

## Research Article



## Comparative Pharmacological Evaluation of *Hibiscus rosa sinensis* Leaf Extract and *Eclipta alba* Bark Extract

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### ABSTRACT

At present scenario the herbal formulations are widely used due to their efficacy and minimum side effects compared with the allopathic medications. Based on several research studies *Hibiscus rosa-sinensis* and *Eclipta alba* have several medicinal properties and these two are mostly used in combination in hair tonics due to their significant impact on the hair growth. These two plants are mostly used in combinations in many poly herbal formulations to enhance the therapeutic activity. This article is based on the study and comparison of antioxidant activity and antidiabetic activity between leaf extract of *Hibiscus rosa-sinensis* and bark extract of *Eclipta alba*. The comparison of the antioxidant and antidiabetic activities are done at a particular concentration respectively which shows maximum effect from the survey of other Research and review papers. For the antidiabetic activity evaluation starch iodide method was used and for the antioxidant activity evaluation phosphomolybdate assay method was used. Based on the experimental study we conclude that the *Hibiscus rosa-sinensis*(L) has more antidiabetic activity than *Eclipta alba* and *Eclipta alba* has more antioxidant activity than *Hibiscus rosa-sinensis*.

**Keywords:** Aqueous extract, Leaf extract of *Hibiscus rosa-sinensis*, Bark extract of *Eclipta alba*, Antioxidant activity, Antidiabetic activity, Starch iodide method, phosphomolybdate assay.

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### INTRODUCTION

Antioxidants is the word in which anti means against and oxidants are the substances which are responsible for the oxidative reactions in the body. Hence antioxidants are the substance which prolongs, inhibits or removes oxidative damage in the body. Generally human body has the antioxidant defence mechanism. Antioxidants are generally obtained to humans through their dietary intake such as from fruits and vegetables as well as through endogenous production of antioxidants.

Now-a-days based on the present scenario, the enthusiasm for finding the natural antioxidants rather than the synthetic natural antioxidants has been increased due to increase in the health consciousness among the people, Antioxidants not only prevent the formation of harmful free radicals through oxidation but also prevent and cure many diseases such as Neoplasm. Antioxidants which are synthetic in origin are generally not preferable because they produce severe toxic effects which naturally increases the use of some antioxidants like vitamin C. Antioxidants like vitamin c are mostly preferred even though it has lesser antioxidant activity compared to the antioxidants which are synthetic in origin. So, the research regarding

antioxidants has been increased due to their interest in treating many complications.

Diabetes mellitus is the most common disease in the present world. The disease occurs mostly all over the world due to change in the lifestyles and is expected to further increase in the incidence rate in the future based on the survey of several health departments. At present scenario there is increase in the research regarding the use of herbal medications to treat the diabetes mellitus even though there are advanced synthetic medications in the market. The Research regarding the herbal drugs is progressing for further reduction or treatment of the complications caused by Diabetes mellitus<sup>2</sup>.



**Figure 1:** Morphological view of *Hibiscus rosa-sinensis*

*Hibiscus rosa-sinensis* L which belongs to the Malvaceae family from figure 1. shows the morphological view of the *Hibiscus rosa-sinensis*. the flowers of *Hibiscus rosa-sinensis* are often used for decoration purpose. It is mostly seen in China, India and Philippines but it is originally from China. The *Hibiscus rosa-sinensis* is widely distributed through out the tropical and subtropical region. The Genus Hibiscus with several species are widely grown specifically in the



Tropical region. Different species of Hibiscus genus flowers varies that they are observed in different colors. Among the same species also the color varies such as *Hibiscus mutabilis* L. The basic anatomy of leaves of Hibiscus are simple, lobed, alternate or spiral and have paired stipules<sup>3</sup>. The anatomy of Flowers is radially symmetrical with cup-shaped calyx, five petals joined at the base, style bearing many stamens and stigma with five hairy lobes. Different parts of *Hibiscus rosa-sinensis* are widely used in traditional medicine. Leaves and flowers of Hibiscus rosa-sinensis leaves are used in skin infections, inflammations and used in treatment of ulcers which acts as antiseptic<sup>4</sup>. Based on the literature review of Ayurveda from olden days *Hibiscus rosa-sinensis* has several pharmacological properties the evidence regarding their pharmacological activities is gathered now-a-days and it is still an ongoing process.

*Eclipta alba* which belongs to the Asteraceae from **FIG 2** shows the morphological view of the *Eclipta alba*. Similar to *Hibiscus rosa-sinensis* it is also widely distributed throughout the tropical and sub-tropical region. It belongs to a sub family of Heliantheae itself, commonly known as false daisy in English and bhringoraj or bhringraj ayurvedic, and unani systems of medicine. *Eclipta alba* is mostly used to treat liver-based problems. It has several pharmacological properties including antivenom property, for treating alopecia, for treatment of GI Tract diseases, for respiratory diseases, against cut and wounds, neuroprotection, to treat inflammation and many other diseases<sup>1</sup>. In Africa, it is uses for its neuropharmacological properties. It also has antimicrobial, antifungal and anticonvulsant properties.



**Figure 2:** Morphological view of *Eclipta alba*

In present study the aqueous extracts of *Eclipta alba* and *Hibiscus rosa-sinensis* are subjected to analysis for their antioxidant activities and antidiabetic activities.

## EXPERIMENTAL SECTION

### Chemicals

Iodine, potassium iodide, starch, Alpha Amylase, potassium dihydrogen phosphate, Disodium hydrogen phosphate, Ammonium molybdate, sodium phosphate, Sulphuric acid, Distilled water.

### Plant Materials

*Hibiscus rosa-sinensis* leaves and *Eclipta alba* bark were collected from the medicinal garden of Sir CRR college of pharmaceutical sciences, Eluru, Andhra Pradesh.

Identification and authentication of the samples was done by using standard botanical monographs. They were further confirmed at the Department of Pharmacognosy, Sir CRR college of pharmaceutical sciences, India.

### Preparation of Aqueous Extract<sup>5</sup>

The dried plant material was powdered and was packed well in Soxhlet apparatus and was subjected to continuous hot extraction with distilled water until the completion of extraction. The hot extract was filtered and dried using rotary evaporator. Obtained extract was weighed and percentage yield was calculated in terms of air-dried powder extract.

### Phytochemical analysis of plant extracts of *Hibiscus rosa-sinensis* and *Eclipta alba*<sup>6</sup>

A small portion of the dry extract was used for the phytochemical tests for compounds which include tannins, flavonoids, alkaloids, saponins and steroids in accordance with the methods with little modifications. Exactly 1.0 g of the plant extract was dissolved in 10ml of distilled water and filtered (using Whatman No.1 filter paper). A blue colouration resulting from the addition of ferric chloride reagent to the filtrate indicated the presence of tannins in the extract. Exactly 0.5 g of the plant extract was dissolved in 5 ml of 1% HCl on steam bath. About 1 ml of the filtrate was treated with few drops of Dragendorff's reagent. Turbidity or precipitation was taken as indicative of the presence of alkaloids. About 0.2g of the extract was dissolved in 2 ml of methanol and heated. A chip of magnesium metal was added to the mixture followed by the addition of a few drops of concentrated HCl. The occurrence of a red or orange colour was indicative of flavonoids. About 2ml of the extract was vigorously shaken in the test tube for 2 min. It was observed for frothing. To about 1 ml of the extract 5 drops of concentrated H<sub>2</sub>SO<sub>4</sub> was added in a test tube. Red coloration was observed which is indicative for the presence of steroids

### Antioxidant Activity

#### *Phosphomolybdate assay (total antioxidant capacity)*<sup>7</sup>

The total antioxidant capacity assay can be used to determine the capacity of antioxidants through the formation of phosphomolybdenum complex. The assay is based on the reduction of Mo (VI) to Mo (V) by using sample analyte, which subsequently produces a green phosphate Mo (V) complex at acidic condition.

Briefly, 1 ml of the sample extract containing concentration 100 µg is added to 10 ml of reagent solution that contains 0.6 M of sulfuric acid, 28 mM of sodium phosphate, and 4 mM of ammonium molybdate. The test tube is covered and incubated at 95°C for 90 min. After that, the mixture is cooled at room temperature and the absorbance is measured at 695 nm. The blank solution that functions as control contains both the reagent solution and the solvent.



The total antioxidant capacity is calculated using the formula:

Total antioxidant capacity (%) =

$$[(A695 \text{ Control} - A695 \text{ Sample}) / A695 \text{ Control}] \times 100$$

#### Anti-Diabetic Activity

##### *Alpha amylase inhibitory test by using starch iodide method<sup>8</sup>*

Iodine solution was prepared by dissolving 0.254 g I<sub>2</sub> and 4.0 g KI in 1L of distilled water. Starch solution was prepared by dissolving 1 g of starch in 10 ml of distilled water, gently boiling, cooling and completing to 100 ml with distilled water. Amylase solution was prepared by transferring 6 µl of the standard porcine pancreatic amylase suspension (40 mg/ml) to 8 ml of phosphate buffer (pH 6.9). Alpha amylase inhibitory activity was based on the starch-iodine method described by Hansawasdi [11] with some modifications. Briefly, control and test solutions were prepared as follows: 0.3 ml of amylase solution were transferred to a sample tube containing 0.3 ml of the extract concentration 300 µg to be tested (substituted by the solvent of

extraction in the case of control) and 0.6 ml phosphate buffer (pH 6.9). The mixture was incubated at 37 degree centigrade for 15 minutes. 0.4 ml aliquots of that incubate were transferred to sample tubes containing 3 ml starch (1g%) and 2 ml of phosphate buffer (pH 6.5) and the mixture was re-incubated for 60 minutes. At zero time and at the end of the incubation period 0.1 ml of the reaction mixture was withdrawn from each tube after mixing and discharged into 10 ml of iodine solution. Solutions were thoroughly mixed and the absorbance measured immediately at 565 nm. Percentage inhibition

was calculated according to the formula:

$$\{[(A_0 - A_t)_{\text{control}} - (A_0 - A_t)_{\text{sample}}] / (A_0 - A_t)_{\text{control}}\} \times 100$$

where **A<sub>0</sub>** and **A<sub>t</sub>** are the absorbance values at zero time and at the end of the incubation, respectively. Each experiment was repeated three to four times and the average value was used for obtaining the relevant plots.

## RESULTS AND DISCUSSION

### Antioxidant Activity

The phospho-molybdenum method was based on the reduction of MO (VI) to MO (V) by the antioxidant compound and the formation of green phosphate/ MO (V) complex at acidic pH with maximal absorption at 695 nm 33. As shown in **Table 1**, the samples exhibit antioxidant activity.

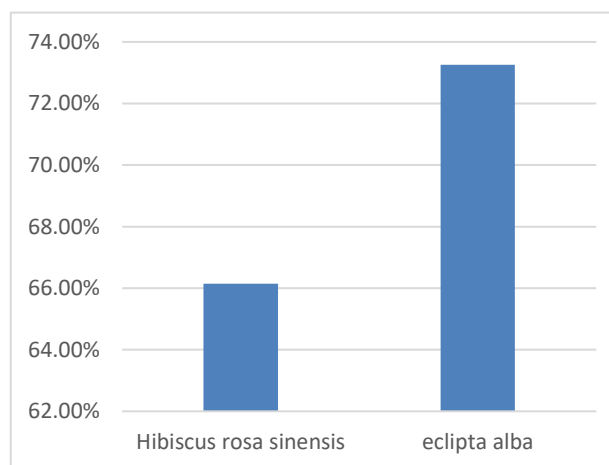
The absorbance of control was found to be 0.65 whereas the absorbance of *Hibiscus rosa-sinensis* was found to be 0.22 and the absorbance of *Eclipta alba* was found to be 0.17 both the samples were at the concentration of 100µg.

The % Antioxidant capacity of *Hibiscus rosa-sinensis* was found to be 66.15% and the % Antioxidant capacity of

*Eclipta alba* was found to be 73.26%. So, the antioxidant activity of *Eclipta alba* was more than the antioxidant capacity of *Hibiscus rosa-sinensis* from the **Graph 1**.

**Table 1:** Comparison of antioxidant activity of *Hibiscus rosa-sinensis* and *Eclipta alba*

Sample	% Antioxidant capacity	Concentration
<i>Hibiscus rosa-sinensis</i>	66.15%	100µg
<i>Eclipta alba</i>	73.26%	100µg



**Graph 1:** Antioxidant activity graphical comparison

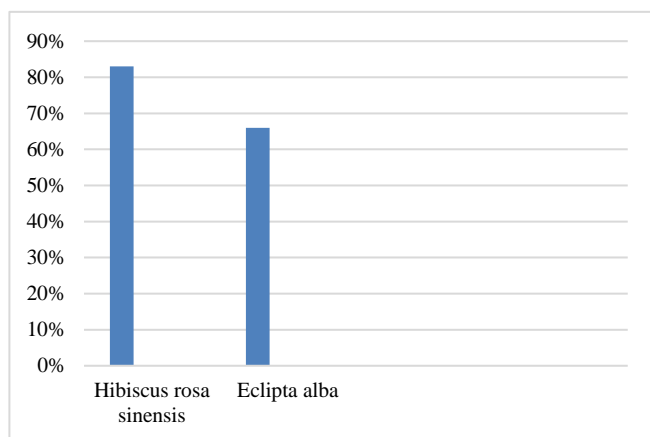
### Anti-Diabetic Activity

The plant extracts tested for alpha amylase inhibitory activity using a literature method. Results were calculated as percentage inhibition (under experimental conditions) and summarized in Table 2. Of the two plant species tested the two extracts exhibited significant (more than 50%) alpha amylase inhibitory activity; namely, *Hibiscus rosa-sinensis* and *Eclipta alba*. For these plants the inhibitory activity was also shown to be concentration dependent. However, the two plant extracts are considered to have a significant inhibitory activity that might contribute to their claimed anti-diabetic activities.

Therefore, alpha amylase inhibition is responsible (at least in part) to the hypoglycemic activity of *Hibiscus rosa-sinensis* and *Eclipta alba*. The absorbance of control was found to be 0.06 because **A<sub>0</sub>** is 0.57 and **A<sub>t</sub>** is 0.51 and the absorbance of *Hibiscus rosa-sinensis* was found to be 0.01 because **A<sub>0</sub>** is 0.28 and **A<sub>t</sub>** is 0.27. The absorbance of *Eclipta alba* was found to be 0.02 because **A<sub>0</sub>** is 0.39 and **A<sub>t</sub>** is 0.37. Hence the percentage inhibition activity of *Hibiscus rosa-sinensis* was found to be 83% and *Eclipta alba* was found to be 66%. From the above data the antidiabetic activity of *Hibiscus rosa-sinensis* was more than the antidiabetic activity of *Eclipta alba* **Graph 2**.

**Table 2:** Comparison of antidiabetic activity of *Hibiscus rosa-sinensis* and *Eclipta alba*

Sample	% Inhibitory activity	Concentration
<i>Hibiscus rosa-sinensis</i>	83%	300µg
<i>Eclipta alba</i>	66%	300µg

**Graph 2:** Antidiabetic activity graphical comparison

## CONCLUSION

This research article looked into comparison of *Hibiscus rosa-sinensis* and *Eclipta alba* to evaluate antioxidant activity using phosphomolybdate assay.

*Hibiscus rosa-sinensis* and *Eclipta alba* offers a remarkable activity for curing of many diseases. It has a wide range of chemical constituents. Alpha amylase inhibitory activity was done on *Hibiscus rosa-sinensis* and *Eclipta alba*. It concluded that *Hibiscus rosa-sinensis* have a significant impact on antidiabetic property than *Eclipta alba*.

From this we conclude that upon using the *Hibiscus rosa-sinensis* and *Eclipta alba* in combinations in many herbal formulations the therapeutic activity increases due to their agonistic activity of both the plants in some clinical conditions. But we cannot conclude that the combinations of these two drugs are suitable for all the therapeutic activities in some situations they also cause antagonistic activity in certain situations.

Hence therefore we conclude that the combination of *Hibiscus rosa-sinensis* and *Eclipta alba* in many hair tonics which shows significant impact also shows certain impact in antidiabetic as well as antioxidant properties.

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