STUDY ON NUTRITIONAL QUALITY AND ANTIMICROBIAL, ANTIOXIDANT AND ANTI-PROLIFERATIVE ACTIVITIES OF *DIOSCOREA ALATA* L. (MYAUK U)

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Abstract

In this study, one of the *Dioscorea* species, *Dioscorea alata* L. (Myauk U) has been chosen to study nutritional values, chemical composition and some biological activities such as antimicrobial, antioxidant and anti-proliferative activities. The phytochemical constituents were screened by test tube method indicating the presence of alkaloids, α-amino acids, carbohydrates, glycosides, phenolic compounds, saponins, starch and terpenoids, however, cyanogenic glycosides, reducing sugars and tannins were absent. The nutritional values of the selected rhizomes were determined by AOAC methods. The antimicrobial activity of the different crude extracts such as pet-ether, ethyl acetate, ethanol and watery extracts was investigated against six microorganisms: *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans and Escherichia coli* by agar well diffusion method. The total phenolic contents (TPC) of ethanol extract (469.1 ± 0.01 μg GAE/mg extract) and watery extract (441.1 ± 0.00 μg GAE/mg extract) were determined by Folin-Ciocalteau assay method. The antioxidant activity of ethanol and watery extracts was evaluated by 2, 2 - diphenyl -1- picryl – hydrazyl free radical scavenging assay. Both ethanol and watery extracts were found to possess antioxidant activity. However, both extracts possess weaker in antioxidant activity than standard ascorbic acid. The anti-proliferative activity or cytotoxicity of methanol extract of the rhizome of *D. alata* was evaluated by MTT assay using Hep G2 (human liver cancer cell).

Keywords: *Dioscorea alata* L., antimicrobial activity, antioxidant activity, anti-proliferative activity, MTT assay

Introduction

A number of wild crops remain unexplored in our country, Myanmar and among them some have excellent medicinal and nutritional properties. In this study, one of the *Dioscorea* species, also called yams, *Dioscorea alata* L. (Figure 1) has been chosen. Yam is a rich source of carbohydrate and also contributes to vitamins and minerals. It has many bioactive substances, such as phenolic compounds, alkaloids, steroidal saponins and proteins. *D. alata* has been used as a moderate laxative and vermifuge, hypertension (blood pressure), in skin diseases and for fever, gonorrhea, leprosy, tumors and inflamed hemorroids. *Dioscorea* species have been reported to have antifungal, antioxidant, anti-inflammatory, hypoglycemic and anticancer activity (Conlan *et al.*, 1998).

The tuber flesh is white or purplish and loose in texture. *Dioscorea* species are perennial through root system but are grown as annual crops, which serves as a staple food for millions of people in tropical and subtropical countries (Shewry, 2003). Its origin is Southeast Asia and is grown throughout the tropics and temperate regions of the world. It also grows in West India and West Africa (Acevedo-Rodriguez and Strong, 2012).

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Botanical Aspects of *Dioscorea alata* L.

Family	:	Dioscoreaceae
Genus	:	Dioscorea
Species	:	alata
Botanical Name	:	*Dioscorea alata* L.
English Name	:	Water yam, greater yam
Myanmar Name	:	Myauk U
Common Names	:	White yam, Water yam, Greater yam, Guyana arrowroot, winged yam, or simply yam
Part used	:	Rhizomes of *D. alata*

(a) Rhizome (b) Leaf (c) Plant

Figure 1 Rhizome, leaf and plant of Myauk U

Aim

The main aim of this research is to study on nutritional quality and to investigate antimicrobial, antioxidant and anti-proliferative activities of the crude extracts of *Dioscorea alata* L. (Myauk U).

Materials and Methods

Collection and Preparation of Plant Samples

The rhizome of *D. alata* was collected from Thapaung Township, Ayeyawady Region. The selected plant had been identified at Botany Department, University of Yangon. The collected sample was washed with water, sliced into small pieces and allowed to air - dried at the room temperature and then made into powder by using the grinding mill. The dried powdered sample was stored in the air-tight container for chemical and biological investigation.

Preliminary Phytochemical Investigation

Investigation of preliminary phytochemical screening was carried out on the dried powdered sample with a view to investigate the presence or absence of phytochemical constituents such as alkaloids, α-amino acids, carbohydrates, cyanogenic glycosides, organic acids, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, starch, steroids, tannins and terpenoids (M-Tin Wa, 1972), (Harbone, 1984).

Analysis of Nutritional Values

The analysis of the nutritional values on the dried powdered sample of *D. alata* was performed according to the procedures described by AOAC, 2000. The moisture content was determined by oven drying method, protein content by micro Kjeldahl distillation method, fat content by Soxhlet extraction method, fiber content by fiber cap method, ash content by ashing in furnace method and carbohydrates content by Phenol - sulphuric acid method (Agrawal *et al.*,2015).

Investigation of Antimicrobial Activity

Antimicrobial activities of various crude extracts such as PE, EtOAc, EtOH, and H_2O extracts were investigated by using agar well diffusion method (Cruickshank, 1960) at the Pharmaceutical Research Department (PRD), Ministry of Industry, Yangon. Fluconazole and tetracycline were taken as a positive control for antifungal and antibacterial tests. Bacterial cultures of *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *Escherichia coli* were used as the tested organisms for antimicrobial activities.

Determination of Total Phenolic Content

One of the antioxidative factors, total phenolic content (TPC) was measured by spectrometrically according to the Folin- Ciocalteu reagent method (Saxena, 2013).

Folin- Ciocalteau reagent (5 mL) was mixed with 0.5 mL of each sample in a test tube. After incubation for 5 min at room temperature, 4 mL of 10 % Na_2CO_3 was added, mixed and incubated for 30 min at room temperature. The absorbance was measured at 765 nm and then the TPC contents were calculated by the following equation,

$$C\ (GAE) = c \times \frac{V}{M}$$

where, c = concentration determined from standard curve (μg/mL),
V = volume used during the assay (mL),
M = mass of the extract during the assay (mg).

Results were expressed as microgram of gallic acid equivalents (GAE) per milligram of extract (GAE; μg/mg extract).

Investigation of Antioxidant Activity

One of the free radical scavenging assays, DPPH (2,2-diphenyl-1-1-picryl hydrazyl) free radical scavenging assay has been chosen to evaluate the free radical scavenging effectiveness of various flavonoids and polyphenols in crude extracts of *D. alata* (Marinova and Batchvarov, 2011).

Preparation of solutions

(i) Preparation of 0.002 % (w/v) DPPH solution

0.002 % DPPH solution was prepared by dissolving (2 mg) of DPPH powder in the 100 mL of ethanol.

(ii) Preparation of standard solutions (Ascorbic acid)

2 mg of ascorbic acid was dissolved with 100 mL of ethanol to obtain the stock solution. The standard solutions (20, 10, 5, 2.5, 1.25 and 0.625 μg/mL concentrations) were prepared from this stock solution by dilution with appropriate amount of EtOH.

(iii) Preparation of test sample solutions

2 mg of respective crude extract was prepared by dissolving 100 mL of ethanol and the stock solution was obtained. The sample solutions with the concentrations of (20, 10, 5, 2.5, 1.25, 0.625 μg/mL) were prepared from the stock solution by dilution with appropriate amount of EtOH.

(iv) Preparation of blank solution

Blank solution was prepared by mixing the sample solution (1.5 mL) with ethanol (1.5 mL).

Procedure

In this assay, the control solution was prepared by mixing 1.5 mL of 0.002 % DPPH solution and 1.5 mL of ethanol in the brown bottle. The sample solution was also prepared by mixing 1.5 mL of 0.002 % DPPH solution and 1.5 mL of test sample solution. These bottles were incubated at room temperature and were shaken on shaker for 30 min. After 30 min, the absorbance of different concentrations (0.625, 1.25, 2.5, 5, 10, 20 μg/mL) of the tested sample was measured at 517 nm using UV-1800 spectrophotometer. Absorbance measurements were used to calculate the percentage of radical scavenging activity (% RSA) by the following equation:

$$\% \text{ RSA} = [A_{DPPH} - (A_{Sample} - A_{blank})/ A_{DPPH}] \times 100$$

where,

% RSA = % radical scavenging activity of test sample
A_{DPPH} = absorbance of DPPH in EtOH solution
A_{Sample} = absorbance of sample + DPPH solution
A_{Blank} = absorbance of sample + EtOH solution

The antioxidant power (IC_{50}) is expressed as the test substances concentration (μg/mL) that results in a 50 % reduction of initial absorbance of DPPH solution and that allows to determine the concentration. IC_{50} (50 % inhibition concentration) values were calculated by linear regressive excel program.

Determination of Cytotoxicity or Anti-proliferative Activity by MTT Assay

The cytotoxicity or anti-proliferative activity of methanol extract of rhizomes of

D. alata was determined against human liver cancer cell line, Hep-G2 by 3- (4,5-dimethylthiazoyl - 2)- 2, 5- diphenyl tetrazolium bromide (MTT) assay at the College of Pharmacy and Natural Products Research Institute, Seoul National University, Seoul (Bahuguna *et al*., 2017).

Cytotoxic activity of crude extract was screened in 1 x 10^4 cells/well seeded in a 96 swell plate (30 mm) and incubated for 24 h for attachment and treated with MeOH extract at

concentration of 0 – 300 µg mL^{-1} and then kept for 24 h. Thereafter, 10 µL of MTT solution (5 mg mL^{-1}) were added to each well, and then incubated in darkness at 37 ºC for 4 h. The culture medium was discarded. The formazan crystals were solubilized by adding 100 µL DMSO per well and then mixed by gently shaking for 10 min. The amount of MTT – formazan is proportional to the number of living cells and the absorbance was measured in a microplate reader at 595 nm. The fractional absorbance was calculated by the following equation:

$$\% \text{ Cell survival} = \frac{\text{Mean absorbance in test well}}{\text{Mean absorbance in control wells}} \times 100$$

Results and Discussion

Phytochemical Constituents and Nutritional Values of *D.alata*

Phytochemical investigation was carried out to know the types of phytochemical constituents present in the rhizomes of *D. alata* by using test tube methods. According to these results, alkaloids, α-amino acid, carbohydrates, flavonoids, glycosides, phenolic compounds, saponins, starch, steroids, organic acids and terpenoids were found to be present but tannins, reducing sugars and cyanogenic glycosides were absent in the selected sample. Therefore, this study may provide the valuable scientific base for the use of herbs in the traditional medicine.

Nutritional values determined by standard AOAC methods were found to be protein (7.97 %), fat (1.98 %), fiber (22.61 %), ash (8.87 %), moisture (10.20 %) and carbohydrate (51.03 %). According to the observation, carbohydrate content has the highest composition than other nutrients. The corresponding energy value calculated is 253.82 kcal/100 g of the sample.

Antimicrobial Activity of *D. alata*

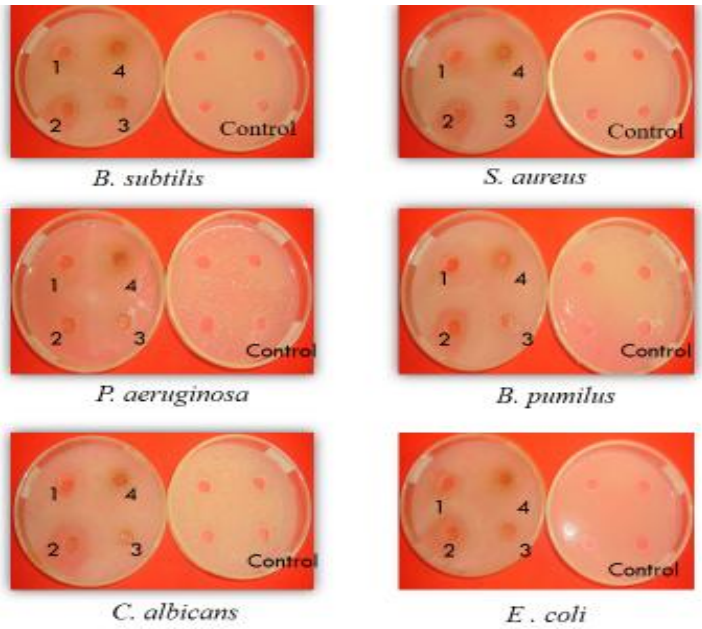
Screening of antimicrobial activity by agar well diffusion method, the measurable clear zone diameter, including the well diameter, shows the degree of antimicrobial activity. The larger the inhibition zone diameters, the higher the antimicrobial activity. The well diameter in the present study is 10 mm.

The results of antimicrobial activity are shown in Table 1 and Figures 2 and 3. It was found that all crude extracts possessed the antimicrobial activity against all tested microorganisms. Among them, the EtOAc extract exhibited the highest activity against all tested microorganisms, inhibition zone ranging from 20 - 24 mm. the PE extract and ethanol extract also showed antimicrobial activity on all tested microorganisms with inhibition zone diameter ranging from 18 - 23 mm and 12 – 13 mm, respectively. It was found that, the PE extract has the lowest antimicrobial activity against five strains except *Bacillus subtilis*. Therefore, the selected plant may be used in the treatment of diseases caused by microorganisms such as diarrhea, dysentery, food poisoning, boils, wound sepsis, respiratory tract infections and skin infections.

Table 1 Antimicrobial Activity of Four Crude Extracts of *D. alata* by Agar Well Diffusion Method

Microorganisms	Inhibition zone diameters of different crude extracts (mm)			
	PE	EtOAc	EtOH	H_2O
B. Subtilis	23 (+++)	20 (+++)	12 (+)	-
S. aureus	20 (+++)	22 (+++)	12 (+)	11 (+)
P. aeruginosa	12 (+)	24 (+++)	12 (+)	11 (+)
B. pumilus	22 (+++)	20 (+++)	12 (+)	11 (+)
C. albicans	23 (+++)	22 (+++)	12 (+)	11 (+)
E. coli	18 (++)	22 (+++)	13 (+)	11 (+)

Agar well - 10 mm
10 mm ~ 14 mm (+) 20 mm above (+++)
15 mm~19 mm (++) No activity (-)



1. PE extract 2. EtOAc extract 3. EtOH extract 4. H_2O extract

Figure 2 Effect of antimicrobial activity of four crude extracts of the rhizomes of *D. alata* on six microorganisms

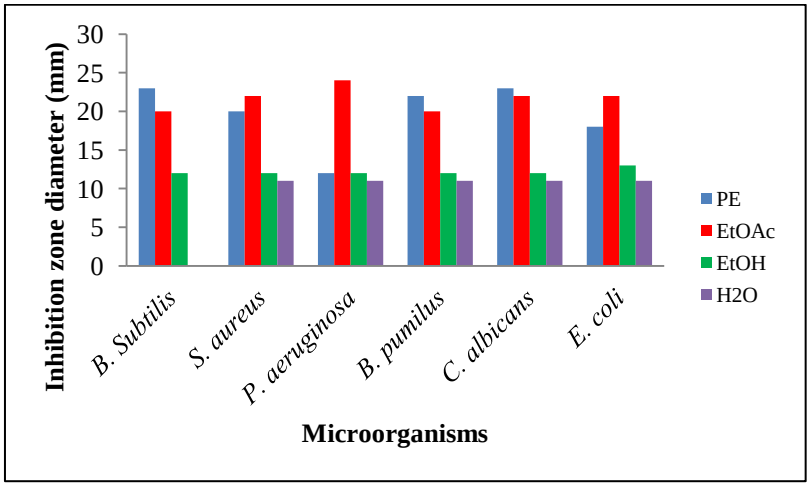


Figure 3 A bar graph of inhibition zone diameters of four crude extracts of the rhizomes of *D. alata* against six microorganisms

Total Phenolic Contents of *D. alata.*

In determination of the total phenolic contents by FCR method, phenols react with an oxidizing agent phosphomolybdate in F-C reagent under alkaline conditions and results in the formation of blue coloured complex which is measured at 765 nm. Gallic acid (3, 4, 5-trihydroxy-benzoic acid) was used to construct the standard calibration curve for total phenol estimation. Total phenolic content (TPC) was expressed as microgram of gallic acid equivalent (GAE) per milligram of crude extract (µg GAE/mg). The TPC content of ethanol extract (469.11 ± 0.01 µg GAE/mg) was found to be higher than that of watery extract (441.18 ± 0.00 µg GAE/mg). These correlations indicated that high total phenol contents in ethanol extract contributed to high in free radical scavenging activity of ethanol extract. The results are shown in Table 2 and Figure 4.

Table 2 Total Phenol Contents (TPC) of Ethanol and Watery Extracts of the rhizomes of *D. alata*

No.	Extracts	TPC (µg GAE/mg ± SD)
1	Ethanol extract	469.11 ± 0.01
2	Watery extract	441.18 ± 0.00

Antioxidant Activity of Crude Extracts of *D. alata*

In determining the antioxidant activity using DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay, a stable free radical from DPPH tends to capture hydrogen from the antioxidant. Due to its free radical, the ethanolic DPPH solution is violet and absorbance is measured at 517 nm. The colour changes upon neutralization of this free radical from violet to pale yellow by daylight. The decolouration of the initial colour is proportional to the test substances having antiradicalizing power.

It was found that as the concentration increased, the absorbance values decreased. The larger % RSA indicates the higher antioxidant activity. In contrast, the lower IC_{50} value indicates the more effective antioxidant activity. The antioxidant activity is expressed as % radical scavenging activity (% RSA) and 50 % inhibition concentration (IC_{50}) value was calculated by linear regressive excel program. The results of % RSA of two crude extracts of the rhizomes of *D. alata* and standard ascorbic acid are tabulated in Table 3 and Figure 5. Furthermore, their respective IC_{50} values are shown in Table 4.

According to the results, the ethanol extract (IC_{50} = 7.28 µg/mL) was found to be more potent than watery extract (7.32 µg/mL), in antioxidant power. Antioxidant potency of ethanol and water extracts were concluded to be weak by compared with the potency of standard ascorbic acid (IC_{50} = 1.73 µg/mL).

Table 3 Radical Scavenging Activity (%RSA) of Ethanol and Watery Crude Extracts of *D. alata* and Ascorbic acid

Sample	%RSA ± SD at Different Concentrations (µg/mL)					
	0.625	1.25	2.5	5	10	20
EtOH	13.46 ± 0.00	18.77 ± 0.01	26.93 ± 0.01	40.61 ± 0.03	61.12 ± 0.01	90.91 ± 0.00
H_2O	18.60 ± 0	22.44 ± 0.01	28.93 ± 0.00	41.92 ± 0	59.25 ± 0.00	65.64 ± 0.02
Ascorbic acid	26.80 ± 0.00	41.06 ± 0.00	61.27 ± 0.00	78.51 ± 0.00	88.93 ± 0.00	97.02 ± 0.00

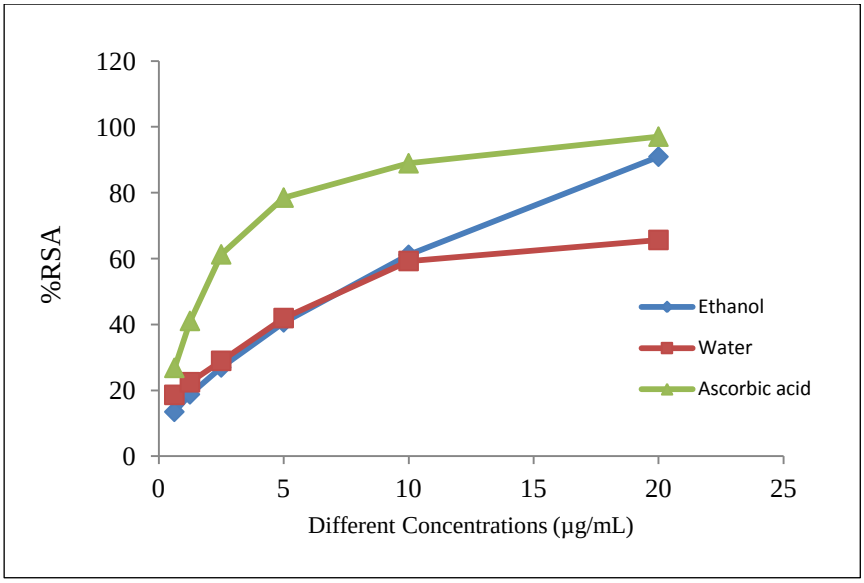


Figure 5 A plot of % RSA vs. concentrations of ethanol and watery extracts of the rhizomes of *D. alata* and standard ascorbic acid

Table 4 IC_{50} Values of Crude Extracts of the rhizomes of *D. alata* and Ascorbic acid

Samples	IC_{50} (µg/mL)
95 % ethanol extract	7.28
Watery extract	7.32
Ascorbic acid	1.73

Anti-proliferative Activity of *D. alata*

The anti-proliferative activity or cytotoxicity of methanol extract of the rhizome of *D. alata* was evaluated by MTT assay using Hep G2 (human liver cancer cell). The anti-proliferative activity or cytotoxicity of the crude extract is measured in terms of cells viability

and IC_{50} value (inhibitory concentration at which 50 % cells are died). Below IC_{50} range, the crude extract is toxic for mammalian cells. DMSO is used as a standard (Moo-Puc *et al*, 2009). The methanol extract of *D. alata* was found to possess the low anti-proliferative activity against Hep G2 (human liver cancer cell) having the IC_{50} value of 195.21 μg/mL, above this concentration the cell mortality rate is increased and below this, the cell survival rate is higher. The results are shown in Table 5.

Table 5 Anti-proliferative Activity of Crude Extract against Human Liver Cancer Cell Lines (Hep G2)

Sample	Cell viability ± SD at different concentrations (μg/mL)			IC_{50}(μg/mL)
	50	100	200	
MeOH - extract	0.8435 ± 0.0479	0.6808 ± 0.0549	0.4908 ± 0.0416	195.21

Conclusion

These observations provide the valuable suggestions for the study of phytochemical constituents concerned with the antimicrobial, antioxidant and anti-proliferative activities of the rhizome of *Dioscorea alata*. Due to the high content of carbohydrate, *D. alata* may be used as a functional food to supplement the fiber and carbohydrate. All extracts except petroleum ether extract, exhibited moderately antimicrobial activity on all tested microorganisms. According to the results, the high total phenol content in *D. alata* contributes to high in free radical scavenging activity. The methanol extract was found to possess low anti-proliferative activity against Hep G2 having the IC_{50} value of 195.21 μg/mL.

According to these observations, the traditional medicinal plant, *D. alata* may, therefore, be useful in the prevention of free radical reactions associated with the degenerative diseases like tumor or cancer and age related problems. In addition, the selected plant may be effectively used in the formulation for treatment of skin disease, wound infections, diarrhea, oxidative stress related diseases, anti-bacterial, anti-inflammatory, diabetic disease and other diseases infected by the microorganisms tested.

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References

Acevedo-Rodriguez, P. and M.T. Strong. (2012). "*Catalogue of the Seed Plants of the West Indies*". Washington DC: Smithsonian Institution. Smithsonian Contributions to Botany, vol 98, pp. 1192

Agrawal, N., D. K. Minj and K. Rani. (2015). "Estimation of Total Carbobydrate Present in Dry Fruits". *J. Enviro. Sci. Toxi. Food. Tech.*, vol 1(6), pp. 24-27

AOAC. (2000). *Official Methods of Analysis*, Association of Official Analytical Chemist International, USA: pp. 7

Bahuguna, A., Khan, I., Bajpai, V. K. and Kang, S. C. (2017). "MTT Assay to Evaluate the Cytotoxic Potential of a Drug". *Bangladesh J. Pharmacol*, vol. 12, pp. 115-118

Conlan, S., Griffiths, L. A., Turner, M., Fido, R., Tatham, A., Ainsworth, C. and Shewry, P. R. (1998). "Characterisation of Yam Tuber Storage Protein Discorin". *Journal of Plant Physiology*, vol. 153, pp. 25-31

Cruickshank, L. (1960). *Handbook of Bacteriology.* London: 10th Ed., E & S Livingstone Ltd., pp. 84-89

Harborne, J. B. (1984). *Phytochemical Methods; A Guide to Modern Techniques of Plant Analysis*. New York: 2nd Ed., Chapman and Hall, pp.154

Marinova, G. and Batchvarov, V. (2011). "Evaluation of the Methods for Determination of the Free Radical Scavenging Activity of DPPH". *Bulgarian Journal of Agricultural Science*, vol. 17**,** pp. 11-24

Moo-Puc, R., Robledo D. and Freile-Pelegrin, Y. (2009). "*In vitro* Cytotoxic and Antiproliferative Activities of Marine Macroalgae from Yucatan, Mexico". *Cienc Mar*, vol. 35, pp. 345-358

M-Tin Wa. (1972). "Phytochemical Screening Methods and Procedures". *Phytochemical Bulletin of Botanical Society of America*, vol. 5(3), pp. 4-10

Saxena, M., Saxena, J., Singh, D. and Gupta, A. (2013). "Phytochemistry of Medicinal Plants". *Journal of Phamacognosy and Phytochemistry*, vol. 1 (6), pp. 168-172

Shewry, P. (2003). "Tuber storage proteins". *Ann. Bot*. (Lond), vol. 91, pp. 755-769