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PHYTOCHEMICAL INVESTIGATION AND EVALUATION OF ANTIOXIDANT AND ANTIDIABETIC ACTIVITIES OF SEEDS OF *SARACA ASOCA* ROXB. DE WILDE

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ABSTRACT: Diabetes mellitus (DM) is a chronic and major endocrine disorder caused by an inherited and/or acquired deficiency in the production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. It is a growing health problem in most countries, and its incidence is considered to be high (4%-5%) all over the world. 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. This study focuses on the *in-vitro* investigation of the antioxidant and *in-vivo* antidiabetic activity of the Methanol and aqueous extracts of *Saraca asoca* (Roxb.) De Wild seeds in healthy adult Wistar albino rats weighing 200-250 g and Swiss albino male mice (25-35 g). Swiss albino mice were made diabetic by a single dose of streptozotocin (150 mg/kg i.p.). Blood glucose levels and body weights of mice were measured using weekly intervals, i.e, day 0, 7, 14, and 21 after daily administration of all extracts at doses of 250 and 500 mg/kg. Other biochemical parameters such as serum cholesterol, triglycerides, HDL-cholesterol, VLDL-cholesterol, LDL-cholesterol, urea, creatinine, and protein levels were also measured at the end of the study. Phytochemical analysis identified the presence of alkaloids, glycosides, carbohydrates, steroids, and flavonoids in both extracts. The antioxidant activity of all the extracts was measured using DPPH and H₂O₂ assays. Additionally, glucose production was assessed through the inhibition of the α -amylase enzyme. *S. asoca* possesses antioxidant and antidiabetic properties as well as improves body weight, liver profile, renal profile, and total lipid levels.

INTRODUCTION: In this modern era for primary health care, most of the world's population still uses herbal medicine because of its better cultural acceptability, better compatibility with the human body, and fewer side effects. Diabetes is one of the major health problems in developing countries and the third major cause of death in the world.

Diabetes mellitus (DM), also known as simply diabetes, is a group of metabolic diseases in which high blood sugar levels over a prolonged period can be seen. Hyperglycemia (elevated blood sugar) is a common symptom of diabetes mellitus (DM), a chronic disorder affecting the metabolism of carbohydrates, fats, and proteins, stemming from issues with insulin levels, insulin function, or both.

Currently, diabetes is among the most significant global health concerns, leading to serious microvascular and macrovascular complications. By 2040, it is estimated that around 700 million people worldwide will be affected by diabetes. Chronichyperglycemia causes complications linked

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to diabetes, such as heart disease, retinopathy, kidney disease, and neuropathy. Medicinal plants have consistently served as key sources of medicine around the world. Several drugs, such as sulfonylureas, metformin, and α -glucosidase inhibitors, are presently used to reduce hyperglycemia. Despite the use of many hypoglycemic agents, diabetes and its linked complications are still an important medical problem. Excessive oxidative stress has been implicated in the pathology and complications of diabetes mellitus^{1,2,3,4}.

Free radicals are implicated in numerous conditions such as diabetes, inflammation, and cancer, which has increased the interest in antioxidant therapies. Diabetes, a metabolic disorder caused by insufficient insulin or its improper metabolism, has seen a global rise in prevalence, with projections indicating further increases in future generations. Among the various therapeutic approaches to manage diabetes, regulating blood glucose levels through different mechanisms is critical. These radicals further damage other important biomolecules including carbohydrates, proteins and DNA. Streptozotocin (STZ) selectively destroy β -cells of pancreas by generating excess ROS and carbonium ion (CH_3^+) leading to DNA breaks by alkylating DNA bases. The N-nitroso-N-methylurea portion of the molecule exhibits diabetogenic activity. Glucose may act as a carrier for this cytotoxic group. Plants have been commonly used to treat diabetes since ancient times and have served as an exemplary source of medicine^{5,6,7}.

We have recently reviewed the Indian plants that may have an antidiabetic potential. The treatment of DM in clinical practice has been confined to use of oral hypoglycemic agents and insulin, the former being reported to be endowed with characteristic profiles of serious side effects. This leads to increasing demand for herbal products with antidiabetic factor but little side effects. A large number of plants have been recognized to be effective in the treatment of DM^{8,9,10}.

Although synthetic antidiabetic drugs, such as insulin, are available, they do not offer long-term glycemic control without causing undesirable side effects. This has led to an increased interest in

using herbal remedies for managing DM. The World Health Organization has advocated for the use of traditional medicinal plants in diabetes treatment due to their safety, effectiveness, low side effect profile, and affordability^{11,12}. The *S. asoca* plant is known for a range of pharmacological effects, including analgesic, antipyretic, anti-inflammatory, antispasmodic, anthelmintic, antimicrobial and antioxidant activities. Thus, extracts from the plant parts were analyzed for them *in-vitro* antioxidant and antidiabetic potential. Our results demonstrated that *Saracaasoca* extracts exhibited significant *in vitro* antioxidant and antidiabetic activity, indicating that these extracts warrant further investigation in pharmacological studies.

MATERIAL AND METHOD:

Collection of Plant Material: The seeds of *S. asoca* were freshly collected from the hilly areas of the Nanded district and authenticated by botanists from the Department of Botany. It was then dried in the shade, ground into a powder, and subjected to extraction using petroleum Ether, Ethyl acetate, methanol and aqueous extract.

Preliminary Phytochemical Screening: A preliminary phytochemical screening of the active extracts was conducted using established phytochemical procedures^{13,14}. Preliminary phytochemical screening of the extract has revealed the presence of carbohydrates, flavonoids, polyphenols, tannins, and saponins. However, no alkaloids, proteins and amino acids were found in the extracts.

Estimation of Total Phenolic Content: The total phenolic content in the crude extracts was measured using the Folin-Ciocalteu method. Various concentrations of the extracts (100 μl) were placed into test tubes, followed by the addition of 1 ml of Folin-Ciocalteu reagent and 0.8 ml of sodium carbonate solution (7.5%). The tubes were thoroughly mixed and left to sit for 30 minutes. Absorbance was then recorded at 765 nm using a UV-visible spectrophotometer.

Estimation of Total Flavonoids: The total flavonoid content in the extracts was assessed as follows: 0.5 mL of a 2% AlCl_3 ethanolic solution was mixed with 0.5 mL of the extracts and allowed

to stand for 1 hour at 25°C. After this period, the absorbance was measured at 420 nm. The development of a yellow color indicated the presence of flavonoids. The total flavonoid content was quantified as quercetin equivalent (mg/g) using the equation derived from the calibration curve.

Animals: Experiments were performed using Swiss albino male mice (25-35 g), and Healthy adult Wistar albino rats weighing 200-250 g were used for the pharmacological studies. The animals were housed in polypropylene cages, maintained under standard conditions (12/12 h light and dark) at 25 ± 3°C and 35-60% humidity. They were fed with a standard rat pellet diet (Amrut, India) and water *ad libitum*. The Animal Ethical Committee of the Institute approved all the protocols of the study before conducting the experiments.

Acute Toxicity Studies: An acute oral toxicity study was performed according to the OECD-423 guidelines. Through random sampling, albino rats of both sexes were selected and used for an acute toxicity study. Animals were fasted for 3-4 h before dosing. Following the period of fasting, all extracts at doses of 200, 300, 600, 1000, 1500, and 2000 mg/kg b.w. were administered to six groups with 6 rats each. Animals were observed individually after dosing at least once during the first 30 min periodically during the first 24 h, with special attention given during the first 4 h time of onset and length of recovery period were observed. Additional observations include changes in skin and fur, eyes and mucous membranes, and also somatomotor activity and behaviour patterns. Attentions were given to observations of tremors, convulsions, salivation, diarrhoea, sleep, and coma 15, 16, 17.

Biochemical Estimation: Blood samples were collected by cardiac puncture and the retro-orbital plexus method from all animals into EDTA-anticoagulated tubes and were centrifuged at 3000 rpm for 20 minutes. Serum was separated and stored at -20°C until analysis was performed. Serum samples were analyzed for cholesterol, HDL, total proteins, urea, creatinine, and triglycerides using the diagnostic kit (ERBA Diagnostics Mannheim, Germany) in an Autoanalyzer. LDL and VLDL were calculated using the following formulae:

$$\text{LDL} = \text{TC} - \text{TG}/5 + \text{HDL}$$

$$\text{VLDL} = \text{TC} - \text{LDL} + \text{HDL}$$

In-vitro Evaluation of Anti-Oxidant Activity: Evaluation of Scavenging Activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH) Radicals: The radical scavenging activity of all extracts was measured by the DPPH method. Extracts or standard solution 1 ml (10-50 µg/ml) was added separately to 2 ml of DPPH in methanol (100 mM). After incubation at 37°C for 30 min, the final volume was made up to 4 ml with methanol and the absorbance of the individual reaction mixture was measured at 517 nm using a spectrophotometer. A reaction mixture without test sample served as a control. Ascorbic acid was used as a standard and antioxidant activity was measured in terms of ascorbic acid equivalents⁸⁻¹⁰. The percentage of inhibition was calculated by comparing the absorbance values of the control and the sample using the formula;

$$\% \text{ Inhibition} = \frac{\text{A517 (Control)} - \text{A517 (Sample)}}{\text{A517 (Control)}} \times 100$$

Where, A517 (Control) - Absorbance of solution mixture without extract, A517 (Sample) - Absorbance of sample solution.

In-vitro Antioxidant Activity: A broad class of chemical substances known as antioxidants is essential for preserving cellular health because it prevents oxidation reactions and neutralizes reactive oxygen species (ROS) and dangerous free radicals. Naturally occurring by products of regular cellular metabolism, especially during aerobic respiration, are these reactive compounds. Even while ROS are necessary for some cell signalling pathways, an excessive build-up of them can result in oxidative stress, which damages proteins, lipids, DNA, and other important macromolecules.

Antioxidants are therefore essential for maintaining homeostasis and cellular defense. ROS are mostly produced by aerobic metabolic processes in higher plants, particularly in the mitochondria, peroxisomes, and chloroplasts, results in the production of partially reduced forms of molecular oxygen, including hydrogen peroxide (H₂O₂), superoxide anions (O₂⁻), and hydroxyl radicals (•OH).

Although ROS cannot be prevented, plants have developed a sophisticated and very effective antioxidant defense mechanism to mitigate their negative effects.

DPPH Radical Scavenging Assay (1, 1-diphenyl-2-picrylhydrazyl): The antioxidant activity of the different *Saraca asoca* extracts was evaluated by their ability to decolorize the purple DPPH methanol solution. 1 mL of a 0.3mM DPPH methanol solution was mixed with 1 mL of the extracts at varying concentrations (0.2–1.0 mg/mL), and the mixture was kept in the dark at 37°C for 30 min. The absorbance of the mixture was then measured at 517 nm against a blank using a UV-visible spectrophotometer. The percentage of DPPH radical inhibition (I %) was calculated using the following formula:

$$\text{Percentage inhibition (I\%)} = (A_{\text{control}} - A_{\text{extract}}) / A_{\text{control}} \times 100$$

Where A_{control} is the absorbance of the control, A_{extract} is the absorbance of the extract.

Hydrogen Peroxide (H₂O₂) Assay: The hydrogen peroxide scavenging ability of the *S. asoca* extract was assessed using a standard procedure. A 2 mM hydrogen peroxide solution was prepared in a 50 mM phosphate buffer (pH 7.4). Aliquots (0.1 mL) of the extract at various concentrations (50, 100, 150 µg/mL) were placed into test tubes, and their volumes were adjusted to 0.4 mL with the same buffer. After adding 0.6 mL of the hydrogen peroxide solution, the tubes were vortexed, and absorbance at 230 nm was measured after 10 minutes, using a blank for reference. The hydrogen peroxide scavenging activity was calculated using the equation.

In-vivo Anti-Diabetic Activity:

α-amylase Activity (DNSA): Five different concentrations of the plant extract were prepared by dissolving it in double-distilled water, set at 2 µg/mL, 4 µg/mL, 6 µg/mL, 8 µg/mL, and 10 µg/mL. A mixture of 500 µL of the plant extract and 500 µL of 0.02 M sodium phosphate buffer

was incubated for 10 minutes at 25°C. Following this pre-incubation, 500 µL of a 1% starch solution in 0.02 M sodium phosphate buffer was added to each tube in 5-second intervals. The mixture was then incubated again for 10 minutes at 25°C. To halt the reaction, 1 mL of DNSA color reagent was added, and the tubes were incubated for 5 minutes before cooling to room temperature. Finally, the reaction mixture was diluted with 10 mL of distilled water, and absorbance was recorded at 540 nm.

Streptozotocin-induced Diabetic Male Albino

Rats: The individual dose of streptozotocin (STZ) to be injected into overnight-fasted animals was prepared fresh in ice-cold citrate buffer (pH 4.5). STZ (150 mg/kg) was injected intraperitoneally into the animals. Control mice received an equivalent amount of citrate buffer. Twelve days after the STZ injection, mice with fasting blood glucose levels greater than 200 mg/dL were considered diabetic. The studies were conducted on four groups of animals. Group I: Normal rats; Group II: Diabetic (STZ-induced) control rats; Group III: Short-term (ST): Diabetic animals kept for 7 days; Group IV: Long-term (LT): Diabetic animals kept for 25 days. The diabetic animals in both the ST and LT groups received seed extract in increasing doses of 250 mg, 350 mg, and 450 mg/kg body weight to assess the therapeutic effects of the extracts. Separate batches were maintained within each group for each dose level. Plasma insulin levels were estimated using a RIA assay kit for rats supplied by Ljico Research Inc. (Stat Diagnostics, Mumbai). The results were expressed as mean ± standard deviation. Statistical analysis was performed using one-way ANOVA with the standard SPSS software.

RESULTS & DISCUSSION:

Phytochemical Screening and Standardization of Extracts: Phytochemical classes characterized in different extracts of the seeds of *Saraca asoca* have been presented in following **Table 1**.

TABLE 1: PHYTOCHEMICAL SCREENING OF DIFFERENT EXTRACTS OF SARACA ASOCA SEEDS

| Extract | Petroleum ether | Ethyl acetate | Methanol | Water |
|-------------------------------|-----------------|---------------|----------|-------|
| Test for phytochemical | | | | |
| Flavonoids | + | + | + | + |
| Tannins | + | + | + | + |
| Alkaloids | - | - | - | - |

| | | | | |
|-----------------------|---|---|---|---|
| Saponins | + | + | + | + |
| Carbohydrates | - | + | + | + |
| Protein & amino acids | - | - | - | - |
| Oils and Fats | + | + | + | - |

(-) absence of phytoconstituents, (+) presence of phytoconstituents.

Preliminary phytochemical screening of extract has shown the presence of carbohydrates, flavonoids, polyphenols, tannins and saponins. However, no alkaloids, proteins and amino acids were found in the extracts.

Estimation of Total Phenolic Content: Total phenolic content was determined using Folin-Ciocalteu reagent. From the analysis it was observed that methanol extract of seed showed highest phenolic content (189.12±0.27 mg/ml) **Table 2**. Hence, it was observed that methanol is a good solvent for the extraction of phenols and these results are in agreement with the study where methanolic extracts showed highest total phenolic content.

Estimation of Total Flavonoid Content: Methanolic extract of *Saraca asoca* seeds (7.45±0.25mg/ml) exhibited highest flavonoid content **Table 2**. The results indicate that flavonoid content was mostly abundant in the seed of *Saraca asoca*. Flavonoids occur naturally in plants which not only have positive effect on human health but also possess antibacterial, antiviral and anti-inflammatory, anticancer, and anti-allergic activities²⁰.

Flavonoid also show strong antioxidant activity, hence in our study we observed a strong positive correlation between total flavonoid content and DPPH radical scavenging activity

TABLE 2: PHYTOCHEMICAL STANDARDIZATION OF SARACA ASOCA EXTRACTS

| Plant | Extract | Total phenolic content* | Total flavonoid content [§] |
|---------------------|---------|-------------------------|--------------------------------------|
| <i>Saraca asoca</i> | PE-SA | 35.14±0.16 | 1.42±0.15 |
| | EA-SA | 150.12±0.12 | 4.12±0.18 |
| | ME-SA | 189.12±0.27 | 7.45±0.25 |
| | WE-SA | 134.55±0.24 | 5.10±0.16 |

All the determinations were carried out in triplicates and expressed in µg/mg of crude extracts. Values are representatives of mean ± standard deviation (SD).

*-µg/mg of GAE, §-µg/mg of RE. Among SA phenolic content 189 µg/mg of GAE, total extracts ME-SA exhibit highest amount of total flavonoid content and 7.45 µg/mg of RE

TABLE 3: ANTI-OXIDANT ACTIVITY OF THE EXTRACTS BY DPPH ASSAY

| Sr. no. | Concentration (µg/ml) | BHA | Ascorbic acid (% inhibition) | Ethyl acetate extract (% inhibition) | Methanolic extract (% inhibition) | Aqueous Extract (% Inhibition) |
|---------|-----------------------|-------|------------------------------|--------------------------------------|-----------------------------------|--------------------------------|
| 1 | 50 | 78.30 | 78.20 % | 59.51% | 56.25 % | 48.50 % |
| 2 | 100 | 83.50 | 85.21 % | 62.20% | 61.10 % | 57.30 % |
| 3 | 150 | 85.36 | 87.25 % | 82.36% | 83.50 % | 76.51 % |

TABLE 4: ANTI-OXIDANT ACTIVITY OF THE EXTRACTS BY H₂O₂ ASSAY

| Sr. no. | Concentration (µg/ml) | BHA | Ascorbic acid (% inhibition) | Ethanol extract (% inhibition) | Methanolic extract (% inhibition) | Aqueous extract (% inhibition) |
|---------|-----------------------|-------|------------------------------|--------------------------------|-----------------------------------|--------------------------------|
| 1 | 50 | 63.80 | 61.48 % | 57.10% | 55.21 % | 54.80 % |
| 2 | 100 | 65.31 | 95.16 % | 60.83% | 62.50 % | 59.10 % |
| 3 | 150 | 68.50 | 97.54 % | 85.11% | 83.35 % | 77.21 % |

TABLE 5: IN-VITRO ANTIDIABETIC ACTIVITY OF THE METHANOL EXTRACT USING A-AMYLASE METHOD AND COMPARISON WITH STANDARD DRUG ACARBOSE

| Sr. no. | Concentration µg/ml | % of Inhibition of Acarbose | IC ₅₀ µg dry extract | % of Inhibition <i>S. asoca</i> Extract | IC ₅₀ µg dry extract |
|---------|---------------------|-----------------------------|---------------------------------|---|---------------------------------|
| 1 | 2 | 7.98±0.02 | 0.37 | 3.35±0.01 | 0.76 |
| | 4 | 12.55±0.03 | | 6.11±0.02 | |
| | 6 | 27.44±0.01 | | 11.85±0.02 | |
| | 8 | 42.63±0.04 | | 14.63±0.05 | |
| | 10 | 60.12±0.03 | | 20.14±0.02 | |

The methanol extract (2-10 µg/ml) of the plant exhibited potent α -amylase inhibitory activity in a dose-dependent manner. The extracts showed inhibitory activity with an IC₅₀ value of 0.76 µg dry extract, respectively **Table 5**. Acarbose is a standard drug, and its concentration of (2-10 µg/ml) showed α -amylase inhibitory activity with an IC₅₀ value 0.37µg dry extract.

The ethanol extracts of the plant showed maximum α -amylase inhibitory activity (IC₅₀ = 0.76µg dry extract), which could be attributed to the presence of polyphenols and flavonoids, because polyphenols are not only capable of reducing oxidative stress but also of inhibiting carbohydrate-hydrolysing enzymes because of their ability to bind with proteins.

TABLE 6: ANTIDIABETIC EFFECT OF METHANOLIC SEED EXTRACT OF MEDICINAL PLANT, *S ASOCA* SERUM INSULIN LEVELS (µ/ML)

| Term | Normal (N) | Diabetic control | Experiment groups (mg/kg body wt) | | |
|------------|-------------|------------------|-----------------------------------|-------------|-------------|
| | | | 250 | 350 | 450 |
| Short Term | 17.27 ±1.67 | 12.10±1.30 | 13.13±1.40* | 15.30±1.08* | 16.26±1.40* |
| Long term | 17.27±1.67 | 7.32±1.21 | 8.61±0.83* | 8.80±1.10* | 9.17±0.92* |

Data are expressed as Mean±SD of 6 individual observations. Statistical significance * P<0.001.

To study the antidiabetic effect of the methanolic seed extract, diabetes was induced in the male albino rats by the intraperitoneal injection of streptozotocin. After 48 hrs of injection of STZ to normal rats, diabetes was evidenced. The blood glucose levels were significantly elevated (+343 %) in the STZ-injected rats when compared with those of normal (Placebo), and therefore considered as diabetic animals control.

The seed extract of *S asoca* that was fed to the diabetic animals recorded significantly lower blood glucose levels of 291, 185, and 103 mg/dL at I hr, II hr, and IV hr of time intervals, respectively. However, the percentage of decrement was elevated at IV hr after the extract was fed to the diabetic animals. Studies were designed to assess the impact of aqueous seed extract of *S. asoca* on serum insulin levels in STZ-induced diabetic male albino rats. The studies were conducted in two groups of STZ-induced diabetic animals. Group I was short-term (7 days-diabetic animals). Group II long-term (25 days-diabetic animals). The animals of both groups were fed with plant extracts in increasing doses, i.e., 250 mg, 350 mg, 450 mg/kg body weight, to assess the insulin-augmenting effect in the diabetic animals. The serum insulin levels were recorded as a significant depletion in both groups, short-term as well as long-term diabetic animals, when compared to those of normal animals. The serum insulin, a significant augmenting effect of the seed extract was recorded in both short-term as well as long-term groups, and also recorded as dose-dependent on the extract **Table 6**. Further investigation was carried out to

assess the hypoglycemic effect of the above plant extract on serum insulin levels in STZ-induced diabetic animals. It was correlated and assessed that higher serum glucose levels were due to lower insulin levels in the diabetic animals, which might be due to lower secretion of insulin from the beta-cells of islets of Langerhans.

In the present study, the plant extract augmented the serum insulin levels, suggesting an improved state of availability of serum insulin to control blood sugar. This might be due to higher secretion of the hormone in seed extract-fed animals. The present study showed that the insulin serum augmenting effect was recorded highest at the dose of 450 mg/kg body weight, suggesting that the serum insulin effect of the seed extract is dose-dependent.

However, the therapeutic effect of the seed extract was recorded as higher in short-term group animals when compared to that of the long-term group. This might be due to the inability of the beta cells to recover from the STZ effect in the long-term animals. The insulin-augmenting effect of the *Saraca asoca* methanolic seed extract was significantly higher in short-term STZ-induced diabetic animals when compared to the long-term group. This suggests that the beta cells of Langerhans regenerating effect of the *Saraca asoca* seed extract was higher in the short term group. However, we suggest that further work should be carried out at the molecular level to find out the absolute mechanism of action of plant *S. asoca* in experimental diabetes.

CONCLUSION: In the present study, the successive extraction of *Saraca asoca* seeds powder has been carried out in petroleum ether, ethyl acetate, methanol and water. Antioxidant study is carried out by estimation of DPPH radical scavenging activity and H₂O₂ assay method. The methanol extract shows potent antioxidant activity. Additionally, glucose production was assessed through the inhibition of the α -amylase enzyme. *S. asoca* possesses antioxidant and antidiabetic properties as well as improves body weight, liver profile, renal profile, and total lipid levels. Our results showed that the oral administration of the aqueous seed extract of *Saraca asoca* has a beneficial effect on diabetic rats. The study revealed that a potent drug with hypoglycemic and insulin-augmenting effects may be formulated from the methanolic seed extracts of *Saraca asoca*.

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CONFLICT OF INTEREST: We declare that we have no conflict of interest.

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