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PHARMACOGNOSTICAL AND ANTIDIABETIC ACTIVITY OF LEAVES OF CALOTROPIS PROCERA WILD

Vivek Tiwari[1]* and Vimal Kumar Yadav[2]

[1]Assistant Professor, Department of Pharmacy, Shri Ramdham Mahavidyalay, Mauranipur, Jhansi, U.P.

[2]Assistant Professor, Institute of Pharmacy, Dr. Rammanohar Lohia Avadh University, Ayodhya, U.P.

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***Corresponding Author**
Vivek Tiwari
Assistant Professor,
Department of Pharmacy,
Shri Ramdham
Mahavidyalay, Mauranipur,
Jhansi, U.P.

ABSTRACT

Herbal plants are known for their anti-diabetic and other various therapeutic activities. Mostly the active chemical moieties of the herbal plants are flavonoids in nature. The side effects associated with the usage of synthetic antidiabetic drugs make it imperative to search for alternative drugs from medicinal plants. Pharmacological studies have revealed that aqueous and organic extracts various parts of Calotropis procera and its phytoconstituents such as cardenolides, terpenes, flavonoids enzymes and other chemical constituents possess a wide range of pharmacological activities such as cytotoxic, antidiabetic, antioxidant, antiarthritic, antimicrobial, wound healing. Preliminary phytochemical identification was done along with measurement of the leaf constants, fluorescence characteristics, and extractive values. The presented research summarizes the information about the phytochemical and pharmacological activity of the Calotropis procera.

KEYWORDS: Herbal plants, Calotropis procera, Antidiabetic, Antioxidant, Antimicrobial, Antidiabetic.

INTRODUCTION

Herbal medicines and their preparations have been widely used traditionally, for the thousands of years in developing and developed countries owing to its natural origin and lesser side effects or dissatisfaction with the results of synthetic drugs. One of the characteristics of oriental herbal medicine preparations is that all the herbal medicines, either

presenting as single herbs or as collections of herbs in composite formulae (Balammal et al., 2012). Attention is being focused on the investigation of efficacy of plant based drugs used in the traditional medicine because they are economy, have a little side effects and according to W.H.O, about 80% of the world population rely mainly on herbal remedies (Patel et al., 2012). The uses of traditional medicines are widely spread and plants represent a large source of natural chemicals that might serve as leads for the development of the novel drugs. Scientists have devised different ways of alienating the problem and one of the easy and cheapest options is herbal medicines. Herbs have been in use since long time to treat various diseases. Almost one fourth of pharmaceutical drugs are derived from botanicals (Brown et al., 1959)

Herbal medicine

Herbal medicines are being used by about 80% of the world population primarily in the developing countries for primary health care. They have stood the test of time for their safety, efficacy, cultural acceptability and lesser side effects. Ancient literature also mentions herbal medicines for age-related diseases namely memory loss, osteoporosis, osteoarthritis, diabetes, immune and liver disorders, etc (Partap et al., 2012).

Antioxidants

Antioxidants are an inhibitor of the process of oxidation, even at relatively small concentration and thus have diverse physiological role in the body. Antioxidant constituents of the plant material act as radical scavengers, and helps in converting the radicals to less reactive species. A variety of free radical scavenging antioxidants is found in dietary sources like fruits, vegetables and tea, etc. Oxygen is absolutely essential for the life of aerobic organism but it may become toxic if supplied at higher concentrations (Hall, 2001). Antioxidants that have traditionally been used to inhibit oxidation in foods also quench dreaded free radicals and stop oxidation chains in-vivo, so they have become viewed by many as nature's answer to environmental and physiological stress, aging, atherosclerosis, and cancer. The nutraceutical trend towards doubling the impact of natural antioxidants that stabilize food and maximize health impact presents distinct challenges in evaluating antioxidant activity of purified individual compounds, mixed extracts, and endogenous food matrices and optimizing applications (Sudhir et al., 1986).

Diabetes mellitus

Diabetes mellitus is a combination of heterogeneous disorders commonly presenting with episodes of hyperglycaemia and glucose intolerance, as a result of lack of insulin , defective insulin action, or both (Sicree et al., 2006). Such complications arise due to derangements in the regulatory systems for storage and mobilization of metabolic fuels, including the catabolism and anabolism of carbohydrates, lipids and proteins emanating from defective insulin secretion, insulin action, or both (Shillitoe, 1988; Votey and Peters, 2004). The debilitating effects of diabetes mellitus include various organ failures, progressive metabolic complications such as retinopathy, nephropathy, and/or neuropathy (Piero, 2006). Diabetics are accompanied by risk of cardiovascular, peripheral vascular and cerebrovascular diseases. Several pathogenetic processes are involved in the development of diabetes, including destruction of pancreatic β-cells that lead to lowered sensitivity of insulin action (WHO, 1999; Votey and Peters, 2004).

Classification

Type 1 diabetes mellitus

The American Diabetes Association provides clear definitions of the various types of diabetes and classification, diagnosis, and clinical care of diabetes. Type 1 DM, which results from destruction of beta cells in the pancreas, accounts for approximately 10 percent of all patients with DM in the United States. It leads to absolute insulin deficiency. There are two forms of type 1 DM. One is an immune mediated disease with autoimmune markers such as islet cell antibodies (ICAs), insulin autoantibodies (IAAs), and autoantibodies to glutamic acid decarboxylase (GAD). As many as 85−90 percent of patients with fasting hyperglycemia are positive for one or more of these markers. (Katsarou et al., 2017)

Type 2 diabetes mellius

Type 2 is the most common form of DM worldwide, and its prevalence is increasing. Its underlying defects can vary from predominant insulin resistance with relative insulin deficiency to a predominant insulin secretory defect with insulin resistance. A great deal of heterogeneity exists, and most patients with type 2 DM do not initially require insulin therapy. Accounting for approximately 90 percent of all cases of DM in the United States, type 2 DM occurs more frequently in adults than in children, and the incidence increases with age, especially after age 40. However, the prevalence of type 2 DM in children is increasing, especially in the high-risk ethnic groups, such as Native Americans, Hispanic Americans,

African Americans, and Asian Americans. Most of these children are between 10 and 19 years old, have had symptoms longer, have infrequent or mild diabetic ketoacidosis, are obese, and have a strong family history of diabetes. A characteristic finding is darkening of the skin (acanthosis nigricans) and there is an increased incidence of insulin resistance (Fagot-Campagna et al., 2000; American Diabetes Association, 2009).

Diabetes in india

According to recent estimates, approximately 285 million people worldwide (6.6%) in the 20–79 year age group will have diabetes in 2010 and by 2030, 438 million people (7.8%) of the adult population, is expected to have diabetes. India leads the world with largest number of diabetic subjects earning the dubious distinction of being termed the "diabetes capital of the world". According to the Diabetes Atlas 2006 published by the International Diabetes Federation, the number of people with diabetes in India currently around 40.9 million is expected to rise to 69.9 million by 2025 unless urgent preventive steps are takenHigher prevalence of diabetes mellitus often results from in changes in dietary patterns and decreased physical activity in the urban population (Ramchandran et al., 2010). Diabetes is fast gaining the status of a potential epidemic in India with more than 62 million diabetic individuals currently diagnosed with the disease (Joshi and Parikh, 2007)

Herbal treatment of diabetes In the last few decades eco-friendly, bio-friendly, cost effective and relatively safe, plant-based medicines have moved from the fringe to the main stream with the increased research in the field of traditional medicine. There are several literature reviews by different authors about anti-diabetic herbal agents, but the most informative is the review by Atta-ar-Rahman who has documented more than 300 plant species accepted for their hypoglycae micproperties. This review has classified the plants according to their botanical name, country of origin; parts used and nature of active agents. One such plant is Momordica charantia (Family: Cucurbitaceae). WHO has listed 21,000 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 the botanical garden of the world (Modak et al., 2007)

MATERIAL AND METHOD

Collection of plant material

Leaves of Calotropis procera were collected from local area of Bhopal in the month of December, 2020.

Selection

The plants have been selected on the basis of its availability and folk use of the plant.

Drying

Drying of fresh plant parts was carried out in sun but under the shade.

Storage

Dried leaves of Calotropis procera were preserved in plastic bags, closed tightly and powdered as per the requirements.

Extraction procedure

Following procedure was adopted for the preparation of extracts from the shade dried and powdered herbs:

Defatting of plant material

56.5 gram of leaves dried powdered of Calotropis procera were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place.

Extraction by maceration process

Defatted dried powdered of Calotropis procera has been extracted with hydroalcoholic solvent (ethanol: water, 70:30) using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40º C (Mukherjee, 2007; Kokate, 1994).

Determination of percentage yield The percentage yield of each extract was calculated by using following formula:

$$\text{Weight of Extract Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powdered drug}} \text{x } 100$$

Quantitative studies of phytoconstituents

Estimation of total phenol content

Principle: The total phenol content of the extract was determined by the modified folin-ciocalteu method (Olufunmiso and Anthony, 2011).

Preparation of standard: 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10- 50µg/ml was prepared in methanol.

Preparation of extract: 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol.

Procedure: 2 ml of extract and each standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Estimation of total alkaloids content

The plant extract (1mg) was dissolved in methanol, added 1ml of 2 N HCl and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (40, 60, 80, 100 and 120 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/100mg of extract (Shamsa et al., 2008).

Antioxidant activity of hydroalcoholic extract of leaves of calotropis procera

DPPH method

DPPH scavenging activity was measured by the spectrophotometer. Stock solution (6 mg in 100ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance. Decrease in the absorbance in presence of sample extract at different concentration (10- 100 µg/ml) was noted after 15 minutes. 1.5 ml of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks andfinal volume was adjusted to 3 ml with methanol. Three test samples were taken and each processed similarly (Olufunmiso and Anthony, 2011). Finally the mean was taken. Absorbance at zero time was taken for each concentration. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm.

$$\text{Calculation of \% Reduction} = \frac{\text{Control Absorbance} - \text{Test absorbance}}{\text{Control Absorbance}} \text{x } 100$$

Antimicrobial activity of hydroalcoholic extract of calotropis procera

Pathogenic microbes used

The pathogenic bacteria used in the current study obtained from microbial culture collection, national centre forcell science, Pune, Maharashtra, India.

Media preparation (broth and agar media)

Table 1: Composition of nutrient agar media.

Agar	**1.5 gms.**
Beef extract	0.3 gms.
Peptone	0.5 gms.
Sodium chloride	0.5 gms.
Distilled water	to make 100 ml.
pH	7 6.8.3

Method of preparation

This agar medium was dissolved in distilled water and boiled in conical flask of sufficient capacity. Dry ingredients are transferred to flask containing required quantity of distilled water and heat to dissolve the medium completely.

Sterilization culture media

The flask containing medium was cotton plugged and was placed in autoclave for sterilization at 15 lbs /inch2 (121o C) for 15 minutes.

Preparation of plates

After sterilization, the media in flask was immediately poured (20 ml/ plate) into sterile petri dishes on plane surface. The poured plates were left at room temperature to solidify and incubate at 37o C overnight to check the sterility of plates. The plates were dried at 50o C for 30 minutes before use.

Revival of the microbial cultures

The microbial cultures used in the study were obtained in lyophilized form. With the help aseptic techniques the lyophilized cultures are inoculated in sterile nutrient broth than incubated for 24 hours at 37o C. After incubation the growth is observed in the form of turbidity. These broth cultures were further inoculated on to the nutrient agar plates with loop full of microbes and further incubated for next 24 hours at 37o C to obtain the pure culture and stored as stocks that are to be used in further research work.

Antibiogram studies

Broth cultures of pure culture isolates of those test microorganisms which are sensitive towards leaves of Calotropis procera used in present study were prepared by transferring a loop of culture into sterile nutrient broth and incubated at 37o C for 24 hours. A loop full was taken from these broths and seeded onto sterile nutrient agar plates through sterile cotton swab to develop diffused heavy lawn culture. The well diffusion method was used to determine the antimicrobial activity of leaves of Calotropis procera using standard procedure (Bauer, 1966). There were 3 concentration used which are 25, 50 and 100 mg/ml in antibiogram studies. It's essential feature is the placing of wells with the antibiotics on the surfaces of agar immediately after inoculation with the organism tested. Undiluted over night broth cultures should never be used as an inoculums. The plates were incubated at 37o C for 24 hr. and then examined for clear zones of inhibition around the wells impregnated with particular concentration of drug.

In vitro anti diabetic activity of hydroalcoholic extract of calotropis procera

Inhibition of alpha amylase enzyme

Preparation of standard: 10 mg acarbose was dissolved in 10 ml methanol, and various aliquots of 100- 1000µg/ml were prepared in methanol.

Preparation of sample: 10 mg of dried extract was extracted with 10 ml methanol. 500 µl of this extract solution was used for the estimation of enzyme inhibition.

Method: A total of 500 µl of test samples and standard drug (100-500µg/ml) were added to 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5mg/ml) solution and were incubated at 25°C for 10 min. After these, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 dinitrosalicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm. Control represent 100% enzyme activity and were conducted in similar way by replacing extract with vehicle

RESULT

Determination of percentage

Yield yield of extraction: To obtain the percentage yield of extraction is very important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a

particular plant, different parts of same plant or different solvents used. The yield of extract obtained from samples using hydroalcoholic solvent is depicted in the table 2.

Table 2: % Yield of calotropis procera.

S. No. Extract % Yield (w/w)
Pet. ether 0.567
Hydroalcoholic 2.542

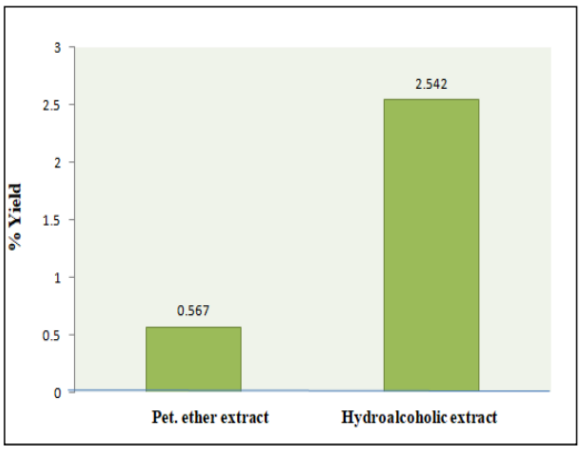

Figure 1: Comparative graph of % yield of calotropis procera.

Percentage yield of Pet. ether and hydroalcoholic extract of Calotropis procera exhibited in 0.567 and 2.542% respectively.

Results of estimation of total Phenol and Alkaloid content of Calotropis procera extract

Estimation of total phenol content

Total phenol content was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: Y = 0.011X+0.011, R2 = 0.998, where X is the gallic acid equivalent (GAE) and Y is the absorbance.

Table 3: Preparation of calibration curve of gallic acid.

S. no.	Concentration (µg/ml)	Absorbance
1.	10	0.135
2.	20	0.247
3.	30	0.364
4.	40	0.474
5.	50	0.581

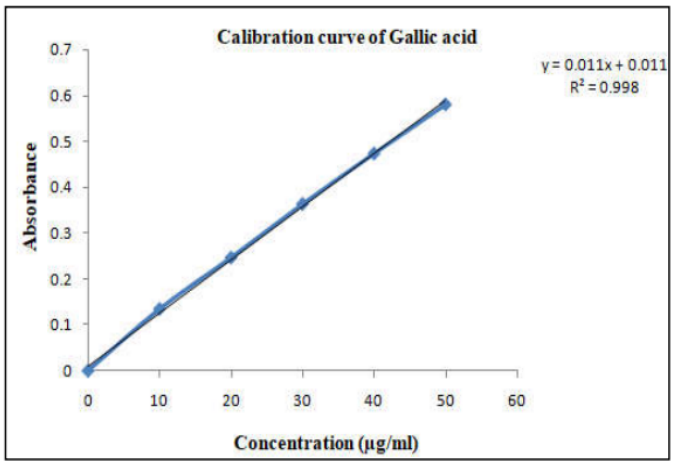

Figure 2: Graph of calibration curve of gallic acid.

Estimation of total alkaloid content (TAC)

Total alkaloid content was calculated as atropine equivalent mg/100mg using the equation based on the calibration curve: Y=0.007X+ 0.024, R2 =0.995, where X is the Atropine equivalent (AE) and Y is the absorbance.

Table 4: Preparation of calibration curve of gallic acid.

S. no.	Concentration (µg/ml)	Absorbance
1.	40	0.325
2.	60	0.457
3.	80	0.609
4.	100	0.721
5.	120	0.849

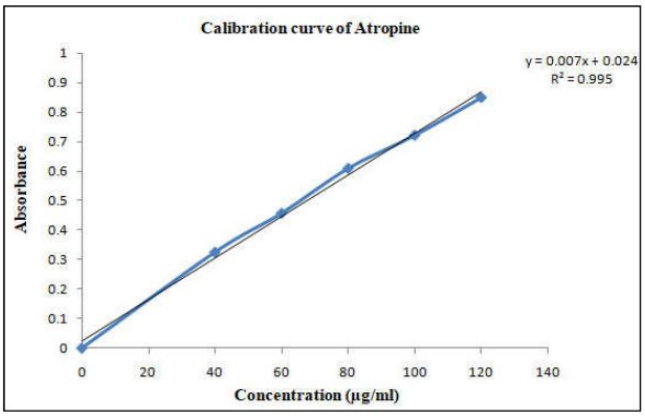

Figure 3: Graph of calibration curve of atropine.

Table 5: Estimation of total Phenolic and Alkaloid content of calotropis.

S. No.	Extract	Total phenol content (mg/100mg of dried extract)	Total alkaloid content (mg/ 100 mg of dried extract)
1.	Hydroalcoholic	1.024	1.125

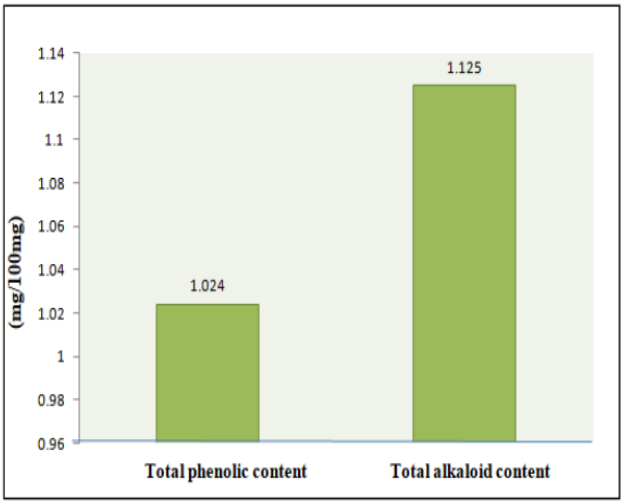

Figure 4: Comparative graph of total Phenolic and Alkaloid content.

The presence of phytochemicals (Phenols, Alkaloid) was quantitatively screened. The extract quantitative analysis revealed total phenolic content (equivalent to gallic acid) of 1.024mg/100 mg. The total content of alkaloid (equivalent to Atropine) was found 1.125mg/100 mg in Calotropis procera.

Results of antioxidant activity of hydroalcoholic extract of Calotropis procera There is increasing evidence that indigenous antioxidants may be useful in preventing the deleterious consequences of oxidative stress and there is increasing interest in the protective biochemical functions of natural antioxidants contained in herbs and medicinal plants. Antioxidant activity of hydroalcoholic extract of Calotropis procera was measured by free radical scavenging activity. The tested plant extract showed strong antioxidant activity in Table 6.

Table 6: % Inhibition of ascorbic acid and Hydroalcoholic extract of Calotropis procera using DPPH method.

S. No.	Concentration ((µg/ml)	% Inhibition	
		Ascorbic acid	Hydroalcoholic extract
1.	10	44.65	18.35
2.	20	48.62	26.47
3.	40	65.34	37.66

4.	60	69.65	46.74
5.	80	77.41	59.24
6.	100	84.13	65.55
IC50 (µg/ml)		17.68	66.28

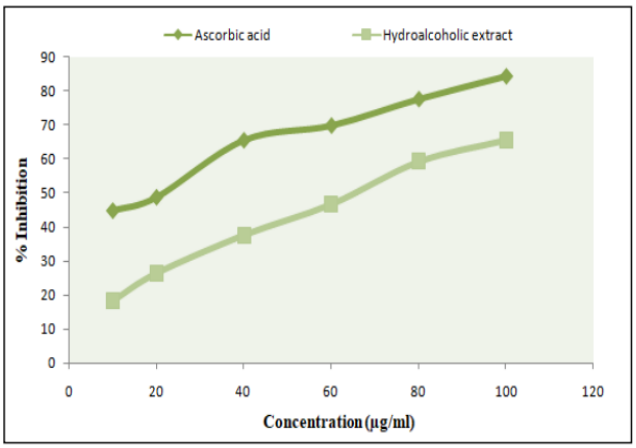

Figure 5: % Inhibition of ascorbic acid and hydroalcoholic extract of Calotropis procera using DPPH method.

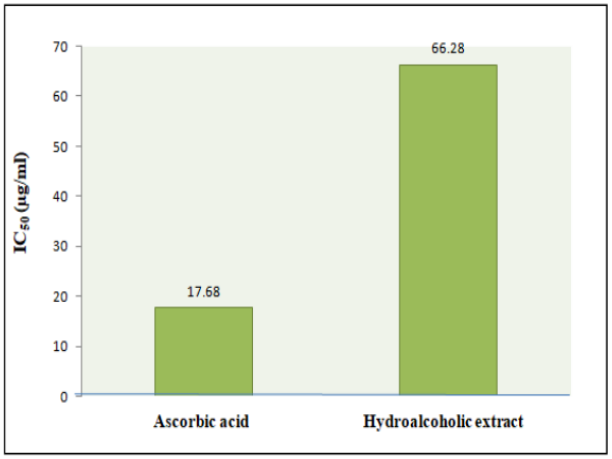

Figure 6: Comparative graph of IC50 value of ascorbic Acid and Hydroalcoholic extract Antimicrobial activity of hydroalcoholic extract of Calotropis procera.

Results of the experiment are being concluded in the table 7, which clearly shows the antimicrobial activity of hydroalcoholic extract of Calotropis procera.

Table 7: Antimicrobial activity of standard drug against selected microbes.

S. No	Name of Drug	Microbes	Zone of inhibition		
			10 µg/ml	20 µg/ml	30 µg/ml
1.	Ciprofloxacin	Staphylococcus Aureus	17±1.69	18±2.62	22±2.16
		Klebsiella pneumoniae	7±0.86	22±0.57	30±0.5

Table 8: Antimicrobial activity of hydroalcoholic extract of calotropis procera against selected microbes.

S. No	Name of Microbes	Zone of inhibition		
		25 mg/ml	50 mg/ml	100 mg/ml
1.	Staphylococcus Aureus	7±0	8±4.49	11±0.47
	Klebsiella pneumoniae	8±0.94	10±0.5	13±0.94

Figure 7: Photoplates of Antimicrobial activity of standard drug against selected microbes.

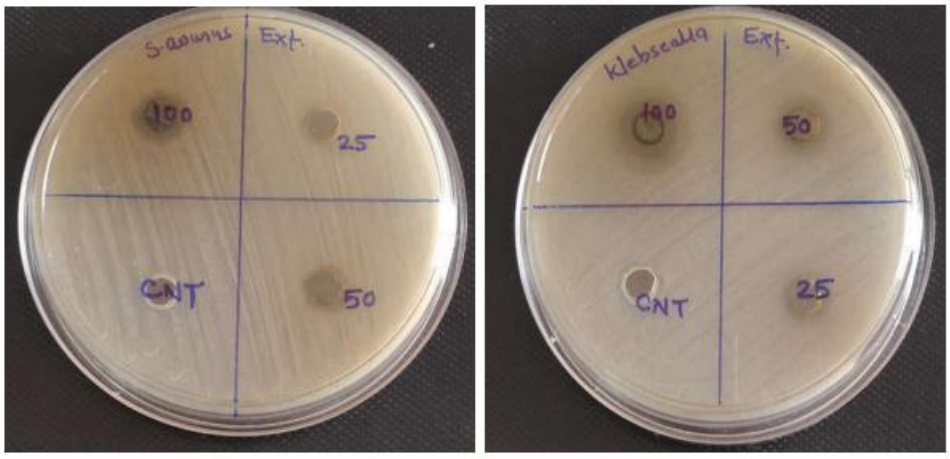

Figure 8: Photoplates of antimicrobial activity of hydroalcoholic extract of Calotropis procera against selected microbes Results of in vitro anti diabetic activity of hydroalcoholic extract of Calotropis procera.

Table 9: % Inhibition of acarbose and Hydroalcoholic extract of calotropis.

S. No	Concentration ((µg/ml)	% Inhibition	
		Acarbose	Calotropis procera extract
1.	100	51.19	30.26

2.	200	70.10	37.52
3.	300	74.20	45.95
4.	400	85.18	55.32
5.	500	88.75	65.32
IC50 (µg/ml)		35.33	338.62

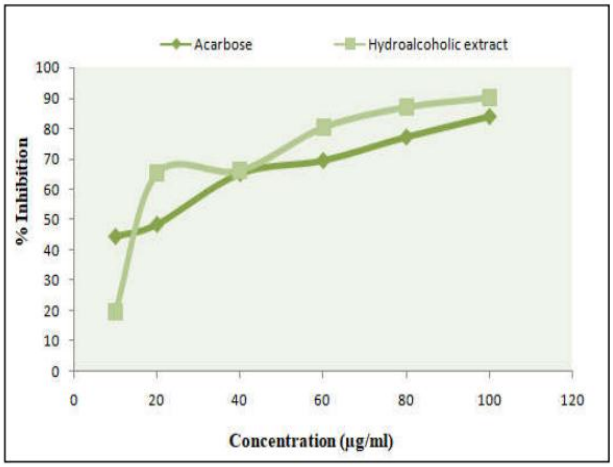

Figure 9: % Inhibition of Acarbose and Hydroalcoholic extract of calotropis procera.

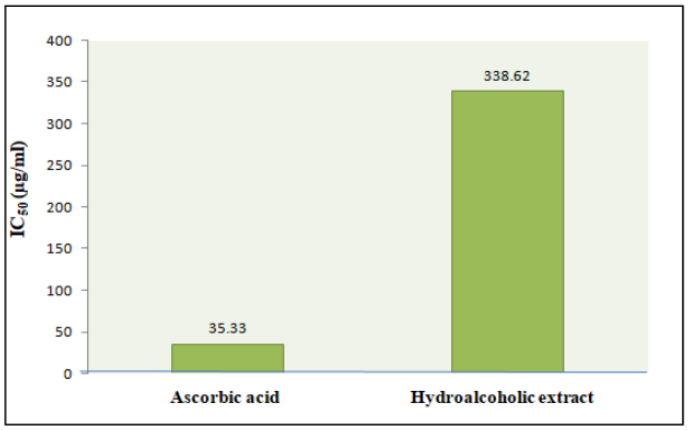

Figure 10: Comparative graph of IC50 value of Acarbose and Hydroalcoholic extract.

CONCLUSION

Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. % Yield of leaves of hydroalcoholic extract of Calotropis procera was found 2.542%. Phytochemical analysis revealed that tannins, proteins, alkaloids, phenol, carbohydrates, saponins were found to be present in crude hydroalcoholic extract of Calotropis procera and flavonoids, glycosides and diterpenes are absence. The phenolic content with respect to gallic acid was found to be 1.024 (mg /100mg of extract) for of leaves of hydroalcoholic extract. The total alkaloid content of the extract was estimated taking Atropine as standard. The alkaloid content was found to be as:

1.125 (mg /100mg of extract) in hydroalcoholic extract. The ascorbic acid and extracts have shown dose dependent scavenging of DPPH radicals. The radical scavenging effect of standard and extracts was in the order ascorbic acid> leaves extract IC50 (µg/ml) was found to be 17.68 and 66.28 respectively. Extract of Calotropis procera showed antibacterial activity against Staphylococcus aureus (11, 8, 7 mm) and Klebsiella pneumonia (13, 10, 8 mm) at the concentration of 100, 50, 25mg/ml. The result suggests that the presence of bioactive compounds could be responsible for the versatile medicinal properties of this plant including diabetes.

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Conflict of interest

The Authors declare no conflict of interest.

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