

A STUDY ON PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT  
POTENTIALS OF PLANTS USED IN THE TREATMENT OF  
VITILIGO

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### ABSTRACT

Vitiligo is an acquired disorder of pigmentation, caused by decreased production of melanin as a result of dysfunction of melanocytes. Antioxidants are compounds that help to inhibit many oxidation reactions caused by free radicals such as singlet oxygen, superoxide, peroxy radicals, hydroxyl radicals etc, thereby preventing or delaying the damage to the cells and tissues. Plant derived natural products such

as flavonoids, terpenoids and steroids etc have diverse pharmacological properties including antioxidant activity. The present work antioxidant potentials of some medicinal plants were used in treatment of Vitiligo. *Cassia fistula*, *Eclipta alba*, *Plumbago zeylanica*, *Lawsonia inermis*, and *Indigofera tinctoria* were used in this investigation as they are known to be having antioxidant properties. The methanolic and aqueous extracts of all four plants were investigated. It revealed the presence of phenols, flavonoids, tannins, alkaloids, terpenoids and steroids in the extracts. Reducing power was also high in aqueous extract. The results of this study show that the aqueous extract of all the plants studied can be used as easily accessible source of natural antioxidants in the treatment of Vitiligo.

**KEYWORDS:** Vitiligo, antioxidants, plant extracts, phytochemical analysis.

### INTRODUCTION

Vitiligo is an acquired disorder of pigmentation, characterized by depigmented skin, as a result of the disappearance of melanin from the epidermis. It affects 0.1 to 2% of the world's population, irrespective of gender and race.<sup>[1]</sup> Vitiligo is a multifactorial skin disorder caused

probably by the three main factors: genetic, immunological, and environmental. Unfortunately even today there is no complete and definite cure for this disorder, which has baffled the medical practitioners worldwide. In the traditional system more than 29 plant species have been found to be effective in the treatment of Vitiligo. Unfortunately there is no concrete conclusion regarding the role played by these plants in the treatment and the specific role of the phytochemicals which have been extensively investigated.<sup>[2]</sup> Most of the plants that have been used in the treatment of vitiligo are known to be antioxidants. Experimentally it has been proven that keratinocytes obtained from the vitiligo areas generate a big amount of superoxide anions - reactive forms of oxygen and nitric oxide. It is known that in patients suffering from generalized form of vitiligo there is a disbalance between oxidative and antioxidative systems. Plant derived natural products such as flavonoids, terpenoids, steroids *etc* have diverse pharmacological properties including antioxidant activity. Antioxidants are compounds that help to inhibit many oxidation reactions caused by free radicals such as singlet oxygen, superoxide, peroxy radicals, hydroxyl radicals *etc*, thereby preventing or delaying the damage to the cells and tissues. As antioxidants play an important role in inhibiting and scavenging radicals, thereby providing protection to humans against several diseases including vitiligo.<sup>[3]</sup> *Cassia fistula*, *Eclipta alba*, *Plumbago zeylanica*, *Lawsonia inermis*, and *Indigofera tinctoria* were used in this investigation as they are known to be anti-inflammatory<sup>[4]</sup>, antihyperglycemic<sup>[5]</sup> and besides having antioxidant properties.<sup>[3,6]</sup> Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) Propylgallate (PG) and test butylated hydroquinone are the most commonly used synthetic antioxidants but they have side effects such as liver damage and carcinogenesis.<sup>[2]</sup> This investigation deals with the study of antioxidant potentials of some medicinal plants used in treatment of Vitiligo.

## MATERIALS AND METHODS

### Plant Materials

Fresh plant parts of *Cassia fistula*, *Eclipta alba*, *Plumbago zeylanica*, *Lawsonia inermis*, and *Indigofera tinctoria* were collected from outskirts of Bangalore city. They usually grow as weeds in the open areas. The taxonomic identification was confirmed by Dr V.K.Haridasan, Associate Professor, St. Joseph's College, Bangalore and the specimens of the plants were preserved in the herbarium at St. Joseph's College, Bangalore. Fresh plant materials were washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles at room temperature.

**Extraction of the sample**

10 grams of plant material was extracted with 500 ml (1:50, w/v) of solvent A, 99% methanol; B Distilled water. For methanolic extract dried powder samples (10 g) were extracted in a Soxhlet apparatus as described AOAC (1980) with methanol (99%) until becoming colorless. The extract was filtered. The filtrate was centrifuged at 1500 rpm for 20 minutes to remove any plant debris. For aqueous extract 10 gm of powder samples were extracted by soaking in 5000ml of water for a day in a glass container at room temperature followed by filtration with Whatman No. 1 paper. Filtrates were centrifuged at 1500 rpm for 20 minutes. Supernatants were stored at 22°C and used for further studies.

**Phytochemical Analysis**

The methanolic extract and the aqueous extract of the four plants namely *Cassia fistula*, *Eclipta alba*, *Plumbago zeylanica*, *Lawsonia inermis*, and *Indigofera tinctoria* were subjected to preliminary phytochemical screening for the detection of the major chemical groups.

**Test for Tannins:** 2-3ml of methanolic and aqueous extracts of the plants were taken in test tubes separately followed by addition of 1ml of alcoholic ferric chloride in each test tube. Dark blue or greenish grey coloration of the solution indicated the presence of tannins in the sample.

**Test for Flavonoids:** 2-3ml of methanolic and aqueous extracts of plants were taken separately in test tubes added with few pieces of magnesium turnings with 1ml of conc. HCl. Pink red or red coloration of the solution indicated the presence of flavonoids in the sample.

**Test for Alkaloids:** 3ml of methanolic and aqueous extracts of plants were taken separately in test tube with the Wagner's reagent. Brown precipitate indicated the presence of alkaloid in the sample.

**Test for phenols:** The methanolic and aqueous extracts of plants were spotted on a filter paper. A drop of phosphomolybdic acid was added to the spots and was exposed to ammonia vapours. Blue coloration of the spot indicated the presence of phenols.

**Test for steroids and terpenoids:** To 1 ml of extract of plants , 1 ml of chloroform, 2–3 ml of acetic anhydride and 1 to 2 drops of concentrated sulphuric acid were added. Dark green coloration of the solution indicated the presence of steroids and dark pink or red coloration of the solution indicate the presence of terpenoids. Analysis of phytochemicals was done by using both methanolic and aqueous extracts.

### Hydrogen peroxide scavenging activity

Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), a biologically relevant, non-radical oxidizing species, may be formed in tissues through oxidative processes, but there has been limited information regarding its scavenging by polyphenolic antioxidants. Ability of the plant extracts to scavenge hydrogen peroxide was determined as described by Govindarajan *et al* [7] and Gulcin *et al.*[8] One ml of the extract was rapidly mixed with 2 ml of 10 mM phosphate buffered (0.1M, pH 7.4) hydrogen peroxide solution. The absorbance was measured at 230 nm in the UV spectrophotometer after 10 min of incubation at 37°C against a blank (without hydrogen peroxide). The percentage of inhibition of hydrogen peroxide was calculated using the following formula.

$$\% \text{ inhibition H}_2\text{O}_2 = ([A0]-[A1]) / [A0] \times 100$$

Where, (A0 – Absorbance of control; A1 – Absorbance of sample)

### Total antioxidant capacity

The total antioxidant capacity of the crude extracts of plant materials was evaluated by the method of Prieto *et al.*[9] The antioxidant capacity of the extracts was measured spectrophotometrically using phosphomolybdenum method, based on the reduction of Mo (VI) to Mo(V) by the sample analyze and the subsequent formation of green phosphate / Mo(V) compounds. A 0.3 ml aliquot of sample solution was combined with 2.7 ml of the TAC reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Both samples were capped and incubated in a boiling water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance was measured at 695 nm. A typical blank solution contained 2.7 ml of reagent solution and the appropriate volume of Methanol was used for the dissolution of the samples and it was incubated under the same conditions as the rest of the samples.

### Reducing power assay

The reducing power of the extracts was determined according to the method of Oyaizu [10]. 1 ml of both extracts and standard antioxidant (ascorbic acid) were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml; 10 g/l). The mixtures were incubated at 50°C for 20 min. Then, a portion of Tri Chloro Acetic acid (10%; 2.5 ml) was added to each mixture and centrifuged at 3000 rpm for 20 min. Finally, the supernatants (2.5 ml) were mixed with distilled water (2.5 ml) and FeCl<sub>3</sub> (0.5 ml; 0.1%). The absorbance

of the solutions was measured at 700 nm. A higher absorbance of the reaction mixture indicated that the reducing power had increased.

## RESULTS

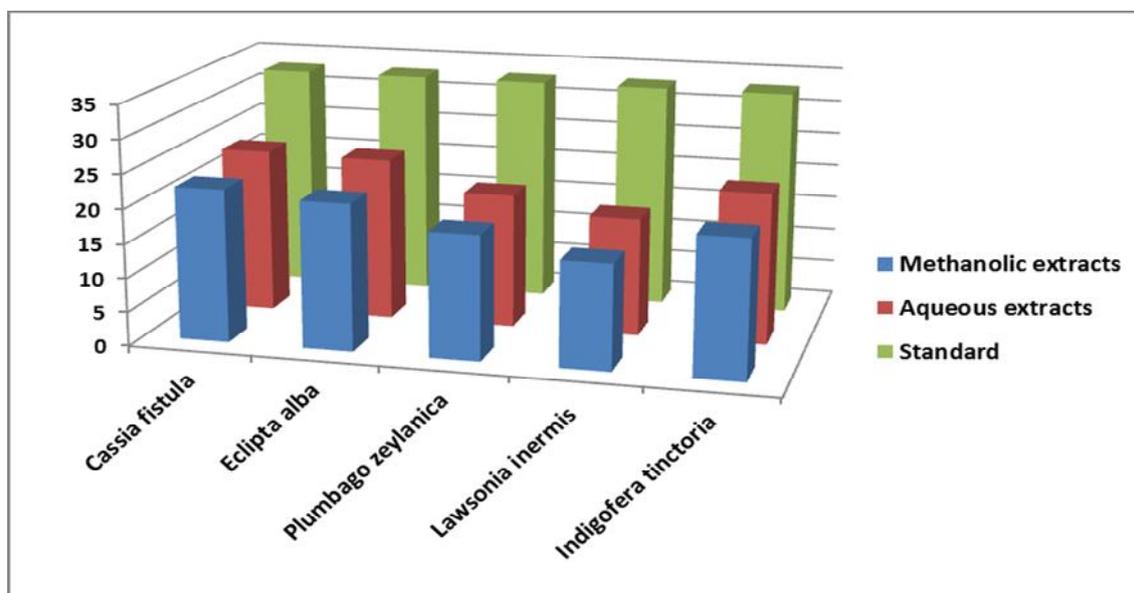
**Table-1: Preliminary results of the phytochemical tests**

Phytochemical Tests	<i>Cassia fistula</i>	<i>Eclipta alba</i>	<i>Plumbago zeylanica</i>	<i>Lawsonia inermis</i>	<i>Indigofera tinctoria</i>
Alkaloids	++	++	++	+++	X
Flavonoids	+	+	+	++	+++
Steroids	+	+	+	++	++
Tannins	++	++	++	+	+
Terpenoids	++	++	++	+++	+

(++: present, +:moderately present, X: absent)

**Table-2: Hydrogen peroxide scavenging activity of plant extracts (Standard using ascorbic acid=33%)**

% Inhibition	<i>Cassia fistula</i>	<i>Eclipta alba</i>	<i>Plumbago zeylanica</i>	<i>Lawsonia inermis</i>	<i>Indigofera tinctoria</i>
Methanolic extracts	22.4	21.5	18.3	15.5	20.3
Aqueous extracts	24.1	23.8	19.6	17.3	22.1
Standard	33	33	33	33	33



**Figure-1 Hydrogen peroxide scavenging activity of plant extracts (Standard using ascorbic acid=33%)**

## DISSCUSION

Free radicals, together with secondarily formed radicals, are known to play an important role in the pathogenesis of Vitiligo and other skin diseases <sup>[11]</sup>. Hence, the study of antioxidant status during a free radical challenge can be used as an index of protection against the development of these degenerative processes in experimental condition for therapeutic measures. Antioxidants block the effects of oxidizing compounds such as reactive oxygen species and harmful free radicals. Essentially, antioxidants prevent the oxidation of cellular structures. This action protects cells from destruction. Therefore, in the case of Vitiligo, antioxidants prevent melanocytes from being destroyed by harmful free radicals and reactive oxygen species produced from cellular metabolism in the skin. Since oxidative stress is an important cause of Vitiligo, this medicinal property is important. Therefore, remedies with antioxidant properties can contribute to the protection of melanocytes and ensure continual production of melanin. Plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical scavengers. Flavonoids are one of the most diverse and widespread group of natural compounds and are one of the most important natural phenolics. These compounds possess a broad spectrum of activities including radical scavenging properties <sup>[12]</sup>. Therefore it was necessary to determine total amounts of phenolics, flavonoids, and flavanols in the selected extracts. Tannins have astringent properties, and they hasten the healing of wounds and inflamed mucous membranes <sup>[13]</sup>. Alkaloids and their synthetic derivatives are used as basic medicinal agents for their antispasmodic and bactericidal effects <sup>[14]</sup>. The measurement of H<sub>2</sub>O<sub>2</sub> scavenging activity is one of the useful methods of determining the ability of antioxidants to decrease the level of pro-oxidants such as H<sub>2</sub>O<sub>2</sub>. It can cross membranes and may slowly oxidize a number of compounds. H<sub>2</sub>O<sub>2</sub> itself is not very reactive, but sometimes it can be toxic to cells because of rise in the hydroxyl radicals in the cells. However weed extracts showed less percentage inhibition in the study.

In the present study, different types of phytochemicals present in weed extracts from different plants namely *Cassia fistula*, *Eclipta alba*, *Plumbago zeylanica*, *Lawsonia inermis*, and *Indigofera tinctoria* have been identified. The aqueous extract demonstrated a very high total antioxidant capacity in terms of percentage inhibition as flavanoid has been detected in the extracts. The reducing capacity of a compound may serve as a significant indicator of potential antioxidant activity. Reducing potential is generally associated with the presence of reductants such as antioxidant substances, which cause a reduction in the Fe<sup>3+</sup>/ferricyanide complex to Fe<sup>2+</sup>. Aqueous extract showed high absorbance i.e. high reducing power as

compared to methanolic extract. The results indicated that the antioxidant activity of all the plants studied seems to be more due to the presence of polyphenols and flavonoids that may act by donating electrons and free radicals. Flavonoids have been shown to have potent antioxidant activity <sup>[15]</sup>. The antioxidants break the free radical chain by donating a hydrogen atom <sup>[16]</sup>. The reducing capacity of a compound may serve as a significant indicator of potential antioxidant activity. Reducing potential is generally associated with the presence of reductants such as antioxidant substances, which cause a reduction in the Fe<sup>3+</sup>/ferricyanide complex to Fe<sup>2+</sup>. Accordingly, Fe<sup>2+</sup> can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. An electron-donating reducing material deals with antioxidant activity to donate an electron to free radicals from the solution <sup>[17]</sup>. The weed showed significant reducing power at the same level as standard antioxidant may be because of some degree of electron donating capacity in a concentration dependent manner. Extracts containing the highest amounts of total phenolics had weaker reducing power. Similar relations between Fe<sup>3+</sup> reducing activity and total phenol content have been reported in the literature <sup>[18]</sup>. Vitiligo is seen all the races of people and has become a social stigma. Its instances are on the rise at present. Its occurrence was found to 0.71% in Bangalore city, India according to the survey made by Dr. Jayarama Reddy <sup>[19]</sup>. Hence a comprehensive method of treatment is required to be developed. The results of this study show that the aqueous extract of all the plants studied can be used as easily accessible source of natural antioxidants in the treatment of Vitiligo. However this is not the only method of treatment because vitiligo is multifunctional disorder. The causes and modes of manifestation are several and it is highly complex. Treatment suggested herewith in the form of antioxidants from plants is one of the many methods that can be safely used.

## CONCLUSION

The preliminary phytochemical investigation indicates the presence of phenols and flavonoids in the plant, In addition, the aqueous extract of all the plants studied found to contain a noticeable amount of total phenols and tannins also flavonoids type components, which plays a major role in controlling antioxidants. The results of this study show that the aqueous extract of all the plants studied can be used as easily accessible source of natural antioxidants. However, the components responsible for the antioxidant activity of aqueous extract of all the plants studied are currently unclear. Therefore, further works have been performed on the isolation and identification of the antioxidant components present.

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