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Antioxidant Properties, Total Phenolic and Flavonoid Content of Ethanolic Extract of *Saraca asoca* Leaf

Mahar D and Kar A*

School of Life Sciences, Devi Ahilya Vishwavidyalaya, India

***Corresponding author:** Anand Kar, School of Life-Sciences, Devi Ahilya Vishwavidyalaya, Takhshila campus, Indore-MP, India, Tel: +91-0731-2477166; Email: karlife@rediffmail.com

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Abstract

Background: Plants are believed to regulate different diseases through their antioxidant property. The primary aim of this investigation was to reveal the unknown antioxidant property of the leaf extract of *Saraca asoca* (SA).

Method: Two different experiments were performed to evaluate the lipid peroxidation (LPO) inhibitory activity of SA leaf extract. The first experiment was conducted on carbon tetrachloride (CCl_4)-induced hepatic LPO. Second experiment was conducted to examine the DPPH free radical scavenging activity of the test plant.

Results: A dose-specific effect was observed with 0.1gm and 0.05gm/ml of the extract appearing more effective than the remaining doses showing its ability to inhibit the tissue LPO. Finding of DPPH assay indicated that 0.008 gm/ml of the extract showed the highest antioxidant property, followed by 0.016gm and 0.032 gm/ml, both being more or less equally effective, further supporting the antioxidative property of *Saraca asoca* leaves.

Conclusion: The total phenolic and flavonoid contents were found to be high. It appears that the observed antioxidant property of the SA extract might be associated with the high phenolic and flavonoid contents in the plant leaf extract. It is suggested that the antioxidant potential of the SA leaf extract may help in preventing or curing some LPO –induced diseases.

Keywords: Antioxidant Activity; *Saraca asoca*; DPPH Assay; Phenol; Flavonoid

Abbreviations: SA: *Saraca Asoca;* LPO: Lipid Peroxidation; PBS: Phosphate Buffered Saline; RSA: Radical Scavenging Activity; DPPH: Diphenyl-2-Picryl Hydrazyl.

Introduction

Most of the diseases are believed to be associated with the production of excessive free radicals leading to oxidative problems in different organs including the liver, the major site of metabolism [1]. To reduce lipid peroxidation (LPO) vary often plant extract or plant derived bio-active compounds are considered to be effective. Therefore, for the regulation of many diseases, plant extracts are used as supplements [2-4]. For reducing LPO in the liver many plants have been used from time to time [5,6]. *Saraca asoca* (SA) is one such plant, one of the most ancient medicinal plants known in India. SA has been regarded as one of the most useful medicinal plants in old Indian ayurvedic text. It belongs to the family Caesalpiniaceae and has been used for the regulation of different diseases such as coronary artery disease, diabetes mellitus, thyroid problems and cancer [7-10]. Extract of *Saraca asoca* is also known to be effective for different health problems including menstrual disorders, uterine infections, antimutagenic activity and bacillary dysentery [11-14]. Primarily, its bark, flower, and seed are used for different diseases [15-18]. However, with respect to its leaf extract

nothing much has been done so far, although reports are available on its antimicrobial properties [19,20]. Therefore, the present research work was conducted to evaluate the efficacy of its leaf extract with respect to the inhibition of hepatic LPO in *in-vitro* conditions and then the possible involvement of its phenolic and flavonoid compounds was studied.

Materials and Methods

Chemicals Used

Thiobarbituric acid (TBA), 1, 1 Diphenyl-2-picryl hydrazyl (DPPH), Gallic acid (GA), Quercetin, Carbon tetra chloride (CCl_4),Sodium nitrite (NaNO2), and Aluminum chloride ($AlCl_3$) were purchased from E. Merk [India], Bombay, India. All other chemicals [reagent grade] were obtained from Loba Chemie Pvt. Ltd., India.

Plant material and Extract Preparation

Leaf powder of the test plant *Saraca asoca* was purchased from the local market Indore, India. This powder was extracted with 70% ethanol for 2days in room temperature [21]. The process was repeated thrice and the entire clear fraction were pooled and evaporated in water bath. The leaf extract yield was found to be 2.8-4.97gm per 100 gm.

Preparation of Tissue Homogenate

The overnight fasted Wistar rat was anesthetized with mild chloroform and then sacrificed. The liver was taken out and kept in phosphate-buffered saline (PBS), and homogenized followed by centrifugation for 15 min at 15000 rpm. This tissue homogenate was used to estimate hepatic LPO following the method as standardized in our laboratory [22].

Study of hepatic LPO in CCl4 -Induced Tissue

The LPO was studied in liver tissue samples as done earlier in our laboratory [23]. In brief, the excised liver was washed in 10% PBS, sliced into fine pieces, and then homogenized in 10% PBS. To 1ml of supernatant from the homogenate, 50 µl of CCl4 was added on one set of test tube, while in the control tubes the same amount of distilled water was added in place of CCl_4. All the tubes were incubated for 2hr at 37°C and then 1ml of TBA was added in all the tubes.

Finally the absorbance was noted at 532nm and the LPO was calculated considering the protein content of each sample.

Evaluation of Antioxidative Efficacy Using DPPH

DPPH is widely used to evaluate the free radicals scavenging activity of any material. The method is based on changes in DPPH concentration due to the presence of antioxidants in the plant extract. This DPPH free radical activity of the test extract was studied using the earlier method of our laboratory [24].

In brief, different concentrations of the plant leaf extract were taken in separate test tubes. The volume was adjusted to 100 µl with methanol. After the addition of 5 ml of 0.1 mM ethanolic solution of DPPH, tubes were allowed to stand at 37 °C for 20 min. Control tubes were prepared by keeping all the chemicals but without the plant extract. Ethanol was used for baseline correction. Changes in the absorbance of the sample were measured at 517nm. The result was expressed in percent inhibition.

Determination of Total Polyphenols

The total polyphenolic content of the test leaf extract was estimated by using an earlier protocol [25]. In brief, 0.123 ml of plant extract of known concentration (100 mg/ml) was diluted with 0.5 ml of distilled water, and then 0.125 ml of Folin- Ciocalteu reagent was added to the mixture. After 6min incubation at room temperature, 1.25ml of 7% sodium carbonate was added to the mixture. The final volume was made up to 3 ml with distilled water and then incubated at room temperature for another 90 min. Finally, the absorbance was measured against the prepared blank at 765 nm in comparison with the standard of known concentration of Gallic acid. The mean of the three readings was considered and results were expressed as mg Gallic acid equivalent / 100 g dry weight of the extract.

Determination of Total Flavonoid

For this an earlier published method was followed [25]. In brief, stock solution (4 mg/mL) in methanol of the extract was prepared and followed by serial dilution to make different concentrations. A similar procedure as described for quercetin was followed for the extract also, and the absorbance was measured by spectrophotometer at 510 nm. All the readings were taken in triplicates and the average value was considered for the final presentation. Flavonoid content was expressed as Quercetin equivalent (mg QE/g) using the linear equation based on the standard calibration curve.

Results

Change in Hepatic LPO in *in-vitro*

Following the administration of 50 µl CCl_4 there was a significant increase in the hepatic LPO as compared to the control value ($p<0.001$). However, with the addition of different concentrations of test plant extract along with CCl_4 there was a significant decrease in the CCl_4-induced LPO at

all concentrations used ($p<0.001$ or $p<0.005$) as compared to the value of only CCl_4 added homogenate. Interestingly out of all the concentrations of plant extract, in 0.05 gm/ml maximum 52% inhibition of LPO was observed.

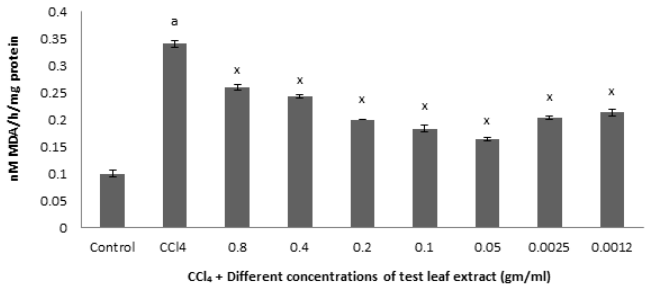

Figure 1: Effect of *Saraca asoca* leaf extract of different concentrations (gm/ml) in CCl_4 (50 µl) - induced hepatic LPO (nM MDA formed/h/mg protein). Data are in mean ± SEM (n=3). a,P <0.001 as compared to the respective control value. x,P <0.001 as compared to the respective CCl4 treated value.

Changes in DPPH Activity

DPPH radical scavenging activity (RSA) was markedly enhanced by the addition of different concentrations of the *Saraca asoca* leaf extract and the maximum (94%) radical scavenging activity was observed in 0.008gm/ ml. However, after 0.008gm/ml, there were no further significant changes in the free radical scavenging activity.

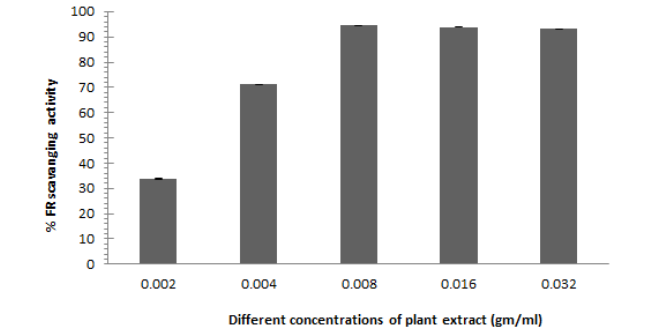

Figure 2: DPPH radical scavenging activity (RSA) expressed in % inhibition. Data are in mean ± SEM (n=3).

Determination of Total Phenol and Flavonoid Content

The total flavonoid content of the test leaf extract was calculated out to be 289 mg/100 gm of the dry weight. The equation of the calibration curve of Quercetin standard at 510 nm wavelength was y=0.001x + 1.051, R2= 0.980. The total phenolic content of the test plant was 435 mg/100gm of dry weight. The equation for the calibration curve of the gallic acid standard at 765nm wavelength was y=0.001x + 0.339, R2= 0.979.

Discussion

From the results of the present experiment it is quite evident that *Saraca asoca* leaf extract has the potency to inhibit LPO in hepatic tissues. This is because of the fact that different concentrations of the test leaf extract could inhibit the CCl4-induced hepatic LPO as well as DPPH free radicals. However, the observed positive effects were concentration dependent. Maximum inhibition in LPO was exhibited by 0.1gm/ml of SA leaf extract.

Although some investigations have already reported the antioxidant property of *Saraca asoca*, they were primarily done on its bark or root extracts [26,27]. On its leaf extract, mainly antimicrobial effects were reported [19]. Therefore, the present study appears to be the first one on the antioxidant activity of the leaf extract of *Saraca asoca*.

CCl_4 is a common toxicant that is known to induce hepatic LPO and is activated by cytochrome P450 and 3A to produce trichloromethyl radicals (CCl_3), which can lead to hepatotoxicity by increasing oxidative stress and cellular damage [28]. In this investigation, CCl_4 was used to induce tissue LPO to find out the hepato-protective potential of the test plant extract, as was done earlier with some plants and drugs [29]. Even for vegetable peels, this CCl_4 was used in *in-vitro* study [30]. Interestingly, our findings indicate the strong antioxidant property of *Saraca asoca* leaf extract clearly suggesting that the leaf extract may be used as a supplement for the control of different LPO-induced diseases including coronary artery disease, diabetes mellitus, and cancer [31]. An interesting finding is that with respect to its bark extract maximum inhibition in DPPH free radicals was reported to be 74% at 350 µg / ml [32], and with its leaf extract, we found 94% scavenging of DPPH free radicals suggesting that leaf extract may be more appropriate in reducing free radicals as compared to the bark of *Saraca asoca*.

Lipidperoxidation is the process in which macromolecules like lipids having double bond between two carbon atoms are attacked by oxidants/free radicals [33]. Free radicals produced by various endogenous/ exogenous processes, possess unpaired electron due to which they become highly reactive molecules. They are referred as derivatives of oxygen and behave as oxidants or reductants [34]. Free radicals are regularly produced during various physiological processes of living organisms and they cause lipidperoxidation of different cellular molecules leading to the damage of the cell. In last 2

to 3 decades, the study of LPO has increased rapidly as it has been found to play an important role in cellular physiology and in human health. It is believed that most of the metabolic disorders are linked with the enhanced LPO [35,36]. In our study, we found that SA leaf extract has a strong antioxidant property. This antioxidant/free radical scavenging activity of the test extract may be related to its bio-active compounds. We also found that its leaf extract possesses a good amount of flavonoid & phenolic compounds which could be responsible for the antioxidant activity of the test extract. Further studies involving the *in-vivo* experiment may throw more light on its usefulness in relation to the regulation of different diseases.

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Conflict of Interest

There are no conflicts of interest between the two authors.

Conclusion

From this investigation it is emphasized that the ethanol extract of *Saraca asoca* leaf has the potential to act as antioxidant, which may have positive impact on human health through the mitigation of several diseases. This positive effect of the test extract might be related to its high phenolic and flavonoid content. Further research using *in-vivo* system will certainly help in revealing its potential application in the amelioration of different diseases that are induced by oxidative process.

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