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ANTIDIABETIC ACTIVITY OF AQUEOUS EXTRACT OF *CAESALPINIA BONDUCELLA* LEAVES IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

The aim of present study was to evaluate the antidiabetic activity of aqueous extract of *Caesalpinia bonducella* leaves in Streptozotocin induced diabetic rats. The extract was obtained by cold maceration process and the animals were induced diabetes by single dose streptozotocin method. A comparison was made between the action of different concentration of leaves extracts of *Caesalpinia bonducella* in a dose of 100 and 200 mg/kg of body wt. and a known antidiabetic drug glibenclamide (4mg). Results shows successive decline in blood glucose level in diabetic animal. Antioxidants have been reported to prevent oxidative damage caused by free radical and may prevent the body from various diseases. The enzymatic antioxidants like catalase, superoxide dismutase have also been evaluated in the aqueous leaves extracts of *Caesalpinia bonducella* they were found to be the good sources of natural antioxidants and can be useful in treating the diseases associated with oxidative stress.

KEY WORDS: *Caesalpinia bonducella*, Antidiabetic activity, Antioxidant, Streptozotocin, Superoxide dismutase, Catalase.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder that occurs when blood glucose or blood sugar, is too high. The word diabetes is Greek for a siphon, referring to the discharge of an excess quantity of urine; and mellitus is Latin for honey. Thus it means the passage of large amount of sweet urine, either because the pancreas does not produce enough insulin, or because cells do not respond to the insulin that is produced.[1] Normal blood glucose level while fasting should be between 3.9 - 7.2 mmol/L (70–130 mg/dL) and post meal glucose levels must be less than10 mmol/L (180 mg/dL).[2]

Type 1 diabetes**,** also known as insulin-dependent diabetes mellitus (IDDM), juvenile onset, characterized by deficient insulin production. Whereas type 2 diabetes also known as non- insulin dependent diabetes mellitus (NIDDM), or adult-onset since it typically develops after age 35. It is because either our body doesn't make or the body's ineffective use of insulin.

Gestational diabetes develops during pregnancy, the time when body can become less sensitive to insulin. It does not occur in all women and most of the time, disappears after delivery. Other less common types of diabetes include monogenic diabetes, which is an inherited form of diabetes, and cystic fibrosis-related diabetes.[4]

Complications of Diabetes mellitus includes Acute Complications like Hypoglycaemia, Diabetic ketoacidosis, Hyperosmolar Hyperglycaemia State (HHS), Cystic Fibrosis, Lactic acidosis and chronic complications includes retinopathy, nephropathy, neuropathy, coronary artery disease, peripheral vascular disease and cerebrovascular disease. Others include Gastrointestinal, Genitourinary, Infectious, Dermatologic, Gum disease, Depression. Current Therapies of Diabetes mellitus includes insulin and oral hypoglycemic agents like Sulfonylureas, biguanides, meglitinides, saccharides, DPP-IV inhibitor, Thiazolidinediones which work on following mechanism,[5]

- Stimulating the release of insulin by the pancreas.
- Slowing the absorption of glucose from the intestines.
- Decreasing synthesis and release of glucose by liver.
- Making cells more sensitive to insulin (e.g. muscle and liver).

Screening Methods for Anti-Diabetic Drugs includes Models for Insulin dependent Diabetes Mellitus like Alloxan Induced Diabetes, Streptozotocin Induced Diabetes, Virus Induced Diabetes, Insulin Antibodies Induced Diabetes, Hormone Induced Diabetes, Genetic Models: Non-Obese Diabetic Mouse, Bio-Breeding Rat. As well as Models for Non-Insulin Dependent Diabetes Mellitus like Streptozotocin Induced Neonatal Model for NIDDM, Adrenaline Induced Acute Hyperglycemia, Chelating Agents: Dithizone Induced Diabetes.

Plant Profile[6,7]

Caesalpinia bonducella (Linn.) belonging to family Caesalpiniaceae, Leguminosae.

The plant is also called as Gajga, Karanju in Hindi, fever nut, bonduc nut, nicker nut in English and Kantakikaranja, Kuberakshi, in Sanskrit.

Caesalpinia bonducella is a prickly shrub widely distributed all over the world specially, in tropical regions of India, Sri Lanka, Burma and Andaman and Nicobar Islands.

Flower are simple or branched racemes yellow flowering from August-December. Fruits are oblong-obovoid pod, inflated, apex beaked, densely prickled; seed(s) 1 or 2, subglobose, shiny. Fruiting October onwards. Alternate-spiral arrangement of Bipinnate leaf.

Whole plant contains Triterpenoids, Flavonoids, glycosides, saponins, tannins, alkaloids, amino acids, isoflavones, steroidal saponin, phenolic compound etc. The twigs and young leaves of *Caesalpinia bonducella* are traditionally used for tumours, inflammation and liver disorders. In addition various parts of plant has been reported to possess multiple therapeutic properties like Adaptogenic, antimicrobial, antiproliferatve, antidiabetic, antifilarial, contractility on uterus, hepatoprotective, antitumor and antioxidant activities.

MATERIALS AND METHODS

Collections and authentication of Plant material- The leaves of *Caesalpinia bonducella* were collected from the field around the roadside area and authenticated as *Caesalpinia bonducella* belonging to Family.

Preparation of Extract-[8,9] The air dried leaves of *Caesalpinia bonducella* were finely powdered and passed through mesh size 20 to get uniform powder. The 50gm of powder was placed in, flat-bottomed glass container and soaked in 500ml distilled water for 48 hrs. With occasional shaking and stirring the above extraction is carried out by cold maceration method. Then filtered using Whatman filter paper no.42. The concentrated liquid was dried on boiling water bath in a porcelain evaporating dish. The semi-solid dark brown coloured residue obtained was dried, weighed and was stored in air tight container for further use.

Preliminary Phytochemical Studies[10]**-** The solvent extracts were subjected to standard qualitative chemical analysis to identify the phytochemical constituents present in them.

Pharmacological Study

Animals[11] **-** Adult Wistar albino rats weighing between 150 - 200 g of either sex were used. The animals were allowed to acclimatize for seven days before being used for the studies. The experimental protocol was approved by the Institutional Animal Ethical Committee of our institute. **Approval No: 1888/PO/Re/S/16/CPCSEA/2019/01.**

Acute Oral Toxicity Study[12]**-**Acute toxicity study was performed according to OECD guideline No 423. The animals were fasted overnight and provided water only.

Procedure- After the period of fasting, the animals were weighed and extract was administered. We used single dose toxicity method, different doses of the drug starting from 5, 50, 100, 200, 400, 1000, 2000mg/kg of body weight were administered to different groups. The animals were observed for any change in skin and fur, eyes and mucous membranes, grooming, respiration, hyperactivity and behaviour pattern, tremors, convulsions, salivation, diarrhoea etc. periodically for 48 hrs. following drug administration.

Induction of Diabetes- Rats were injected by streptozotocin at the dose of 60 mg/kg body weight prepared in citrated buffer (pH: 4.5) solution by IP route. After three days fasting blood sugar level was measured

and the animal having blood glucose level beyond 200 mg/dl was considered as diabetic and selected for the further investigation.

Evaluation of Antidiabetic Activity[13]- The animals were further divided into four groups of four animals each. All the test & standard drug was administered 30 minutes prior to test using oral feeding tube in a volume of 1ml.
Group 1 (Control): Vehicle i.e. 1 ml, CMC 0.5%.
Group 2 (Standard): 10 mg/kg standard drug Glibenclamide in CMC 0.5% orally.
Group 3 (Test 1):100 mg/kg plant extract in CMC 0.5%.
Group 4 (Test 2): 200 mg/kg plant extract in CMC 0.5%.

Blood samples were collected on 1st, 7th, 14th and 21st day respectively by tail prick method using insulin syringe, sample was collected and glucose level was estimated using glucometer. (Dr. Morepen Glucometer). At the end of 21 days of treatment, animals were dissected and liver was transferred into 10% formalin for further studies.

Preparation of dose: Glibenclamide tablet was crushed to fine powder. Exactly 5 mg of powdered drug was dissolved in 10 ml of CMC 0.5% prepared in distilled water to get the stock solution of concentration 0.5 mg/ml. similarly for 100mg and 200mg/kg body weight 40mg of extract was dissolved in 10 ml of CMC 0.5% to get 4mg/ml stock solution. Similarly 50mg in 10ml of CMC 0.5% prepared in distilled water we get 5mg/ml stock solution.

Preparation of Tissue Homogenate: The tissue was weighed then 10 % v/v of tissue homogenate was prepared in 1 ml, 0.1 M Phosphate buffer solution (pH 7.0), centrifuged at 10000 rpm for 20 min at 4^0C and the clear supernatant liquid was used for the estimation of catalase and superoxide dismutase.

Estimation of catalase[14,15]- This antioxidant facilitates the breakdown of hydrogen peroxide to water and oxygen.

Procedure- To 0.1 ml of diluted tissue homogenate, 1.0 ml of phosphate buffer and 0.4 ml of distilled water was added. In the test sample 0.5 ml of H_2O_2 solution was added to start the reaction, while the H_2O_2 solution was not added in control tubes. After incubation for 1 min. at 37^0C the reaction was stopped by adding 2 ml of potassium dichromate acetic acid reagent. The sample was boiled in water for 15 minutes, cooled and the absorbance of the sample was measured at 570 nm against control. H_2O_2 solution of known concentration i.e. (0-400 µM) was processed to prepare a standard curve and the slope of the standard curve was determined. The amount of µmole of H_2O_2 consumed/mg protein in the sample was calculated using the standard curve generated from known H_2O_2 solution.
Total catalase concentration was calculated according to formula,

$$\text{Catalase (U/L)} = \frac{\text{RSample Blank} - \text{RSample}}{\text{Slope (µM-1) x 30 min}} \text{ x n}$$

Where RSample Blank and RSample are optical density readings of the Sample Blank and Sample respectively. 30 min. is the catalase reaction time. n is the sample dilution factor. One unit is defined as the quantity of catalase that decomposes 1µmole of H_2O_2 per min. at pH 7.0 and room temperature.

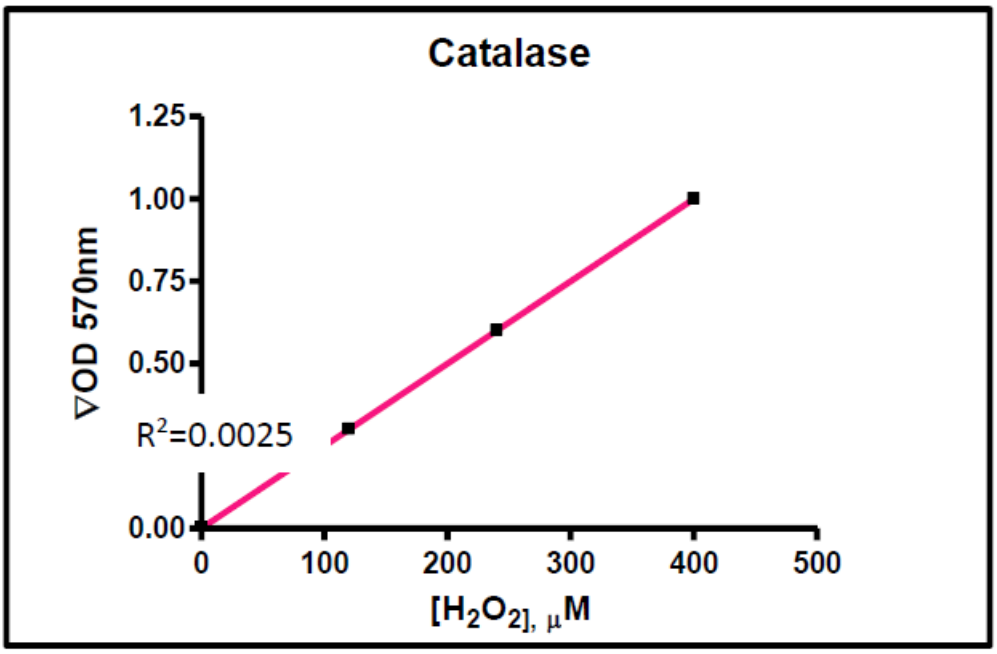

Fig.1 Graph of H_2O_2 µM against ΔOD at 570nm.

The slope of the curve is 0.0025 means for each positive change of 1 unit in X variable; the Y variable will increase by 0.0025 units.

Estimation of superoxide dismutase- The dismutation of superoxide into molecular oxygen and H_2O_2.

Procedure- In a test tube, 0.5 ml of supernatant of tissue homogenate was taken. 160 µL Assay buffer, 5 µL Xanthine and 5µL water soluble tetrazolium salts was added to the supernatant liquid and mixed well. Finally 20 µL diluted XO enzyme solution was added to the mixture and immediately measured the optical density at 440nm (OD_0). The mixture was incubated for 60 min at room temperature (25°C) in the dark and optical density was again taken at 440nm (OD_{60}). Superoxide dismutase enzyme solution of known concentrations (0-3µmol) was processed to prepare a standard curve and the slope of the standard curve was determined. The amount of µmol of SOD consumed /mg protein in the sample was calculated using the standard curve. Total SOD concentration of sample was calculated as per formula,

$\Delta OD_{60} = OD_{60} - OD_0$.

OD_0 is the optical density of the sample and standard solutions measured prior to incubation, whereas $OD_{60}$is the optical density of the sample and standard solutions measured after incubation period of 60 min.

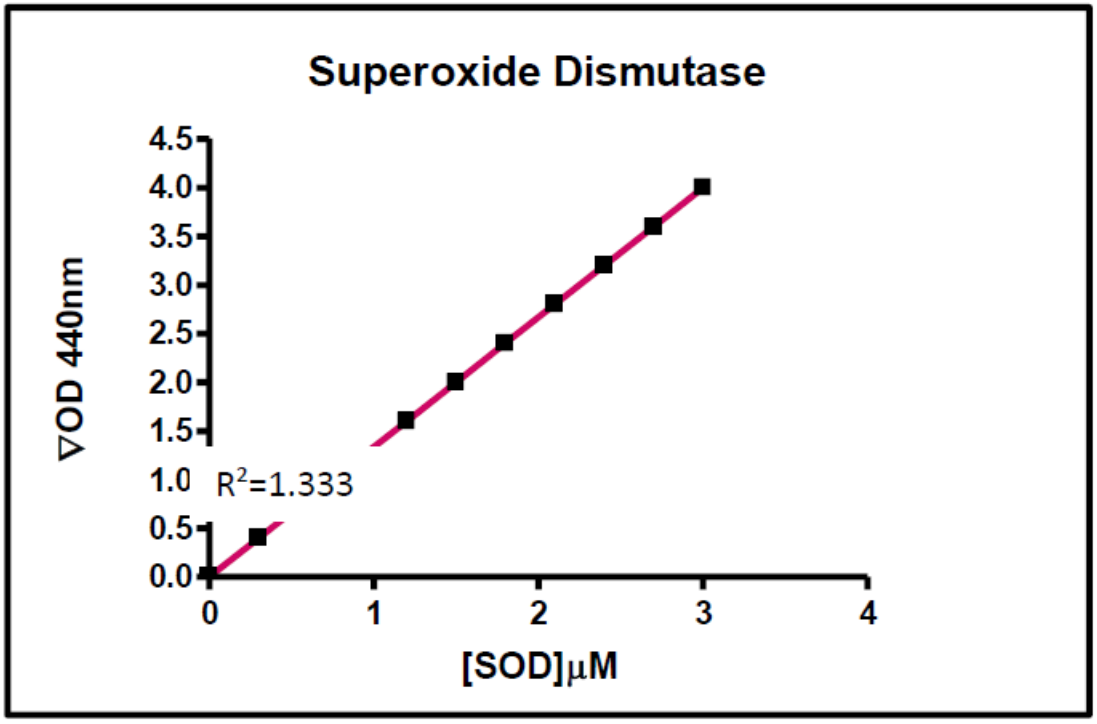

Fig. 2: Graph of SOD µM against ΔOD at 440nm.

The slope of the curve is 1.33 means for each positive change of 1 unit in X variable; the Y variable will increase by 1.333 units.

RESULT AND DISCUSSION

Plant Extraction- The percentage yield of aqueous extract of *Caesalpinia bonducella* leaves was found to be 3.4 gm. (6.8%) in Semi solid consistency and darkish brown in colour.

Phytochemical Analysis- The extract revealed the presence of Saponins, Tannins, Amino acids, Proteins, Glycosides, Alkaloids, Carbohydrates and Flavonoids.

Acute Oral Toxicity Studies- Acute toxicity study of aqueous leaves extract of *Caesalpinia bonducella* was carried out for 24 hours with maximum dose of 2000 mg/kg. During the study period it was observed that the extract did not produce any behavioural changes such as excitement, restlessness, respiratory distress, convulsions or coma. Also no mortality was observed during the study period.

Evaluation of Antidiabetic Activity- dose dependent hypoglycemic effect was observed in streptozotocin induced diabetic rats. Treatment with standard drug glibenclamide, demonstrated a significant decline in the level of serum glucose on 7th, 14th and 21st day of drug administration as compared to the diabetic control group. On administration of aqueous extracts of the plants, the blood glucose was significantly decreased on day 7th, 14th and 21st in contrast to the diabetic control group.

Table 1: Effect of *Caesalpinia bonducella* on blood glucose in diabetic rats.

S. No.	Treatment	Blood glucose(mg/dl)			
		1st Day	7th Day	14th Day	21st Day
1	Diabetic control	221.5±3.69	229±4.29	238.33±2.36	249.5±2.47
2	Glibenclamide	234.16±3.38**	145±2.73**	114±3.18**	85±4.50**
3	*C. bonducella* Ext. (100mg/kg)	220.33±2.04**	158.33±2.83**	131.66±3.54**	128.66±1.49**
4	*C. bonducella* Ext. (200mg/kg)	218.5±2.43**	151.16±1.30**	130±1.18**	100.66±1.11**

The values are mean ± SEM. (n=6). **p<0.01; when compared with diabetic control group

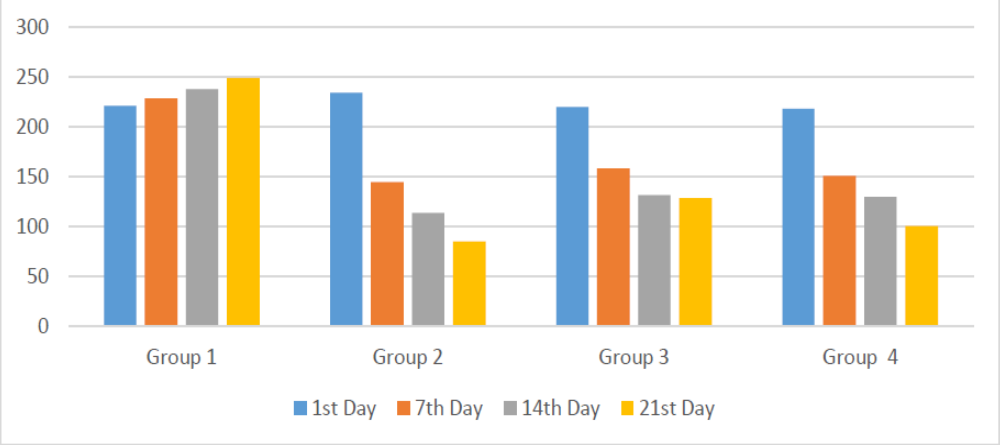

Figure 3: Effect of *Caesalpinia bonducella* leaves extract on blood glucose in diabetic rats.

Evaluation of Antioxidant Activity- Streptozotocin induced diabetic control group shows decrease in levels of antioxidant enzymes SOD, CAT. The glibenclamide treated group significantly increase the level of SOD, CAT when compared with diabetic control group. Whereas treatment with aqueous extract of *Caesalpinia bonducella* shows significant increase in these enzyme levels when compared with diabetic control group.

Table 2: Effect of Caesalpinia bonducella on antioxidant's level in diabetic rats.

S. No.	Treatment	CAT (µmol of H_2O_2/mg protein) 21st Day	SOD (µmol/mg protein) 21st Day
1	Diabetic control	34.88±2.71	2.48±0.15
2	Glibenclamide	60.59±1.90**	4.71±0.22**
3	*C. bonducella* Ext. (100 mg/kg)	43.40±1.89*	3.17±0.08*
4	*C. bonducella* Ext. (200 mg/kg)	48.66±1.99**	4.09±0.15**

The values are mean ± SEM. (n=6). *p<0.05 & **p<0.01; compared with diabetic group

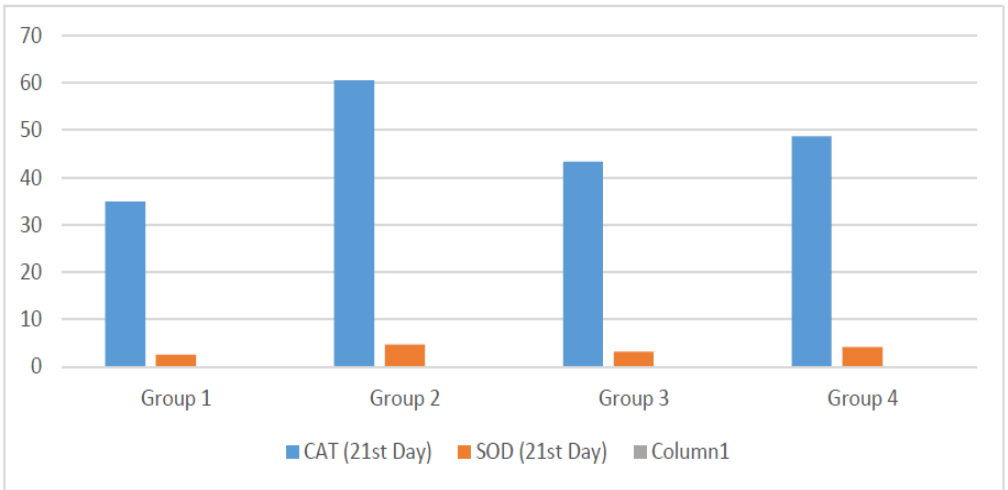

Figure 4: Effect of *Caesalpinia bonducella* leaves extract on antioxidant activity in diabetic rats.

DISCUSSION

The aqueous leaves extract of *Caesalpinia bonducella* treated test group show significant decrease in blood glucose level on 21st day respectively as compared to that of control group. This shows that the extract treatment could reduce the blood glucose level towards the normal level within 21 days of study. Though the detailed mechanism of action of the test samples has not been known it is assume that it may lead to normalize the lipid profile and also stimulate the secretion of insulin from beta cells of islets of Langerhans or by increase in peripheral glucose uptake. The presence of phytoconstituents like Flavonoids, sterols/triterpenoids, alkaloids and phenolic compounds are also known to play a vital role for anti-diabetic principles which could act synergistically or independently in lowering the blood sugar level.

In this study we get to know that increase in the blood glucose level lead to the generation of free radicals by glucose auto-oxidation and this increase of free radicals may lead to liver cell damage. We can say that *Caesalpinia bonducella* leaves extract showed strong antioxidant activities with increase in catalase and superoxide dismutase enzyme concentration. Antioxidants are substances that delay the oxidation process, by inhibiting the polymerization chain initiated by free radicals and other subsequent oxidizing reactions. In which superoxide dismutase is one of the most indispensable enzyme for protecting the cells from the toxicity of the reactive oxygen radicals. While catalase is a tetrahedrical protein, constituted by four heme groups which catalyze the dismutation of hydrogen peroxide in water and oxygen.

CONCLUSION

Hypoglycemic agents from plant origin used in medicine are important as they may show the effect due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or a decrease in the intestinal absorption of glucose. The plants found to contain substances like glycosides, alkaloids, terpenoids, flavonoids etc. that are reported to have anti diabetic effects. The presence of flavonoids may be responsible for the observed antidiabetic activity. The various enzymatic antioxidants level has also been evaluated with aqueous leaves extracts of *Caesalpinia bonducella.* They were found to be the good sources of natural antioxidants and can be useful in treating the diseases associated with oxidative stress. Thus it is concluded that aqueous extract of *Caesalpinia bonducella* leaves has effective antidiabetic effect.

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BIBLIOGRAPHY

1. Petit, William A, "The Encyclopedia of diabetes", Library of Congress cataloging in Hermitage, Printed by Sheridan,2011, vii.
2. International Diabetes Fedration (IDF) Atlas, III (ed.), 2006.
3. Powers A.C. and David D' Alessio, "Goodman Gilman's: The Pharmacological Basis of Therapeutics", Brunton L.L, Chabner B. A. and Knollmann B.C. (Ed.), XII (ed.), The McGraw-Hill Companies, Inc., Printed in China., 2011: 1237-73.
4. Sleevi M, "Burger's medicinal Chemistry Drug Discovery", Abraham D J (Ed.), IV (ed.), vol. 4(4), Published by John Wiley and Sons, Inc., Hoboken, New Jersey, Printed and bounded in India by Replika Pree Pvt. Ltd.,2007, 1-44.
5. Konan A B, Bleyere M N, Amonkan A K, Bouafou M K G, Datte J Y, "Why african traditional birth attendants used *Caesalpinia Bonducella* leaves to facilitate childbirth in women," International Journal of Pharmacy Review & Research[Internet]. 2014; 4(1): 11-6.
6. Shukla Shruti, Mehta A, Mehta P, Vyas S P, Shivaprasad H N, "In vivo immunomodulatory activities of the aqueous extract of *Caesalpinia bonducella* seeds", Pharmaceutical Biology taylor and francisco, January 2010; 48(2): 227-230.
7. Manikandaselvi S, Vadivel V, Brindha P, "Studies on Nutraceutical Properties of *Caesalpinia bonducella* L.: An Indian Traditional Medicinal Plant", Research Journal of Medicinal Plant, January 2016; 10(2): 127.139.
8. Dr. K R Khandelwal, Dr. Varunda Sethi, "Practical Pharmacognosy, techniques and experiments", Nirali prakashan, edition no. 25th, 2015: 25.1-25.9.
9. OECD guidelines 423, https://ntp.niehs.nih.gov/iccvam/suppdocs/oecd/oecd_gl423.pdf.
10. S S Agrawal, M Paridhavi, "Herbal drug technology", 2nd E dition, Universities press (India) private limited, 2012: 321-341 & 512-515.