) - A_{bs}\,(\text{blank})}$$

Results and Discussion

Preliminary Phytochemical Present in of *Dredge volubilis* Leaf

According to the phytochemical test results, α-amino acids, alkaloids, carbohydrates, flavonoids, glycosides, phenolic compound, reducing sugars, saponins, steroids, tannins, terpenoids and organic acids are present in the sample but cyanogenic glycosides and starch are absent (Table 1).

Table 1 Results of Phytochemical Investigation on *Dregea volubilis* Leaf

Sr. No.	Tests	Extract	Test Reagents	Observation	Remark
1.	α-amion acids	H_2O	Ninhydrin reagent	Pink colour	+
2.	Alkaloids	1 % HCl	Mayer's reagent	White ppt	+
			Dragendorff's reagent	Orange ppt	+
			Wagner's reagent	Brown ppt	+
			Sodium picrate solution	Yellow ppt	+
3.	Cyanogenic glycosides	H_2O	Conc:H_2SO_4	No brick red	-
4.	Carbohydrates	H_2O	10 % α-naphthol & H_2SO_4 (conc.)	Red ring	+
5.	Flavonoids	EtOH	Mg ribbon & HCl (conc.)	Pink colour	+
6.	Glycosides	H_2O	10 % lead acetate	White ppt	+
7.	Phenolic compounds	H_2O	1 % $FeCl_3$	Deep blue colour	+
8.	Reducing sugars	H_2SO_4 (dil)	Benedict's solution	Brick-red ppt	+
9.	Starch	H_2O	Iodine solution	No blue colour	-
10.	Saponins	H_2O	Distilled water	Frothing	+
11.	Steroids	PE	Acetic anhydride & H_2SO_4 (conc.)	Green colour	+
12.	Tannins	H_2O	1 % gelation solution	White ppt	+
13.	Terpenoids	$CHCl_3$	Acetic anhydride & H_2SO_4 (conc.)	Pink colour	+
14.	Organic acids	H_2O	Bromocresol	Green colour	+

Presnce = (+) , Absence = (-), ppt=precipitate

Elemental Analysis of *Dregea volubilis* Leaf by Energy Dispersive X-Ray Fluorescence

X-ray spectrometer permits simultaneous analysis of light element to heavy metal. Shimadzu EDX-720 spectrometer can analyze the elements from $_{11}$Na and $_{92}$U under vacuum condition. The ED XRF spectrum of the sample results was reported in Table 2. It can be seen that essential minerals for human health such as calcium and potassium in leaf were the most predominant. Ca is key for the health of bone 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 muscle strength. According to ED XRF, no toxic element was found in leaf sample.

Table 2 Relative Abundance of Some Elements in Leaf of *Dregea volubilis* (Gwe-dauk-nwe) by ED XRF Method

No.	Elements	Relative Abundance (%)
1.	Calcium (Ca)	38.316
2.	Potassium (K)	33.721
3.	Chlorine (Cl)	12.308
4.	Aluminium (Al)	11.238
5.	Sulphur (S)	1.947
6.	Zinc (Zn)	0.980

Detection of Organic Compounds in Essential Oil of *Dregea volubilis* Laf by GC-MS Method

Gas chromatographic mass spectrometry (GC-MS) is the single most important tool for identification of unknown organic compounds by matching with reference spectra. The GC-MS chromatogram and mass spectra of essential oil from the *Dregea volubilis* leaf are shown in Table 3 and Figure 4. Identifications were made by comparison of their retention time and m/z ratio with private reference library data and from the literature.

All the compounds such as 7-chloro benzofuran, Phenol, 2, 4-bis (1, 1-dimethylethyl), 6,10,14-trimethyl-2-pentadecanone, dibutyl-phthalate,1-eicosene, phytol and uridine compounds detected in the essential oil were found to possess the pharmacological activity. Among them, benzofuran compound used as recreational drugs producing sympathetic system stimulation and euphoria. Phenol was widely used as an antiseptic. It was used as soap, known as carbolic soap. Pentadecan-2-one is flavor and fragrance agents. Phthalates are used in hundreds of products in homes, hospitals, cars and businesses, because of their strong performance, durability and stability. Eicosene are used in lubricants, lubricant additives and viscosity adjustors (eg. lubricant). Phytol is used in the fragrance industry and used in cosmetic, shampoos, toilet soap, household cleaners, and detergents. Uridine is cognitive enhancer, helps protect the brain and improve memory, and reduces pain and inflammation.

Antioxidant Activity of crude Extracts from leaf of *Dregea volubilis* (Gwe-dauk-nwe)

DPPH (1,1-diphenyl-2-picryl-hydrazyl) method is the most widely reported method for screening of antioxidant activity on many plant drugs. This method is based on the reduction of colored free radical DPPH in 95 % ethanol solution by different concentration of the samples. The antioxidant activity was expressed as 50 % oxidative inhibitory concentration (IC_{50}).

Table 3 Chemical Compositions of Essential Oil from *Dregea volubilis* Leaf

Chemical compound	MW	Retention time (min)	Formula
7-Chloro benzofuran	152	5.94	C_8H_5OCl
Phenol, 2,4-bis(1,1-dimethylethyl)	206	10.36	$C_{14}H_{22}O$
6,10,14-trimethyl-2-pentadecanone	268	13.87	$C_{18}H_{36}O$
Dibutyl-phthalate	278	14.76	$C_{16}H_{22}O_4$
1-eicosene	280	15.03	$C_{20}H_{40}$
Phytol	298	15.84	$C_{20}H_{40}O$
Uridine,2'-5'-dideoxy-5'-(3-4-dihydro-5-methyl-2,4-dioxo-1(2H)-pyrimidinyl	336	17.996	$C_{14}H_{16}O_6N_4$

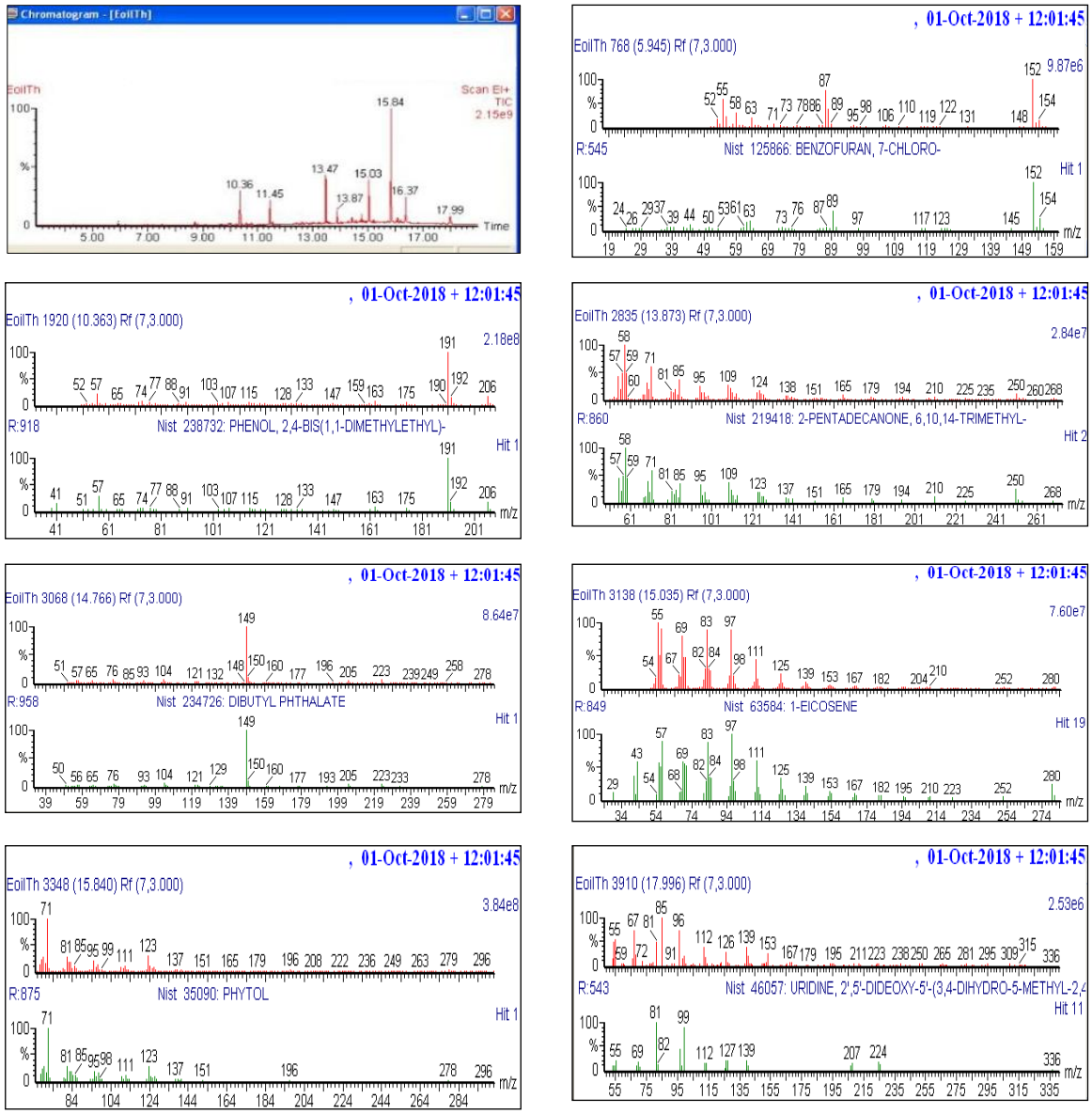


Figure 4 Gas chromatogram-Mass spectra of compounds from essential oil of *Dregea volubilis* leaf

The percent oxidative inhibition values of crude extracts measured at different concentration and the results are summarized in Table 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 IC_{50} values were found to be > 1000 µg/mL for ethanol extract and 582.12 µg/mL for water extract. The lower the IC_{50} showed the higher the free radical scavenging activity. Water extract was found to be more effective than ethanol extracts in free radical scavenging activity. However, it was observed that all of these extracts have the lower antioxidant activity than standard ascorbic acid (IC_{50}=8.99 µg/mL).

Table 4 Present Oxidative Inhibition and IC_{50} Values of 95 % Ethanol and Aqueous Extracts of *Dregea volubilis* Leaf and Standard Ascorbic Acid

Extracts	% Inhibition (mean±SD) in different concentration (μg/mL)					IC_{50} (μg/mL)
	200	400	600	800	1000	
Water	22.41 ± 4.10	26.62 ± 5.06	52.60 ± 4.45	62.86 ± 2.08	73.15 ± 4.40	582.12
EtOH	3.75 ± 1.03	4.03 ± 2.16	6.71 ± 2.86	14.84 ± 2.04	21.16 ± 0.00	> 1000
	6.25	12.50	25.0	50	100	
STD Ascorbic acid	24.50 ± 7.28	82.58 ± 4.02	94.46 ± 0.44	94.24 ± 2.68	96.16 ± 0.13	8.99

Detoxification Activity of Crude Extracts and Essential oil by Potato Dextrose Agar Well Diffusion method

Screening of detoxification activity of various crude extracts such as PE, $CHCl_3$**,** MeOH, watery extract and essential oil of *Dregea volubilis* leaf samples was done by using potato dextrose agar well diffusion method. In this study, the microorganisms were firstly cultured from agricultural products. From this process, it can be clearly seen that agricultural products are the sources of aflatoxin –producing fungi such as yeast, *Aspergillus flavus* and *Aspergillus niger*. The morphology of all cultured species could be confirmed by under the microscope (40X magnification). The samples were tested on these three species of microorganisms. The inhibition zone diameter shows the degree of detoxification activity.

The larger the inhibition zone diameter, the higher the detoxification activity. The resulting inhibition zone diameters are described in Table 5 and Figure 5.

Table 5 Inhibition Zone Diameters of Crude Extracts and Essential Oil against three Microorganisms by Potato Dextrose Agar Well Diffusion method

No.	Microorganisms	Inhibition Zone diameter (mm)				
		PE extract	$CHCl_3$ extract	MeOH extract	Water extract	Essential oil
1.	Yeast	11	15	19	14	16
2.	*Aspergillus flavus*	-	14	11	-	15
3.	*Aspergillus niger*	12	12	12	12	15

Agar well diameter – 10 mm
10mm – 14 mm (+); 15 mm – 19 mm (++); 20 mm – above (+++)

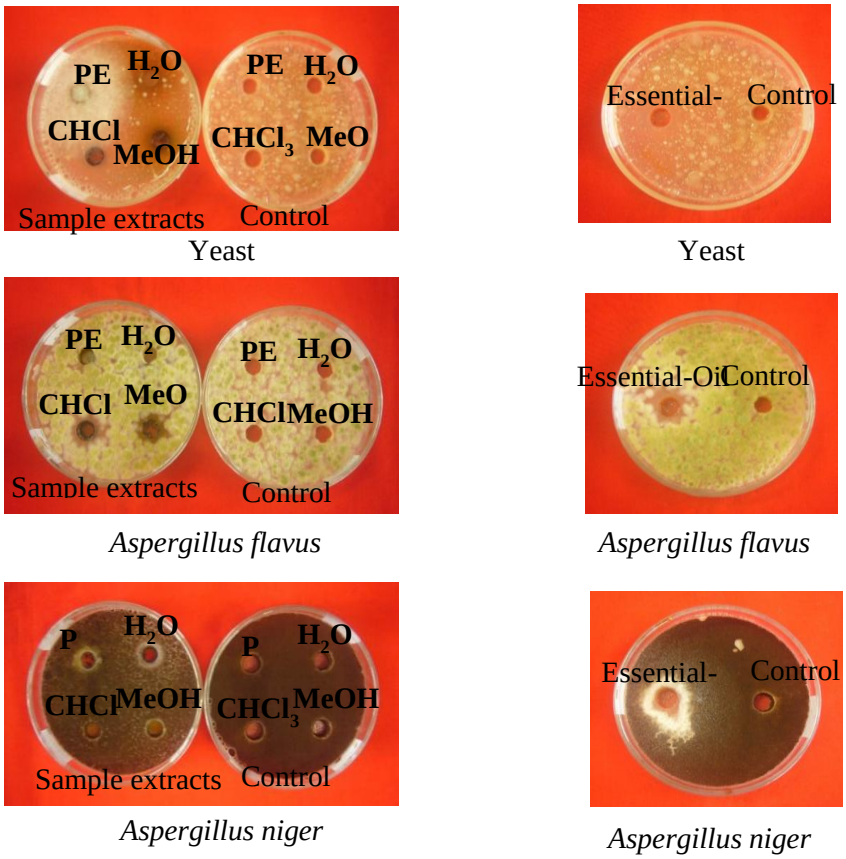


Figure 5 Screening of Detoxification activity on three microorganisms (Yeast, *Aspergillus flavus* and *Aspergillus niger*)

From these results, it was found that MeOH and essential oil are found to possess detoxification activity against three microorganisms; yeast, *Aspergillus flavus* and *Aspergillus niger* with the inhibition zone diameter range between (11 mm to 19 mm).

In addition, PE extract (inhibition zone diameter 11 mm to 12 mm), $CHCl_3$ extract (inhibition zone diameter 12 mm to 15 mm) and water extract (inhibition zone 12 mm to 14 mm). But PE and water extract were not active against *A. flavus*. According to the results the crude extracts the essential oil of *Dregea volubilis* leaf showed the detoxification properties against Yeast, *A. flavus* and *A. niger*.

Minimum Inhibitory Concentration (MIC) by Potato Dextrose Agar Well Diffusion Method

MIC values of essential oil of *Dregea volubilis* were determined by agar well diffusion method. In this study, the essential oil was tested on two species of microorganisms, *Aspergillus niger* and *Aspergillus flavus* with different concentrations. The concentrations of essential oil were range from 1×10^5 µg /mL to 0.2 µg/mL to determine the MIC value. The lowest MIC values for essential oil of *Dregea volubilis* was found to be 2.5×10^4 µg/mL against *A. niger* and *A. flavus*. These results are reported in Table 6 and Figure 6. According to literature survey, the essential oil from other plants; *Styrax tonkinensis, Lavandula angustifolia, Melaleuca alternifolia, Pelargonium graveolens* and *Rosmarinus officinalis* were also reported significantly inhibited growth of *A. niger* and to a lesser extent that of *A. flavus* with MIC (minimal inhibitory concentrations) in the range 0.078-1.25 x 10^4 µg/mL (Shine, 2003). The results showed that five

plant essential oil possess lower minimal concentrations inhibition on *A. niger* and *A. flavus* than *D. volubilis* leaf.

Table 6 Minimum Inhibitory Concentration (MIC) of Essential Oil from *Dredge volubilis* Leaf

Sample No.	Concentrations (µg/mL)	Inhibition zone diameter (mm)	
		A. niger	***A. flavous***
1	1×10^5	15 (++)	15 (++)
2	5×10^4	13 (+)	13 (+)
3	2.5×10^4	12 (+)	12 (+)
4	1.25×10^4	-	-
5	6.25×10^3	-	-
6	3.12×10^3	-	-
7	1.56×10^3	-	-
8	7.8×10^2	-	-
to	to	-	-
20	0.2	-	-

Agar well diameter – 10 mm 10 mm – 14 mm (+); 15 mm – 19 mm (++); 20 mm – above (+++)

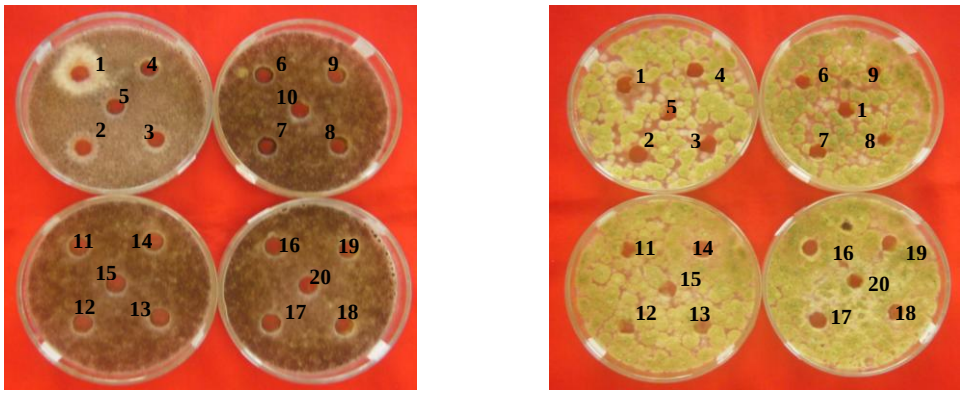


Figure 6 Minimum inhibitory concentration (MIC) of essential oil for detoxification activity on (a) *Aspergillus niger* and (b) *Aspergius flavus*

Cytotoxicity of Methanol Extract of *Dregea volubilis* leaf

The cytotoxicity of methanol extract of *Dregea volubilis* was evaluated by MTT assay. The cytotoxicity of methanol extract was expressed in terms of mean ± SDC standard deviation and IC_{50} (50 % Inhibitory Concentration) and the results are shown in Table 7 and Figure 7**.** There are many plants extracts have been used as anticancer agents even vegetables and fruits many help reduce the risk of cancer in humans. Some Thai plants namely *Glochidion daltonii, Cladogynos orientalis, Catimbium speciosum, Acorus tatarinowii, Amomum villosum and Pinus kesya* showed the highest selectivity and potent cytotoxicity in the HepG2 cell line, with an IC_{50}value of 52.0 ± 5.8 µg/mL (mean ±standard deviation) Figure 8.Extract of *Catimbium speciosum* exerted cytotoxicity with an IC_{50} value of 55.7 ± 8.1 µg/mL. Crude extracts from *Glochidion daltonii, Cladogynos orietialis, Acorus tatarinowii and Amomum villosum* exhibited cytotoxicity with IC_{50}values ranging 100 – 500 µg/mL (Machana *et al*., 2011).

In this study, the local plant extract showed moderate IC_{50} value (168.05 µg/mL) for cytotoxicity against HepG2 cell line and is recommended for medical cuisine beyond traditional cuisine.

Table 7 Viability of HepG2 Cell by MeOH Extract of *Dregea volubilis* leaf using MTT Assay

Extract	Cell Viability (mean ±SD) in different concentrations (µg/mL)						IC_{50} (µg/mL)
	0	100	150	200	250	300	
MeOH	1.0 ± 0.0	0.70 ± 0.17	0.63 ± 0.08	0.27 ± 0.10	0.19 ± 0.03	0.17 ± 0.04	168.05 ± 4.32

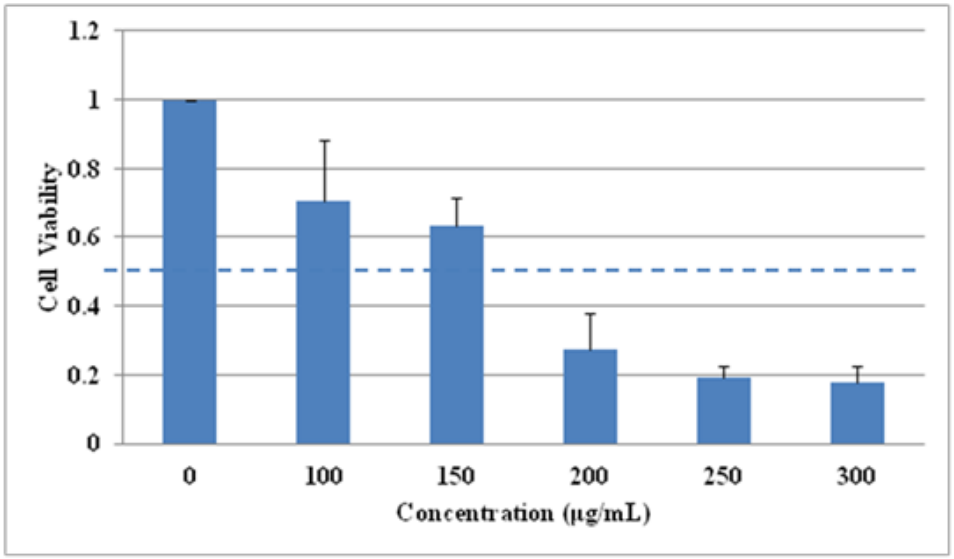
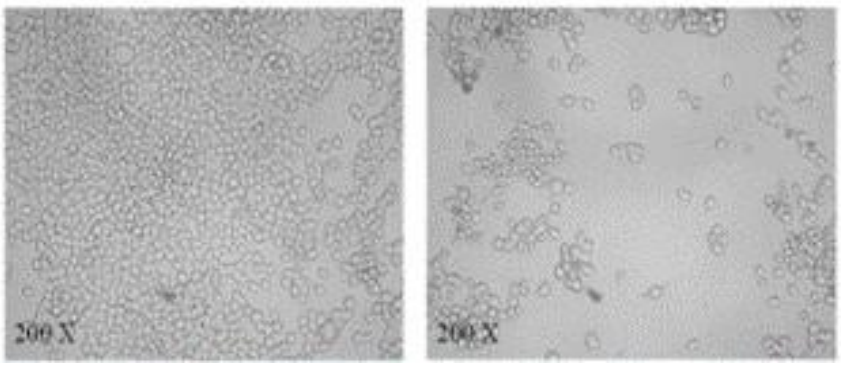


Figure 7 A bar graph of cell viability for 24 hours treated by MeOH extract



(a) (b)

Figure 8 Morphology of HepG2 cells after 24 hours treatment of **(a)** 0 µg/mL and **(b)** 250µg/mL of sample

Conclusion

From the overall assessments of the present work concerning with the phytochemical constituents, detoxification activity and cytotoxicity of *Dredge volubilis* (Gwe-dauk-nwe) leaf, the following inference could be deduced.

The GC-MS analysis results have revealed that the presence of benzofuran, phenol, pentadecan-2-one, phthalate, eicosene, phytol and uridine compounds in *D. volubilis* indicating

potential to protect the brain and improve memory, reduces pain and inflammation. Qualitative elemental analysis of plant sample by ED XRF method showed that Ca and K were the highest amount of elements in the sample. According to the elemental result, this sample was found to be effective for good help by regulating fluid balance and controlling the electrical activity of the heart and other muscles. The leaf sample also possesses antioxidant and cytotoxicity. The radical scavenging activity of water extract was found to be more effective than EtOH extract by DPPH assay. The IC_{50} value of MeOH extract against human liver cancer line (Hep G2) was observed 168.05 µg/mL. Essential oil of *D.volubilis* leaf showed detoxification activity against *A. flavus* and *A. niger* (MIC values was 2.5 x 10^4 µg/mL) whereas PE and water extracts did not show activity against *A. flavus.* According to these observations, *D. volubilis* leaf extracts could be applied not only for nutrition but also for pharmacological properties.

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