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Original Article

Phytochemical screening and bioassay of *Casssia alata* leaf extract to study its skin hyperpigmentation activity

J. Meenupriya[1], A. Sahaya Vinisha[2] and P. Priya[2]

[1]Research Head, White Lake Organics Private Limited, Kanyakumari -629 703
[2]Research Associate, White Lake Organics Private Limited, Kanyakumari -629 703, India

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ABSTRACT

Medicinal plants of Indian origin play a significant role in treating a broad spectrum of human diseases. With the increase in awareness about the side effects associated with the modern medicines, natural products seem a reliable alternate that's considered safe and effective. The present study investigates the phytochemical screening and analysis of *Cassia alata* leaf extract against hyperpigmentation of skin due to spray allergy and under eye darkness. The present study reveals the efficacy of hydroethanolic extract of *Cassia alata* applied to under eye dark circles and hyper pigmented under arms due to regular usage of body spray. The results obtained are highly promising on regular application twice a day for 20 days. Visible difference is observed within one week of application and normal skin color is obtained within a month's time.

Keywords: Hyperpigmentation, phytochemical, *Cassia alata*, skin allergy.

INTRODUCTION

Natural product is a chemical compound or substance produced by a living organism. More than 40% of commonly prescribed medicines throughout the world found their origins directly or indirectly in plants or animals. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids etc. which have been found in-vitro to have antimicrobial properties [16].These include several major life-saving drugs such as reserpine, atropine, quabin, vinblastine, vincristine and taxol. According to the estimate of World Health Organization (WHO), approximately 80% of the people in developing countries rely chiefly on traditional medicines for the primary health care [8]. Nearly 80% of the world's population relies on traditional medicines for primary health care [20]. Recently, some higher plant products have attracted the attention of microbiologists to search for some phytochemicals for their exploitation as antimicrobials, such plant products would be biodegradable and safe to human health [12,13,14,22]. Furthermore, the increasing awareness on the use of medicinal plants in industrialized countries has been traced to the development of several drugs and chemotherapeutics from the traditionally used herbs [26]. Research has shown that collectively, plants produce a remarkably diverse array of over 500,000 low molecular mass secondary metabolites [9]. In India, almost 95% of the prescriptions are plant based in the traditional systems of Unani, Ayurveda, Homeopathy and Siddha [19].

Infectious diseases, particularly skin and mucosal infections are common in most of the tribal inhabitants due to lack of sanitation, potable water and awareness of hygienic food habits. Skin diseases like wounds, furuncles, sepsis, atopic dermatitis, cellulitis, gas gangrene, acne and candidiasis can be caused by a variety of the microbes. Plants produce a diverse range of bioactive molecules, making them rich sources of different types of medicine. Many hundreds of medicinal plant species worldwide are used in the traditional medicine as treatment for skin diseases caused by bacteria and fungi [5]. Plants act generally to stimulate and supplement the bodies healing forces [1]. Current research on natural molecule and products primarily focuses on plants and selected based on their ethno-medicinal uses [2]. One of such medicinal plants is *Cassia alata*. It belongs to the family of Caesalpiniaceae is a pantropical, ornamental shrub distributed from tropical America to India and some parts of Africa

Corresponding Author Address: J. Meenupriya, Research Head, White Lake Organics Private Limited, Kanyakumari -629 703, India;
E-mail: meenupriya.j@gmail.com

[11]. In the present study qualitative analysis was performed in *Cassia alata* leaf powder by different solvents ethanol (50%), methanol (50%) and aqueous extract. Quantitative analysis was performed in 50% ethanol extract. And the preclinical study was performed in antiblackening activity of *Cassia alata* against spray allergy and undereye darkness.

MATERIALS AND METHODS

Collection of Plant Material: Fresh leaves of *Cassia alata* were collected from kanyakumari district and transported in sterile polythene bags. The leaves were washed thoroughly with running tap water to remove dirt and rinsed with distilled water to ensure sterility and shade dried at room temperature. The dried leaf obtained was pulverized using a sterile electric blender, to a fine powder and stored in airtight dark bottles at room temperature for future analyses.

Aqueous and Solvent Extraction: The aqueous extract of the plant samples were prepared by soaking leaf powder and solvents of varying polarity in the ratio 1:2 for 12 hours. The extracts were then filtered through Whatman filter paper and concentrated in a Rotary Evaporator (SUPERFIT^{TM} Rotavap –PBV 7D). The concentrated extracts were used for qualitative and quantitative analysis.

Preliminary Qualitative and Quantitative Phytochemical Analysis: The different qualitative chemical tests were carried out on the ethanol, methanol and aqueous extract using standard procedures to identify the constituents as described by [3,10,15,18,21,23,24,25]. Quantitative analysis of 50 % ethanol extract were determined using the standard methods by [4,10,17].

In vitro study of skin hyperpigmentation due to spray allergy: Healthy volunteers with skin hyperpigmentation due to regular application of body spray were selected. *Cassia alata* based herbal formulation was applied in the affected area twice a day and usage of bath soap in the affected area was avoided during analysis.

In vitrounder eye skin hyperpigmentation study: Healthy volunteers with under eye skin hyperpigmentation due to irregular sleep pattern and lifestyle changes were selected. *Cassia alata* based herbal formulation was applied in the affected area twice a day and usage of soap/facewash in the affected area was avoided during analysis.

Herbal cream formulation: Oil phase components (olive oil and Cetostearyl alcohol) were weighed (quantity given in Table 1) and heated in a double boilersystem until the components melted evenly. Aqueous phase components were subjected to moderate heat and slowly oil phase was added to the aqueous phase with continuous stirring. Saponification process mixes oil with water to form a creamy consistent paste cream.

Table 1: Table showing the component used in herbal cream formulation

S.No	Ingredients	Quantity/volume
Oil phase		
1.	Olive oil	12 gm
2.	Emulsifying wax (Cetostearyl)	14 gm
3.	Polysorbate 20	12
Aqueous phase		
4.	Leaf ethanolic extract	15 gm
5.	Distilled water	35gm

RESULTS

Screening of phytoconstituents from Cassia leaf extract shows the following result listed in Table 2.

Table 2: Qualitative phytochemical screening of powdered leaf aqueous, ethanol and methanol extracts of *Cassiaalata*.

S.No	Phytochemical test	50% Ethanol Extract	50% Methanol Extract	Aqueous extract
1.	**Test For Alkaloids**			
	Dragendroff's Test	+	+	+
2.	**Test For Flavonoids**	+	+	+
3.	**Teast For Saponins**			
	Foam Test	+	_	+
4.	**Test For Tannins**			
	Lead Acetate Test	+	+	+
5.	**Test For Coumarins**	+	+	+
6.	**Test For Terpenoids**	+	+	+
7.	**Test For Steroids**			
	Salkowski's Test	+	+	+
8.	**Test For Glycosides**			
	Molisch Test	+	+	+
9.	**Test For Carbohydrates**			
	Benedict's Test			
	Fehling's Test	+	+	+
	Test For Phenols	+	+	+
10.	Ferric Chloride Test			
	Test For Proteins	+	+	+
11.	Xanthoproteic Test	_	_	+
	Test For Aminoacids			
12.	Ninhydrin Test	_	_	+

Key= + present; - absent

Table 3: Quantitative analysis of the major phytochemical contents of 50% ethanol leaf extracts of *Cassia alata*.

S.No	Phytochemicals	%
1.	Alkaloids	20
2.	Saponin	50
3.	Flavonoid	25

In vitro study of skin hyperpigmentation due to spray allergy: After regular application of cream twice daily,mild color change was observed on the second and third day.After Seven day of application,the colour of the skin gradually changed and within twenty days the skin regained the normal color.

Fig 1 : Figure showing Hyperpigmentation of skin due to spray allergy before treatment

Fig 2 : Figure showing color channge after 7 days of cream application.

Fig 3 : Figure showing complete color channge after 20 days of cream application.

In vitro under eye skin hyperpigmentation study: Cassia alata formulation was regularly applied twice daily and color change was observed in 7-10 days. Within 15-20 days, the skin regained its normal color.

Fig 4 :Figure showing under eye hyper pigmentation before treatment cream application

Fig 5 :Figure showing change in colorafter 20 days of regular cream application

DISCUSSION

The investigations on the phytochemical and biochemical screening of the crude extract of *S. alata* leaves revealed the presence of alkaloid, flavonoid, and saponins. These compounds have been known to be biologically active and therefore aid the antimicrobial activities of *Cassia alata*. The present study reveals that the hydroethanolic cream of *Cassia alata* applied to the hyperpigmented area for 15-20 days provides visible results. Documented literature assures the role of *Cassia* in treating the chronic fungal disease, Pityriasis versicolor [6,7] revealed that the extracts incorporated into the ointment bases showed better activity than that of the crude extract of *Cassia alata*. This herbal cream contains no preservatives and hence free of any chemical intervention. The "all – organic" preparation will definitely pioneer the world of holistic therapy in this modern world.

CONCLUSION

In this study, the hydroethanolic cream of *Cassia alata* leaf extract showed good result against hyperpigmented under arms and under eye circles within 20 days. The phytochemical studies revealed the presence of important secondary metabolites (alkaloids, flavonoids, saponins etc.,) thus indicating the therapeutic potential of *Cassia alata* leaf. However, this finding provides an insight into the usage of this plant in treatment of other dermatophytic infections. This extract can be safely used to remove dark circles and darkening due to skin allergies. Hence, this research opens up new avenues in hyperpigmentation and cosmetology.

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