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Study on the Antioxidant Status of Some Myanmar Medicinal Plants

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Abstract

The present study evaluated the antioxidant capacity of fifteen plant samples, which are used as medicinal plants in Myanmar traditional medicine. The crude ethanolic extracts of fifteen plant parts of eleven medicinal plants were screened for their activities. In phytochemical analysis and some mineral examination, the selected plant samples did not contain cyanogenic glycosides and arsenic. The antioxidant activities of the extracts were examined by two different methods, dot-blot DPPH staining assay and in vitro DPPH free radical scavenging assay. The qualitative analysis of antioxidant activity of selected plant extracts using dot-blot DPPH staining was revealed the presence of significant antioxidant activity in the extracts of Cledronum siphonanthus (Leaves and Stalk), Curcuma longa, Garcinia mangostana, Heliotropium indicum (Stalk) indicated by the presence of yellow or white spot on TLC plate. The scavenging activity of ascorbic acid, positive control, was found about 94.25%. In in vitro DPPH free radical scavenging assay, Heliotropium indicum (Leaves) and Calotropis procera (Leaves) showed 84.54% and 82.96% in free radical scavenging activity, other selected plant extracts exhibited moderate antioxidant activities. In two methods, the plant extract of Heliotropium indicum (Leaves) showed the best activity for antioxidant activity in this study. Therefore, the selected plant extracts can lead to discovery of natural antioxidant agent.

Keywords: phytochemical; antioxidant; dot-blot; in vitro DPPH; Heliotropium indicum (Leaves)

1. Introduction

According to World Health Organization, more than 80% of the world's populations rely on traditional medicine for their primary health care needs [1]. Plants contain a wide variety of free radical scavenging molecules, such as flavonoids, anthocyanins, cartenoids, dietary glutathionine, vitamins and endogenous metabolites and such natural products are rich in antioxidant activities [2- 5].

The ability of the plant extracts as antimicrobial agent is due to their antioxidant properties which are correlated with their phenolic contents. The phenolic compounds are common in many plants and exhibited antioxidant properties due to their high redox potential. They also showed a wide range of biological activity as antimicrobial activity, anticarcinogenicity and antiproliferation and many biological activities of these compounds can be attributed to their antioxidant properties [6].

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In an aerobic environment, all animals and plants require oxygen and hence reactive oxygen species (ROS) are omnipotence. It is well known that excess generation of ROS is involved in structural alternations of cellular molecules leading to cytotoxicity and cell death. The antioxidants have been reported to prevent oxidative damage caused by free radical; it can interfere with the oxidation process by reacting with free radicals, chelating, catalytic metals and also by acting as oxygen scavengers [7].

The potentially reactive derivatives of oxygen, attributed as reactive oxygen species (ROS), are continuously generated inside the human body. The generated ROS are detoxified by the antioxidants present in the body. However, overproduction of ROS and/or inadequate antioxidant defense can easily affect and persuade oxidative damage to various biomolecules including proteins, lipids, lipoproteins and DNA. This oxidative damage is a critical etiological factor implicated in several chronic human diseases such as diabetes mellitus, cancer, atherosclerosis, arthritis and neurodegenerative diseases and also in the ageing process. The enhanced generation of ROS in vivo could be quite deleterious, since they are in-volved in mutagenesis, apoptosis, ageing, and carcino-genesis [8].

Free radicals also cause DNA strand breaks and chromosome deletions and rearrangements. Further, activated oxygen species most likely play an important role in tumor promotion and progression [9]. As crude extracts of herbs and spices and other plant materials, rich in phenolics are of increasing interest in the food industry. While, flavonoids are a group of polyphenolic compounds' with known properties, which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action [10].

The use of traditional medicine is wide spread and plants are still a large source of natural antioxidants that might serve as leads for the development of novel drugs.

Medicinal plants constitute one of the main sources of new pharmaceuticals and healthcare products. A whole range of plant-derived dietary supplements, phytochemicals and pro-vitamins that assist in maintaining good health and combating diseases are now being described as functional ingredients and nutraceutical. In this study, the antioxidant activity of eleven Myanmar medicinal plants was evaluated.

2. Materials and Method

2.1. Sample Collection

Eleven traditional Myanmar medicinal plant samples were collected in Shan State and Kyaukse District, Mandalay Region. Reconfirmation of the plant samples were done by authorized botanist from Pharmaceutical Research Department (PRD), Department of Research and Innovation (DRI), Ministry of Education, Yangon. The botanical name, the family name, Myanmar name, English name and the parts of plant used were shown in Table 1.

Table 1. Selected Myanmar Medicinal Plants and Their Parts

No.	Scientific Name	Common name	Family name	Myanmar name	Parts used
1	Allium cepa Linn.	Onion	Lilraceae	Kyet-thun-u-gyi	Bulbs
2	Allium sativum Linn.	Garlic	Lilraceae	Kyet-thun-phyu	Rhizomes
3	Annona reticulata Linn.	Bullock's heart	Annonaceae	Thin-baw-awza	Leaves
4	Capparis horrida Linn.	-	Capparidaceae	Namani-than-lyet	Leaves
5	Clerodendrum siphonanthus R.Br.	-	Verbenaceae	Nga-yant-padu	Leaves
6	Curcuma longa Linn.	Turmeric	Zingiberaceae	Sa-nwin	Rhizomes
7	Calotropis procera R.Br.	Swallow-wort, Madar	Asclepiadaceae	Mayo	Leaves, Stalk
8	Garcinia mangostana Linn.	Mangosteen	Guttiferae	Min-gut	Fruit-rind
9	Heliotropium indicum Linn.	Heliotrope	Boraginaceae	Sin-hna-maung-gyi	Leaves, Stalk
10	Morinda citrifolia Roxb.	Noni	Rubiaceae	Ye-yo	Leaves, Fruit
11	Vitis repens Wight & Arn.	-	Vitaceae	Dabindaing-myanan	Rhizomes

2.2. Mineral Analysis of Plant Samples

Mineral contents of the plant samples were analysed by Atomic Absorption Spectrophotometer (AAS), at Department of Analysis, Department of Research and Innovation (DRI), Yangon.

2.3. Preliminary Phytochemical Examination of Plant Samples

Preliminary phytochemical examinations for the selected medicinal plants were carried out. Presence of some classes of compounds were determined namely, glycosides, alkaloids, reducing sugar, carbohydrates, tannins, steroid, flavonoids, cyanogenic glycosides, saponin glycosides, α-amino acids and acid, base or neutral.

2.4. Determination for Antioxidant Activity

Antioxidant activity of ethanolic extracts of samples was determined qualitatively and quantitatively on the basis of their scavenging potential of the stable DPPH free radical.

2.4.1. Qualitative determination

The antioxidant activity of plant extracts were qualitatively determined by using dot-blot DPPH staining procedure [11]. According to Soler Rivas et al. (2000), the dot- blot test is to compare radical scavenging capacity (RSC) of various products. This assay was used to establish whether different extracts of plant samples had radical scavenging activity. All the extracts were dried and redissolved in ethanol. Aliquots of 5 µL (of a 10 mg/mL final concentration) of each extract was applied on TLC (Merck Silica gel F254 plate) and allowed to dry for a few minutes. 0.4 mM DPPH solution in methanol was sprayed on the plates until they were evenly covered. L-ascorbic acid (Vitamin C) was used as positive control for comparative study of plant extracts and only DPPH solution was used as control. The results of dot- blot assay showed coloured spots where the purple area on the plate indicates no free radical scavenging (antioxidant) activity and the yellow or white area indicates free radical scavenger or antioxidant activity. The more intense the yellow colour, the greater the antioxidant activity.

2.4.2. Quantitative determination

The antioxidant activity of plant extracts were quantitatively determined by using in vitro DPPH free radical scavenging assay [12]. For antioxidant (AOX) activity, the samples were determined spectrophotometrically using DPPH free radical scavenging assay. In this study, 1 mL of five different concentrations (200,400,600,800 and 1000 ppm) of ethanol extracts of plant samples was mixed with 2 mL of 0.006 mM DPPH solution in methanol. The mixture was allowed to react at room temperature in the dark for 30 min. Blank solutions were prepared with each test sample solution only when negative control was only DPPH solution. L-ascorbic acid (Vitamin C) was used as reference antioxidant and/or as positive control. The decrease in absorbance was measured at 518 nm using UV-Vis spectrophotometer.

Inhibition of free radical DPPH in percent (I %) or the DPPH free radical scavenging activity (%) was calculated from the absorption according to the following equation:

% DPPH radical Scavenging = [(Absorbance of control – Absorbance of test sample)/ Absorbance of control] X 100

3. Results and Discussion

For evaluation of antioxidant activity, eleven Myanmar medicinal plants have been selected and studied in this research work.

3.1. Yield Percentage of Selected Crude Plant Extracts

In this study, the yield percentage of crude plant extracts of the selected plant sample was calculated on the basis of the dried materials. The results are shown in Table 2.

Table 2. Yield (%) of Ethanolic Crude Plant Extracts

No.	Selected Medicinal Plants	Dry Sample Weight (g)	Extracted Amount (g)	Yield (%)
1	Allium cepa Linn.	100	4.93	4.93

2	Allium sativum Linn.	100	7.92	7.92
3	Annona reticulata Linn.	100	4.68	4.68
4	Capparis horrida Linn.	100	8.16	8.16
5	Clerodendrum siphonanthus R.Br. (Leaves)	100	3.42	3.42
6	Clerodendrum siphonanthus R.Br. (Stalk)	100	3.26	3.26
7	Curcuma longa Linn.	100	3.39	3.39
8	Calotropis procera R.Br. (Leaves)	100	2.44	2.44
9	Calotropis procera R.Br. (Stalk)	100	1.22	1.22
10	Garcinia mangostana Linn.	100	3.37	3.37
11	Heliotropium indicum Linn. (Leaves)	100	4.61	4.61
12	Heliotropium indicum Linn. (Stalk)	100	5.34	5.34
13	Morinda citrifolia Roxb.(Leaves)	100	1.70	1.70
14	Morinda citrifolia Roxb.(Fruit)	100	2.33	2.33
15	Vitis repens Wight & Arn.	100	4.52	4.52

3.2. Preliminary Phytochemical Examination of Selected Plant Samples

Before preparing the crude plant extracts, the identification for types of compound containing in the selected plant samples was made by employing phytochemical tests and the results are shown in Table 3. All the selected plant samples did not contain cyanogenic glycosides. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins, saponin glycosides and phenolic compounds. And the presence of flavonoids, phenolic compounds and tannins have antioxidant activity. Due to the results of preliminary phytochemical examination, the presence of these compounds indicated that all plant samples could have antioxidant activity.

Table 3. Preliminary Phytochemical Examination of Selected Medicinal Plants

Constituents	**Selected Medicinal Plants**														
	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o
Alkaloidos	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Reducing sugars	-	+	+	-	+	+	+	-	-	+	-	+	+	+	+
Carbohydrates	+	-	-	+	+	+	+	-	-	-	+	+	+	+	+
α-amino acids	-	+	-	+	+	+	+	+	-	+	+	+	+	+	+
Flavonoids	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Saponin glycosides	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acid/Base/ Neutral	A	B	B	B	B	B	N	N	N	A	B	B	A	B	B
Cyanogenic glycosides	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phenolic compounds	+	-	+	-	+	+	-	+	+	+	+	+	+	+	+

+ = Present - = Absent A = Acidic B = BasicN = Neutral

a = Allium cepa Linn. (Bulbs)

b = Allium sativum Linn. (Rhizomes)

c = Annona reticulata Linn. (Leaves)

d = Capparis horrida Linn. (Leaves)

e = Clerodendrum siphonanthus R.Br. (Leaves)

f = Clerodendrum siphonanthus R.Br. (Stalk)

g = Curcuma longa Linn. (Rhizomes)

h = Calotropis procera R.Br. (Leaves)

i = Calotropis procera R.Br. (Stalk)

j = Garcinia mangostana Linn.(Fruit-rind)

k = Heliotropium indicum Linn.(Leaves)

l = Heliotropium indicum Linn.(Stalk)

m = Morinda citrifolia Roxb.(Leaves)

n = Morinda citrifolia Roxb. (Fruit)

o = Vitis repens Wight & Arn.(Rhizomes)

3.3. Mineral Contents of Selected Plant Samples

The mineral contents of the selected Myanmar medicinal plants samples are shown in Table 4. According to the resulting data, the selected plant samples were found to lack of arsenic. Therefore, it could assume that these samples are potentially safe. Iron (Fe) content was observed in Allium cepa Linn., Allium sativum Linn. And Heliotropium indicum Linn.(Stalk). It was assumed that these samples were potentially used to support for iron deficiency. Macronutrients were potassium (K), magnesium (Mg), calcium (Ca) and iron (Fe). Zinc (Zn) was micronutrient.

Table 4. Mineral Contents of Selected Plant Samples

No.	Plant Samples	Ash (%)	Fe (%)	Zn (%)	Ca (%)	Mg (%)	As (%)	K (%)
1	Allium cepa Linn.	NT	0.01	ND	0.18	NT	ND	3.00
2	Allium sativum Linn.	3.03	0.01	ND	0.14	0.27	ND	1.10
3	Annona reticulata Linn.	6.35	ND	ND	2.52	0.23	ND	1.10
4	Capparis horrida Linn.	2.78	ND	ND	0.08	0.10	ND	1.40
5	Clerodendrum siphonanthus R.Br. (L)	8.58	ND	ND	2.10	0.47	ND	1.94
6	Clerodendrum siphonanthus R.Br. (S)	2.83	ND	ND	0.92	0.08	ND	0.80
7	Curcuma longa Linn.	5.92	ND	ND	0.19	0.17	ND	1.84
8	Calotropis procera R.Br. (Leaves)	10.83	ND	ND	1.82	0.95	ND	2.20
9	Calotropis procera R.Br. (Stalk)	8.31	ND	ND	0.14	NT	ND	NT
10	Garcinia mangostana Linn.	2.92	ND	ND	0.14	0.07	ND	1.00
11	Heliotropium indicum Linn. (Leaves)	17.01	ND	ND	0.08	0.10	ND	1.40
12	Heliotropium indicum Linn. (Stalk)	11.92	0.004	ND	0.92	0.31	ND	3.90
13	Morinda citrifolia Roxb. (Leaves)	11.82	ND	ND	1.18	0.16	ND	1.95
14	Morinda citrifolia Roxb. (Fruit)	44.70	ND	ND	0.74	0.25	ND	1.11
15	Vitis repens Wight & Arn.	9.54	ND	ND	0.08	0.10	ND	1.40

ND = Not Detected NT = Not Tested

3.4. Determination of Antioxidant Activity of Selected Myanmar Medicinal Plants

The antioxidant activity of selected medicinal plants was determined by using dot-blot DPPH staining and in vitro DPPH free radical scavenging assay.

3.4.1. Dot- Blot DPPH Staining

The antioxidant capacities of the crude plant extracts were eye-detected semi-quantitatively by a rapid DPPH staining TLC method. Each diluted crude extracts were applied as a dot on a TLC layer that was stained with DPPH solution and shown in Plate 4. L-ascorbic acid (Vitamin C) was used as positive control for comparative study of plant extracts and only DPPH solution was used as negative control. The results of dot- blot assay showed coloured spots where the purple area on the plate indicates no free radical scavenging (antioxidant) activity and the yellow or white area indicates free radical scavenger or antioxidant activity. The more intense the yellow or white colour, the greater the antioxidant activity. The

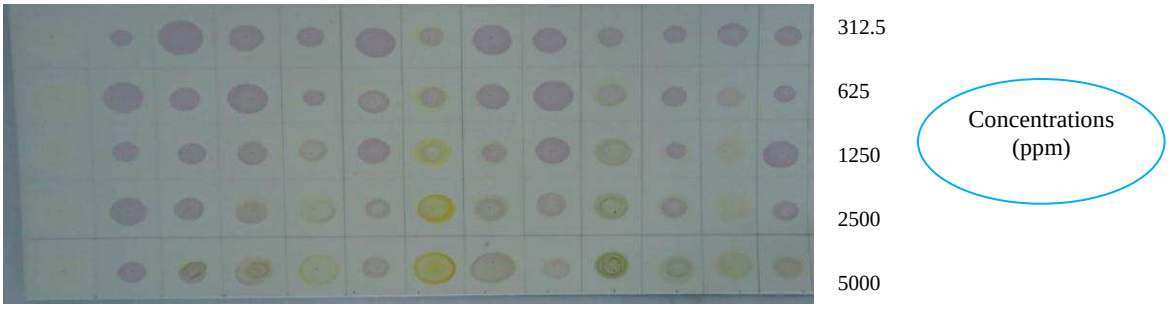
results of Dot-blot DPPH staining on a TLC plate are shown in Fig 1. These white or yellow spots with strong intensity appeared at the concentration of 1000ppm of each extract. In this study, The TLC based qualitative dot-blot DPPH spray revealed that the selected plant extracts showed antioxidant activity.

3.4.2. In Vitro DPPH Free Radical Scavenging Assay

The DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolorize in the present of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 518 nm and also for a visible deep purple colour. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolourized which can be quantitatively measured from the changes in absorbance. The DPPH (1,1 diphenyl 2-picrylhydrazyl) radical scavenging activity of crude plant extracts at different concentrations (200,400,600,800,1000 ppm) were determined by UV-Vis spectrophotometer at 518 nm. This activity was found to increase by increasing concentrations of the extracts. Table 3.4 shows free radical scavenging activity of selected plant extracts at 1000 ppm.

The extracts of all the selected medicinal plant materials possessed free radical scavenging properties, but to varying degree, ranging from 22% to 84% DPPH scavenging at concentrations of 200 to 1000 ppm of plant extracts. A maximum scavenging activity was offered by Heliotropium indicum (Leaves) (84.54%), followed by Calotropis procera (Leaves) (82.96%) at 1000 ppm. As expected, a higher percent of DPPH scavenging is correlated to a higher antioxidant activity while the other plant extracts showed moderated antioxidant properties. The scavenging activity of ascorbic acid, positive control, was found about 94.25% because it acts as a chain breaking antioxidant impairs with the formation of free radicals in the process of formation of intracellular substances throughout the body including collagen, bone matrix and tooth dentine[13]. The therapeutic potential of natural medicinal plants as an antioxidant in reducing such free radical induced tissue injury, suggests that many plants have antioxidant activity that can be therapeutically useful.

Fig 1. Dot-Blot DPPH Staining Assay of Selected Myanmar Medicinal Plant Extracts on a TLC Plate



S1 = Allium sativum Linn.
S2 = Vitis repens Wight & Arn.
S3 = Garcinia mangostana Linn.
S4 = Morinda citrofolia Roxb.(Fruit)
S5 = Curcuma longa Linn.
S6 = Annona reticulata Linn.(Leaves)
S7 = Capparis horrida Linn.
S8 = Clerodendrum siphonanthus R.Br. (Leaves)
S9 = Heliotropium indicum Linn.(Leaves)
S10 = Clerodendrum siphonanthus R.Br. (Stalk)
S11 = Allium cepa Linn.

Table 4. Free Radical Scavenging Activity of Selected Plant Extracts at 1000 ppm

Plant Samples	DPPH Radical Scavenging (%)
Allium cepa Linn.	72.51
Allium sativum Linn.	56.88
Annona reticulata Linn.(Leaves)	74.10
Capparis horrida Linn.	79.77
Clerodendrum siphonanthus R.Br.(Leaves)	73.50

Clerodendrum siphonanthus R.Br.(Stalk)	61.68
Curcuma longa Linn.	62.73
Calotropis procera R.Br.(Leaves)	82.96
Calotropis procera R.Br. (Stalk)	58.67
Garcinia mangostana Linn.	61.69
Heliotropium indicum Linn.(Leaves)	84.54
Heliotropium indicum Linn.(Stalk)	70.19
Morinda citrifolia Roxb.(Leaves)	75.24
Morinda citrifolia Roxb.(Fruit)	51.95
Vitis repens Wight & Arn.	74.81
Ascorbic Acid (Positive Control)	94.25

4. Conclusion

The present research work has studied that eleven selected Myanmar medicinal plants that possess antioxidant potential were the best supplements for the diseases associated with oxidative stress. In this study, the selected ethanol extracts did not show cyanogenic glycosides and arsenic. The major groups of the phytochemicals obtained from plants showed antioxidant activities and was known to prevent several degenerative diseases. The antioxidant activities of the extracts were screened by two different methods, dot-blot DPPH staining assay and in vitro DPPH free radical scavenging assay. In these two methods, the highest antioxidant activity was demonstrated by the leaves extract of Heliotropium indicum (84.54 %) and followed by the leaves extract of Calotropis procera (82.96 %) at 1000 ppm. Therefore, further studies should be needed for in vivo studies for better understanding of their mechanism of action as antioxidant.

Statistical analysis

Presented data are mean ± standard deviation of three independent replicates.

Conflict of Interest

None declared.

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