Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2015; 7(4); 793-801

ISSN: 0975-4873

Research Article

Phytochemical Potential of *Pavonia procumbens* (Wall. Ex Wight & Arn.) Walp. with Reference to Antioxidant and Antidiabetic Activity

Rajesh V[1], Bharathi P[2], Priya M[2], Arumugam P[3], P Jayaraman[1*]

[1]*PG and Research Department of Botany, Government Arts College for Men (Autonomous), Nandanam, Chennai 600 035, Tamil Nadu, India.*
[2]*Karpaga Vinayaga College of Engineering and Technology, Maduranthakam Taluk, Kanchipuram 603 308, Tamil Nadu, India*
[3]*Armats Biotek Pvt. Ltd., No.14, Mettu street, Maduvankarai, Guindy, Chennai 600 032.*

Available Online: 21st July, 2015

ABSTRACT
Dried Leaves of *Pavonia procumbens* (wall.ex wight & Arn.) walp. were collected from Damanur hills, Villupuram District, Tamil Nadu, India for the comparative analysis of phyto-chemical potential with reference to antioxidant and anti-diabetic activity. The active plant compounds were extracted with ethyl acetate, hexane and methanol solvents. Among the solvents used for extraction, methanol found to be a suitable solvent and hence used for further extraction and analysis of biocompounds. The preliminary qualitative phyto-chemical screening of *Pavonia procumbens* has revealed the presence of Phenols, Flavanoids and Terbenoids and absence of Alkaloids, Tannins and Reducing sugars in methanol extract. The quantitative determination of phenol and flavanoids were carried out and found that the total phenolic content with 1.6095µg GAE/ml and flavonoids 2.071µg QE/ml respectively. The IC_{50} value of DPPH assy, Nitric oxide scavenging assay and Fe^{2+} Chelation assay of methanol extract were recorded as 200.41 µg/ml, 108.81 µg/ml and 386.72 µg/ml respectively. The Phosphomolybdenum assay and reducing power assay indicate that the plant extract is a potential antioxidant. The results for the antidiabetic assay by α-amylase inhibition exhibited from 27% - 79% in the concentration of 5 - 25 µg/ml and non enzymatic glycosylation of hemoglobin shows 33% - 73% in the concentration of 250 - 1000 µg/ml. The inhibitory activity of Glucose uptake by yeast cells in methanol extract of *Pavonia procumbens* were found and compared with the standard drug acarbose. The details of the above results are discussed in the present study.

Keywords: Phytochemical analysis, antioxidant activity, DPPH assay, radical scavenging activity, antidiabetic assay, alpha amylase assay, *Pavonia procumbens.*

INTRODUCTION

Medicinal plants have a long history of use in the treatment of many diseases in human since many decades. India is the largest producer of medicinal plants and is called as "Botanical garden of the world". The medicinal plants, besides having natural therapeutic values against various diseases, also provide high quality of food and raw materials. Several reports describe that the anticancer activity of medicinal plants is due to the presence of antioxidants in them. In recent years, the use of traditional medicine information on plant research has received considerable interest[1]. Free radicals, produced as a result of normal biochemical reactions in the body, are implicated in contributing to cancer, atherosclerosis, aging, immunosuppressant, inflammation, ischemic heart disease, diabetes, hair loss and neurodegenerative disorders such as Alzheimer"s disease and Parkinson's disease[2]. The human body possesses innate defence mechanisms to counter free radicals in the form of enzymes such as superoxides dismutate, catalase, and glutathione peroxidase. Vitamin C, vitamin E, selenium, β-carotene, lycopene, lutein and other carotenoids have been used as supplementary antioxidants. Apart from these,

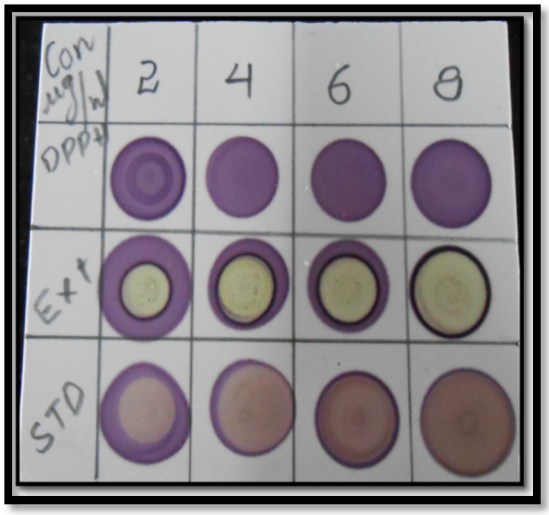

Figure 1 **:** Dot blot assay

**Author for Correspondence*

plant secondary metabolites such as flavonoids and terpenoids play important role in the defence against free radicals[3]. Free radicals, namely reactive oxygen species (ROS) and reactive nitrogen species (RNS), are known to cause damage to lipids, proteins, enzymes and nucleic acids leading to cell or tissue injury implicated in the process of ageing. Several evidences indicate that oxidative stress can lead to cell and tissue injury. The term oxidative stress indicates that the antioxidant status of cells and tissues is altered by exposure to oxidants and there occurs depletion of antioxidants during oxidative stress.

ROS and RNS contribute in different ways to carcinogenesis and the malignant progression of tumor cells, enhancing their metastatic potential. In fact, they are now considered a distinctive characteristic of cancer. These species lead to genomic damage and genetic instability and they participate as intermediaries in mitogenic and survival signals via growth factor receptors and adhesion molecules, promoting cell mobility, inducing inflammation/repair and angiogenesis in the tumor microenvironment[4,5,6,7,8,9,10,11,12]. Antioxidants are also widely used as ingredients in dietary supplements in the hope of maintaining health and preventing diseases such as cancer and coronary heart disease or many years chemists have known that free radicals cause oxidation which can be controlled or prevented by a range of antioxidants substances[13]. A healthy cell has a mortal enemy which is called a "free radical." free radicals are known to cause defects in normal RNA as well as in life perpetuating DNA, the genetic material of the cells[14]. A good anti-oxidant complex supplement actually has advantages over diet sources in that the complex has many different specific types of anti-oxidants which seek out and destroy free radicals at many various cellular sites[15].

Medicinal plant parts are commonly rich in phenolic compounds, such as flavonoids, phenolic acids, stilbenes, tannins, coumarins, lignans and lignins. These compounds have multiple biological effects including antioxidant activity[16]. Over the past decade, herbal medicines have been accepted universally, and they have an impact on both world health and international trade. Hence, medicinal plants continue to play an important role in the healthcare system of a large number of the world's population[17].

Diabetes mellitus (DM) is the commonest endocrine disorder and it becomes one of the biggest problems in the modern world and there is a growing and urgent need to control it. About 170 million of the population suffers from diabetes disease throughout the world and it has no known permanent cure[18]. The characteristic high blood sugar levels result from either lack of the hormone insulin (type 1 diabetes T1D) or because body tissues do not respond to the hormone (type 2 diabetes T2D) and both is common and serious metabolic disorder throughout the world. Diabetes acquiring around 2 .8 % of the world's population and is anticipated to cross 5 .4 % by the year 2025. It is a growing health concern worldwide and now emerging as an epidemic world over. India has a rich source of indigenous medicinal plants which are traditionally being used in various health care purposes. India has the largest number of diabetic patients in the world and has been infamously known as the 'diabetic capital of the world'[19]. The prevalence of diabetes mellitus is on increase and needs to be addressed appropriately.

Throughout the history natural products have afforded a rich respiratory of remedies with diverse chemical structure and bioactivities against several health disorder including cancer. It is estimated that 122 drugs from 92 plant species have been discovered through ethnobotanical leads. Additionally the use of herbs complementary and alternative medicine has increased dramatically in the last 20-25 years[20]. According to World Health Organization (WHO)[21] in a number of resolutions emphasized the need to ensure the quality control of plant products by using modern techniques and applying suitable standards[22]. Phyto-chemical potential, anti-hyphoglycemic activity and antioxidant activity of *Memecylon umbellatum* Burn.F. *Cleistanthus collinus* Roxb., *Polygonum glabrum* Wild., *Melia azedarch* Linn., *Indigofera trifoliata* Linn., *Cassia absus* Linn., *Cassia auriculata* Linn. and *Cassia fistula* Linn. of our earlier studies showed the methanol extracts of the plant parts like leaves and seeds had moderate anti-diabetic and antioxidant acticvity[23,24,25,26,27]. These studies support for further research on exploration of various plant species in control of tumour and diabetics. With reference to the above scientific information the present study was planned to assess the anti-diabetic efficacy of *Pavonia procumbens* collected from Damanur hills of Gingee, Villuppuram district of Tamil Nadu.

MATERIALS AND METHODS

Cleaning of glasswares and laboratory procedures

The cleaning and preparation of glassware were followed after Mahadevan and Sridhar[28]. General laboratory techniques recommended by Purvis *et al*.[29] was followed for the preparation of media inoculation and maintenance of cultures. All the chemicals and reagents used were laboratory grade.

Collection of samples and preparation

Fresh leaves of *Pavonia procumbens* (Wall. ex Wight & Arn.) Wallp., were collected from Damanur hillocks of Gingee fort in Villupuram district of Tamil Nadu, India. The plant was identified and authenticated from Institute of Herbal Science & Plant Anatomy Research Centre (PARC), Chennai (Ref. PARC/2015/3114 dated 18.05.2015). This plant belongs to class magnoliopsida of dicotylodons and family of Malvaceae. The leaves of *Pavonia procumbens* were carefully washed with tap water, rinsed with distilled water and air-dried in room temperature for few days till the leaves were completely dried. Then the dried leaves were crushed and grinded in to fine powder.

Extraction of samples

The powdered leaf samples were subjected to direct extraction with Chloroform, Ethyl acetate and Methanol in the ratio of 1:10 (w/v) by repeated extraction. The extracts were filtered through the Whatmann No. 1 filter paper and the solvent was condensed by steam batch to obtain concentrated sample[30]. These extracts were diluted with respective solvents and used to perform various assays like *in vitro* anti-diabetic and antioxidant including other

Table 1: DPPH scavenging assay of *Pavonia procumbens* Methanol Ethylacetate and Hexane extracts

Sl.No	Concentration (µg /mL)	Percentage of Inhibition		
		Methanol	Ethyl Acetate	Hexane
1.	100	24.740	22.161	19.818
2.	200	49.896	41.941	23.636
3.	300	76.091	45.421	29.454
4.	400	80.457	65.934	43.818
5.	500	85.239	84.615	51.454

Ic 50 value of methanol extract = 200.41 µg /mL

Table 2: Phosphomolybdenum assay of *Pavonia procumbens*

Sl.No	Concentration (µg /mL)	Absorbance		
		Methanol	Ethyl Acetate	Hexane
1.	100	0.883	0.479	0.125
2.	200	1.04	0.567	0.146
3.	300	1.088	0.637	0.614
4.	400	1.191	0.844	0.656
5.	500	1.158	1.028	0.745

Table 3: Reducing power assay of *Pavoniaprocumbens* (Methanol, Ethylacetate and Hexane extracts)

S.No	Concentration (µg /mL)	Absorbance		
		Methanol	Ethyl Acetate	Hexane
1.	100	0.046	0.041	0.046
2.	200	0.072	0.044	0.049
3.	300	0.103	0.059	0.055
4.	400	0.126	0.064	0.056
5.	500	0.248	0.222	0.218

Table 4: Nitric oxide scavenging assay

Sl.No	concentration (µg /mL)	Percentage of Inhibition		
		Methanol	Ethyl Acetate	Hexane
1.	100	45.959	33.447	18.466
2.	200	75.727	68.941	32.752
3.	300	84.848	78.498	68.292
4.	400	85.521	82.764	71.777
5.	500	88.888	86.518	78.745

Ic 50 value = 108.81 µg /mL

phytochemical parameters.

Antioxidant Assays

Dot blot assay

The samples were applied on a 2.5 mm silica gel 60 F254 TLC plate. After the plate had been developed, it was dried at room temperature and then sprayed with 0.2 mM DPPH in methanol. The DPPH solution was freshly prepared and stored in darkness. After the plate was dried at room temperature, an active spot indicating antioxidant was a colorless spot on the purple background.

DPPH assay: (2 2-diphenyl-1-picrylhydrazyl)

The Radical Scavenging Activity of different plant extracts was determined by using DPPH assay according to Chang *et al*.[31] with little modification. The decrease of the absorption at 517nm of the DPPH solution after the addition of the antioxidant was measured in a cuvette containing 2.96ml of ethanolic DPPH (0.1 mM) solution and 20 to 200 µg/ml of plant extract. The setup was left at dark in room temperature and the absorption was monitored after 20 minutes. Ascorbic acid was used as standard. The ability of the plant extract to scavenge DPPH radical was calculated by the following equation:

% of DPPH Radical Scavenging Activity (% RSA) = (Abs. control – Abs. sample * 100) / (Abs. control)

Phosphomolybdenum assay

The antioxidant activity of samples was evaluated by the green phosphomolybdenum complex formation according to the method of Prieto *et al*.[32]. An aliquot of 100µl of sample solution was combined with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in a 4 ml vial. The vials were capped and incubated in a water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The results reported (Ascorbic acid equivalent antioxidant activity) are mean values expressed as g of ascorbic acid equivalents/100g extract.

Reducing power assay

The reducing power of the extracts was evaluated according to Oyaizu *et al*.[33]. Different amounts of methanol extracts were perched in methanol solvent and

Table 5: Details showing Fe2+ chelation assay in different solvents

S.No	Concentration (µg /mL)	Percentage of Inhibition		
		Methanol	Ethyl Acetate	Hexane
1.	100	29.977	26.888	20.571
2.	200	33.180	34.444	31.843
3.	300	41.647	38.444	38.547
4.	400	51.716	53.555	40.502
5.	500	61.327	57.777	49.441

Ic 50 value of = 386.72 µg /mL

Table 6: Alpha amylase inhibition assay

S.No	Concentration (µg/mL)	Percentage of inhibition
1	5	27.95
2	10	33.75
3	15	51.17
4	20	66.07
5	25	79.15

The IC_{50} value = 14.65µg /mL

diverse with 2.5 ml of 0.2 M phosphate buffer (pH 6.6), and 2.5 ml of 1% $K_3Fe(CN)_6$. This mixture was incubated at 50°C for 20 min, 2.5 ml of 10% TCA was added to the blend and centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was assorted with methanol (2.5 ml) and $FeCl_3$ (0.5ml, 0.1%), and the absorbance was measured at 700 nm. Increase in absorbance of the reaction mixture indicated increased reducing power.

Nitric oxide scavenging assay[34]

10mg each of methanol, hexane and ethyl acetate extracts were separately dissolved each in 1ml of methanol. A given volume of 10, 20, 30, 40 and 50µl were taken in separate test tubes for the three different extracts. A given amounts of Sodium Nitro Prusside reagent was added to each test tube making each tube to contain 1.5ml (sample + sodium nitro Prusside reagent), the setups were incubated at 25°C for 30 minutes. 1.5ml of Greiss reagent was added to each test tube there by making each test tube of 3ml by volume of its constituents, the control was made without sample containing only 1.5ml of both sodium nitro Prusside reagent and Greiss reagent. The optical density was measured at 546 nm. The percentage of radical scavenging activity was found by the following equation:

[(OD of control – OD of sample)/ OD of control] x 100

Fe2+ chelation assay[35]

1mg each of methanol, hexane and ethyl acetate extracts were separately dissolved each in 1ml of methanol. A given volume of 10, 20, 30, 40 and 50µl were taken in separate test tubes for the three different extracts. And concentrations of 10, 20, 30, 40 and 50µg/ml were obtained. Appropriate amounts of methanol were added to each test tube there by making the contents of each tube 1ml by volume. 500µl each of 0.05% 1, 10-phenanthroline and 500 µM Fe_2Cl_3 were subsequently added to each test tube, the setup was incubated at normal temperature for 10 minutes and the optical densities were measured at 510nm. The percentage of radical scavenging activity was found by the following equation:

[(OD of control – OD of sample)/ OD of control] x 100

Antidiabetic assays

Alpha amylase assay[36]

Briefly, appropriate dilutions (0-200 µL) of the extracts and 500 µL of 0.02 M/L sodium phosphate buffer (pH 6.9 with 0.006 M/L sodium chloride) containing porcine pancreatic alpha-amylase (EC 3.2.1.1, 0.5 mg/mL) were incubated at 25 °C for 10 min. Then, 500 µL of 10 g/L starch solution in 0.02 M/L sodium phosphate buffer (pH 6.9 with 0.006 M/L sodium chloride) was added to each tube. The reaction mixtures was incubated at 25 °C for 10 min and stopped with 1.0 mL of dinitrosalicylic acid colour reagent. Thereafter, the mixture was incubated in a boiling water bath for 5 min and cooled to room temperature. The reaction mixture was then diluted by adding 10 mL of distilled water, and absorbance measured at 540 nm. The alpha-amylase inhibitory activity was expressed as percentage inhibition. The percentage inhibition was calculated using the following formula:

% inhibition of α-amylase = (Abs of control – Abs sample/Abs control)* 100

Non-enzymatic glycosylation of Haemoglobin

To measure non-enzymatic glycosylation test 1 ml each of Glucose (2%) haemoglobin (0.06%) and Gentamycin (0.02%) in phosphate buffer 0.01M at pH 7.4. were taken and mixed in a test tube. The methanol extract was weighed and dissolved in DMSO to obtain stock solutions of 1-5 µg/ml. Then 1 ml of each concentration was added to above mixture. The Mixture was incubated in dark at room temperature for 72 hrs. The degree of glycosylation of haemoglobin was measured calorimetrically at 520 nm[37]. Metformin was used as a standard drug for assay and % inhibition was calculated using the formula;

% inhibition of glycosylation = $(A_{sc} - A_{c}) / A_{sc}) * 100$

Where Abs control is the absorbance of the control reaction (containing all reagents except the test sample) and Abs sample is the absorbance of the test sample.

Glucose uptake by Yeast cells

Yeast cells were prepared according to the method of *Cirillo* (1962)[38]. Commercial baker's yeast was washed by repeated centrifugation (3,000×g; 5 min) in distilled water until the supernatant fluids were clear and a 10% (v/v) suspension was prepared in distilled water. Various concentrations of extracts (1–5 mg) were added to 1ml of glucose solution (5, 10 and 25 mM) and incubated together for 10 min at 37 °C. Reaction was started by adding 100 µl of yeast suspension, vortex and further incubated at 37 °C for 60 min. After 60 min, the tubes were centrifuged (2,500 × g, 5 min) and glucose was estimated in the

Table 7: Non enzymatic glycosylation of haemoglobin

Sl.No	Concentration (µg/mL)	Percentage of inhibition
1	Con	
2	250	33.33
3	500	51.29
4	750	53.41
5	1000	62.68
6	1250	73.49

Ic 50 = 487.42µg /mL

Table 8**:** Glucose uptake by yeast cells **–** ***Pavonia procumbens***

Sl.No	Concentration (µg /mL**)**	Percentage of Glucose uptake by yeast cells		
		5mM	10mM	25mM
1	250	4.12	2.69	2.13
2	500	13.49	7.28	5.83
3	750	28.07	9.01	9.37
4	1000	31.79	12.87	13.23
5	1250	48.47	20.53	14.09

supernatant. Metronidazole was taken as standard drug[39]. The percentage increase in glucose uptake by yeast cells was calculated using the following formula;

Increase in glucose uptake (%) = sample – control/sample*100

where, Abs control is the absorbance of the control reaction (containing all reagents except the test sample),and Abs sample is the absorbance of the test sample.

Qualitative photochemical screening

Detection of alkaloids: Solvent free extract (50mg) was stirred with 2 ml of diluted hydrochloric acid (1mL HCL + 1mL H_2o) and filtered. The filtrate was tested carefully with various alkaloid reagents.

Mayer's Test: To small quantity of the extracts Mayer's reagent was added. Presence of creamy white precipitate indicates the presence of alkaloids[40].

Detection of phenolic compound (Ferric chloride test by Mace 1963)[41]*:* The extract (50 mg) was dissolved in 5mL of distilled water. To this few drops of neutral 5% ferric chloride solution were added. A dark green color indicated the presence of Phenol.

Detection of glycosides: About 50 mg of extract was hydrolysed with 5mL of concentrated hydrochloric acid for 2h on a water bath filtered and the hydrolysate 2 mL and 3mL of chloroform were taken and shaken. Choloroform layer was separated and 10% ammonia solution was added to it. Pink colour indicated the presence of glycosides.[40]

Detection of terpenoids (Salkowski test): About 0.5 g of the extract was added in 2 ml of chloroform. Concentrated H_2S0_4 (3ml) was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

Detection of flavonoids: The sample extract 0.5g was dissolved in 5mL of Distilled water and filtered. Dilute ammonia (5mL) was added to 1mL of the extract filtrate. Concentrated sulphuric acid (1mL) was added. Yellow colorations that disappear on standing indicate the presence of flavonoids.

Detection of tannins: To 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration

Detection of reducing sugars[42]*:* The extract (100mg) was dissolved in 5mL of water and filtered. The filtrate was subjected to the Fehling's test for identification of reducing sugars. For this test 1 mL of filtrate was boiled on water bath with 1mL each of Fehling's solution I and II and a red precipitate indicated the presence of sugar.

Detection of saponins[43]**:** About 50mg of extract was diluted with 5mL distilled water. The suspension was shaken in a graduated cylinder for 15 min. A 2cm layer of foam indicated the presence of saponins.

Millon's test[44]*:* About 100mg of extract was dissolved in 10mL distilled water and filtered through Whatmann No.1 filter paper and the filtrate was subjected to tests of proteins. To 2 mL of filtrate few drops of millon's reagent were added. A white precipitate indicated the presence of proteins. To prepare a Millon's reagent Mercury (0.1g) was dissolved in 0.9mL of fumic nitric acid. When the reaction was completed equal volume (0.9mL) of distilled water was added.

Quantitative Phytochemical analysis

Determination of Flavonoids - Aluminium chloride test[45]

To 1 ml of varying concentrations of extract 3 ml of methanol 0.2ml of 1 M potassium acetate 0.2ml of10% aluminium chloride and 5.6ml of distilled water were added and left at room temperature for 30 minutes. Absorbance of the mixture was read at 415 nm using UV–VIS spectrophotometer. Calibration curve was prepared using Quercetin as standard.

Determination of Phenolic compound – Folin Ciocaltaeu's method[46]

The total phenol content of the extract was measured at 765 nm by Folin-Ciocalteu reagent (McDonald *et al.* 2001). The dilute methanolic extract (0.5 ml of 1:10 g ml-1) and or gallic acid (standard phenolic compound) was mixed with 5ml of Folin-Ciocalteu reagent (1:10 diluted with distilled water) and added 4ml of aqueous sodium carbonate (1 M). The mixture was allowed to stand for 15 min and the total phenols were determined by spectrophotometer at 765 nm. The standard curve was prepared using 0 50 100 150 200 250 mg/ml^{-1} solutions of gallic acid in methanol: water (50:50 v/v). Total phenol values are expressed in terms of gallic acid equivalent (mg per gm of dry mass) which is a common reference compound.

Thin Layer Chromatography (TLC)

Thin layer chromatography (TLC) technique for separation of active compounds extracted from *Pavonia procumbens* was achieved after the method of Hao *et al.*[47]**.** Silica gel TLC plate obtained from Emerck laboratories about 1.5cm wide and 5cm long was used. A small spot of solution containing the sample is applied to a plate and dried. A small amount of an appropriate solvent (eluent) poured in to a TLC chamber to a depth of less than 1 centimeter. The

Table 9: Details of Phytochemical compounds present

Sl.No	Compound	Test	Result
1	Phenols	Ferric chloride	+ + + 1.6095µg GAE/mL
2	Flavonoids	Alkaline reagent	+ + + 2.071µg QE/mL
3	Tannins	Neutral ferric chloride	_ _
4	Reducing sugars	Fehling solution test	_ _
5	Alkaloids	Wagners test	_ _
6	Terpenoids	Salkowski test	+

+++ (Presence in major amount)
+ (Present in less amount)
- (presence not detected)

Table 10: Details of compounds separated in Thin layer chromatography

No of the Spot	UV 254nm (Rf value)
1	0.12
2	0.28
3	0.36
4	0.40
5	0.60
6	0.74
7	0.92

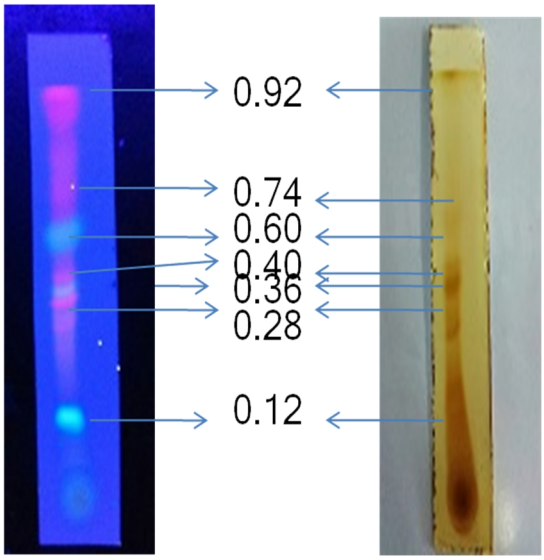

Figure 2: Compounds showing in Thin layer chromatography from *Pavonia procumbens*

container is closed with a cover glass or lid and is left for 10 minutes for saturation. The TLC plate is then placed in the chamber and allowed to run the chromatogram. The solvent moves up on the plate by capillary action meets the sample mixture and carries it up the plate (elutes the sample). The dried plate is placed in a chamber containing a few crystals of iodine. The iodine vapor in the chamber oxidizes the substances in the various spots making them visible to the eye. Once the spots are visible they may be outlined with a pencil before the iodine coloration fades.

RESULTS AND DISCUSSION

Collection of plant

Pavonia procumbens leaves were collected from Damanur hillocks, Gingee taluk, villupuram district of Tamil nadu, India.

Evaluation of in vitro antioxidant Potential

The result of dot- plot assay showed (Fig 1) that the Methanolic extracts of *Pavonia procumbens* appeared the purple colour of DPPH reaction to yellow, which indicates that the leaves of *P.procumbens* has active compounds responsible for antioxidant activity .

Antioxidant Activity

The DPPH radical scavenging activity of *P.procumbens* showed (Table 1) maximum activity of 85.2% compared with Ethyl acetate (84.6%) and Hexane (51.4%) extract at 500 µg/ml concentration. The degree of discoloration indicates that the P.procumbens showed free radical scavenging potentials due to the hydrogen donating ability. The Phosphomolypdenum assay and Reducing power assay of methanolic extract of P.procumbens showed (Table 2 & 3) high absorbance of 1.158 and 0.248 at 500 µg/ml concentration respectively.In nitric oxide scavenging activity and $Fe2^{+}$ Chelation activity of methanolic extracts *Pavonia procumbens* showed (Table 4 & Table 5) maximum activity of 88% and 61% at 500 µg/ml concentration respectively. The IC50 value of methanolic extracts of *Pavonia procumbens* for DPPH radical scavenging activity, Nitric oxide scavenging activity and Fe2+ chelation activity 200.41, 108.81 and 386.72 µg /ml respectively.

Antidiabetic activity

The antidiabetic activity of methanolic extract of *Pavonia procumbens* showed in Alpha amylase inhibition activity, Non enzymatic glycosylation of Haemoglobin and Glucose uptake by yeast cells with maximum activity of 79.15% at 25 µg/ml, 73.49% at 1250 µg/ml and 48.47% at 1250 µg/ml respectiviely. The IC50 value of methanolic extracts of *Pavonia procumbens* for Alpha amylase inhibition assay and non-enzymatic glycosylation of haemogolbin was 14.65 and 487.42 µg /ml respectively. Table 6, 7 and 8 indicate the percentage inhibition of methanolic extract from *Pavonia procumbens* at different antidiabetic assays.

Phytochemical Analysis

The qualitative and quantitative phytochemical analysis of methanolic extracts of *Pavonia procumbens* showed the presence of Phenol and flavonoid at significant level and terbenoid at moderate level. The quantitative estimation

of the methanolic extract show the phenol with 1.6095µg GAE/mL and the flavanoides with 2.071 µg QE/mL which indicates that the presence of higher amount of pheol and flavonoids play a vital role in antioxidant and antidiabetic activity. Table 9 shows the detailed picture of phytochemical profile of *Pavonia procumbens*.

Separation and isolation of biocompounds by TLC

The biocompounds present in the methanolic extract of *Pavonia procumbens* separated by Thin Layer Chromatorgraphy and it was found that 7 compounds were separated. The Rf value of the eluted spots for the *Pavonia procumbens* were 0.12,0.28,0.36,0.40,0.60,0.74 and 0.92 which exposed under UV light at 254 nm in the solvent ratio of 1:9 of methanol: chloroform. Table 10 indicates the details of biocompounds separated by Thin Layer Chromatography and it Rf values. In Fig. 2, the fluorescence of different compounds under UV light and iodine vapour were seen.

CONCLUSION

Among the solvents like Methanol, ethyl acetate and hexane used for the extraction and isolation of bioactive compounds from *Pavonia procumbens*, the methanolic extract show significant level of activity for antioxidant and antidiabetic compound which is in accordance with the earlier report of Gokani *et al*.[48]. The findings suggest that the DPPH radical scavenging ability of pavonia procumnbens methanolic leaf extract was significant when α-tocopherol was used as standard; it reveals the proton donating capacity of the extract. Velioglu *et al*.[49] studied the total phenolics and the antioxidant activity in selected fruits, vegetables and grain products which showed at significant level. The reducing capacity of the plant extract components as a significant indicator of its potential antioxidant activity was reported by Gupta *et al*.[50] which corroborates the present investigation. The presence of phenolic compounds which are dietary constituents widely existing in plants have been considered to have high antioxidant capacity and free radical scavenging capacity[51,52]. Research studies from many laboratories showed that the phenolic compounds were the main antioxidant ingredients in various medicinal plants[53]. Moreover, the flavonoids also are well-known antioxidant constituents of plants and possess a broad spectrum of chemical and biological activity, including radical scavenging properties[54]. The presence of significant level of phenolic compounds in *Pavonia procumbens* proves the high degree of antioxidant activity and moderate level of antidiabetic activity.

The Phyto-chemical screening of the crude extracts of *Pavonia procumbens* revealed that the presence of secondary compounds such as Phenols, Flavanoids and Terbenoids from methanolic extract attributed to their excellent antioxidant activity. The scavenging activities observed against DPPH, phosphomolybdenum, Fe 2^{+} reducing power, hydroxyl radicals and anti gluycosylation activities for alpha amylase, non enzymatioc glycosylation, glucose uptake by yeast cells, lead us to propose the above plant leaves as promising natural sources of antioxidants and antidiabetic which is suitable for application in nutritional/pharmaceutical fields. Further studies are needed to explore in vivo and clinical response for better understanding and exploration of further work.

ACKNOWLEDGEMENTS

The authors are very much thankful to M/s. Armats Biotek Pvt. Ltd., for the laboratory facilities to carry out the present project study.

REFERENCES

1. UmadeviM , Sampath KumarKP , Debjit Bhowmik, Duraivel.S Traditionally Used Anticancer Herbs In India .*Journal of Medicinal Plants Studies* 2013 :(3)1 ; 56-74.
2. Beal MF, Aging, energy and oxidations stress in neurodegenerative diseases. *Annals of Neurology* ;1995 38, 357-366.
3. Olajide AO, Eziyi AK, Olajide FO, Beyioku BK. Novel surgical extrication of penile constricting metal ring: A case report. *International Journal of Case Reports and Images* 2013; 4(9):511–514.
4. Klaunig JE, Kamendulis LM. The role of oxidative stress in carcinogenesis. *Annu Rev Pharmacol Toxicology* 2004; 44:239–267.
5. Valko M, Rhodes C, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-biological interactions*2006; 160(1):1–40.
6. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol.* 2007; 39(1):44–84.
7. Roberts RA, Laskin DL, Smith CV, Robertson FM, Allen EM, Doorn JA, Slikker W. Nitrative and oxidative stress in toxicology and disease. *Toxicological sciences* 2009; 112(1):4–16.
8. Pani G, Galeotti T, Chiarugi P. Metastasis: cancer cell's escape from oxidative stress. *Cancer Metastasis Rev*. 2010; 29(2):351–378.
9. Klaunig JE, Kamendulis LM, Hocevar BA. Oxidative stress and oxidative damage in carcinogenesis.*Toxicologic pathology* 2010; 38(1):96–109.
10. Pande D, Negi R, Khanna S, Khanna R, Khanna HD. Vascular endothelial growth factor levels in relation to oxidative damage and antioxidant status in patients with breast cancer. *Journal of breast cancer* 2011; 14(3):181–184.
11. Fuchs-Tarlovsky V. Role of antioxidants in cancer therapy. *Nutrition (Burbank, Los Angeles County, Calif)* 2013; 29(1):15–21.
12. Pervin S, Tran L, Urman R, Braga M, Parveen M, Li S, Chaudhuri G, Singh R. Oxidative stress specifically downregulates survivin to promote breast tumour formation. *Brit J Cancer* 2013; 108(4):848–858.
13. Bjelakovic G, Nikolova D, Gluud L L, Simonetti R G and Gluud C. Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis.

JAMA J. Am. Med. Assoc., 2007 ;297: 842–857.
14. Warner J, Epstein M, Sweet A, Singh D, Burgess J, Stranks S, Hill P, Perry-Keene D, Learoyd D, Robinson B, Birdsey P, Mackenzie E, Teh BT, Prins JB,Cardinal J. Genetic testing in familial isolated hyperparathyroidism: unexpected results and their implications. *J Med Genet*. 2004; 41(3):155-60.
15. Hamid N, Bukhari N and Jawaid F. Physiological responses of Phaseolus vulgaris to different lead concentrations. *Pak J Bot,*. ;2010 42: 239-246.
16. Suruchi Singh ,S.K Sharma, Lalit Singh .A review on medicinal plants having antioxidant potential. *Indian Journal of Research in Pharmacy and Biotechnology* .409-404:(3)1 ;2013
17. Rajandeep Kaur, Jagpreet Singh, Gagandeep Singh, Harpreet kaur. Anticancer plants: A Review. J. Nat. Prod. *Plant Resour*. 2011; 1 (4): 131-136.
18. Vineeta Tripathi and Janeshwer Verma. Current updates of indian antidiabetic medicinal plants. *International Journal of Research in Pharmacy and Chemistry* 2014; 4(1): 114-118.
19. Abate N. Chandalia M. Ethnicity type 2 diabetes & migrant Asian Indians. *The Indian Journal of Medical Research* 2007; 125(3): 251-258.
20. Plengsuriyakarn T, Viyanant V, Eursitthichai V, Picha P, Kupradinun P, Itharat A and Na-Bangchang K. Anticancer activities against cholangiocarcinoma toxicity and pharmacological activities of Thai medicinal plants in animal models. *BMC Complementary and Alternative Medicine* 2012; 1-19.
21. WHO,Monographs on selected medicinal plants, 1999.Vol.1.
22. Aliyu AB, Musa AMO, shanimi JA, Ibrahim HA, Oyewale AO. Phytochemical analyses and mineral elements composition of some medicinal plants of northern Nigeria. *Nigerian Journal of Pharmaceutical Sciences* 2008; 7(1):119-125.
23. Rajesh V, Sarthaki R, Palani R and Jayaraman P. In vitro evaluation of *Memecylon umbellatum* Burm.F for antihyperglycemic activity and phytochemical potential. *Int. Journal of Pharmacognsoy And Phytochemical Research* 2014**;** 6:(4).
24. Palani R, Karunakaran D, Rajesh V, Mathivanan K and Jayaraman P. Analysis of Antioxidant Antimicrobial Activity and Phytochemical Potential of *Cleistanthus colli nus* Roxb. *Polygonum glabrum* Wild. and *Melia azedarch* Linn. *Asian J. of Medical and Pharmaceutical Sciences* 2014; 2(2):108-118.
25. Rajesh V, Mohanraj G, Palani R, Mathivanan K, Sindhu S and Jayaraman P. Investigation of Antioxidant and Antiproliferative activity of *Indigofera trifoliata* Linn. against Hep G2 cell line. *Asian J. of Medical and Pharmaceutical Sciences* 2014; 2(2):119-127.
26. Jayaraman P, Sivaprakasam E, Rajesh V, Mathivanan K, Arumugam P. Comparative analysis of antioxidant activity and phytochemical potential of *Cassia absus* Linn. *Cassia auriculata* Linn. and *Cassia fistula* Linn. *Ind. J. of Drugs & Diseases* 2014; 3(1):298-304.
27. Mathivanan.K., E.Sivaprakasam, R.Palani, V.Rajesh and P.Jayaraman, 2014. Analysis of antimicrobial activity and phytochemical potential of *Cassia absus* Linn., *C. auriculata* Linn. and *C. fistula* Linn. *Indian Journal of Medicine and Healthcare*, 3 (1): 314-321.
28. Mahadevan A and Sridhar R. Methods in physiological plant pathology.4th Edn. Sivakami Publications Chennai India, 1996, 154-161.
29. Purvis MJ, Collier DC and Walls D. Laboratory techniques in Botany. Second edition. Butterworth Company Ltd. London, 1966, 439.
30. Eloff JN. Which extractant should be used for the screening and isolation of antimicrobial components from plants? Journal of Ethnopharmacology 1998; 60:1-8. Chang ST, Wu JH, Wang SYand Kang PL. Antioxidant activity of extracts from *Acacia confesa* barkand heartwood. *Journal of Agricultural and Food Chemistry* 2008; 49:3420-3424.
31. Chang ST, Wu JH, Wang SYand Kang PL. Antioxidant activity of extracts from Acacia confesa barkand heartwood. Journal of Agricultural and Food Chemistry 2008; .3424-49:3420
32. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal. Biochem*. 1999; 269:337–341.
33. Oyaizu M. Studies on product of browning reaction prepared from glucose amine. *Jpn. J. Nutr*. ;198644: 307- 315.
34. Sreejayan N and Roa MNA. Nitric oxide scavenging by curcuminoids. *Journal of Pharmacy and Pharmacology* 1997; 49: 105- 107.
35. Dinis TCP, Madeira VMC, Almeida MLM. Action of phenolic derivates (acetoaminophen, salycilate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Arch. Biochem. Biophys.* ;1994315: 161-169.
36. Worthington V. Worthington Enzyme Manual. Freehold: Worthington Biochemical Corp; 1993. Alpha amylase; pp. 36–41.
37. Narkhede MB, Ajimire PV, Wagh E, Manoj M, Shivashanmugam AT. *In vitro* antidiabetic activity of *Caesalpina digyna*(R.) methanol root extract. *Asian Journal of Plant Science and Research* 2011; 1(2):101-106.
38. Cirillo VP.Mechanism of Glucose Transport Across .the Yeast Cell Membrane *J. Bacteriol*. ;1962 84491-485:(3).
39. Chandrashekar, Daksha G, Lobo R, Yogendra, Nilesh G. In-vitro Antidiabetic activity of stem bark of Bauhinia purpurea Linn. *Der Pharmacia Lettre* 2012; 4(2):614–619.
40. Evans WC. Trease and Evans Pharmacognosy. 14th Edition. Harcourt Brace and company. Asia Pvt. Ltd. Singapore, 1997, 343.
41. Mace ME, Histochemical localization of phenols in healthy & diseased tomato roots. *Phytopathology 16* 1963; 915-925.
42. Ramakrishna GN, Prasad BL and Azeemoddin G.

Cold-processing of neem seed. Proceedings of the World Neem Conference (WNC'93) Bangalore India, 1994, 54-54.
43. Kokate CK. Practoca Pharmacognosy. 4th edition, Vallaph Prakash Publication, New Delhi India, 1999, 115.
44. Rasch E and Swift H. Microphotometric analysis of the cytochemical Millon reaction. *Journal Histochemistry Cytochemistry* 1960; 8: 4–17.
45. Yadav RD, Jain SK, Alok S, Mahor A, Bharti JP and Jaiswal M. Herbal plants used in the treatment of urolithiasis: a review. *Intl. J. Pharm. Sci. Res* 2011; 2(6): 1412-1420.
46. McDonald S, Prenzler PD, Autolovich M, Robards K. Phenolic content and antioxidant activity of olive extracts. *Food Chem*. 2001; 73:73-84.
47. Hao T, Quach Robert L, Steeper G, William Griffin. An Improved Method for the Extraction and Thin-Layer Chromatography of Chlorophyll a and b from Spinach. Journal of Chemical Education 2004**;** 38:(3)81
48. GokaniRH , RachchhMA , PatelTP , LahiriSK , SantaniDD and ShahMB Evaluation of anti-oxidant activity (in vitro) of Clerodendrum phlomidis Linn.f. suppl. Root. *Journal of Herbal Medicine and Toxicology*. ;2011 **..**53-47:(1) 5
49. VeliogluYS **,** MazzaG , GaoL and Oomah **.**BD Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. *Journal of Agricultural and Food Chemistry.*;1998 -46:4113 **.**4117
50. GuptaR**.**, Bruce Bleakley and Gupta**.**RK Phytochemical analysis and antioxidant activity of herbal plant Doronicum hookeri Hook (Asteraceae). *Journal of Medicinal Plants*. ;2011 :(13)5 .2742-2736
51. KahkonenMP , HopiaAI and Heinonen .M Berry phenolics and their antioxidant activity. *Journal of Agricultural and Food Chemistry*.;2001 :49 -4076 **.**29 .29 .4082
52. RobardsK , PrenzlerPD **,** TuckerG , SwatsitangP and Glover**.**W Phenolic compounds and their roles in oxidative process in fruits. *Food Chemistry*.:66 ; 1999 .436-401
53. CaiYZ , LuoQ , SunM and Corke**.**H Antioxidant activity and phenolic compounds of 112traditional Chinese medicinal plants associated with anticancer. *Life Sciences.* .2184-2157 : 74; 2004
54. Miliauskas G , VenskutonisR P and Van Beek**.**TA Screening of radical scavenging activity of some medicinal and aromatic plants. *Food Chemistry*.;2004 :85 .237-231