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Invitro Evaluating of Antidiabetic and AntiLithiatic Activity of AnacardiumOccidentale Leaf Extract

G. Jayasri, R. Sathya, P. Nirmala Devi

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Abstract: *In this present study we investigated to the in vitro evaluation of antidiabetic and antilithetatic activity of anacardium occidental leaf extract. Diabetes is a clinical syndrome characterized by hyperglycimia due to a relative insulin deficiency absolute. The dried leaf was powdered and extracted cold method with water (mixed with ethanol & petroleum ether) to recover complete secondary metabolites the aqueous extracted 29.62 evaluated for flavonoid gravimetrialy and the flavonoid content retained to be 2.146g and the secondary metabolite crude and the flavonoid to be evaluated for the efficiency in treating antidiabetics and anti lithiatic property. The potiential antidiabetic activity of Anacardiumoccidentale might be due to the phytochemical alkaloids, flavonoids, phenol&tannin, Ellagic acid, glycosides present in the extract.*

Keywords: *Anti-diabetic, Anti lithatic, alkaloids, flavonoids, Anacardiumoccidentale*

INTRODUCTION

Diabetes has become a global health problem. More than 176 million people suffer from this disease globally, as estimated by the world health organization (1). It is a disorder of carbohydrate fat, and protein metabolism it's associated with increased blood sugar it may caused with vascular complication including Nephropathy and neuropathy (2). Urolithiasis describes the prevalence of kidney stone as a stone that occurs in the urinary tract and may contain different combinations of chemicals such as calcium in mixture with either oxalate or phosphate(3).

Medicinal plants are rich in antioxidant activity and contain a wide variety of free radical scavenging molecules like phenolic derivatives flavonoids, anthocyanis, carotenoids, dietry glutathione vitamins furan derivatives and endogenous metabolites. (4) Anacardium occidental is ausally identified as cashew it has been various pharmacological activities such as Antioxideant, anesthetic, antibacterial, antidiabetics and anti lithiatics.(5). Previous studies reported that the studies of acute, subacute toxicity and genotoxic effect and hypoglycemic effect of Cashew in mice and rats (*A. occidentale* L.). Therefore, we undertook this study to investigate the in vitro effect of evaluation of antidiabetic and anti lithaticactivity of anacaridumoccidentale leaf extract.

MATERIALS AND METHODS

Plant Material Collection

The leaves of *Anacardiumoccidentale* were gathered from Cashew farm, Marungur, Panruti, Tamilnadu.

Preparation of plant extract Plant Material Collection

The fresh leaves of a *Anacardiumoccidentale* were collected. Leaves were dried with sunlight and ground in to powder 61.91g of plant powder sample was extracted in 40ml ethanol and 40ml peteroleum ether, 160 ml distilled water in cold method. The macerated sample was filtered using muslin cloth at room temperature. The sample in water bath in 70^0C in 3days. The dried extract was weighed.

G. Jayasri, R. Sathya, P. Nirmala Devi
Assistant Professor, Department of Biochemistry, Dhanalakshmi Srinivasan College of Arts and Science for Women, Perambalur-621212

Total extract weight,

$$\text{yield } \% = \frac{\text{Total weight} - \text{Empty weight}}{Initialsamplequantity} X\ 100$$

Screening of Phytoconstituents

1. Alkaloids:

Wagner's test:(iodine in potassium iodide)

Treat the acid layer with few drops of Wagner's reagent. Formation of reddish brown precipitate indicates presence of alkaloids.

2. Flavonoids:

Shinoda test:

Add a few fragments of magnesium ribbon to the extract and focus hydrochloric acid. Flavonoid appearance after a few minutes of red to pink colour.

3. Phenol and tannin:

Nitric acid test:

The extract was treated separately with dilute nitric acid. The reddish to yellowish colour formation indicates the presence of tannins and phenolic compounds.

4. Volatile oil:

Mix 0.5g of powder sample with 1ml of 0.1m NaOH solution and 1% aqueous HCl. Formation of white precipitate indicates the volatile oil.

5. Fixed oil:

Adding 1 ml of 1% copper sulphate solution and a few drops of 10% sodium hydroxide to 5 drops of the sample. The creation of a clear blue solution does not confirm the test.

6.Coumarins:

Added 10% of sodium hydroxide and chloroform to the test sample. Yellow colour formation shows volatile oil presence.

7.Iridoids test:

To the 5 drops of the sample was added 2 drops of Trion Hcl reagent then heat. Blue green colour is formed.

8.Ellagic acid test:

To the 5 drops of the sample then 5% acetic acid is added and 5% sodium nitrate is added turns yellow and olive brown (or) deep chocolate is formed.

9.Glycosides test:

Keller-Kilanitest:

To the extract, add 2 ml of glacial acetic acid and 2 drops of 2% $FeCl_3$. Finally add s 2ml concentrate H_2SO_4 along the side of test tube. A brown ring indicates presence of glycoside.

Flavonoid Determination (Gravimetric)

The flavonoid content of the leaves of the plant was determined by the gravimetric method as was described by Harbone(1973) (6)

5g of the powder sample was placed into a conical flask and 50 ml of water and 2 ml of HCL solution was added. The solution was allowed to boil for 30 minutes. The boiled mixture was allowed to cool before it was filtered through whatman filter paper (N042). 10 ml ethyl acetate extract which contained flavonoid was recovered, white the aqucous layer was discarded. A pure weighed whatman filter paper was used to filter second (ethyl-acetate layer), the residue was then placed in an oven to dry at 60^0 c. It was cooled in a desiccator and weighed. The quantity of flavonoid was determined using the formula.

% flavonoid = $\underline{w_2\text{-}w_1}$×100 Weight of sample

Where:

W_1=weight of empty filter papers

W_2=weight of paper+flavonoid extract

Antilithiatic Activity

Preparation of experimental kidney stone (synthetic)

Equimolar solution of sodium oxalate 1g calcium chloride 1g in 20 ml of (2N sulphuric acid) were allowed to react in sufficient quantity of distilled water in a beaker. Stirring was done using magnetic stirrer for 1 hour.

Estimation of calcium oxalate by titrimetry

Exactly 1 mg of calcium oxalate and 10 mg of the extract/standard were weighed and packed it together in semi-permeable membrane by suturing. This was allowed to suspend in a conical flask containing a 100 ml 0.1 M TRIS buffer. The conical flask of all groups in an incubator were placed, preheated to 37°C for 2 hrs, for about 7-8 hrs and then, removed the contents of the semi - permeable membrane from each group into a test tube. It was added 2 ml of 1 N sulfuric acid and titrated with 0.9494 N KMnO4 till a light pink color end point obtained (1 ml of 0.9494 N KMnO4 equivalents to 0.1898 mg of calcium).

Control

2 ml of sodium oxalate and 20 ml of Tris buffer was kept for 1 hour incubation at room temperature and 2ml H_2So_4 added and titrated against potassium permanganate.

Extract

2 ml of sodium oxalate and 1 ml of extract, 20 ml of Tris buffer was kept 1 hour incubation at room temperature and titrated 2ml H_2So_4 added against potassium permanganate.

Flavonoid

2 ml of sodium oxalate and 1 ml of flavonoid, 20 ml of Tris buffer was kept 1 hour incubation at room temperature and titrated 2ml H_2So_4 added against potassium permanganate.

Formula

mg/100g= (Titer value×0.2004×100) / 2

Anti Diabetic Activity

(Acharya AS, et al, 1980) (7)

Procedure

Antidiabetic activity of leaves of *Anacardiumoccidentale*. were investigated by estimating degree of non-enzymatic haemoglobin glycosylation, measured calorimetrically at 520nm. Glucose (2%), haemoglobin (0.06%) and Gentamycin (0.02%) solutions were prepared in phosphate buffer 0.01 M, pH 7.4.1 ml each of above solution was mixed. Solvent extract of leaves *Anacardiumoccidentale* was weighed and dissolved in DMSO to obtain stock solution and then 1-5 µg/ml solutions were prepared. 1 ml of each concentration was added to above mixture. Mixture was incubated in dark at room temperature for 72hrs. The degree of glycosylation of haemoglobin was measured calorimetrically at 520nm. Alpha-Tocopherol was used as a standard drug for assay. % inhibition was calculated as-

$$\% \text{ inhibition} = \frac{As - Ac}{As} * 100$$

Ac is Absorbance of Control
As is Absorbance of Sample

RESULT

Table 1: Moisture Content of A*nacardiumoccidentale* leaf

Fresh leaf(g)	Dried leaf(g)	Moisture %
89.8	61.91	27.89

Table-1 The leaves of anacardiumoccidentale weight 89.8g. Dried leaf weight is 61.91g. The leaf moisture content weight 27.89 were presented in table 1.

Cold Extraction

Table 2: Extractive Value of A*nacardiumoccidentale* leaf Extract

Final weight (g)	Initial weight (g)	Extract quantity(g)	Yield %
121.4565	114.0511	7.405	**29.62**

Table 3: Qualitative analysis of Phytochemicals

S.No.	Sample	Observation	Inference
1	Wagner's test	Precipitate was formed	Presence of alkaloid
2	Shinoda test	Appearance of red to pink colour formed	Presence of flavonoids
3	Nitric acid test	Reddish to yellowish colour is formed	Presence of tannin & phenol
4	Volatile oil	White precipitate was not obtained	Absence of volatile oil
5	Fixed oil	Clear blue solution was not obtained	Absence of fixed oil
6	Coumarins	Yellow colour was not obtained	Absence of Coumarins
7	Iriodoids	Blue colour was not obtained	Absence of iriodoids
8	Ellagic acid	Turns yellow colour is formed	Presence of Ellagic acid
9	Keller-killani	Brown ring is formed	Presence of glycosides

Table-3 Represent the qualitative analysis of phytochemicals ***alkaloid, flavonoids, tannin, phenol, Ellagic acid, Ellagic acids glycosides***

QUANTATIVE ANALYSIS

FLAVONOID DETERMINATION

Table 4: Flavinoid estimation of A*nacardiumoccidentale* leaf extract

Extract weight(g)	Weight of paper(g)	Weight of sample(g)	Yield(g)
53.6780	53.5707	5	2.146

Table-4 Shows the flavonoid estimation of *AnacardiumOccidentale*weight of the leaf extract which contains 53.6780g.Flavonoid estimation using the sample weight was5g.The yield of flavonoid estimation content 2.146g.

ANTILITHATIC ACTIVITY

Table 5: Antilithatic activity of Anacardium occidental leaf extract

Sample	Titration value	Antilithatic activity mg/100g
Control	**0.4**	**4.008**
Extract	**20.6**	**206.412**
Flavonoid	**7.6**	**76.152**

Table-5: Shows present antilithatic activity of the leaf extract of *AnacardiumOccidentale.* The activity of the extract is 206.412mg/100g and the activity of flavonoid is 76.152mg/100 when comparing with control flavonoid the antilithatic activity of the extract is high.

ANTIDIABETIC ACTIVITY

Table 6: Antidiabetic activity of A*nacardiumoccidentale* leaf extract

Sample	Absorbance 520nm	% of anti diabetic activity
Control	0.68	0.00
Flavonoid T_1	1.52	55.26
Extract $_{T2}$	1.69	59.76
Extract T_3	2	66

Table-6: The Anti diabetics activity of *anacardiumoccidentale* leaf extract was shown in when compared with the flavonoid content the leaf extract of AnacardiumOccidentale have high antidiabetic activity.

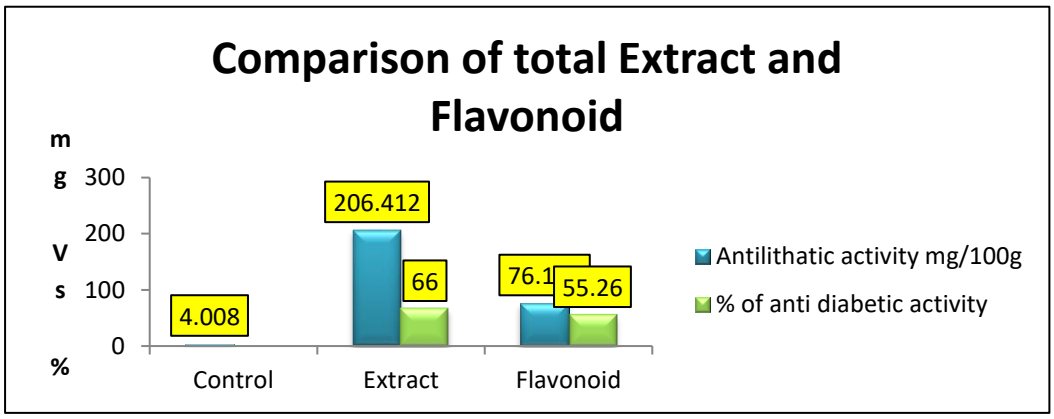

Figure 1: Comparison of Antilithatic and Antidiabetic activity of Extract and flavinoid of A*nacardiumoccidentale* leaf extrac

DISCUSSION

The use of medicinal plants and their phytocemicals as radical scavengers has been reported in various scientific studies(8). In this present study we were analysed the phytochemical screening such as alkaloids, falvonoids, tannins, phenolic content, antidiabetics and anti lithiatics effect of anacardium occidental leaf extract. Radical scavengers are broadly used as active components of phytochemicals to prevent and treat various diseases including diabetes and lithiasis(9). Many studies have reported that phenolic compounds, flavanoids and tannins are the main constituents of antioxidant activity in most medicinal plants and are responsible for the free effect of radical scavenging.

Phenolic compounds are the largest group of phytochemicals and accounts for most of the antioxidant and free radical scavenging activity in plants and plant products (10). Phytoconstituents act as an ant diabetic agent can stimulate the insulin secretion and inhibit the abnormal secretion of glucose used for the treatment of hyperglycemia, oxidative stress, cancer, inflammation (11).

Many naturally occurring triterpenoids exhibit good anti-lithiatic activity and they have been isolated from various plants (12), that results from a succession of multiple phytochemical including supersaturation, nucleation, growth, aggregation, and retention within the kidneys. Several studies reported that anacardium have prevented the kidney stone formation in human. In our study concluded that a strong antioxidant and many phytochemicals may be useful in medicine they are bactericidal, fungicidal, antiviral, cytotoxic, analgesic, anti-inflammatory, anti-cancer and antiallergic (13).

The extract evaluated for antidiabetic property as it showed significant activity for the inhibition of reactive oxygen species produced during glycosylation of the sugar in diabetic patient as an invitro study. The % of antidiabetic property was found to be average of 62 %for the total extract and the purified flavonoid showed 55 % activity.

CONCLUSION

The present study scaled for the invitro determination and evaluation of properties achieved for inhibition (or) solubilization of glucose and calcium oxalate crystals using the natural resource especially *Anacardiumoccidentale* leaf extract for a steady and increasing activity. By making a pilot research study over the extract especially from the leaf of cashew tree showed a significant effect which on further research move its effective metabolic hypothesis and can use as a generic medicine.

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