IJBPAS, March, 2025, 14(3): 1649-1659

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

PHYTOCHEMICAL CHARACTERIZATION AND ANTI HYPERGYCEMIC POTENTIAL OF *CAJANUS CAJAN* LEAF EXTRACTS

ARCHNA J* AND ANUSHA V

Department of Pharmacology, RBVRR Women's College of Pharmacy, Barkatpura, Hyderabad-500027, Telangana, India

***Corresponding Author: Dr. Archana Jorige: E Mail: archanacology@gmail.com**

Received 16th Nov. 2023; Revised 18th Dec. 2023; Accepted 27th May 2024; Available online 1st March 2025

https://doi.org/10.31032/IJBPAS/2025/14.3.8821

ABSTRACT

Aim: To evaluate the phytochemical profile and pharmacological potential of Ethanolic Extract of *Cajanus cajan* (EECC) and Hexane Extract of *Cajanus cajan* (HECC). **Methods:** 8–12-week male albino Wistar rats were used. Nicotinamide (NAD-100 mg/kg) was administered through Intraperitoneal (I.P) route. After 15 mins Streptozocin (STZ-50 mg/kg) was administered through I.P. route. After 72 hrs (3 days) rats showing FBGL (Fasting Blood Glucose Levels) of 230-330 mg/dl were considered as diabetic and divided into the groups as control (vehicle), diabetic control (STZ+NAD), standard (STZ+NAD+glibenclamide-0.5 mg/kg), Ethanolic Extract of *Cajanus cajan* leaves (EECC-500 mg/kg), Hexane Extract of *Cajanus cajan* leaves (HECC-500 mg/kg). **Results:** EECC & HECC consists of high number of flavonoids (20.21% and 30.95%) and phenols (14.98% and 18.57%). OGTT (Oral glucose tolerance test) was performed, the maximum reduction of blood glucose levels by standard (54.26%), EECC (23.19%) and HECC (41.09%) was seen at 120 mins. **Conclusion:** These results shown that the EECC and HECC were able to produce glucose tolerance and hypoglycemic action.

Keywords: *Cajanus cajan*, Flavonoids, Phenols, OGTT, blood glucose levels

INTRODUCTION

Nature can be described as the oldest and most ample pharmacy of all time, and phytomedicine has been practiced for health benefits in different systems of traditional medicine. According to the latest report, around 80% of the world population

relies on some forms of traditional medicines mainly the herbs (WHO, 2012). One of the eldest herbs that has been used for over 3,000 years, for both gastronomic and medicinal purposes.

Herbal medications are known to mankind. Medicinal plants have been used as tradition treatment for several human diseases for centuries in many portions of the world. In developing countries, where the accessibility of modern health services is limited, folk medication, mainly based on plant are common. The rich plant diversity of India is well utilized by the native communities for the treatment of many diseases. The term of medicinal plants includes a various type of plants use in herbalism and some of these plants have medicinal actions. Medicinal plants considered a rich source of ingredients which can be used in drug development and synthesis around the entire world. Medicinal plants play a critical role in the development of human culture. Some plants considered an important source of nutrients these plants are used as therapeutic ethics **[1]**.

Cajanus cajan Linn., a shrub grown in Central India, is a member of the Fabeaceae family, and it's leaves and seeds are utilized in medicine **[2]**. Pigeon pea is its common name and it is a source of protein, vitamin B and minerals. The plant *Cajanus cajan* contains many bioactive constituents such as stilbenes, flavones, phytosterols, coumarins and many more which possess therapeutic applications for diabetes, hepatitis, malaria, cancer, hyperglycaemia etc. This plant is also considered to have anti-oxidant, anti-cancer, anti-tumour, anti-malarial, anti-bacterial properties. Essences of pigeon pea leaves are useful for jaundice, looseness of the bowels, wounds, hack, bronchitis, bladder-stones and diabetes. In China these leaves are used to arrest blood, relieve pain and kill worms. The young leaves of pigeon pea can be gnawed for treating Aphtha. In current times pigeon pea leaves have been used to treat traumatism, bedsore, burnt-infections and also used for anti-inflammatory, anti-biotic and abirritation effects and inhibit capillary permeability **[3]**.

MATERIALS AND METHODS

Instruments: Digital Balance, Blender, Soxhlet glass ware, rotatory evaporator, Glucometer, biochemical analyser.

Chemicals and reagents: All chemicals and drugs which was obtained were analytical grade. Alpha amylase (SD Fine-Chem Ltd), alpha glucosidase (SRL), Streptozocin (Central Drug House Pvt Ltd), Nicotinamide (SD Fine-Chem Ltd), Glibenclamide (Pure powder) and commercial kits for estimation of glucose, cholesterol, triglycerides and High-Density Lipoproteins (HDL) from Erba.

Collection and Authentication

The fresh leaves of *Cajanus cajan* were collected in winter season from cultivated farms of Peddapally district of Telangana, India. The leaves were identified by the Botanist Dr.A. Vijaya Bhasker Reddy, Department of Botany, OU (Osmania University), Hyderabad-07, Telangana and a Voucher specimen of leaf (NO. OUAS-110) was deposited in Department of Botany, O.U. The leaves were shade dried for two weeks and grinded into powder by using a blender. Sunlight was avoided because it consists of ultraviolent radiation which may cause chemical degradation **[4]**.

Preparation of Extracts: The crude powder weight of 100 gms was placed in a bag which is porous, made of strong filter paper and loaded then inserted into the Soxhlet apparatus thimble. The extracted solvent in round bottom flask (RBF) was heated at 30^{o} C using 70% ethanol. The extraction was continued for 72 hrs. The vapour condenses in the condenser. Liquid in the chamber rises to the siphon tube's top and falls into the flask. By using rotary evaporator, the solvent extract was concentrated and air dried **[5]**. The Ethanolic Extract of *Cajanus cajan* (EECC) was fractionized using equal amount of hexane and water in the separating funnel. The obtained Hexane Extract of *Cajanus cajan* (HECC) was dried by rotatory evaporator and stored in desiccators for analysis. The percentage yield of extracts was calculated.

Qualitative Tests

Phytochemical Tests: The phytochemical tests of EECC and HECC were estimated using standard methods **[6, 7]**.

1.Test for Carbohydrates

Molisch's Test: 2 drops of alpha-naphthol added to the extracts and 1ml conc.H_2SO_4 solution added to the test tube by sides, a carbohydrates presence indicated by a ring which is violet colour.

Barfoed's Test: To the extracts added 1ml Barfoed's reagent and heated for 2mins, red precipitate formation shows the Monosaccharides existence in the sample.

2.Test for Alkaloids

Mayer's test: To the extracts added 2%v/v Sulphuric Acid (H_2SO_4) solution and Mayer's reagent to test tube by its sides, formation of precipitate which was yellowish indicates alkaloids in the sample or extract.

Dragendroff's test: 1-2 ml of Dragendroff's reagent added to extracts, formation of reddish-brown precipitate or turbid solution shows the alkaloids presence in the extract.

3.Test for Flavonoids

Lead Acetate Test*:* To the extracts added 10% v/v lead acetate formation of a yellow precipitate indicates the flavonoids present.

Ferric Chloride ($FeCI_3$) Test: 3-4 drops of 10% ferric chloride was added to extracts,

formation of green precipitate shows the presence of flavonoids.

4.Test for Phenols

Iodine Test: To extracts added few drops of dilute Iodine (I_2) solution formation of red colour shows the phenols presence in the sample.

Ferric Chloride Test: To the extracts added few drops of 5% ferric chloride, formation of dark green/bluish green colour indicates the phenols.

Lead Acetate Test: After dissolving the extracts in distilled water and adding three drops of a 10% lead acetate solution, phenols were detected by the appearance of a white precipitate.

5.Test for Reducing Sugars

***Benedict's Test*:** 0.5ml Benedict's reagent added to the extracts and boiled for 2 mins, formation of red or green colour shows non- reducing sugars.

***Fehling's Test*:** Fehling's A and B solutions were added in equal amounts to the extracts and heated in a water bath, the presence of reducing sugars was indicated by the production of a red precipitate.

6.Test for Glycosides

Aqueous NaOH Test: In 1ml of water, extracts were dissolved and few drops of Sodium Hydroxide (NaOH) solution was added, presence of glycosides was confirmed by formation of yellow colour.

Killer-Killian Test: To the extracts add 1.5ml glacial acetic acid, 5% ferric chloride and conc.H_2SO_4 to the test tube by its walls it shows solution which is blue colour indicates the Cardiac glycosides.

7.Test for Proteins

Biuret Test: 2% Copper Sulphate ($CuSO_4$) drops was added to the solution of extracts, then 1ml of 95% ethanol and Potassium Hydroxide (KOH) added which shows pink coloured solution indicates proteins.

Millon's Test: To extracts, Millon's reagent was added which shows a precipitate which was white indicates incidence of proteins.

8.Test for Amino Acids

Ninhydrin Test: Ninhydrin solution drops were added to solution of extracts which shows the purple-coloured solution shows amino acids.

9.Test for Tannins

Braymer's Test: 3 drops of 10% ferric chloride were added to the extract's solution, formation of bluish green colour specifies the existence of tannins.

10.Test for Saponins: To aliquot added 2ml of 5%w/v Sodium bicarbonate ($NaHCO_3$) followed by vigorous shaking and allow it to stand for 3mins which shows the formation of a honey comb like foam indicates saponins.

11.Test for Steroids: Conc.H_2SO_4 and Acetic anhydride were added to the extracts results in development of violet to green or blue colour indicates steroids in the extract.

12.Test for Terpenoids: To the extracts added chloroform ($CHCL_3$) and H_2SO_4 solution which shows reddish brown colour indicates terpenoids.

Quantitative Analysis

Total Phenolic Content (TPC)

Total Phenols present in extract were estimated by using Gallic acid method. Gallic acid was used because it is natural, cheap & stable phenols compared with others compounds. A standard gallic acid curve was constructed by preparing the different dilutions of 1, 5, 10, 15, 20 μg/ml in methanol from standard solution of gallic acid. 100μl each dilution of gallic acid and extracts (EECC, HECC) were mixed with 500μl of water & 100μl of Folin-Ciocalteu reagent and allowed to stand for 6mins. Then 1ml of 7% Sodium Carbonate & 500μl of distilled water was added to reaction mixture. The absorbance recorded after 90 mins at 760nm.Total Phenol content expressed as mg of Gallic acid equivalent per gram of leave extracts (mg GAE/g) **[8]**.

Total Flavonoid Content (TFC)

Total flavonoids present in the extract were estimated by using Rutin. A standard Rutin curve was constructed by preparing the dilutions of 1,2,4,5,6,8,10,20,40 μg/ml in water from standard solution of Rutin. 0.5ml of each dilution of rutin and extracts (EECC, HECC) were mixed with 3ml methanol, 0.2ml of 10% Aluminium Chloride, 5ml of distilled water. Absorbance recorded after 30mins at 415nm. Total Flavonoid content expressed as mg of Rutin equivalent per gram of leave extracts (mg RUE/g) **[9]**.

Experimental Design

Thirty adult male wistar albino rats of 8-12 weeks age were housed in polypropylene cages with sterile padded husk as beddings and maintained with the room temperature of 22 ± 2^0C, 12 hours dark and light condition, with relative humidity at >40%. Animals were fed with pellets and water *ad libitum.* Animals were acclimatized for one week before the conduction of study **[10]**. The Institutional Animal Ethics Committee (IAEC) approved the study with reference number RBVRR/1320/08/2023. Guidelines of the Committee for the Control and Supervision of Experiments on Animals (CCSEA) were followed for the animal's maintenance.

Experimental induction of Diabetes by STZ-NAD Injection

Streptozocin (STZ) was prepared freshly by dissolving in 0.1M ice-cold citrate buffer. Nicotinamide was dissolved in the normal saline. Before the induction, the rats were kept on fast overnight with free access to water. Diabetes was induced by injecting a single dose of Nicotinamide (100mg/kg, i.p) and after 15 minutes, single dose of Streptozotocin (50mg/kg) injection was

given through intraperitoneal route **[11]**. After 3 days (72 hours), fasting blood glucose levels of rats were measured using Biochemical Analyser. Rats showing fasting blood glucose levels (FBG) between 230 - 330 mg/dl were considered as Type II diabetic animals and distributed into different groups as per the experimental design **[Table 1]**.

Oral Glucose Tolerance Test (OGTT)

Glucose tolerance can be determined by OGTT on the day of the treatment, 1 hour after the dose. Administrating glucose (2 mg/kg, orally) to 12 hours fasted animals and collecting the blood samples through retro-orbital puncture at 0, 30, 60 and 120 mins. Estimated blood glucose by using a Biochemical Analyzer **[12, 13]**.

RESULTS

Preparation of Extracts

The Percentage Yield obtained for EECC and HECC was 20.55% and 16.19%.

Qualitative Tests

Photochemical Tests: Both the Extracts EECC and HECC are considered as potential source of numerous photochemical constituents including Carbohydrates, alkaloids, tannins, phenols, glycosides and flavonoid compounds **[Table 2]**.

Total Phenolic Content (TPC)

Phenolic content estimated by folin-ciocalteau reagent method using gallic acid as standard. TPC was found to be 308 mg GAE/gm EECE and HECC and 185.7 mg GAE/gm HECC **[Figure 1]**.

Total Flavonoid Content (TFC)

Flavonoid content estimated by $AICl_3$ (Aluminium chloride) method using standard as rutin. Flavanoid content estimated was 202.1 mg RUE/g EECC and 309.5 mg RUE/g HECC **[Figure 2]**.

Oral Glucose Tolerance Test

During the Oral Glucose Tolerance Test (OGTT), diabetic control animals exhibited a significant ($p<0.0001$) elevation in blood glucose levels when compared to the normal control group. Treated diabetic animals demonstrated significantly lower blood glucose levels (***$p<0.001$, **$p<0.01$) compared to diabetic controls. Peak glucose levels were observed 30 minutes after the oral administration of glucose in all groups. At the 120-minute mark, diabetic control animals still maintained elevated plasma glucose levels, whereas Glibenclamide, EECC, and HECC treated groups showed reductions of 54.3%, 23.2%, and 41.1% in blood glucose levels, respectively. The OGTT's increased glucose Area Under the Curve (AUC) in the diabetic control group indicated severe glucose intolerance in diabetic rats. Diabetic rats treated with EECC and HECC exhibited a reduced AUC. The findings suggest that EECC and HECC significantly improved impaired glucose tolerance,

comparable to the effect observed with Glibenclamide **[Table 3, Figure 3]**.

Table 1: *Invivo* Experimental Design

S. No.	GROUPS	TREATMENT
1.	Group I (Normal)	Vehicle
2.	Group II(Diabetic)	STZ (50mg/kg) + NAD (100mg/kg)
3.	Group III(Standard)	STZ (50mg/kg) + NAD (100mg/kg) + Glibenclamide (0.5mg/kg)
4.	Group IV(EECC)	STZ (50mg/kg) + NAD (100mg/kg) + EECC (500 mg/kg)
5.	Group V(HECC)	STZ (50mg/kg) + NAD (100mg/kg) + HECC (500mg/kg)

Table 2: Phytochemical constituents of EECC and HECC

S. No.	Phytocompounds	EECC	HECC
1	Carbohydrates	+	+
2	Alkaloids	+	+
3	Flavonoids	+	+
4	Phenols	+	+
5	Reducing sugars	+	-
6	Glycosides	+	+
7	Proteins	-	-
8	Amino acids	-	-
9	Tannins	+	+
10	Saponins	-	-
11	Steroids	+	+
12	Terpenoids	+	-

+ indicates Presence and - indicates Absence

Table 3: The effect of EECC and HECC on the blood glucose levels following OGTT

Groups	0 Min	30 Min	60 Min	120 Min
Control	87.66 ±1.72	130.5 ± 1.91	87.5±2.02	89.5±2.94
Diabetic control	236.7±6.59$^{\#\#\#\#}$	394.6±6.907$^{\#\#\#\#}$	309.8±2.38$^{\#\#\#\#}$	246.5±3.51$^{\#\#\#\#}$
Standard	280.16±1.32**	317.16±1.865***	245.66±2.184****	128.16±2.03****
EECC (500mg/kg)	284.5±4.52**	306.16±2.52***	276.33±1.960**	218.5±2.15**
HECC (500 mg/kg)	297.16±5.19**	312.5±3.31***	221.5±4.41***	175±1.76****

Values are Mean ± S.E.M. $^{\#\#}$p<0.01, $^{\#\#\#}$p<0.001 $^{\#\#\#\#}$p<0.0001 compared to Normal control., **p<0.01, ***p<0.001, ****p<0.0001 compared to Diabetic Control.

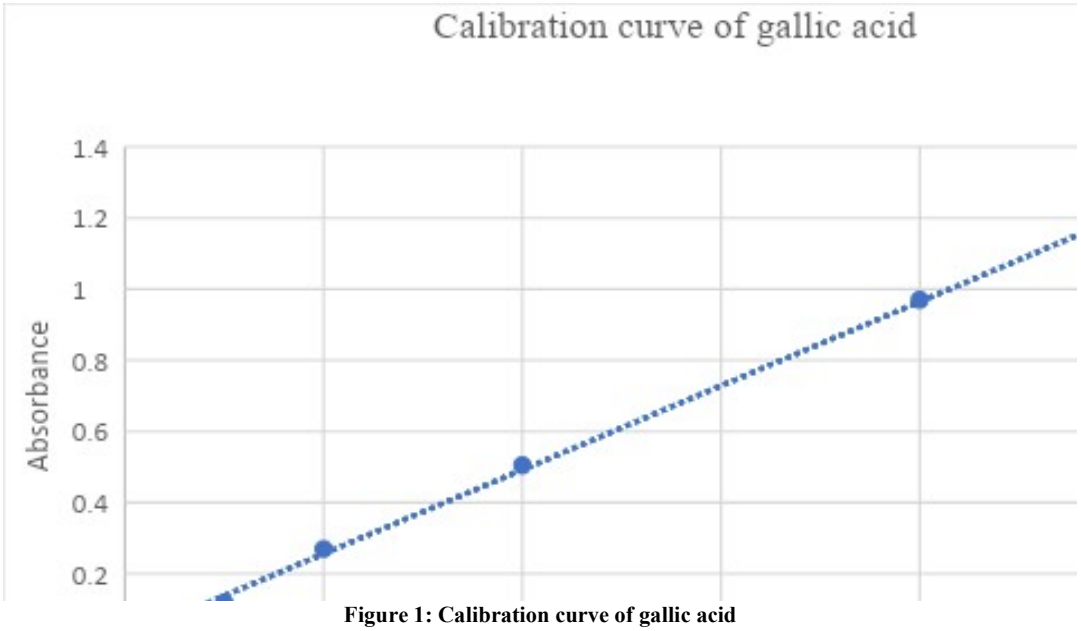


Figure 1: Calibration curve of gallic acid

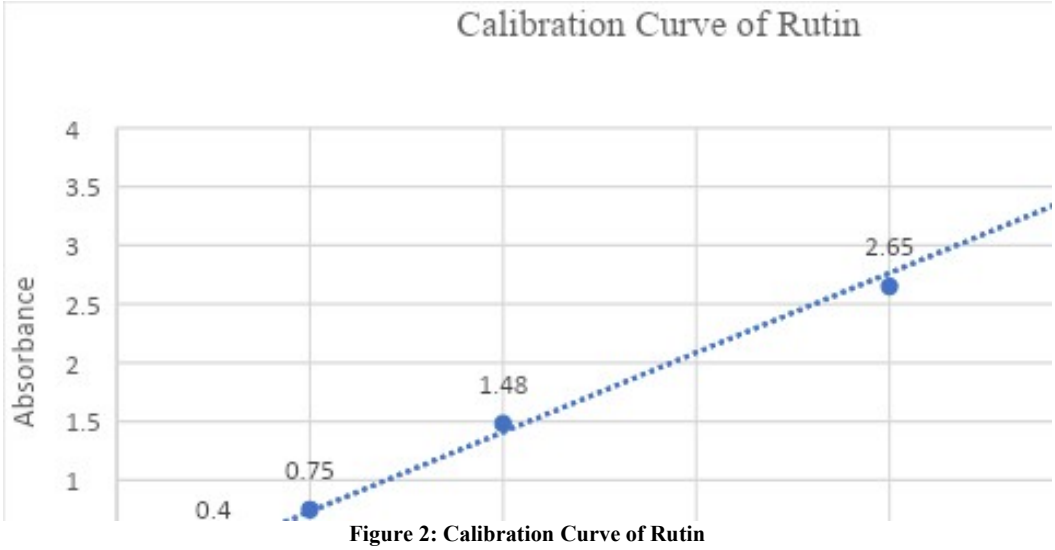
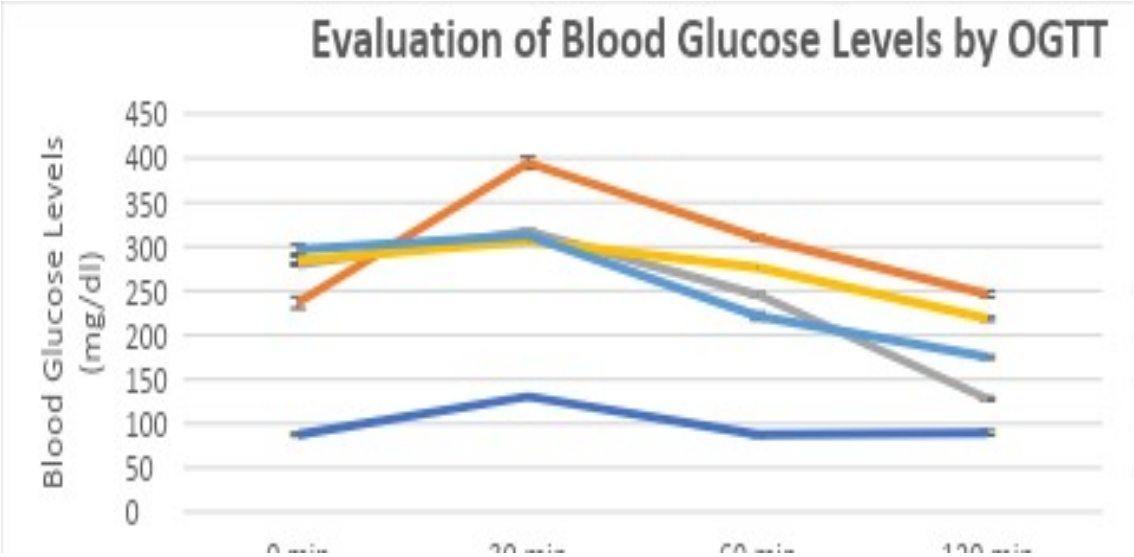


Figure 2: Calibration Curve of Rutin



Values are Mean ± S.E.M

Figure 3: Blood glucose levels blood glucose levels following OGTT

DISCUSSION

Exploring traditional herbs as potential natural remedies for managing Type 2 diabetes proves promising, and pigeonpea leaves emerge as a particularly rich source of flavonoids within the plant. These leaves reportedly contain various flavones, isoflavones, flavonols, flavanones, isoflavanones, and chalcones, with orientin and vitexin being notable abundant flavonoids **[14]**. These constituents contribute to the antioxidant, antimicrobial, hypoglycaemic, antihyper lipidaemic, neuro protective and analgesic actions **[14, 15]**.

In our current investigation, the Ethanolic extract of *Cajanus Cajan* (EECC) and Hexane extract of *Cajanus Cajan* (HECC)

exhibited higher Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) yields compared to ethyl acetate extracts previously documented **[16, 17]**. The Oral Glucose Tolerance Test (OGTT) was conducted to evaluate the animals' ability to handle a glucose challenge. Results indicated that the extracts effectively slowed the rise in blood sugar levels after a high-glucose meal, with the peak effect observed at 120 minutes, consistent with previous findings **[18]**. This observed effect may be attributed to enhanced glucose elimination through improved insulin sensitivity, particularly during fasting states in diabetic animals. The extracts' antihyperglycemic activity might involve an insulin-like action, potentially through increased peripheral glucose consumption or heightened sensitivity of β-cells to glucose. Alternatively, it could be due to the extracts' insulin secretagogue action. The regulation of insulin sensitivity is proposed to occur through the reduction of blood lipids and the alleviation of oxidative stress, facilitated by the abundance of flavonoids present in *Cajanus cajan* leaves.

CONCLUSION

The study on the EECC and HECC highlights thier rich phytochemical composition, which is believed to underlie its pharmacological activity. Results from Oral Glucose Tolerance Test (OGTT) indicate that EECC & HECC can improve glucose tolerance in diabetic animals. These findings are encouraging and warrant further investigation to explore the underlying mechanisms and potential applications of *Cajanus cajan* in human diabetes management.

ACKNOWLEDGEMENTS

The authors are thankful to the principal and management of RBVRR Women's College of Pharmacy, Barkatpura, Hyderabad for providing facilities to conduct this research work.

CONFLICTS OF INTEREST

There are no conflicts of interest.

REFERENCES:

[1] Shrivastava DK. Phytochemical analysis of a miracle herb Coriander sativum. *Indian Journal of Scientific Research*, 2017, 13(2), pp.09-14.

[2] Mohanty PK, Chourasia N, Bhatt NK and Jaliwala, YA. Preliminary phytochemical screening of *Cajanus cajan* Linn. *Asian journal of pharmacy and technology*, 2011, 1(2), pp.49-52.

[3] Orni PR, Ahmed SZ, Monefa M, Khan T and Dash PR. Pharmacological and phytochemical properties of *Cajanus cajan* (L.) Huth. (Fabaceae): A review. *Int J Pharmaceutic Sci Res*, 2018, 3(2), pp.27-37.

[4] Aja PM, Igwenyi IO, Okechukwu PU, Orji OU and Alum EU. Evaluation of anti-diabetic effect and liver function

indices of ethanol extracts of *Moringa oleifera* and *Cajanus cajan* leaves in alloxan induced diabetic albino rats. Global *Veterinaria*, 2015, 14(3), pp.439-447.

[5] Shetty P, Rai M, Ravindran A, Gopalakrishna HN, Pai VR and Kalal BS. Hypoglycemic and hypolipidemic effects of *Garcinia cambogia* extracts in streptozotocin-nicotinamide induced diabetic rat model. *International Journal of Clinical and Experimental Pathology*, 2022,*15*(9), p.380.

[6] Shaikh JR and Patil M. Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies*, 2020, *8*(2), pp.603-608.

[7] Khatib NA, Patil PA. Evaluation of Garcina indica Whole Fruit Extracts For Hypoglycemic Potential in Streptozotocin Induced Hyperglycemic Rats. *Research J. Pharm. and Tech.* 2011, 4(6), 999-1003.

[8] Shirazi OU, Khattak MMAK and Shukri NAM. Determination of total phenolic, flavonoid content and free radical scavenging activities of common herbs and spices. *Journal of pharmacognosy and phytochemistry*, 2014, *3*(3), pp.104-108.

[9] Patel PA, Parikh MP, Johari S and Gandhi TR. Antihyperglycemic activity of *Albizzia lebbeck* bark extract in streptozotocin-nicotinamide induced type II diabetes mellitus rats. *Ayu*, 2015, *36*(3), p.335.

[10] Cruz PL, Moraes-Silva IC, Ribeiro AA, Machi JF, de Melo MD, Dos Santos F, da Silva MB, Strunz CM, Caldini EG, Irigoyen MC. Nicotinamide attenuates streptozotocin-induced diabetes complications and increases survival rate in rats: Role of the autonomic nervous system. *BMC Endocrine Disorders*. 2021, 21(1), 1-0.

[11] Nahar, L, Nasrin F, Zahan R, Haque A, Haque E and Mosaddik A. Comparative study of antidiabetic activity of *Cajanus cajan* and *Tamarindus indica* in alloxan-induced diabetic mice with a reference to in vitro antioxidant activity. *Pharmacognosy research*, 2014, *6*(2), p.180.

[12] Bhargava S and Shah MB. Evaluation of Hypoglycemic activity of different extracts of *Bombax ceiba* L. leaves. *Research J. Pharm. and Tech.* 2016, 9(3), 205-208. doi: 10.5958/0974-360X.2016.00036.6

[13] Nix A, Paull CA and Colgrave M. The flavonoid profile of pigeonpea, *Cajanus cajan*: a review. *Springer Plus,* 2015, 4, 125. https://doi.org/10.1186/s40064-015-0906-x

[14] Subrata Das, Kamalesh Purkait, Kazi Asraf Ali, Pranabesh Chakraborty. Phytochemical Investigation and Hypoglycaemic Effect of *Cajanus cajan*. *Research J. Pharm. and Tech* 2016, 9(9),1341-1343. doi: 10.5958/0974-360X.2016.00256.0

[15] Ghodake RS, Chikhali US, Shinde, AD, Patil SB, Naikwade NS. Experimental Study of Analgesic Activity of *Cajanus cajan* Linn Leaves in Mice. *Research J. Science and Tech.* 2011, 3(3), 161-163.

[16] Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S and Ju YH. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. *Journal of food and drug analysis*, 2014. *22*(3), pp.296-302.

[17] Sandeep B. Patil, Nilofar S.Naikwade, Chandrakant S. Magdum, Vikas B. Awale. Centrally Acting Analgesic Activity and CNS Depressant Activity of *Cajanus cajan* Linn. *Asian J. Res. Pharm. Sci,* 2011, 1(2), 50-51.

[18] Yang SE, Lin YF, Liao JW, Chen JT, Chen CL, Chen CI, Hsu SL and Song TY, Insulin sensitizer and antihyperlipidemic effects of *Cajanus cajan* (L.) mill sp. root in methylglyoxal-induced diabetic rats. *Chinese Journal of Physiology*, 2022, *65*(3), p.125.