Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2014-15; 6(4); 1024-1031

ISSN: 0975-4873

Research Article

Phytochemical Potential of *Euphorbia hirta* Linn. and *Strychnos nux-vomica* Linn. With Reference to Antidiabetic and Antioxidant Properties

Mathivanan K, Rengasamy D, Rajesh V, Palani R, *P.Jayaraman

PG and Research Department of Botany, Government Arts College for Men (Autonomous), Nandanam, Chennai 600 035, Tamil Nadu, India.

Available Online: 1st December, 2014

ABSTRACT

Since the synthetic drugs are more costly and lead to side effects the usage of herbal medicines are getting importance due to promising results and less or no side effects. The plant containing active biological compounds are prepared from various parts of medicinal plants and used as drugs for curing diseases including diabetes and tumor diseases to human and proved for controlling blood glucose level and protect cells against the damaging effects of free radicals. Therefore the present study involved in assessing of antidiabetic and antioxidant efficacy of plants like *Euphorbia hirta Linn.* and *Strychnos nux-vomica Linn.* collected from jawadhu hills of Tamil Nadu India. The assay methods like inhibitory assay of α-amylase non enzymatic glycosylation of hemoglobin for antidiabetic evaluation and DPPH assay for antioxidant were performed. Among the solvents like chloroform ethyl acetate and methanol used for extraction from both the plants the methanol extract showed higher activity. The results for the antidiabetic assay for α-amylase inhibition exhibited from 4.4% - 35.7% and glycosylation of hemoglobin shows 22% - 55% in the concentration of 250 - 1000 µg/ml. The antioxidant potential of the extracts were found moderate to significant in both the samples with remarkable radical scavenging activity. The IC_{50} values were recorded as 108 and 73.41µg/ml for *E.hirta* and *S.nux-vomica* Linn. respectively. The phytochemical analysis of *E.hirta* Linn. and *S.nux-vomica* Linn. extracts revealed the presence of phenols (79.99 GAE/g) flavonoids (388 µg/ml) terpenoids tannins saponins and proteins; the absence of alkaloids glycosides and reducing sugars in the methanol extract. This study presents a review on the *in vitro* anti-diabetic and antioxidant effect of *E.hirta* Linn. and *S.nux-vomica* Linn. which leads to the utilization of these plants as a potential source of bio-compounds for development of herbal medicine..

Key words: Anti-diabetic activity Antioxoidnat activity phytochemical analysis Euphorbia hirta Strychnox nux-vomica α-amylase assay anti-diabetic assays Radical scavenging activity DPPH assay.

INTRODUCTION

From the time immemorial man is trying to control diseases through various ways and different medicinal plants have been contributed a lot in this regard from time to time. Plant derived medicines have been part of traditional health care in most of the world for thousands of years[1]. Herbal medicines are the oldest remedies known to mankind. In the present scenario the demand for herbal products is growing exponentially throughout the world and major pharmaceutical companies are currently conducting extensive research on plant materials for their potential medicinal value. . It also plays an important role in the management of diabetes mellitus especially in developing countries. 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[2]. Diabetes is arising from complex interactions between multiple genetic and environmental factors. 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) both are common and serious metabolic disorder throughout the world. The insulin deficiency results in increased concentration of glucose in the blood. 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. It has the largest number of diabetic patients in the world and has been infamously known as the 'diabetic capital of the world'[3] . The prevalence of diabetes mellitus is on increase and needs to be addressed appropriately.

The prevalence of diabetes mellitus is on increase and needs to be addressed appropriately. More than 400 traditional plant treatments for diabetes mellitus have been recorded but only a small number of these have

*Author for correspondence

Table 1: Details of α – Amylase assay by *E.hirta* and *S.nux-vomica*

Sl. No.	Concentration (mg/ml)	% of inhibition (*E.hirta*)	% of inhibition (*S.nux-vomica*)
1	25	4.411	1.470
2	50	14.705	3.431
3	75	25.490	10.294
4	100	35.784	17.156

received scientific and medical evaluation to assess their efficacy. According to the World Health Organization[4] has recommended the evaluation of traditional plant treatments for diabetes as they are effective non-toxic with less or no side effects and are considered to be excellent candidates for oral therapy [5] and also listed 21000 plants which are used for medicinal purposes around the World. Among these 2500 species are in India out of which 150 species are used commercially on a fairly large scale. India is the largest producer of medicinal herbs and is called as Botanical garden of the World[6]. In this study area herbal remedies are considered convenient for management of type 2 diabetes with postprandial hyperglycaemia due to their traditional acceptability and availability low costs and lesser side effects. Herbal drugs are prescribed due to their good effectiveness fewer side effects in clinical experience and relatively low costs[7]. In the modern therapy of diabetes mellitus insulin sulfonylurea's biguanides α-glycosidase inhibitors and glinides etc provide excellent relief from the acute symptoms of the disease. These drugs lend to cause side effects like nausea vomiting abdominal pain diarrhoea head ache abnormal weight gain allergic reaction low blood glucose darkurine fluid retention or swelling[8]. 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 20-25years[9]. World health organization[4] in a number of resolutions emphasized the need to ensure the quality control of plant products by using modern techniques and applying suitable standards[10]. Hence some of the indigenous herbal plants have been reported to showing efficacy in the treatment and control of diabetes[11,12]. Our earlier studies of phytochemical potential ant-iglycemic activity and antioxidant activitry on *Memecylon umbellatum* Burn.F. *Cleistanthus colli nus* Roxb. *Polygonum glabrum* Wild. and *Melia azedarch* Linn. *Indigofera trifoliata* Linn. *Cassia absus* Linn. *Cassia auriculata* Linn. and *Cassia fistula* Linn. showed the methanol extracts of the plant parts like leaves and seeds had moderate anti-diabetic and antioxidant acticvity[13,14,15,16] which gives further support for the use of plants in control of diabetics. With reference to the above scientific information the present study was planned to assessing the antidiabetic efficacy of plants like *Euphorbia hirta L.* and *Strychnos nux-vomica L.* from jawadhu hills Tamil Nadu India.

MATERIALS AND METHODS

Plant collection: Fresh leaves of *Euphorbia hirta* Linn. and *Strychnos nux-vomica* Linn. were collected from the fields of Jawadhu Hills Thiruvannamalai District Tamil Nadu. These plants belong to class magnoliopsida of Dicotyledons and family of Euphorbiaceae and Logoniaceae respectively.

Preparation of Plant extracts: The leaves of *Euphorbia hirta* Linn. and *Strychnos nux-vomica* Linn 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. 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[17]. These extracts were diluted with respective solvents and used to perform various *in vitro* anti-diabetic assays and other phytochemical parameters.

The cleaning and preparation of glassware were followed after Mahadevan and Sridhar (1996)[18]. General laboratory techniques recommended by Purvis *et al* (1966)[19] was followed for the preparation of media inoculation and maintenance of cultures.

Inhibition of α-amylase activity: The test sample 500µl and standard drug (100-1000µg/ml) were added to 500µl of 0.20 mM phosphate buffer (pH 6.9) which containing α-amylase (0.5mg/ml) solution in individual test tubes. The contents were incubated at 25°C for 10 min period. Then 500µl of a 1% starch solution and 0.02 M sodium phosphate buffer (pH 6.9) were added to the test tubes containing the reaction mixture. The reaction mixtures were then incubated at 25°C for 10 min and the reaction was stopped by adding 1.0 ml of 3 5 di nitro salicylic acid reagent. The test tubes were then cooled to room temperature and the reaction mixture was diluted by adding 10 ml distilled water for measuring the Optical Density (OD) at 540 nm[20].

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

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[20]. Metformin was used as a standard drug for assay and % inhibition was calculated using the formula

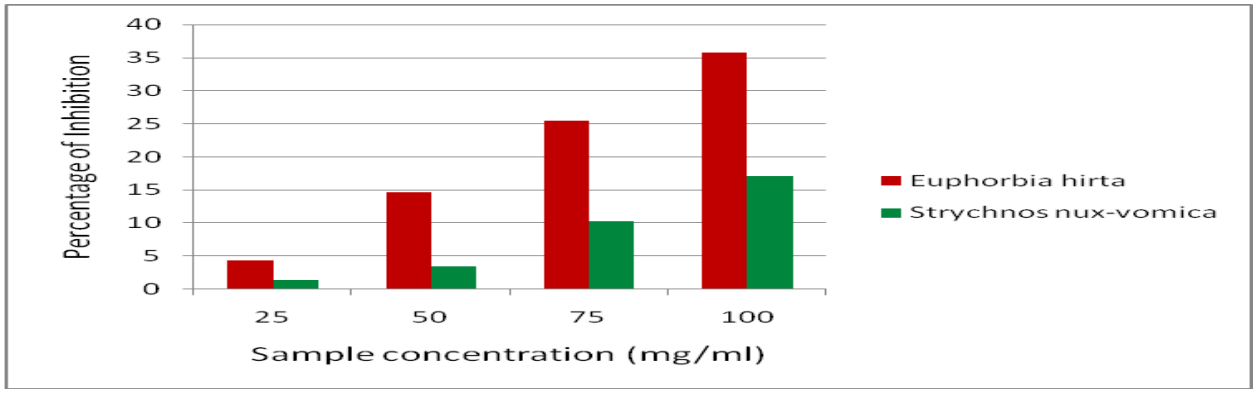


Fig 1. Comparison of α – Amylase activity by E.hirta and S.nux-vomica

Table 2: Non-enzymatic glycosylation of Haemoglobin of *E.hirta* and *S.nux-vomica*

Sl. No.	Concentration (mg/ml)	% of inhibition (*E.hirta*)	% of inhibition (*S.nux-vomica*)
1	250	22.028	25.069
2	500	29.947	26.098
3	750	46.520	29.765
4	1000	55.091	34.866

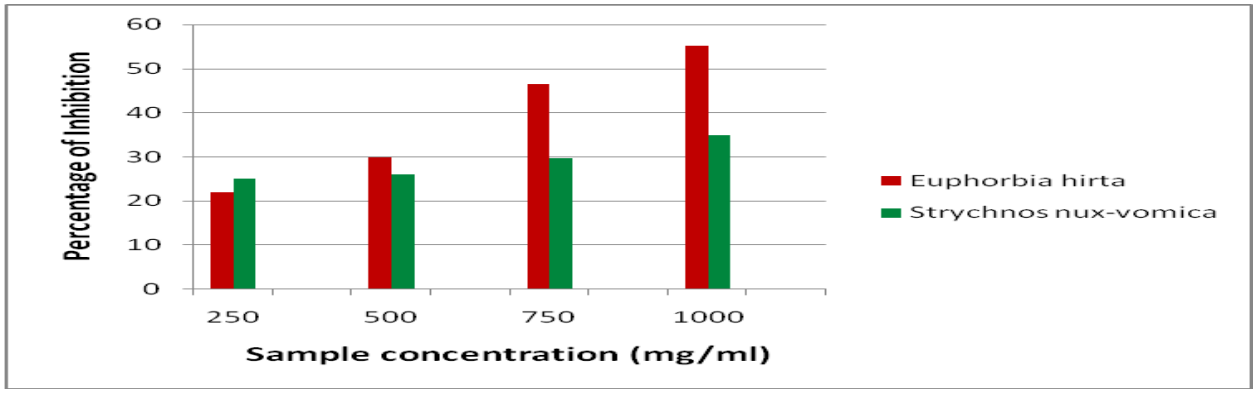


Fig. 2: Comparative analysis of Non-enzymatic glycosylation of Haemoglobin by E.hirta and S.nux-vomica

% inhibition of glycosylation =

$$(Abs\ sample - Abs\ control | Abs\ sample) * 100$$

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.

Antioxidant activity assay - 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* (2008)[21] 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

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[22].

Detection of phenolic compound (Ferric chloride test by Mace 1963[23]**):** 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[22].

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.

Table 3: DPPH Radical Scavenging effect of *E.hirta* and *S.nux-vomica*

Sl. No.	Concentration (µg /ml)	RSA (%) *E.hirta*	*S.nux-vomica*
1	20	10.648	28.62
2	40	18.981	38.045
3	60	30.425	44.942
4	80	38.425	54.482
5	100	46.296	62.643

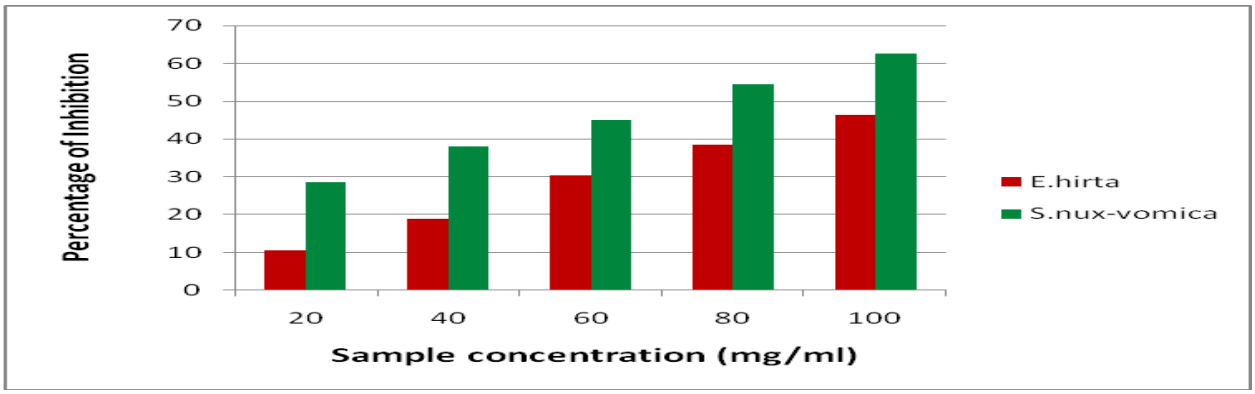


Fig. 3: Comparative Radical Scavenging effect of E.hirta and S.nux-vomica

Table 4: Phytochemical analysis of *E.hirta & Strychnos nux-vomica*

S.no	Phytochemicals	Resuts *E.hirta*	*S.nux-vomica*
1	phenolic compound	+++ (79.99 GAE/g)	++ (82.80 GAE/g)
2	Glycosides	--	--
3	Flavonoids	++ (1234.94 QE/g)	++ (1374.46 QE/g)
4	Terpenoids	++	++
5	Tannins	+++	+++
6	reducing sugars	++	++
7	Saponins	_	-
8	Proteins	--	--

Table 5 : Compounds separated from E.hirta and *S.nux-vomica* in in TLC

S.No	Ratio	*E.hirta* Rf value UV long/ UV short	*S.nux-* Rf value UVlong/ UV short	*vomica*	*E.hirta* Rf value Iodine	*S.nux-vomica* Rf value Iodine
1	1:9	0.35	0.48			
2	1:9	0.50	0.60		0.676	-
3	1:9	0.67	0.71			
4	1:9	0.82	0.82			

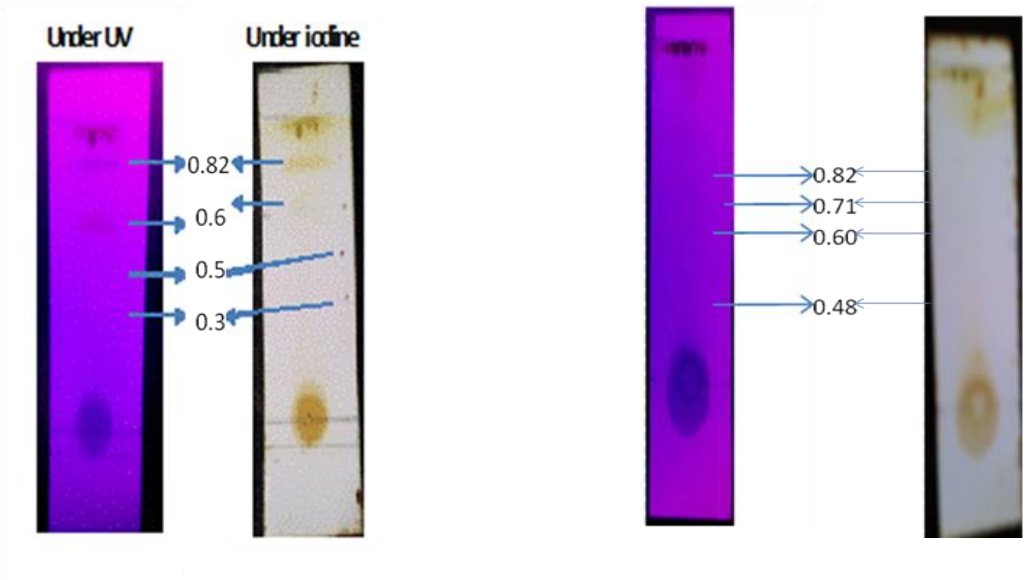
Detection of flavonoids:. The sample extract 0.5g was dissolved in 5mL of Distilled water and filtered. Dilute ammonia (5mL) was added to 1mL of the extract filtrate. Concentrated sulphuric acid (1mL) was added. Yellow colorations that disappear on standing indicate the presence of flavonoids.

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[24]: 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 (Foam test by Kokate 1999[25]): 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[26]: 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



Under UV *Under Iodine* *Under UV* *Under Iodine*
E. hirta *S. nux-vomica*

Fig. 4: Sample spots separated from E.hirta and S.nux-vomica in TLC

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[27]: 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 - FolinCiocaltaeu's method[28]: The total phenol content of the extract was measured at 765 nm by Folin-Ciocalteu reagent. 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 *Euphorbia hirta and Strychnosnux-vomica plants* was achieved after the method of Hao *et al.* (2004)[29]**.** 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 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 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

Extraction: Extracts of the *Euphorbia hirta* and *Strychnos nux-vomica* was obtained using coarse powder of leaves. The dried powder was extracted with different kinds of polar and non polar solvents such as chloroform ethyl acetate and methanol. Among the solvents used for extraction methanol found to be suitable solvent system which shows significant values of various phytochemical parameters tested**.**

Inhibition of α - Amylase Activity: Alpha amylase inhibitors of the sample bind to alpha- bond of polysaccharide and prevent break down of polysaccharide into mono and disaccharide. *In vitro* inhibitory assay of α-amylase was performed using the methanol extracts of *Euphorbia hirta* and *Strychnos nux-vomica*. From the data obtained from the present study it indicates that the methanol extract of *E.hirta* and *S.nux-vomica* showed significant inhibitory activity with inhibition percentage of 4.4 – 35.7% and 1.47 – 17.15% respectively with the concentration of 25 - 100µg/ml. The details of results were obtained presented in Table 1 and Fig. 1. The sample of *E.hirta* showed higher inhibition percentage (35.78%) when compared to *S.nux-vomica* (17.15%) at the higher concentration like 100 mg/ml..

Glycosylation of Haemoglobin: The haemoglobin present in the red blood corpuscles has a tendency to get bound to glucose. The greater the blood-glucose concentration the greater is the amount of glucose-bound haemoglobin. As the concentration of drug increases formation of glucose-haemoglobin complex decreases and free haemoglobin increases which show the inhibition of glycosylated haemoglobin. The inhibitory activity of methanolic extract of *Euphorbia hirta and Strychnosnux-vomica* were found and compared with the standard drug acarbose. Results showed that the individual extracts *E.hirta and S.nux-vomica* having comparable inhibitory activity with the standard drug which is presented in Table 2 and Fig. 2.

Radical scavenging activity (RSA) of *E.hirta* and *S.nux-vomica* extracts (DPPH assay): Radical scavenging activity of the *E.hirta* and *Strychnos nux-vomica* observed that the Methanol extract had higher scavenging activity than ethyl acetate and chloroform (Table 3 and Fig.3). At a concentration of 100µg/mL the scavenging activity of Methanol extract of *E.hirta* reached 46% and *S.nux-vomica* reached 62%. The Methanol leaf extract of *E.hirta* and *S.nux-vomica* showed excellent antioxidant and free radical scavenging activity. In considering this the Methanol leaf extract was chosen for further study. The Ic 50 value for *E.hirta* is 108µg/mL and Ic 50 value for *S.nux-vomica* is 73.41 µg/mL.

Qualitative phytochemical screening: These screening for the phytochemical tests revealed the presence of various plant constituents in the plant extracts. Among the various phytoconstituents phenols flavonoids terpenoids reducing sugars and tannins were present in *E.hirta* and *S.nux-vomica*. Other compounds like glycosides saponins and protiens were absent (Table 4).

Quantitative determination of Flavonoids and Phenolic compounds: Quantitative analysis of the methanol extract of plant was revealed that the flavonoid was present in significant amount as 1234.94 QE/g and 1374.46 QE/g in *E.hirta* and *S.nux-vomica* respectively (Table 4). The phenolic compound shows that the amount of was fairly low when compared to other constituents which is equivalent to the standard Gallic acid found to be 79.99 GAE/g and 82.8 GAE/g in *E.hirta and S.nux-vomica* respectively. The Flavonoid compound shows that the amount of was fairly low when compared to other constituents which is equivalent to the standard Quercetin found to be 1234.94 QE/g and 1374.46 QE/g in *E.hirta and S.nux-vomica* respectively (Table 4).

Separation of Compounds by Thin Layer Chromatography (TLC): The solvent system used for chromatogram in the ratio 1:9 (Methanol:Chloroform v/v) which shows better separation of compounds. Under the influence of UV 4 compounds were recognized and the Rf value (retention factor) were calculated. Iodine was used as a reaction reagent for the compounds present in the chromatogram and 4 compounds were recognized. The Rf values of the spots from *E.hirta* were calculated and found in iodine vapouras as 0.67 fluoresced in UV light as blue colour. Other spots of *E.hirta* in Rf value as 0.35 0.5 0.67 and 0.82 are appeared as red colour indicates which are grouped in same category of compounds (Table 5 and Fig. 4**).** There is no compound fluoresced from *S.nux-vomica* in iodine vapour. Other spots of *S.nux-vomica* showed as Rf value of 0.48 0.60 0.71 and 0.83 are appeared in UV light as red colour indicates which are grouped in same category of compounds (Table 5 and Fig. 4). From the present study it is presumed as these compounds might be responsible for anti-diabetic activity of plant *E. hirta* and *S. nux-vomica*. The specific compound (band) which has anti oxidative properties was found out as the compound with R_f value of 0.82 (Fig. 4). The antioxidant activity was confirmed when the DPPH purple color changed to yellow.

SUMMARY AND CONCLUSION

It is evident that various medicines used at present day for human diseases have been commonly originated from the naturally available plants. These plant and herbal medicines are increasingly used to treat many diseases such as asthma chronic fatigue rheumatoid arthritis premenstrual syndrome irritable bowel syndrome cancer and many others including diabetes[30,31]. Hence it is prudent to find out the various possible ways to treat Diabetes and other diseases due to cellular damages resulted to radical scavengers using the plant extracts. Herbal plant products may even replace the synthetic drugs as they are safe to use effective no side effects and reduce the cost related with the treatment.

In recent times plant remedies have been offering higher promises in the management of Diabetes mellitus. Plants provide large range of natural compounds which has various properties to treat and cure the widespread of diabetes. Many bioactive drugs isolated from the plant showed anti-diabetic activity and more effective than some of the oral hypoglycemic agents such as daonil tolbutamide and chlorpropamide[31] . These findings add evidence and support to present study regarding results of anti-diabetic activity of the plant extracts from *E.hirta and S.nux-vomica.* Research studies have proven that several plant species possess hypoglycemic effects and they can be highly potent in the management of hypoglycemic actions of Diabetes mellitus which is a simple accessible method for the management of

Diabetes. This study presents a review on the *in vitro* anti-diabetic and Antioxidant effect of *E.hirta* and *S.nux-vomica* in the assessment of diabetes and anti-proliferative capability which provides promising results for the utilization of the extracts of the plant as a formulation for the drug. The main aim of this study involves the identification of the active compound in the plant which can play an important role in the hypoglycemic actions for the treatment of diabetes which is similar to that of the results shown from *Memecyon umbellattum* studied by Rajesh *et. al.*[33]. This work highlights the use of chromatogram to identify and purify the desired compound. The basis of the work revolves around the use on desired solvents to extract the active compounds and further investigated by the *in vitro* anti-diabetic assay. *In vitro* anti-diabetic assays involved

several carbohydrate digesting enzymes which prevent the breakdown of carbohydrates into glucose thus lowering the glucose level in the body which provides a remedy for the treatment of diabetes mellitus.

The potential antioxidant properties of the plants *E.hirta and S.nux-vomica* of the present study shows the therapeutic uses of medicinal plants in various ailments including diabetes which is similar to that of the findings of Rajamurugan *et al*[34]. who reported the medicinal importance of plants. Free radicals are known to play a definite role in a wide variety of pathological manifestations. Antioxidants fight against free radicals and protect us from various diseases. They exert their action either by scavenging the reactive oxygen species or protecting the antioxidant defence mechanisms[35]**.** The ROS play an important role in several human diseases such as cancer neurodegenerative disorders cardiovascular diseases atherosclerosis cataracts and inflammation[30]. The electron donation ability of natural products can be measured by 22′-diphenyl-1- picryl hydrazyl radical (DPPH) purple-coloured solution bleaching. The method is based on scavenging of DPPH through the addition of a radical species or antioxidant that decolourizes the DPPH solution. The degree of colour change is proportional to the concentration and potency of the antioxidants. A large decrease in the absorbance of the reaction mixture indicates significant free radical scavenging activity of the compound under test. In the present study among all the fractions tested methanol extract showed significantly higher inhibition percentage and positively correlated with total phenolic content and total flavonoid content. Results of this study suggest that the plant extract contain phytochemical constituents that are capable of donating hydrogen to a free radical to scavenge the potential damage[35,36,37]**.**

Phytochemical screening identified different active compounds such as alkaloid flavonoid phenolic compounds and these compounds were investigated for several in vitro assays and showed inhibitory activities which provide a basis for the plant extract to be used in the treatment of diabetes[13] and anti-proliferative activity[15]. Among the various extracts methanol extract of leaves of *Euphorbia hirta* and *Strychnus nux - vomica* possessed higher anti-diabetic and antioxidant activity and this study offers the use of the plant extract in the management and treatment of diabetes and abnormal cell proliferation. Further other active compounds from the plant must be investigated and its potency to be tested for its safety in order to develop plant remedies on a universal basis and in the development of several other new drugs.

ACKNOWLEDGEMENTS

Authors thankful to Dr.P.Arumugam, Director, Armats Biotek (P) Ltd., Chennai for providing laboratory facilities and valuable suggestions for the above research work.

REFERENCES

1. Modak M, Dixit P, Londhe J, Ghaskabdi S, Devasagayam TPA. Indian herbs and herbal drugs used for the treatment of diabetes. *Journal of Clinical Biochemistry and Nutrition* 2007; 40:163-173.
2. 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.
3. Abate N. Chandalia M. Ethnicity type 2 diabetes & migrant Asian Indians. *The Indian Journal of Medical Research* 2007; 125(3): 251-258.
4. WHO,Monographs on selected medicinal plants, 1999.Vol.1.
5. Patel K and Srinivasan K. Plant foods in the management of diabetes mellitus: Vegetables as potential hypoglycemic agents. *Nahrung* 1997; 41:68-74.
6. Seth SD and Sharma B, Medicinal plants of India. *Indian J. Med. Res*.2004; 120: 9 –11.
7. Ren J, Gintant GA, Miller RE, Davidoff AJ. High extracellular glucose impairs cardiac E-C coupling in a glycosylation-dependent manner. *Am J Physiol*.1997; 273 (6): H2876-83.
8. Anbu N. Musthafa Velpandian V. Anti-Diabetic Activity of Polyherbal Formulation *Aavaraiyathichurnam*in Alloxan Induced Diabetic Rats. *International Journal of Toxicological and Pharmacological Research* 2012; 4(4):77-80.
9. 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.
10. 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.
11. Irshad N, Mohit K. Evaluation of antidiabetic potentiality of methanolic extract of *Wedelia chinensis* whole plant. *Int J Pharm Biomed Res* 2013; 4(4) 197-201.
12. Manikandan R, Vijaya AA and Durai MG . Phytochemical and in vitro anti-diabetic activity of methanolic extract of *Psidium guajava* leaves. *Int. J. Curr. Microbiol. App. Sci*. 2013; 2(2):15-19.
13. 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).
14. 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.
15. 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.
16. 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.
17. Eloff JN. Which extractant should be used for the screening and isolation of antimicrobial components from plants? *Journal of Ethnopharmacology* 1998; 60:1-8.
18. Mahadevan A and Sridhar R. Methods in physiological plant pathology.4th Edn. Sivakami Publications Chennai India, 1996, 154-161.
19. Purvis MJ, Collier DC and Walls D. Laboratory techniques in Botany. Second edition. Butterworth Company Ltd. London, 1966, 439.
20. 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.
21. 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.
22. Evans WC. Trease and Evans Pharmacognosy. 14th Edition. Harcourt Brace and company. Asia Pvt. Ltd. Singapore, 1997, 343.
23. Mace ME, Histochemical localization of phenols in healthy & diseased tomato roots. *Phytopathology 16* 1963; 915-925.
24. 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.
25. Kokate CK. Practoca Pharmacognosy. 4th edition, Vallaph Prakash Publication, New Delhi India, 1999, 115.
26. Rasch E and Swift H. Microphotometric analysis of the cytochemical Millon reaction. *Journal Histochemistry Cytochemistry* 1960; 8: 4–17.
27. 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.
28. McDonald S, Prenzler PD, Autolovich M, Robards K. Phenolic content and antioxidant activity of olive extracts. *Food Chem*. 2001; 73:73-84.
29. 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; *81*(3):38
30. Dinesh TCP, Madeira VMC, Almeida MLM. Action of phenolic derivatives (acetoaminophen salycilate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Arch. Biochem. Biophys* 1994; 315:161-169.
31. Grover JK, Yadav S, Vats V. Medicinal plants of India with anti-diabetic potential. *J. Ethnopharmacol* 2002; 81:81-100.
32. Jung M, Park M, Lee HC, Kang YH, Kang ES, Kim SK. Antidiabetic Agents from Medicinal Plants. *Current Medicinal Chemistry* 2006; 13(10):1203-1218.
33. 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
34. Rajamurugan R, Deepa V, Sivashanmugam M and Raghavan CM. Phytochemistry antioxidant and antibacterial activities of medicinal plants- a comparative study. *International Journal of Current Research and Review* 2013; 5:08-19.
35. Saeed N, Muhammad R, Khan Maria Shabbir. Antioxidant activity total phenolic and total flavonoid contents of whole plant extracts of *Torilis leptophylla. L. BMC Complementary and Alternative Medicine* 2012; 12:221.
36. Soon Huat Tiong, Chung Yeng Looi, Hazrina Hazni , Aditya Arya, Mohammad,javad Paydar, Won Fen Wong, Shiau-Chuen Cheah, Mohd Rais Mustafa and Khalijah Awang. Antidiabetic and Antioxidant Properties of Alkaloids from *Catharanthus roseus* (L.) G. *Don. Molecules* 2013; 18: 9770-9784.
37. Ashok kumar B.S Saleemullakhan Gopi Setty Saran R.Nandeesh &N.K. Manjunath Invitro antidiabetic activity of Nisamalaki Churna. *Sains Malaysiana* 2013; 42(5) 625-628.