WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 6, 1242-1257. Research Article ISSN 2277–7105

PHYTOCHEMICAL SCREENING, ANTIGLYCATION AND ANTIOXIDANT ACTIVITIES OF ETHANOLIC LEAF EXTRACT OF *AZIMA TETRACANTHA* LAM.

S. Josephinol*, T. Nargis Begum[#] and M.H. Muhammad Ilyas[@]

[*]Research Scholar, P.G. and Research Department of Biotechnology, Jamal Mohamed College (Autonomous), Tiruchirappalli - 620 020, Tamil Nadu, India.
[#]Assistant Professor, P.G. and Research Department of Biotechnology, Jamal Mohamed College (Autonomous), Tiruchirappalli - 620 020, Tamil Nadu, India.
[@]Associate Professor, P.G. and Research Department of Botany, Jamal Mohamed College (Autonomous), Tiruchirappalli - 620 020, Tamil Nadu, India.

Article Received on 08 April 2017,
Revised on 29 April 2017,
Accepted on 19 May 2017
DOI: 10.20959/wjpr20176-8622

***Corresponding Author'**
S. Josephinol
Research Scholar, P.G. and Research Department of Biotechnology, Jamal Mohamed College (Autonomous), Tiruchirappalli - 620 020, Tamil Nadu, India.

ABSTRACT

Objective: Assessment of *in vitro* antioxidant and *in vitro* antidiabetic potential of *Azima tetracantha* Lam. **Methods:** Phytochemicals of hexane, aqueous and ethanolic extracts of the *Azima tetracantha* Lam were analyzed. The antioxidant potential of hexane, aqueous and Ethanolic extracts was assessed by 2,2-diphenyl-1-picryl hydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), ferric reducing ability power (FRAP), superoxide ($O2^{-}$), hydrogen peroxide (H_2O_2)and nitric oxide (NO) scavenging assay and antidiabetic activity was assessed by α-amylase and α-glucosidase inhibition. **Results:** Amongst all the three extracts of *A. tetracantha*, ethanol leaf extracts contain significant antioxidant as well as anti-diabetic potential. *A. tetracantha* showed potential antioxidant as well as anti-diabetic activities and have good reputation in traditional Indian medicine. **Conclusion:** Ethanol leaf extracts of *A. tetracantha*is having high medicinal potential in terms of their antioxidant and antidiabetic activities. Studies are required to further elucidate antioxidant and anti-diabetic potentials using *in-vivo* biochemical and molecular biology techniques.

KEYWORDS: *Azima tetracantha*; DPPH; α-amylase inhibition; α-glucosidase inhibition.

INTRODUCTION

Diabetes mellitus [DM] is a metabolic disease describedthroughhyperglycemia and disruptions in fat and protein metabolism that results from deficiency in both insulin secretion and/or insulin action.[1] Various pharmacologicalattitudes have been exhausted to recover diabetes via different modes of action including increasing the number of glucose transporters and reduction of glucose absorption from the intestine, inhibition of gluconeogenesis and stimulation of insulin release.[2] One of the beneficial therapies to impair glucose absorption is through the inhibition of carbohydrate hydrolyzing enzymes such as α-amylase and α-glucosidase in the digestive organs.[3] Inhibitors of these enzymes postponement the carbohydrate digestionand extend the overall time for carbohydrate digestion,subsequently reduce the rate of glucose absorption. Snowballingindication has exposed that extended exposure to raised glucose persuades the manufacture offree radicals, remarkably reactive oxygen species (ROS),through glucose auto-oxidation and protein glycosylation.[4] Oxidative injury by ROS has been recommended toelucidate the surplusoccurrence of vascular hitchesin DM, which may be facilitated by oxidativestress. This destructionin the equilibrium betweenROS and antioxidants results in oxidative stress a destructive process that can be a significant mediator ofharm to cell structures, including lipids and membranes, proteins and DNA. Indeed, a variability of defects inantioxidative status have been described in experimentaland in diabetic patients when associated with normalpopulace.[5]

Conventionally herbal medicines are presently used inshielding and renovating health. *Azima tetracantha* Lam., (Salvadoraceae) commonly known as "mulluchangu" is a glabrous, rigid, rambling, thorny bushc ommonly named Bee sting bush available in Madagascar, Africa and India. Numerous therapeutic properties are accredited to this plant in the Indian system of medicine and included in the specifications of traded medicinal plants. The ethno botanical study discloses the custom of this plant as an exclusive folk medication by the tribes.[6] The leaves, root bark and root are administered with food as a remedy for rheumatism.[7,8] It is an authorative given in rheumatism, dropsy, dyspepsia and chronic diarrhea and as astimulant tonic after confinement.[9] The leaves are found to have carpine, azcarpine, azimineand isorhamnitine-3-O-rutinoside etc.[10] β-sitosterol, lupeol, glutinol and friedelin were isolated from the petroleum ether extract of the leaves of *A. tetracantha.*[11] The seeds of this plant have been found to have novel fatty acids along with other fatty acids.[12] Antimicrobial activity was also reported for this plant.[13] *A. tetracantha* leaf powder was assessed forits anti-inflammatory activity.[14] The benzene, chloroform and aqueous extract of leaves of *A.*

tetracantha were screened for analgesic activity in mice using hot plate method.[15] However, information pertaining to the systematic studies on the antioxidant properties of *A. tetracantha* is lacking. In view of the above fact, in the present study the possible *in vitro* antioxidant activity and *in vitro*anti-diabetic activity of the ethanol extracts were investigated by employing different *in vitro* free radical scavenging models and α-amylase and α-glucosidase inhibition tests.

MATERIALS AND METHODS

The fresh leaves of *A. tetracantha* were collected from Thiruchirappalli District, Tamil Nadu, South India. The leaves were identified and authenticated by Dr. S. John Britto, The Director, the Rabinat Herbarium and centre for molecular systematics, St. Joseph's college Trichy-Tamilnadu. India. A Voucher specimen (EP001) has been deposited at the Rapinat Herbarium, St. Josephs College, Thiruchirappalli, Tamil Nadu, India.

Preliminary phytochemical screening

The leaf powder of *A. tetracantha* and extraction from different solvents (Hexane, Aqueous and Ethanol) were studied for the presence and absence of secondary metabolites like, Pholabatannins, Resins, Tannins, Glycosides, Acid compound, Terpenoids, Reducing sugar, Phenols, Carbohydrates, Anthraquinone, Alkaloids and Volatile oil by qualitative chemical tests.[16]

In vitro antioxidant Activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay

The free radical scavenging capacity of the extract of *Azima tetracantha* was determined by using DPPH.[17] DPPH solution (0.04% W/V) was prepared in 95% methanol. From stock solution 0.2-1.0 ml of solution were taken in five test tubes and serially diluted to concentration such as 20µg/ml to 100µg/ml respectively. Freshly prepared DPPH 0.5ml solution was incubated with test drug and after 10 minutes, the absorbance was taken at 517nm using spectrophotometer. Ascorbic acid was used as reference.

CALCULATION

% scavenging of the DPPH free radical was measured using the following equation

$$\text{DPPH Scavenging activity (\%)} = \frac{A_{Control} - A_{Test}}{A_{Control}} \times 100$$

The antioxidant activity of the leaf extract was expressed as IC_{50} and compared with standard. The IC_{50} value was defined as the concentration of extract that inhibit the formation of DPPH radicals by 50%.

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) Radical Scavenging Assay

ABTS solution was freshly prepared by adding 5ml of a 4.9mM potassium persulphate solution to 5ml of a 14mM ABTS solution and the resulting solution was kept for 16h in dark at room temperature (25±1°C). This solution was diluted with methanol to yield an absorbance of 0.700±0.02 at 734nm and the same solution was used for the antioxidant assay. One milliliter of reaction mixture of standard and extracts comprised 950µL of ABTS solution and 50µL of the samples. This solution was vortexed for 10s and the absorbance was recorded at 734nm after 6 minutes using a UV-visible spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) which was compared with the control ABTS solution.[18] The calibration curve of vitamin C was prepared by plotting the percentage inhibition of vitamin C at various concentrations (5-100µg mL^{-1}). The percentage inhibition was calculated using the following formula.

$$\text{Percentage inhibition of vitamin C and extracts} = \frac{A_0 - A_1}{A_0} \times 100$$

where, A_0 is the absorbance of the control and A_1 is the absorbance of samples. The results were expressed as mg Vitamin C Equivalent Antioxidant Capacity (VCEAC) per gram extract.

Ferric Reducing Antioxidant Power (FRAP) Assay

The capability of the plant extracts to reduce ferric ions was evaluated through the FRAP assay.[19] FRAP reagent (900µL), prepared freshly and incubated at 37°C, was mixed with 90µL of distilled water and 30µL of test sample or methanol (for the reagent blank). The test samples and reagent blank were incubated at 37°C for 30 minutes in a water bath. The final dilution of the test sample in the reaction mixture was 1/34. The FRAP reagent was prepared by mixing 2.5mL of 20mM TPTZ in 40mM HCl, 2.5mL of 20mM $FeCl_3.\ 6H_2O$ and 25mL of 0.3M acetate buffer (pH-3.6). At the end of incubation, the absorbance readings were taken immediately at 593nm against the reagent blank, using a spectrophotometer. Methanolic solutions of known Fe (II) concentration, ranging from 100 to 2000µM, ($FeSO_4.7H_2O$) were used for the preparation of the calibration curve.

$$\text{Ferric Reducing Power Assay} = \frac{A_{Control} - A_{Test}}{A_{Control}} \times 100$$

Superoxide Radical Scavenging Activity

About 1ml of nitro blue tetrazolium (156µM), 1ml NADH (468µM) 1N 100mM phosphate buffer of pH 7.8 and 0.1ml of different concentration of sample solution were mixed. The reaction started on the addition of 100µl phenazinemethosulfate (60µM). Kept the reaction mixture at 25^0C for 5 minutes and absorbance of the mixture was measured at 560nm against blank samples.[20] The percentage inhibition was determined by comparing the result of control and test samples.

$$\%\ \text{of Superoxide radical Scavenging activity} = \frac{A_{Control} - A_{Test}}{A_{Control}} \times 100$$

Where $A_{Control}$ was the absorbance of the control (blank, without extract) and A_{Test} was the absorbance with extract.

H_2O_2 Radical Scavenging Activity

A solution of H_2O_2 (40mM) was prepared in phosphate buffer (pH 7.4). The extract at the 100µg/ml concentration in 3.4ml phosphate buffer were added to a H_2O_2 solution (0.6ml, 40mM). The absorbance value of the reaction mixture was recorded at 230nm. Blank solution was containing the phosphate buffer without H_2O_2.[21] Scavenging of the extract and a standard compound was calculated as

$$\%\ \text{Scavenging of } H_2O_2 = \frac{A_{Control} - A_{Test}}{A_{Control}} \times 100$$

Where $A_{Control}$is the absorbance of the control, and A_{Test} is the absorbance in the presence of the sample.

Nitric oxide (NO) scavenging assays

NO was generated *in vitro* from sodium nitroprussiate dehydrate (SNP) and measured by the Griess reaction. SNP solution (6 mg/mL) was prepared in phosphate buffer (KH2PO4 100 mM, pH 7.4) and mixed with the same volume (100 µL) of different concentrations of extracts, in a 96-wells plate. The mixture was further incubated at room temperature for 1 h under light. After that, 100 µL of Griessreagen t[22] (1:1 mixture (v/v) of1% sulfanilamide and 0.1% N-(1-naphthyl) ethylenediamine in 2% H3PO4) was added and the mixture was further incubated for 10 min in the dark. The absorbance was read at 560 nm. Results were expressed as IC50 and ascorbic acid was used as positive control.

Anti-diabetic study - α-Amylase and α-Glucosidase Inhibitory Activity

Different concentration of each sample extract (100 μL) and 100 μL of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing α-amylase solution (0.5 mg/mL) were incubated at25°C for 10 min. After pre-incubation, 100 μL of a 1% starch solution in sodium phosphate buffer was added to each tube at timed intervals. The reaction mixtures were then incubated at 25°C for10 min. The reaction was stopped with 200 μL of dinitrosalicylic acid color reagent. The test tubeswere then incubated in a boiling water bath for 5 min and cooled to room temperature. The reaction mixture was then diluted after adding 3 mL of distilled water, and absorbance was measured at540 nm. The absorbance of blanks (enzyme solution was added during the boiling) and a control(buffer in place of sample extract) were recorded.[23] Analyses were performed in triplicate and the final extract absorbance (540 nm) was obtained by subtracting its corresponding sample blank reading.

The effect on α-glucosidase was assessed in 96-well plates, using a procedure previously reported. Briefly, each well contained 2.5 mM PNP-G (100 μL) in phosphate buffer pH 7.0(150 μL) and methanol extract at different concentrations (50 μL). The reaction was initiated by the addition of 0.28 U/mL enzyme (20 μL) and the plates were incubated at 37 °C for 10 min. The absorbance at 400 nm was measured in a Multiskan Ascent plate reader (Thermo Electron Corporation). Results were expressed as IC50 calculated from three independent tests, performed in triplicate.

Statistical Analysis

All experiments were repeated at least three times. Results are reported as Mean ± Standard deviation.

RESULTS AND DISCUSSION

As a part of standardization procedure, the leaf sample of *A. tetracantha* was tested for relevant phytochemical parameters and also subjected to *in vitro* antioxidant activity, *in vitro* antidiabetic screening through quality control measures.

Qualitative Phytochemical screening

Preliminary Phytochemical results showed in Table 1indicated the presence or absence of certain phytochemical in the drug and also needed to standardize the crude drugs and it's become very important for identification and authentication of drug. The Hexane leaves extracts of *A. tetracantha* showed that the presence of Glycosides, Terpenoids, Reducing sugar, Carbohydrates and Volatile oil. The aqueous leaves extract of *A. tetracantha* exhibited

the Volatile oil, Glycosides, Resins, Alkaloids and Phenols. The Ethanolic leaves extract exposed the Terpenoids, Tannins, Phenols, Alkaloids, Reducing sugar, Carbohydrates and Acid compound. Results obtained from phytochemical analysis could make the plant useful for treating different ailments and having a potential of providing useful drugs for human use and further work is required to investigate the leaf extracts of *A. tetracantha* for various pharmacological activities.

Table 1: Preliminary phytochemical screening of various extracts

S. No.	Name of the Test	Extraction of different solvents		
		Hexane	Aqueous	Ethanol
1	Pholabatannins	-	-	-
2	Resins	-	+	-
3	Tannins	-	-	+
4	Glycosides	+	+	-
5	Acid compound	-	-	+
6	Terpenoids	+	-	+
7	Reducing sugar	+	-	+
8	Phenols	-	+	+
9	Carbohydrates	+	-	+
10	Anthraquinone	-	-	-
11	Alkaloids	-	+	+
12	Volatile oil	+	+	-

+ve indicates Presence

-ve indicates Absence

RESULT AND DISCUSSION

Radical Scavenging Activity by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Assay

The method is based on the reduction of methanol DPPH solution in the presence of a hydrogen donating antioxidant, due to the formation of thenon-radical form DPPH-H. The extracts were able to reduce the stable radical DPPH to the yellow colored diphenyl picryl hydrazine, in a concentration-dependent manner.[24] Results were expressed as IC_{50} (Table 2)and Ethanol leaf extract showed a fair DPPH scavenging activity, lower than ascorbic acid (used as standard) with an $IC_{50}$of 389.26µg/ml. Aqueous extracts of ($IC_{50}$of 449.77 µg/mL) and Hexane extracts ($IC_{50}$of 728.13 µg/mL), showed the lowest activity when compared to Ethanol extract of *A. tetracantha*. The IC_{50} of ascorbic acid was 396.01µg/mL.The antioxidant activity of *A. tetracantha* against DPPH scavenging assay was observed at maximum dose (500µg/ml) at 62.02±0.38%, and followed by 53.71±1.29, 37.69±0.81, 14.39±0.15 and 28.29±0.09 at 100, 200, 300 and 400 µg/ml respectively. The previous investigations reported that tannins, terpenoids and phenol could be the responsible for

antioxidant property.[25] The reason for the high and moderate scavenging activities of the ethanol and water extract than the hexane extract could be as a result of the solvent polarity.

Table 2: Effect of *A. tetracantha* on the DPPH scavenging activity.

Concentration (µg)	Hexane	Aqueous	Ethanol	Ascorbic acid (10 µg/ml)
100	7.72±0.47	13.02±1.04	19±0.15	8.61±0.53
200	14.96±0.65	22.93±1.21	28.29±0.09	23.26±1.09
300	22.95±1.09	34.54±0.59	37.69±0.81	37.35±0.80
400	25.68±0.73	42.34±2.05	53.71±1.29	51.89±1.47
500	35.78±0.51	56.91±2.57	62.02±0.38	62.93±0.70
IC_{50} Value (µg/mL)	728.13	449.77	389.26	396.01

Values are expressed as Mean±SD (n=3)

Reducing Power by Ferric Reducing Antioxidant Power (FRAP) Test.

Table 3: Effect of *A. tetracantha* on the FRAPscavenging activity.

Concentration (µg)	Hexane	Aqueous	Ethanol	Ascorbic acid (10 µg/ml)
100	13.18±1.25	25.91±1.47	38.62±0.64	35.53±0.62
200	27.01±1.49	51.14±0.96	52.44±0.68	51.23±1.10
300	35.75±6.19	62.58±2.61	64.68±1.84	66.20±0.71
400	51.21±1.10	72.25±2.00	77.17±0.81	75.25±1.00
500	52.04±0.33	79.30±0.97	83.72±1.30	81.23±1.10
IC_{50} Value (µg/mL)	439.04	235.55	184.06	197.03

Values are expressed as Mean±SD (n=3)

The method is simple and rapid; it was originally applied to plasma but has been extended to other biological fluids, foods, plant extracts, juices, etc.In Table 3, ascorbic acid ranged from 78.20±0.97 to 82.41±0.65µg of Ascorbic Acid of dried extract and IC_{50} value was determined as 197.03µg/mL. Extracts of hexane, Aqueous and Ethanol extracts showed the highest reducing power, 77.74±1.89, 83.07±2.30 and 87.38±0.59 µg/ml of dried extract, respectively. The IC_{50} value of all three extracts was determined as 439.04µg/mL (Hexane), 235.55µg/mL (Aqueous) and 184.06µg/mL(Ethanol). The Ethanol extract showed lowest IC_{50} which indicated that Ethanol extract has high reducing potential than other extracts and also with ascorbic acid. Similar to the antioxidant activity, the reducing power of *A. tetracantha* increased with increasing dosage. The result shows that *A. tetracantha* consists of poly phenolic compounds and alkaloids that may be reason for the greater reducing power.[26]

ABTS Radical Scavenging Assay

Table 4: Effect of *A. tetracantha* on the ABTS scavenging activity

Concentration (µg)	Hexane	Aqueous	Ethanol	Ascorbic acid (10 µg/ml)
100	6.93±0.36	12.26±1.00	16.98±1.04	14.19±0.42
200	13.43±0.70	22.26±0.90	40.70±0.44	29.18±0.56
300	20.50±1.50	35.22±1.00	45.53±0.24	46.41±3.76
400	24.02±1.65	48.47±0.80	58.02±1.33	57.88±0.91
500	30.02±1.92	58.40±1.22	69.76±0.82	73.09±0.62
IC_{50} Value (µg/mL)	846	423.89	330.87	339.93

Values are expressed as Mean±SD (n=3)

The result showed that different concentrations of *A. tetracantha* showed varying degree of scavenging potential for $ABTS^+$ radicals in concentration dependent manner. Table 4 showed the results based on their IC_{50} value total Ethanol extract (330.87µg/mL) was found to be most active, when all three extracts were compared with the trapping potential of ascorbic acid (339.93µg/mL). Maximum inhibition of Ethanol extract was 72.89±0.33% at 500 µg/mL among different concentrations of 100, 200, 400and 500 µg/mL with IC_{50} 330.87µg/mL. Therefore, the ABTS radical scavenging activity of Ethanol extract of leaves of *A. tetracantha*indicates its ability to scavenge free radicals, there by lipid oxidation via chain breaking reaction. The scavenging activity of ABTS radical by the plant extracts justifies the presence of compounds with free radical scavenging activity as well as the possibility of the extracts being used for treating radical-related pathological ailments.[27]

Superoxide Radical ($O2^-$) Scavenging Activity

Table 5: Effect of *A. tetracantha* on the SO scavenging activity

Concentration (µg)	Hexane	Aqueous	Ethanol	Ascorbic acid (10 µg/ml)
100	15.47±0.85	18.47±0.85	27.61±0.59	22.88±0.47
200	32.15±0.31	35.15±0.31	46.67±0.56	44.55±0.58
300	50.18±2.70	53.18±2.70	56.56±0.56	55.22±0.92
400	60.52±1.56	63.52±1.56	65.56±0.38	64.91±1.13
500	75.69±0.50	78.69±0.50	81.49±0.78	79.19±0.49
IC_{50} Value (µg/mL)	321.52	301.36	255.87	274.74

Values are expressed as Mean±SD (n=3)

The superoxide scavenging capacity of methanol extracts was quantified by their ability to inhibit nitrotetrazolium blue chloride (NBT) reduction by superoxide. Scavenging activity was, also in this case, concentration-dependent and the activity of ascorbic acid, used as

reference, was compared with the samples by IC_{50} values. Ethanol extract of *A. tetracantha* exhibited (Table 5) the slowest IC_{50} value among investigated extracts (IC_{50} = 255.87µg/mL), so it was the most active against superoxide radical obtaining a comparable value to ascorbic acid (IC_{50} was 274.74), used as positive control. The Aqueous extract (IC_{50} = 301.36µg/mL) and hexane extract (IC_{50} = 321.52 µg/mL) showed a good superoxide anion scavenging activity. The superoxide scavenging activity of *A. tetracantha* was increased markedly with the increase in concentrations. Thus, higher inhibitory effects of the ethanol leaf extracts on superoxide anion formation noted herein possibly renders them as a promising antioxidants. These results suggested that *A. tetracantha* has a potent superoxide radical scavenging effects.

H_2O_2 Radical Scavenging Activity

Table 6: Effect of *A. tetracantha* on the H_2O_2 scavenging activity

Concentration (µg)	Hexane	Aqueous	Ethanol	Ascorbic acid (10 µg/ml)
100	7.33±0.85	18.23±0.98	21.55±0.59	18.53±1.07
200	15.36±0.79	25.18±0.95	31.48±0.69	31.32±0.97
300	24.00±1.41	37.20±1.06	44.67±0.97	44.75±1.42
400	37.20±1.02	54.21±1.00	54.67±0.79	58.16±0.65
500	41.30±0.91	60.19±0.99	65.95±1.22	61.37±0.99
IC_{50} Value	577.90	397.52	356.57	363.82

Values are expressed as Mean±SD (n=3)

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. H_2O_2 is very important due to its capability to diffuse biological membranes. Table 6 showed the results of H_2O_2 scavenging activity of various extracts of *A. tetracantha* andthe highest percentage of activity was found with ethanol (64.89± 1.32%), followed by Aqueous (57.70± 0.39%) and hexane (43.59± 2.10%) at highest concentrations (500µg/ml). IC_{50} values were determined for three extracts of *A. tetracantha* and reported in Table 5. The IC_{50} ofascorbic acid was found to be 363.82µg/ml. The acidic methanol and water extracts of six herbaceous plants (*Bidensalba*, *Lyciumchinense*, *Menthaarvensis*, *Plantagoasiatica*, *Houttuyniacordata*, *and Centella asiatica*) containing antioxidants can protect against DNA damage in human lymphocytes induced by hydrogen peroxide.[28] Scavenging of OH^- is an important antioxidant activity because of its very high reactivity, which can easily cross the cell membranes at specific sites, react with most biomolecules and furthermore cause tissue damage and cell death. In the present study, all the extracts of *A. tetracantha* at 500µg/ml scavenged H_2O_2 and this may

be attributed to the presence of phenolics, which could donate electrons to $H_2O_2$and thus neutralize it to water.[25]

Nitric Oxide (˙NO) Radical Scavenging Activity

Table 7: Effect of *A. tetracantha* on the NO scavenging activity

Concentrations (µg)	Hexane	Aqueous	Ethanol	Ascorbic acid (10 µg / ml)
100	12.23±0.86	19.16±1.01	33.42±1.70	13.34±0.98
200	25.03±1.05	37.36±0.88	53.54±1.27	30.46±0.51
300	37.10±0.81	46.72±1.34	64.04±1.92	46.07±0.20
400	47.60±1.46	56.01±2.03	71.42±0.37	60.19±1.00
500	60.11±1.97	73.16±1.04	79.33±1.41	72.55±1.47
IC_{50} Value (µg/mL)	414.89	327.70	205.68	336.86

Values are expressed as Mean±SD (n=3)

The ability to inhibit nitric oxide production was concentration-dependent. Ascorbic acid was used as reference, showing a IC_{50} value of 336.86 mg/mL. All extracts demonstrated to possess nitric oxide scavenging activity. The results of Ethanol (IC_{50} = 205.68 mg/mL), Aqueous (IC_{50} = 327.70 mg/mL) and Hexane (IC_{50} = 414.89 mg/mL) extracts of *A. tetracantha* showed the highest scavenging activity; also Ethanol extract (IC_{50} = 205.68 mg/mL) reported a good scavenging activity, lower than ascorbic acid. Hexane extract with the highest IC_{50} value (IC_{50} = 414.89 mg/mL), was the less active. Hence, ethanol extract scavenge more of the radicals than ascorbic acid. This result conforms with previous studies which reported that ethanol leaf extract showed the highest percentage nitric oxide inhibition at the lowest concentration due to existence of bioactive molecules such as tannin, phenols, alkaloid and Terpenoids. The similar result was observed in leaf of *Carpobrotus edulis* (L.).[29]

Inhibition Activity against α-Glucosidase and α-Amylase Enzymes

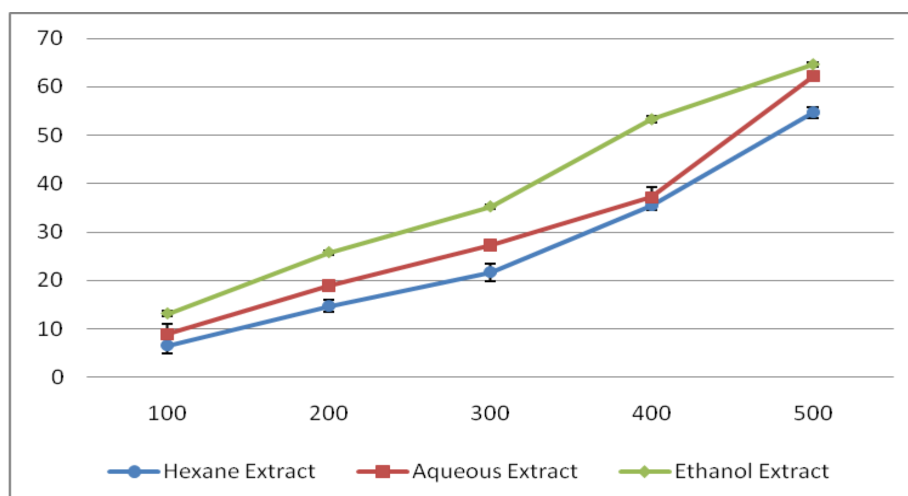

Figure 1: Inhibition of *A. tetracantha* against α - amylase

Medicinal plants and herbal extracts containing secondary metabolites have been reported to demonstrate antioxidant and antidiabetic activities. One therapeutic approach for treating diabetes is to decrease post-prandial hyperglycemia. This is done by hindering the absorption of glucose through inhibition of the carbohydrate hydrolyzing enzymes, α-amylase andα-glucosidase, in the digestive tract. In our investigation, the inhibitory activity of crude hexane, Aqueous and Ethanolextracts against both mentioned enzymes was carried out. Most of the studies reported only theα-glucosidase inhibitory activity of some isolated constituents from leaves of medicinal plants. Data obtained showed that Ethanolextracts of *A. tetracantha*leaves were stronger inhibitors of α-amylase than α-glucosidase, as presented in Figure 1& 2 respectively.

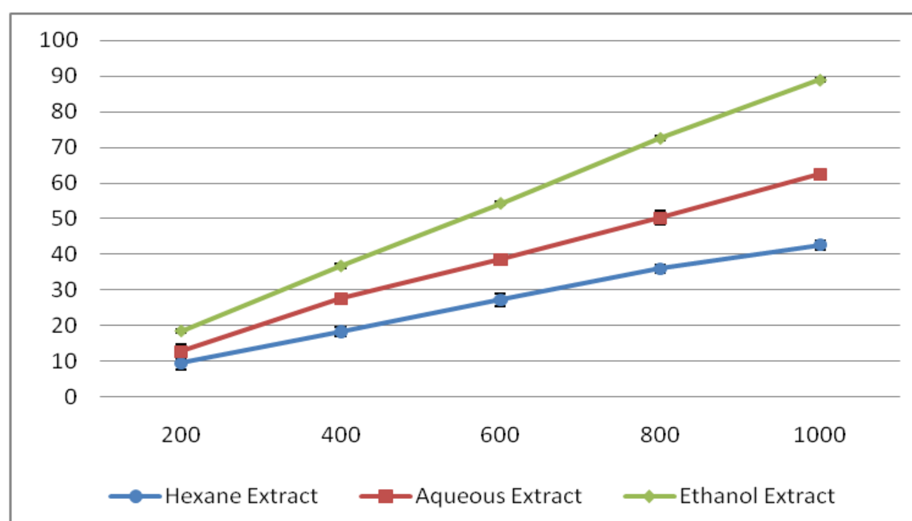

Figure 2: Inhibition of *A. tetracantha* against α - glucosidase

Among *A. tetracantha* extracts, the most active was found to be Ethanol extract for both enzyme inhibitory activities (IC_{50} = 438.21µg/mL for α-amylase inhibition and 574.38µg/mL for α-glucosidase); Hexane and Aqueous extracts act as α-amylase moderate inhibitors. According to previous study[30], our investigation reported that Ethanol extracts highly inhibited α-amylase and α-glucosidase and this result can, at least partially, explain the traditional useof *A. tetracantha* leaves for treating hyperglycemia.

CONCLUSION

In conclusion, the phytochemical, antioxidant and antidiabetic screening of leaves of *A. tetracantha* provides substantial information for the proper identificationand scientific evaluation of the medicinal plant. The present study results showed the phytochemical and its antidiabetic activity of hexane, Aqueous and Ethanol extracts of*A. Tetracantha* exhibited a significant antioxidant and antidiabetic activity which is suggesting the presence of bioactive metabolites and also exhibited the good antioxidant and antidiabetic potentials of the extracts. The observed tendency of the extract to inhibit α-Glucosidase and α-Amylase enzymes showed that *A. tetracantha* contain interesting bio-medicinal substances capable of attracting significant scientific attention against diabetes. This study also revealed that the extracts of *A. tetracantha* exhibited phytochemical, antioxidant and antidiabetic activities of natural origin which may offer promising anti-diabetic agents. Investigation will be carried on the pharmacologic activity of the compounds from *A. tetracantha* responsible for these activities to be elucidated.

REFERENCES

1. Teixeira CC, Rava CA, Da-Silva PM, Melchior R, Argenta R, Anselmi F, Almeida CR, Fuchs FD: Absence of antihyperglycemic effect of jambolan in experimental and clinical models. J Ethnopharmacol, 2000; 71: 343–347.
2. Youn J, Park H, Cho K: Anti-hyperglycaemic activity of *Commelinacommunis* L.: inhibition of α-glucosidase. Diabetic Res ClinPrac, 2004; 665: S149–S155
3. Saito N, Sakai H, Sekihara H, Yajima Y: Effect of α-glucosidase inhibitor (voglibose), in combination with sulphonylureas, on glycaemic control in type 2 diabetes patients. J Int Med Res, 1998; 26: 219–232.
4. Bhandari MR, Jong-Anurakkhun N, Hong G, Kawabata J: α-glucosidase and α-amylase inhibitory activities of Napalese medicinal herb Pakhanbhed (*Bergeniaciliata*, Haw). Food Chem, 2008; 106: 247–252.

5. Rhabasa-Lhoret R, Chiasson JL: Glucosidase inhibitors. In International Textbook of Diabetes Mellitus. 3rd edition. Edited by Defronzo RA, Ferrannini E, Keen H, Zimmet P. UK: John Wiley & Sons Ltd, 2004; 1: 901–914.
6. Ignacimuthu S, Ayyanar M, Sankarasivaraman K. Ethnobotanical study of medicinal plants used by Paliyartribals in Theni district of Tamil Nadu, India. Fitoterapia, 2008; 79(7): 562-8.
7. Kirtikar KR, Basu BD and An ICS. Ind. Med. Plants.1 & 2, 2nd Edn. Bishen Singh Mahendra Pal Singh, Dehra Dun, 1984; 582: 1541.
8. Chopra RN, Nayar SL and Chopra C. Glossary of lndian Medicinal Plants.CSIR, New Delhi, 1956; 32: 218.
9. Nadkarni KM. Indian Meteria Medica, 3rd Edn. Popular Prakhasan, Bombay, 1976; 1: 165.
10. Bennett RN, Rosa EA, Perkins L and Kroon PA. Profiling glucosinolates, flavonoids, alkaloids, and other secondary metabolites in tissues of *Azimatetracantha* L (Salvadoraceae). J. Agric. Food Chem, 2004; 52(19): 5856- 5862.
11. Rao VE and Prasada Rao PRS. Occurrence of triterpenoids in *Azimatetracantha*. Curr. Sci, 1978; 47: 857.
12. Daulatabad CD, Desai VA, Hosamani KM and Jawkhande AM. Novel fatty acids in *Azimatetracantha* seed oil. J. Amer. Oil Chemists Soc, 1991; 68: 978- 979.
13. Siva N, Ganesan N, Banumathy and Muthuchelian. Antifungal effect of leaf extract of some medicinal plants against *Fusariumoxysporum* causing wilt disease of Solanummelogena L. Ethnobotanical Leaflets, 2008; 12: 156-163.
14. Ismail TS, Gopalakrishnan S, Begum VH and Elango V. Anti-inflammatory activity of Salaciaoblonga Wall and *Azimatetracantha* Lam. J. Ethnopharmacol, 1997; 56(2): 145-152.
15. Nandgude TD, Bhojwani AP and Krishna Kinage. Analgesic activity of various extracts of leaves of *Azimatetracantha* Lam. Int. J. Green Pharm, 2007; 1(1): 37-38.
16. Harborne JB. Phytochemical methods: A Guide to Modern Techniques of Plant Analysis. New York: Chapman and Hall, 1984; 1-89.
17. Gyamfi MA and Aniya Y. Antioxidant properties of ThonningianinA, isolated from the African medicinal herb, *Thonningiasanguines*. *Biochemical Pharmacology,* 2002; 63: 1725–1737.

18. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M and Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorizing assay. Free Radical Biol Med, 1999; 26: 1231-1237.
19. Benzie IFF and Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal Biochem*, 1996; 239: 70-76.
20. Halliwell B and Gutteridge JM. The importance of free radicals and catalytic metal ions in human diseases. Mol Aspects Med, 1985; 8(2): 89–193.
21. Ruch RJ, Cheng SJ and Klavning JE. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogens, 1989; 10: 1003-1008.
22. Marcocci, L.; Maguire, J.J.; Droy-Lefaix, M.T.; Packer, L. The nitric oxide-scavenging properties of *Ginkgo biloba* extract EGb 761. Biochem. Biophys. Res. Commun, 1994; 201: 748–755.
23. Ranilla, L.G.; Kwon, Y.I.; Apostolidis, E.; Shetty, K. Phenolic compounds, antioxidant activity and *in vitro* inhibitory potential against key enzymes relevant for hyperglycemia and hypertension of commonly used medicinal plants, herbs and spices in Latin America. Bioresour. Technol, 2010; 101: 4676–4689.
24. Jimenez-Escrig, A.; Jimenez-Jimenez, I.; Sanchez-Moreno, C.; Saura-Calixto, F. Evaluation of free radical scavenging of dietary carotenoids by the stable radical 2,2-diphenyl-1-picrylhydrazyl. J. Sci. Food Agric, 2000; 80: 1686–1690.
25. Wintola, O. A., &Afolayan, A. J. Phytochemical constituents and antioxidant activities of the whole leaf extract of *Aloe ferox* Mill. Pharmacognosy magazine, 2011; 7(28): 325.
26. Nagulendran, K. R., Velavan, S., Mahesh, R., & Begum, V. H. In vitro antioxidant activity and total polyphenolic content of *Cyperusrotundus* rhizomes. Journal of Chemistry, 2007; 4(3): 440-449.
27. Omoruyi, B. E., Bradley, G., &Afolayan, A. J. Antioxidant and phytochemical properties of *Carpobrotusedulis* (L.) bolus leaf used for the management of common infections in HIV/AIDS patients in Eastern Cape Province. BMC complementary and alternative medicine, 2012; 12(1): 215.
28. Kuan-Hung Lin, Yan-Yin Yang, Chi-Ming Yang, Meng-Yuan Huang, Hsiao-Feng Lo, Kuang-Chuan Liu, Hwei-Shen Lin and Pi-Yu Chao. Antioxidant activity of herbaceous plant extracts protect against hydrogen peroxide-induced DNA damage in human lymphocytes. *BMC Research Notes,* 2013; 6: 490.

29. Mathew S and Abraham TE. *Invitro*antioxidant activities and scavenging effects of *Cinnamomumverum*leaf extract assayed by different methodologies. *J. Food. Chem. Toxicol,* 2006; 44: 198-206.
30. Xiang, Z.; He, F.; Kang, T.G.; Dou, D.Q.; Gai, K.; Shi, Y.Y.; Young-Ho, K.; Dong, F. Anti-diabetes constituents in leaves of *Smallanthussonchifolius*. Nat. Prod. Commun, 2010; 5: 95–98.