ORIGINAL RESEARCH PAPER

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KEY WORDS: Antioxidant, *Wrightia tinctoria* DPPH, superoxide.

EVALUATION OF ANTIOXIDANT ACTIVITY OF LEAVES OF *WRIGHTIA TINCTORIA*

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ABSTRACT

The aim of this study was to screen antioxidant activity of *Wrightia tinctoria* extract in vitro to in order to find possible sources for future novel antioxidants in food and pharmaceutical formulations. A detailed study was performed on the antioxidant activity of the standardized leaf extract of *Wrightia tinctoria* using DPPH scavenging assay and superoxide scavenging assay. Data from present results revealed that *Wrightia tinctoria* may act as an antioxidant agent due to its free radical scavenging activity.

INTRODUCTION

Wrightia tinctoria (roxb) R.Br is a small deciduous tree of the family Apocynaceae commonly known as Dudhi (Sweet indrajao) distributed in central India, Burma and Timor and is extensively used in Indian system of medicine. Fresh leaves are pungent and gives relief from tooth ache.[1-2] A decoction of leaves used as astomachic and in treatment of abdominal pain.[1-2] Ethyl acetate, acetone and methanol extract of *Wrightia tinctoria* bark show antinociceptive activity in pinice and wound healing activity.[3] The reported constitution are alkaloids, triterpnoids & wrightial have shown anti-inflammatory activity.[4,5,6]

In our previous studies we have reported anti inflammatory activity of methanolic extract of leaves of *Wrightia tinctoria* in Carrageenan induced paw edema test.[7] Therefore, with the aim to investigate the antioxidant activity *Wrightia Tinctoria* the current study was designed to evaluate the antioxidant activity of extract of leaf by using DPPH scavenging assay and superoxide scavenging assay.

MATERIALS AND METHODS

Plant Material- The leaves *of Wrightia tinctoria* were collected from Jalgaon state Maharashtra in the month of august 2007. The plant was identified and authenticated by T. Chakraborty Joint Director, Botanical survey of India, Western circle, Pune.

Preparation of Extract- The World Health Organization procedure of extraction was adapted for the present study. A total of 100 gram of the powder *of Wrightia tincoria* leaves were subjected to exhaustive soxhlet extraction in 500 ml of distil water for 72 hours. The extract obtained was concentrated in water bath until constant dark sticky residue was obtained which was further dried by vacuum dryers and maintained in desiccators until a constant weight was obtained. In all experiments three doses (25, 50, 100 & 200 µg/ml) of the extract was tested in vitro models for evaluation of antioxidant activity.

Drugs and chemicals- Ascorbic acid was procured from Loba Chemicals Ltd, Mumbai. All the drugs and chemicals were of analytical grade obtained commercially. Double distilled water was used throughout the study.

Determination of antioxidant property

Scavenging activity of DPPH radical[8]-

The DPPH free radical scavenging assay was carried out for the evaluation of the antioxidant activity. This assay measures the free radical scavenging capacity of the investigated extract. DPPH is a molecule containing a stable free radical. In the presence of an antioxidant, which can donate an electron to DPPH, the purple color typical for free DPPH radical decays and the absorbance change is measured at =517 nm. The antiradical activity of the plant extract was examined based on the scavenging effect of the stable DPPH free radical activity. Briefly, in 3 mL of each diluted extract, 1 mL of methanol solution of DPPH (0.1 mmol/L) was added. The mixture was kept in the dark at room temperature for 30 min and the absorbance was measured at 517 nm against a blank. The following equation was used to determine the percentage of the radical scavenging activity of each extract.

Percentage of radical scavenging activity=[(OD control- OD sample)/OD control] × 100
Where OD is the optical density.

The IC50 value (µg/mL) is the effective concentration at which DPPH radicals were scavenged by 50% and the value was obtained by interpolation from linear regression analysis.

Superoxide scavenging assay[8]- The activity was evaluated using nitro blue tetrazolium (NBT) reduction method given by Nishikimi et al., (1972)15. The reaction mixture consisted of 1ml of NBT solution (156µM) and sample solution at different concentrations. The reaction was started by adding 100µl of phenazine methosulfate solution (60µM, PMS) in phosphate buffer (pH 7.4) to the reaction mixture followed by incubation at 25oC for 5 min and the absorbance at 560mm was measured against blank. Ascorbic acid was used as the standard.

RESULTS

DPPH scavenging activity- *Wrightia tincoria* extract possess significant in-vitro antioxidant activity. DPPH radical scavenging activities of *Wrightia tincoria* extract are presented in Fig 1. Results showed that the radical scavenging activity of *Wrightia tincoria* extract increased with increasing concentration. However Ascorbic acid is used as a standard and its radical scavenging activity was found to be more potent. The IC50 Values of standard ascorbic acid was found to be 9.57 and of *Wrightia tincoria* extract 18.54 at the concentration of 100 µg/ml (see figure 2).

Superoxide radical scavenging activity

The *Wrightia tincoria* extract show potent free radical scavenging activity. The antioxidant potential of the *Wrightia tincoria* extract is expressed in terms of percentage inhibition of superoxide radical (figure 3). The antioxidant potential of *Wrightia tincoria* extract was found to be concentration dependent. Superoxide radical scavenging activities of *Wrightia tincoria* extract are expressed as IC50 value (figure 4). *Wrightia tincoria* extract showed good superoxide radical scavenging activity. Ascorbic acid used as a standard and was more potent.

DISCUSSION

In this study we assessed the antioxidant potential of *Wrightia tincoria* extract in two invitro assays. The results from the current study clearly justified the antioxidant potential of *Wrightia tincoria* extract in various pathologic conditions. Two different models have been used to evaluate the antioxidant activity of Wrightia tincoria species. The *Wrightia tincoria* extract gives protection against various free radicals by inhibiting the DPPH radical and superoxide anion.

The findings of the present study are in accordance with the earlier report on total phenolic and antioxidant activity.[8,9] The phenolic compounds may contribute directly to antioxidative actions. The radical scavenging efficacy of *Wrightia tincoria* extract may be due to their retention of antioxidant phytochemicals in these extract.

Strong evidence supports these findings that the tannins and flavonoids from the *Wrightia tincoria* extract enhance the antioxidants effects in–vitro assays.

An antioxidant can be broadly defined as any substance that delays or inhibits oxidative damage to a target molecule[8,9]. The main characteristic of an antioxidant is its ability to trap free radicals. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases[8,9].

CONCLUSION

The evaluation of *Wrightia tincoria* extract using DPPH free radical assay and Superoxide radical scavenging method suggest good antioxidant potential of the extract. These results suggest that phytochemicals from *Wrightia tincoria* extract may offer effective protection from free radicals and supports its promise as a natural antioxidant.

Figure 1. Effect of Wrightia Tinctoria on DPPH radical (Values are represented as mean, n=5)

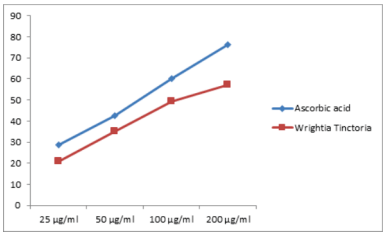


Figure 2 . IC 50 values of *Wrightia Tinctoria* on DPPH radical (Values are represented in histograms as geometric mean, n=5)

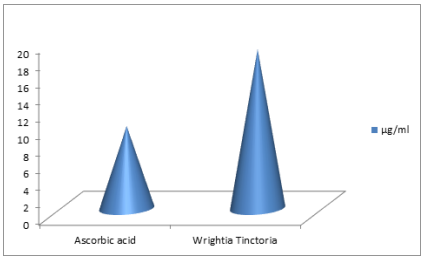


Figure 3. Effect of *Wrightia Tinctoria* on Superoxide radical (Values are represented as mean, n=5)

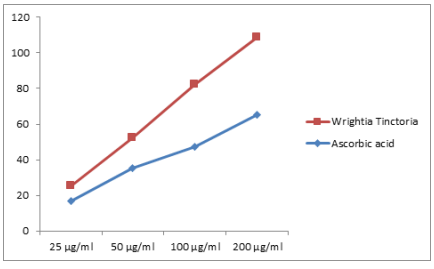
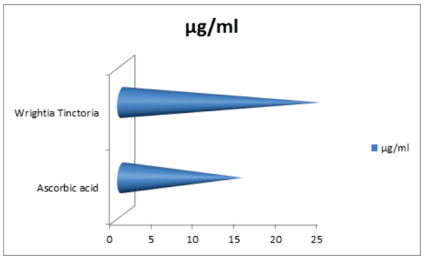


Figure 4. IC 50 values of *Wrightia Tinctoria* on Superoxide radical scavenging activity (Values are represented in histograms as geometric mean, n=5)



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