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# *In-Vitro* Investigation Of Antioxidant And Antidiabetic Properties Of Plant Extracts

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**ABSTRACT**

Medicinal plants have consistently served as key sources of medicine around the world. India possesses a vast heritage of traditional medicinal plant resources and is home to diverse plant and animal species. 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.
This study focus on the *In-Vitro* investigation of the antioxidant and antidiabetic potential of plant extracts. Phytochemical analysis identified the presence of alkaloids, glycosides, carbohydrates, steroids, and flavonoids in both extracts. Physical properties such as solubility, melting point, ash values, loss on drying (LOD), and extractive values were also examined. The antioxidant activity of the extracts was measured using DPPH and $H_2O_2$ assays. Additionally, glucose production was assessed through the inhibition of the α-amylase enzyme. Our results demonstrated that *Saraca asoca* extracts exhibited significant *in-vitro* antioxidant and antidiabetic activity, indicating that these extracts warrant further investigation in pharmacological studies.

**Keywords**: *S. asoca*, Physicochemical parameter, Antioxidant effect, Anti-diabetic activity, α-amylase enzyme.

## INTRODUCTION:

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 macro vascular complications. By 2040, it is estimated that around 700 million people worldwide will be affected by diabetes. Hyperglycemia is known to trigger the production of reactive oxygen species (ROS), which play a vital role in the progression of diabetic complications. Antioxidants, which are compounds or nutrients present in food, act as scavengers of free radicals, helping to prevent and repair damage caused by ROS and reactive nitrogen species (RNS). Consequently, antioxidants can enhance the immune system and reduce the likelihood of diabetes-related complications.
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. 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.

## MATERIAL AND METHOD

### Collection of Plant Material

The plant material was gathered 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 ethanol, methanol and water through maceration.

### Preliminary Phytochemical Screening

A preliminary phytochemical screening of the active extracts was conducted using established phytochemical procedures.

### 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.

**ANTIOXIDANT ACTIVITY:**

**A. DPPH Radical Scavenging Assay (1, 1-diphenyl-2-picrylhydrazyl)**
The antioxidant activity of the different *S. asoca* extracts was evaluated by their ability to decolorize the purple DPPH methanol solution, following the method outlined. In summary, 1 mL of a 0.2 mM DPPH methanol solution was mixed with 1 mL of the extracts at varying concentrations (0.2–1.0 mg/mL) and incubated for 30 minutes at 25°C. The absorbance of the mixture was then measured at 516 nm against a blank using a UV-visible spectrophotometer. The percentage of DPPH radical inhibition (I %) was calculated using the following formula:
Percentage inhibition (I%) = $[(A_{control} - A_{extract})/A_{control}] \times 100$
where $A_{control}$ is the absorbance of the control, $A_{extract}$ is the absorbance of the extract.

**B. Hydrogen Peroxide ($H_2O_2$) 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 equation.

**C. FRAP Assay**
The reducing power of the extracts was evaluated following the established method. Various concentrations of *S. asoca* extracts were combined with 1 mL of distilled water, then mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferrocyanide. The mixture was incubated at 50°C for 20 minutes, followed by the addition of 2.5 mL of trichloroacetic acid. The solution was then centrifuged at 3000 rpm for 10 minutes. Next, 2.5 mL of the supernatant was mixed with an equal volume of distilled water and 0.5 mL of 0.1% $FeCl_3$. The color change in the solution was measured at 700 nm.

***IN-VITRO* 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 at 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.

**RESULTS & DISCUSSION**

**Table 1:** Anti-oxidant activity of the extracts by DDPH assay.

| Sr. no | Concentration (µg/ml) | Ascorbic acid (% inhibition) | Ethanolic extract (% inhibition) | Methanolic extract (% inhibition) | Aqueous extract (% inhibition) |
|---|---|---|---|---|---|
| 1 | 50 | 60.75 % | 58.21% | 58.11 % | 57.80 % |
| 2 | 100 | 94.21 % | 63.28% | 62.32 % | 61.07 % |
| 3 | 150 | 97.25 % | 84.15% | 80.2 % | 79.31 % |

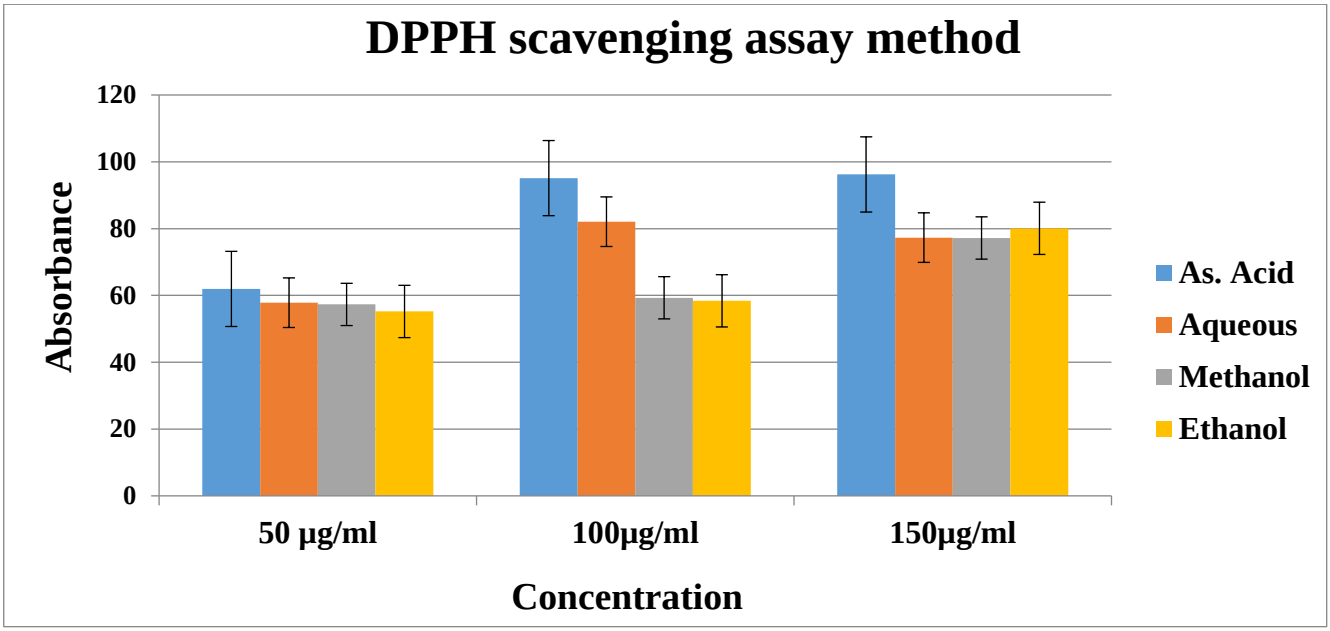


**Table 2:** Anti-oxidant activity of the extracts by $H_2O_2$ assay.

| Sr. no | Concentration (µg/ml) | Ascorbic acid (%inhibition) | Ethanolic extract (%inhibition) | Methanolic extract (%inhibition) | Aqueous extract (%inhibition) |
|---|---|---|---|---|---|
| 1 | 50 | 61.31 % | 57.10% | 57.32 % | 55.78 % |
| 2 | 100 | 97.25 % | 60.83% | 61.13 % | 58.74 % |
| 3 | 150 | 98.22 % | 85.11% | 82.21 % | 78.32 % |

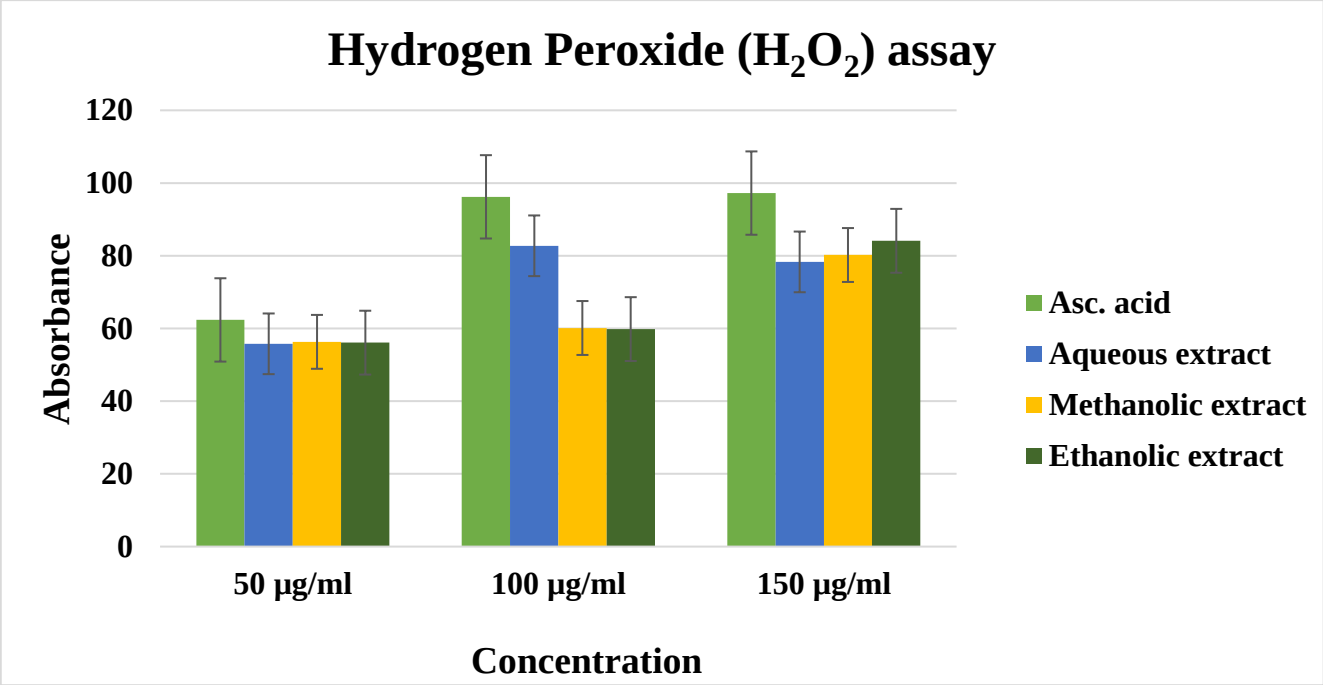


**Table 3:** Anti-oxidant activity of the extracts by FRAP assay

| Sr. no | Concentration (µg/ml) | Ascorbic acid (% inhibition) | Ethanolic extract (% inhibition) | Methanolic extract (% inhibition) | Aqueous extract (% inhibition) |
|---|---|---|---|---|---|
| 1 | 50 | 61.85 % | 58.98 % | 57.69 % | 56.87 % |
| 2 | 100 | 95.22 % | 61.84 % | 62.57 % | 83.21 % |
| 3 | 150 | 98.12 % | 86.33 % | 81.23 % | 79.47 % |

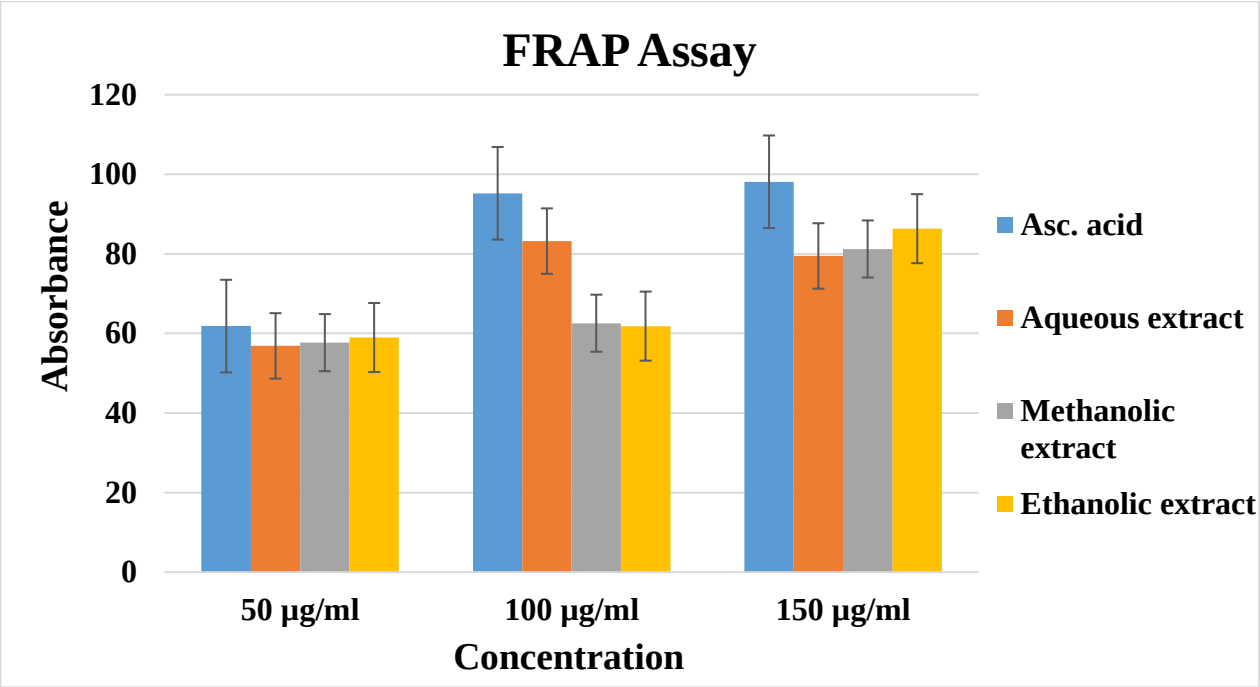


**Table 4**. *In-vitro* antidiabetic activity of the ethanol extract using α-amylase method and comparison with standard drug Acarbose

| Sr. no. | Concentration µg/ml | % of Inhibition of Acarbose | IC 50 µg dry extract | % of Inhibition *S. asoca* Extract | IC 50 µg dry extract |
|---|---|---|---|---|---|
| 1 | 2 | 7.98±0.02 | 0.35 | 3.35±0.01 | 0.74 |
| | 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 ethanol 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 $IC_{50}$ value of 0.74 µg dry extract respectively (Table 4). Acarbose is a standard drug and its concentration of (2-10 µg/ml) showed α-amylase inhibitory activity with $IC_{50}$ value 0.35 µg dry extract. The ethanol extracts of the plant showed maximum α-amylase inhibitory activity ($IC_{50}$ = 0.74 µg 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 hydrolyzing enzymes because of their ability to bind with proteins.

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