The Pharma Innovation

ISSN: 2277- 7695
CODEN Code: PIHNBQ
ZDB-Number: 2663038-2
IC Journal No: 7725

ISSN 2277-7695

Received: 26-01-2013
Accepted: 23-03-2013

Vol. 2 No. 2 2013
Online Available at www.thepharmajournal.com

THE PHARMA INNOVATION - JOURNAL

Antioxidant (In-Vitro) Effect of Methanol& Petroleum Ether Extracts of the Aerva lanata

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The present study was designed to investigate antioxidant activity of methanolic and petroleum ether extracts of the Aerva lanata. In vitro antioxidant activities of the extracts were studied using DPPH radical scavenging, reducing power, total phenol and total flavonoid content determination assays. The antioxidant activities of the extracts were found promising. The results of these studies suggest significant antioxidant activities of different extracts of Aerva lanata. The result that obtained from the Crude extract of the of Aerva lanatahave moderate antioxidant activity.

Keyword: *Aerva lanata* and Antioxidant

1. Introduction

Medicinal plants are various plants used in herbalism and thought by some to have medicinal properties**.**The definition of Medicinal Plant has been formulated by WHO (World Health Organization) as follows-"A medicinal plant is any plant which, in one or more of its organ, contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs." The plants that possess therapeutic properties or exert beneficial pharmacological effects on the animal body are generally designated as "Medicinal Plants". Although there are no apparent morphological characteristics in the medicinal plants growing with them, yet they possess some special qualities or virtues that make them medicinally important. It has now been established that the plants which naturally synthesis and accumulate some secondary metabolites, like alkaloids, glycosides, tannins, volatiles oils and contain minerals and vitamins, possess medicinal properties.

Amaranthaceae family of the genus Aerva that sometimes flowers in the first year. *Aerva lanata* belonging to Amaranthaceae family is a common weed which grows wild everywhere in plains of India and Bangladesh. The root has camphor like aroma. The dried flowers which look like soft spikes are sold under the commercial name as Buikallan or Boor. Decoction of the flowers is said to cure stones in any part of the stomach and that of the root is diuretic and cure for kidney stones.

2. Methods and Materials

a) Collection and Proper Identification:

The parts of the plants must be collected from an authentic source and identified by a taxonomist. A voucher specimen ought to be submitted to the herbarium for further reference.

b) Preparation of Plant Material:

Normally the plant materials are collected in fresh condition. Then these are cut into small pieces if necessary to make it suitable for grinding purpose and sun dried and finally dried in an oven at 40-45^{o}C for 36 hrs. The materials are grinded into coarse powder with the help of a grinder and stored in an air tight container for further use.

A) DPPH Radical Scavenging Assay

Free radical scavenging abilities of the test samples can be determined by measuring the change in absorbance of DPPH (1, 1-Diphenyl-2-picrylhydrazyl radical) at 517 nm by the spectrophotometric method described by Braca *et al*(2001).

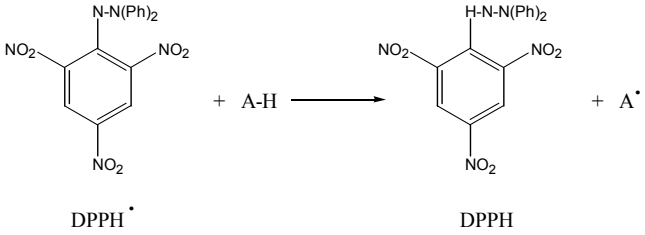

Fig 1: Mechanism of DPPH· with an Antioxidant having Transferable Hydrogen Radical

Principle:

DPPH(1, 1-Diphenyl-2--picrylhydrazyl radical) is a stable free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. The radical scavenging potential of the sample was determined by measuring the decrease in absorbance due to DPPH at 517 nm, representing the formation of its reduced form, 1, 1-Diphenyl-1-2- picrylhydrazine (DPPH), which was yellow in color. Because of the odd electron, the purple colored methanolic solution shows a strong absorption band at 517 nm (Blois, 1958). The mechanism of reaction was presented in Fig.-1.

Reagents:

DPPH, Methanol, Ascorbic acid as standard.

Procedure: 0.1ml of extract at various concentrations (10, 50, 100 and 500 µg/ml) was added to 3ml of a 0.004% methanol solution of DPPH After30min, absorbance of the resulting solution was measured against a blank at 517nm.The percentage DPPH radical scavenging activities (%SCV) were calculated by comparing the results of the test with the control (not treated with extract) using following formula:

$$\% \text{ SCV} = \frac{A_0 - A_1}{A_0} \times 100$$

Where,

SCV = Radical scavenging activity, A_0 = Absorbance of the control and A_1 = Absorbance of the test (extracts / standard).

Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted % SCV versus concentration curve. Test carried out in duplicate and ascorbic acid was used as standard.

B) Determination of total Phenolic Content

Total phenolic content of different extractives of Aerva lanata were determined employing the method as described by Singleton *et a*l. (1965) involving Folin-Ciocalteu reagent as oxidizing agent and Asocorbicacid as standard.

Principle:

The content of total phenolic compounds of different fractions in the plant was determined by Folin–Ciocalteu Reagent (FCR). The FCR actually measures a sample's reducing capacity. The exact chemical nature of the FC reagent is not known, but it is believed to contain heteropoly phosphorus states - molybdates. Sequences of reversible one-or two-electron reduction reactions lead to blue species, possibly $(PMoW_{11}O_{40})_4$. In essence, it is believed that the molybdenum is easier to be reduced in the complex and electron-transfer reaction occurs between reductants and Mo.

Materials:

Folin – ciocalteu reagent, Sodium carbonate (Na_2CO_3), Methanol, Ascorbic acid Micropipette (10-100 µl), Pipette (1-10 ml) , UV-spectrophotometer.

Procedure:

0.5 ml of plant extract or standard of different concentration solution was taken in a test tube. 2.5 ml of Folin – Ciocalteu (Diluted 10 times with water) reagent solution was added into the test tube.2.5 ml of Sodium carbonate (7.5%) solution was added into the test tube. The test tube was incubated for 20 minutes at 25^0C to complete the reaction. Then the absorbance of the solution was measured at 760 nm using a spectrophotometer against blank. A typical blank solution contained all reagents except plant extract or standard solution. The total content of phenolic compounds in plant methanol & petroleum ether extracts and in different fractionates in Gallic acid equivalents (GAE) was calculated by the following formula:

$$C = (c \times V)/m$$

Where,

C = total content of phenolic compounds, mg/g plant extract in GAE,

c = the concentration of asocrbic acid established from the calibration curve mg/ml,

V = the volume of extractml,

m = the weight of different pure plant extracts, gm;

C) Determination of total Flavonoids Content:

Total flavonoid content of different extractives ofAerva lanatawas determined by aluminum chloride colorimetric method. Quercetin was used as standard and the flavonoid content of the extractives was expressed as mg of quercetin equivalent/gm of dried extract.

Principle: The content of total flavonoids in different fractionates of plant extract was determined by the well-known aluminum chloride colorimetric method. In this method aluminum chloride forms complex with hydroxyl groups of flavonoids present in the samples. This complex has the maximum absorbance at 420 nm.

Materials:

Aluminum Chloride ($AlCl_3$), Potassium Acetate, Methanol, Quercetin, Micropipette (10-100 µl),Pipette (1-10 ml),UV-spectrophotometer

Procedure:

1 ml of plant extract or standard of different concentration solution was taken in a test tube.3 ml of methanol was added into the test tube.200µl of 10% aluminum chloride solution was added into the test tube. 200µl of 1M potassium acetate solution was added into the test tube. 5.6 ml of distilled water was added into the test tube. The test tube was then incubated at room temperature for 30 minutes to complete the reaction. Then the absorbance of the solution was measured at 420 nm using a spectrophotometer against blank.A typical blank solution contained all reagents except plant extract or standard solution. The total content of flavonoid compounds in plant extracts in Quercetin equivalents was calculated by the following

Formula equation:

$$C = (c \times V)/m$$

Where,

C = total content of flavonoid compounds, mg/g plant extract in Quercetin equivalent (GAE).

c = the concentration of Quercetin established from the calibration curve, mg/ml; V = the volume of extract, ml;

m = the weight of pure plant extracts, gm**;**

D) Reducing Power Capacity Assessment:

The reducing power of different extractives of Aerva lanata was evaluated by the method of Oyaizu (1986).

Principle:

In this assay, the yellow color of the test solution changes to various shades of green and blue depending on the reducing power of antioxidant samples. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The presence of reductants such as antioxidant substances in the samples causes the reduction of the Fe^{3+}-ferricyanide complex to the ferrous form by donating an electron. The amount of Fe^{2+}complex can then be monitored by measuring the formation of Perl's Prussian blue at 700 nm.

$$Fe^{3+}\text{-ferricyanide} + e^- \longrightarrow Fe^{2+}\text{ferricyanide}$$

Materials:

Potassium ferricyanide [$K_3Fe(CN)_6$] ,Trichloro Acetic acid ,Ferric Chloride ($FeCl_3$)Phosphate buffer [K_2HPO_4 + KH_2PO_4],Ascorbic acid (Analytical or Reagent grade) ,Water bath, Centrifuge machine, Pipette (1-10 ml), UV spectrophotometer

Procedure:

1.0 ml of plant extract or standard of different concentration solution was taken in a test tube.2.5 ml of potassium buffer (0.2 M) and 2.5 ml of Potassium ferricyanide [K_3Fe (CN) $_6$], (1%) solution were added into the test tube. The reaction mixture was incubated for 20 minutes at 50^0C to complete the reaction.2.5 ml of trichloro acetic acid, (10%) solution was added into the test tube. The total mixture was centrifuged at 3000 rpm for 10 min. 2.5 ml supernatant solution was withdrawn from the mixture and mix with 2.5 ml of distilled water. 0.5 ml of ferric chloride ($FeCl_3$), (0.1%) solution was added to the diluted reaction mixture. Then the absorbance of the solution was measured at 700 nm using a spectrophotometer against blank. A typical blank solution contained the same solution mixture without plant extract or standard and it was incubated under the same conditions as the rest of the samples solution. Also the absorbance of the blank solution was measured at 700 nm against the solvent used in solution preparation. Increased absorbance of the reaction mixture indicated increase reducing power.

3. Result & Discussion

A) DPPH Radical Scavenging:

The results of DPPH radical scavenging assays on plant extracts and ascorbic acid were given in Table**s** and IC_{50} value of the samples were presented in Figure-2. IC_{50} of the standard and methanol extract & Petroliem ether extract of Aerva lanata are 10µg/ml, 360µg/ml and 344µg/ml respectively. Among the samples, Petroliem ether extractshowed highest radical scavenging activity with IC_{50} value 344µg/ml.

Table 1: Percentage of DPPH Radical Scavenging Activity of Ascorbic Acid at Different Concentration

Concentration (µg/ ml)	Absorbance	% SCV	IC_{50} (µg/ml)
10	0.529	51.90909	
50	0.429	61	
100	0.399	63.72727	10
300	0.280	74.54545	
500	0.268	75.63636	

Table 2: Percentage of DPPH radical scavenging activity **o**f methanol extract of Aerva lanata at different concentration

Concentration (µg/ ml)	Absorbance	% SCV	IC_{50} (µg/ml)
10	0.995	9.545455	
50	0.808	26.54545	
100	0.808	32.36364	360
300	0.589	46.45455	
500	0.487	55.72727	

Table 3: Percentage of DPPH Radical Scavenging Activity of Petroleum Ether Extract of Aerva lanata at Different Concentration

Concentration (µg/ ml)	Absorbance	% SCV	IC_{50} (µg/ml)
10	1.01	8.181818	
50	0.922	16.18182	
100	0.721	34.45455	344
300	0.629	42.81818	
500	0.322	70.72727	

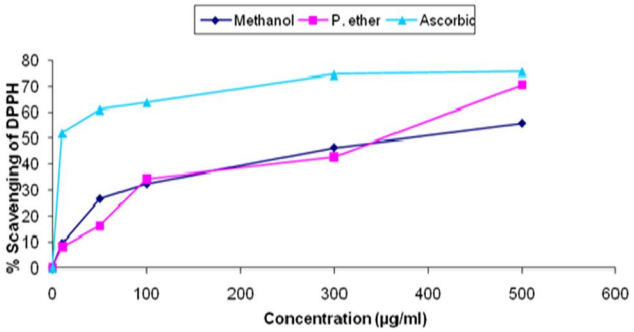

Fig 2: %-Scavenging Activity of Ascorbic Acid and Methanol & Petroleum Ether Extract of Aerva lanata at Different Concentration

B) Total Phenol Content

The total phenol contents of methanol and petroleum ether extract of Aerva lanata were 108.9125mg/ml and 147.5025 mg/ml respectively. Petroleum ether of Aerva lanata with total phenol contents of 147.5125 mg/ml had the highest amount among the samples in this study.

Table 04: Absorbance of Ascorbic Acid at Different Concentration

Conc. (µg/ml)	Absorbance
0	**0**
10	**0.334**
50	**0.890**
100	**1.464**
200	2.328

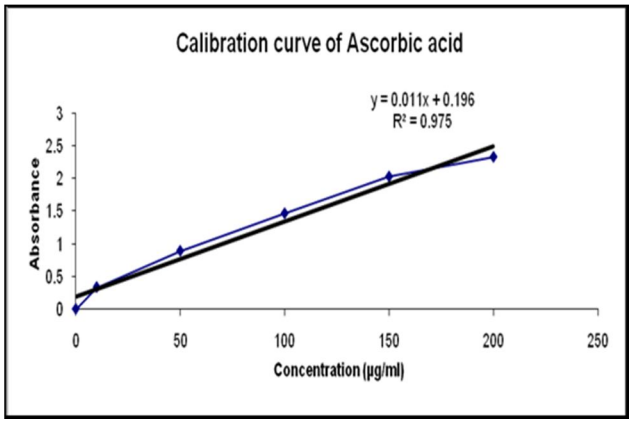

Fig 3: Calibration Curve of Ascorbic Acid for Total Phenol Content Determination

The concentration of ascorbic acid in samples was determined by using an equation that was obtained from standard ascorbic acid graph. The equation is given below:

$$y = 0.011x + 0.196$$

Where,

y = absorbance, x = ascorbic acid concentration µg/ml, m = slope = 0.011 c = Intersection = 0.196

∴ x =0.11/0.196-Y

Now,

The concentration of total phenolic compounds in sample was determined as milligram ofascorbicacid equivalent by using the following equation:

A = (c×v)/m

Where,

A = Total phenol content (mg/ml ascorbic acid equivalent), c = X/1000= Concentration of ascorbic acid in mg/ml, v = Volume of extract, m = Mass of the extract (gm).

So, the total phenol content of the samples with necessary data is shown in the following table.

Table 5: Total Phenol Content of all Four Samples with Necessary Data

Sample	Conc. (µg/ ml)	Absorbance	m (gm)	c (mg/ml)	c(mg/ml)	A= (cxv)/m	A mean
Methanol-1	200	0.454	0.0002	22.43478	0.022435	112.175	108.9125
2-Methanol	200	0.439	0.0002	21.13043	0.02113	105.650	
1-P.ether	200	0.543	0.0002	30.17391	0.030174	153.70	147.5025
2-P.ether	200	0.521	0.0002	28.26087	0.028261	141.305	

C) Total flavonoid contents:

The Total flavonoid contents of the methanol and petroleum ether extracts of Aerva lanata, in terms of quercetin equivalent were 140.357 mg/ml and 287.807 mg/ml respectively From Fig.-4, it is found that petroleum ether extract with total flavonoid content of 287.8075 mg/ml had the highest amount among the samples in this study.

Table 6: Absorbance of Quercetin (Standard) at Different Concentration

Conc. (µg/ml)	Abs	Abs	Abs	AVG
100	1.060	0.939	0.985	0.995
50	0.342	0.352	0.339	0.344
25	0.210	0.183	0.195	0.196
12.5	0.117	0.120	0.112	0.116

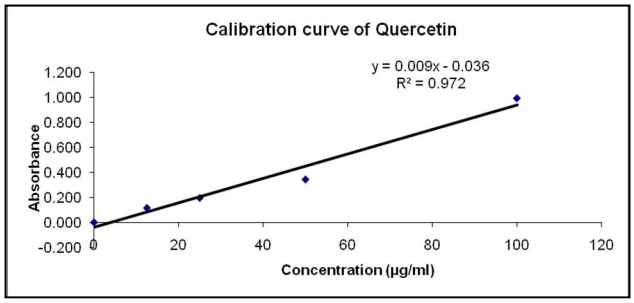

Fig 4: Calibration Curve of Quercetin for total Flavonoid Content Determination

The concentration of quercetin in samples was determined by using an equation that was obtained from standard quercetin graph. The equation is given below:

y = 0.0098x – 0.0366

Where,

y = absorbance, x =*quercetin*concentration µg/ml

m = slope = 0.0098, c = Intersection = 0.0366

∴ x =Y+0.366/0.0098

Now, the total flavonoid content in sample was determined as milligram of quercetin equivalent by using the following equation:

A = (c×v)/m

Where,

A = Total flavonoid content (mg/ml quercetin equivalent)

c = X/1000= Concentration of quercetin in mg/ml, v = Volume of extract = Mass of the extract (gm).

So, the total flavonoid content of the samples with necessary data is shown in the following table.

Table 7: Total Flavonoid Content of all Four Samples with Necessary Data

Sample	Conc. (µg/ml)	Absorbance	m (gm)	c (mg/ml)	c(mg/ml)	A=(cxv)/m	A mean
Methanol-1	200	0.233	0.0002	27.5102	0.02751	137.55	140.3575
Methanol-2	200	0.244	0.0002	28.63265	0.028633	143.165	
P.ether-1	200	0.544	0.0002	59.2449	0.059245	296.225	287.8075
P.ether-2	200	0.511	0.0002	55.87755	0.055878	279.39	

D) Reducing Power Capacity:

The reducing power of extract of these plants was found remarkable and the reducing power of the extract was observed to raise as the concentration of the extract gradually increases.

Table 8: Absorbance of Ascorbic Acid (Standard) at Four Concentrations

Concentration(µg/ml)	Absorbance
0	0
125	1
250	1.504
500	1.598
1000	1.878

Table 9: Absorbance of Methanol Extract of Aerva lanata at Four Concentrations

Concentration(µg/ml)	Absorbance
0	0
125	0.716
250	0.902
500	1.429
1000	1.562

Table 10: Absorbance of Petroleum Ether Extract of Aerva lanata at Four Concentrations

Concentration(µg/ml)	Absorbance
0	0
125	0.842
250	1.012
500	1.146
1000	1.321

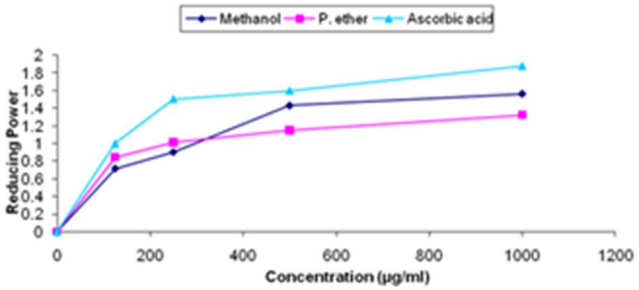

Fig. 5: Reducing Power of Methanol, Petroleum ether Extracts of Aerva lanata and Ascorbic Acid

4. Conclusion:

In conclusion, the present study, using in vitro experiments established that petroleum ether and methanol extract of Aerva lanata has moderate antioxidant effect.The both extracts of Aerva lanata showed moderate 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, total phenol content, total flavonoid content and reducing power. The antioxidant property depends upon concentration and increased with increasing amount of the extract in all the models. IC_{50} value of the standard and methanol extract &Petroleum ether extract of Aerva lanata, are 10 µg/ml, 360 µg/ml, 344 µg/ml respectively. The total phenol contents of methanol and petroleum ether extract of Aerva lanata were 108.9125 mg/ml and 147.5025 mg/ml respectively. The flavonoid contents of methanol & petroleum ether extracts Aerva lanata in terms of quercetin equivalent were 140.3575 mg/ml and 287.8075 mg/ml respectively. The reducing power of the extract was linearly proportional to the concentration of the sample. Ascorbic acid was taken as reference standard.This is only a preliminary study but the plant can be further screened against various diseases in order to find out its unexplored efficacy and can be a potential source of biologically important drug candidates.

5. Acknowledgement:

I want to express my heartfelt gratitude to my beloved supervisor Mr.Obayed Raihan, department of pharmacy,Jessore Science &

Technology University, who's sincere, active, passionate outstanding guidance and competent supervision during the occupancy of research has enabled me to reach the end. I express my thanks to him.

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