

Protective Role of Superoxide Dismutase Assay and Its Relation with *Wrightia tinctoria* R.Br Leaf Extract and Its Correlation with Bioactive Compounds

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ABSTRACT

Wrightia tinctoria R.Br is a medicinal plant valued for the bioactive compounds and the diverse pharmacological properties referring to anti-inflammatory, skin diseases, acne, anti-bacterial, anti-diabetic, anti-HIV properties. This study aimed to do the pre-extraction process followed by the extraction and perform the preliminary analysis along with the anti-oxidant potential of the defatted ethanolic leaf extract of *Wrightia tinctoria* R.Br using Soxhlet apparatus and the extract was evaporated in rotary evaporator to a syrupy consistency and was concentrated to extract. The phytochemical screening revealed important Phyto constituents like terpenoids, phenols, flavonoids carbohydrates that have role in diverse pharmacological spectrum. The antioxidant assay using Superoxide dismutase unravels the key foundation for many other investigations in relation to complex diseases that rises from oxidative stress. These findings indicate that the leaf extract possesses significant scavenging of superoxide radicals (O_2^-) by converting to less damaging hydrogen peroxide and molecular oxygen, likely due to its phenolic and flavonoid content along with the terpenoid compounds while the study provides insight into several phytoconstituents and anti-oxidant efficacy. Further research into in-silico and in-vivo studies are necessary and the proteomic studies in relation to these actions may untie the different cellular and molecular mechanisms.

I. INTRODUCTION

The lifetime incidence suggests that 1 in 109 people will develop vitiligo, and that incidence is substantially increased in minority ethnic groups, in particular for those of Asian ethnicity (1 in 28 people). Overall, people with vitiligo had an increased risk of depression, anxiety and reduced quality of life (QoL). [1] The psychosocial dimensions of vitiligo have gained increasing

attention in recent literature. Studies suggest that a substantial proportion of patients with vitiligo experience diminished quality of life, with depression prevalence reported as high as 20%. One investigation highlighted varying degrees of quality-of-life impairment among patients: 28.24% reported a small effect, 31.76% experienced a moderate impact, 34.12% endured a very large effect, and 4.12% suffered from an extremely large effect on their daily functioning. These statistics underscore the pressing need for healthcare systems to address not only the physical but also the emotional aspects of this condition. Vitiligo is a chronic, acquired dyschromia that promotes autoimmune aggression against melanocytes, resulting in hypochromic or achromic macules and patches on the skin and mucous membranes, with possible involvement of hair follicles, in different extensions of the skin, which may accompany systemic manifestations (e.g., sensorineural deafness, uveitis, thyroiditis). [2] Vitiligo pathogenesis incorporates both intrinsic defects within melanocytes that activate the cellular stress response, as well as autoimmune mechanisms that target these cells. The available off-label treatments are problematic and often ineffective. Narrow-band UVB (Nb UVB) light is only provided in select facilities and requires time away from work and school multiple times per week, resulting in lost productivity. Topical steroids are impractical when large surface areas are affected, and carry risk of systemic absorption with suppression of the adrenal axis, in addition to striae and skin atrophy. Topical calcineurin inhibitors appear to have fewer risks but are costly, less effective, and treat only focal disease. Systemic immunosuppression has been reported to halt the progression of vitiligo and reverse disease through repigmentation; however, the risks associated with a non-targeted systemic approach are substantial, and usually deemed unacceptable when compared to the benefits.

Therefore, the identification of targeted therapies with an improved safety profile would be a substantial advance.[3] The precise etiology and pathophysiology are complex, and there is still much debate about the various theories on the loss of melanocyte function. Multiple pathophysiological mechanisms, including genetics, autoimmunity, oxidative stress, and neurological system dysfunction, have been proposed.[4] *Wrightia tinctoria* R.Br. (Family: Apocynaceae) commonly called “Indrajau” is distributed throughout the world and occurs abundantly in India. It is a deciduous tree with white fragrant flowers. *Wrightia* is named after a Scottish physician and botanist William Wright (1740-1827). The leaves of this tree yield a blue dye called Pala Indigo. *Wrightia tinctoria* R.Br is an important medicinal plant with diverse pharmacological spectrum. Few novel chemical constituent isolated from the *Wrightia tinctoria* R.Br showed anti-cancer, anti-HIV, and anti-diabetic (type 2 diabetic) properties too. Further evaluation need to be carried out on *Wrightia tinctoria* in order to explore concealed areas and their practical clinical application, which can be used for the welfare of the mankind .[5] Leaves are simple with opposite leaf arrangement, petiole, glands axillary and Glabrous ovate, obtusely acuminate and are 10-20 cm long and 5cm width. Sometimes pubescent beneath. Leaf stalks are very short. Leaves have the properties of anti-bacterial activity, anti-viral activity, anti-fungal activity, anti-inflammatory activity, anti-nociceptive activity, anti-psoriatic activity, anti-helminthic activity, anti-convulsant activity, anti-HIV activity, anti-leishmanial activity, anti-oxidant activity, anti-tumour activity, anti-diabetic activity, and diuretic activity.[6]

PLANT DESCRIPTION

Taxonomical classification

Kingdom: Plantae, Angiosperms, Eudicots, Asterids

Order: Gentianales

Family: Apocyanaceae

Genus: *Wrightia*

Species: *tinctoria*

Vernacular names

Telugu: Akupala, Amkudu, Chittankudu, Dodda pala chettu, Jemma pala chettu.

Hindi: Indrajava

Sanskrit: Svetakuja, Madhuindrajava, Hayamoraka

Marathi: Gode indrajava

Tamil: Erukkalam, Nilambalai

Kannada: Veppale

Malayalam: Ayyapala, Dantappala

Urdu: Indearjao Shireen

II. MATERIALS AND METHODS

A. Plant Materials And Authentication

Fresh and disease-free leaves of *Wrightia tinctoria* R. Br ,were collected from Thiruvalla , India and authenticated by Dr. Rojimon P Thomas from CMS College ,Kottayam and was collected in the month of January.[7]

B. Extraction

The plant leaves were dried for 14 days and 500gm was extracted with 1500 ml of petroleum ether. [8]Extraction was done using ethanol (90% v/v) in a Soxhlet apparatus at 60°C for 72 hours. The solvent was completely evaporated in rotary evaporator to syrupy consistency.The extract was stored in airtight container at 4°C until use. The yield was observed to be 31.02%. [7,9]

C. Phytochemical Screening

Phytochemical examinations were carried out for all the extract as per the standard methods [10]

D. in-vitro Assay

SUPEROXIDE DISMUTASE ASSAY

Superoxide dismutase (SOD) activity was determined by measuring the inhibition in photoreduction of nitro blue tetrazolium (NBT) by SOD enzyme. The reaction mixture contained 50 mM sodium phosphate buffer (pH 7.6), 0.1 mM EDTA, 50 mM sodium carbonate, 12 mM L-methionine, 50 μM NBT, 10 μM riboflavin and 100 μL of crude extract in a final volume of 3.0 ml. A control reaction was performed without crude extract. The SOD reaction was carried out by exposing the reaction mixture to white light for 15 min at room temperature. After 15 min incubation, absorbance was recorded at 560 nm using a spectrophotometer. One unit (U) of SOD activity was defined as the amount of enzyme causing 50% inhibition of photochemical reduction of NBT. (11) % Inhibition = [(Absorbance of Control - Absorbance of Sample) / Absorbance of Control] * 100

III. RESULTS AND DISCUSSION

1, Phytochemical screening of the plant

The ethanolic leaf extract of *Wrightia tinctoria* R. Br was subjected to preliminary

phytochemical screening. The extract was positive for carbohydrate, proteins, tannins, phenols, flavonoids, terpenoids, glycosides & phytosterols. The other tested phytochemicals such as saponins and alkaloids were absent. The results were summarized in the table 1.

Table 1. Preliminary phytochemical screening of ethanolic defatted *Wrightia tinctoria* leaf extract

PHYTOCHEMICAL GROUP	RESULTS
Carbohydrate	Present
Protein	Present
Tannin	Present
Flavonoid	Present
Terpenoids	Present
Glycosides	Present
Phytosterols	Present

2.in-vitro antioxidant activity

The antioxidant potential was evaluated using the superoxide dismutase assay. The defatted ethanolic leaf extract of *Wrightia tinctoria* leaf extract demonstrated that both in the standard and sample, as the concentration increases, the percentage inhibition increases in a concentration dependent manner.

In reference to the standard, sample values are nearly. The values in the sample were equivalent to the standard values but not exceeding the standard values of ascorbic acid.

As an increasing % inhibition indicates a greater ability to neutralize superoxide radicals suggesting a strong antioxidant defence system. The IC₅₀ Value of sample 10.78µg/mL was comparable to that of IC₅₀ value of standard 8.90µg/mL. This indicates that the extract has significant radical scavenging ability. The dose response relationship is illustrated in Figure1

SUPEROXIDE DISMUTASE ASSAY

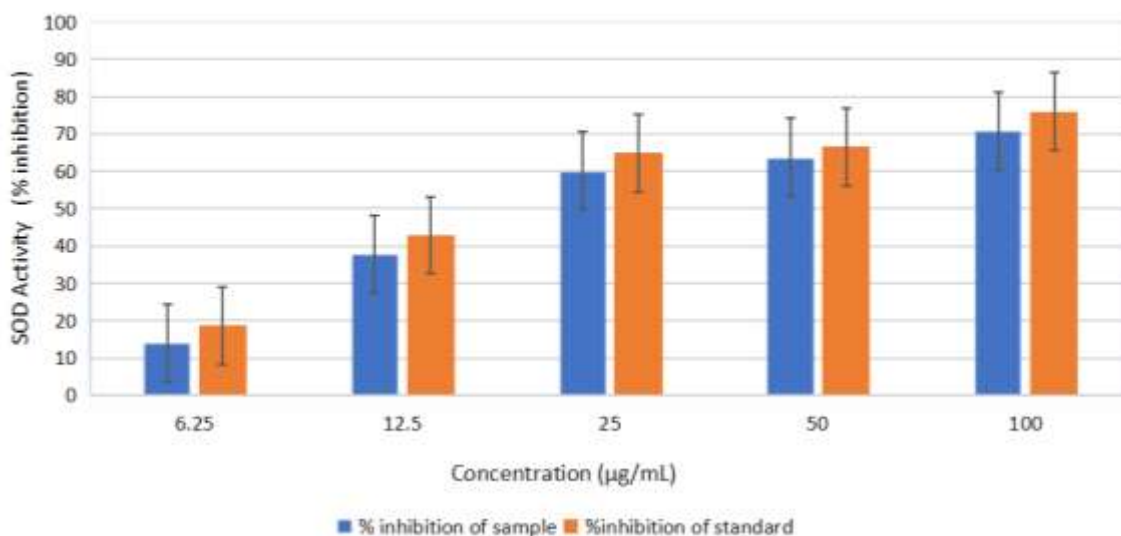


Fig 1. Dose dependent Superoxide dismutase assay of defatted ethanolic *Wrightia tinctoria* R.Br leaf extract compared to ascorbic acid

IV. DISCUSSIONS

The ethanolic leaf extract of the *Wrightia tinctoria* R.Br was evaluated for its phytochemical constituents. The preliminary screening revealed the presence of multiple bioactive constituents that including carbohydrate, proteins, tannins, phenols, flavonoids, terpenoids, glycosides & phytosterols. The wide range of phytochemical constituents and their composition indicate the potential for multiple

biological activities like anti-oxidant, anti-inflammatory, anti-diabetic, anti-psoriatic, anti-acne activities. The compounds like have the ability to reduce the oxidative stress thereby have the ability to scavenge the free radicals by catalyzing the dismutation of the superoxide anion.

The extracts anti-oxidant potential shows the extract the capacity to neutralize the free radicals. The observed activity may be due to the extract's combined effects of the phytoconstituents

present such as phenolics and flavonoids which can as free radical scavengers.

The presence of multiple bioactive compounds with the compounds like triterpenoids, flavonoids along with potential anti-oxidant activity suggests potential relevance in mitigating the oxidative stress and can also be explored for the conditions like vitiligo which is based on oxidative stress theory and increased inflammatory infiltration. While the current study provides the preliminary evidence for the anti-oxidant potential of *Wrightia tinctoria* R. Br, but its suggests the in-vitro evidences only. Future studies including the detailed analysis of in-vitro experiments, in-experiments are necessary to better understand the therapeutic potential of the plant, *Wrightia tinctoria* R.BR leaves. By conducting those studies will help to analyze how these in-vitro studies can be translated to vitiligo related research.

V. CONCLUSION

The present study evaluated the defatted ethanolic leaf extract of *Wrightia tinctoria* R.Br. This revealed the presence of different Phyto constituents and their combined effect demonstrated the anti-oxidant potential. The extract showed the antioxidant potential in superoxide dismutase assay and is capable of scavenging the free radicals. However, as the study is limited to the in-vitro antioxidant assay and the phytochemical screening of the Phyto constituents. This research has laid the foundational work for the further study and extensive investigations will further unravel the potential of this herb.

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