

Cassia auriculata L.: An Ethno Medical Approach in Exploring Anti-solar Efficacy

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ABSTRACT

Objectives: The present study deals with an *in-vitro* screening for the anti-solar potential of aqueous and ethanolic extracts of *Cassia auriculata* using the UV method. **Methods:** Aqueous and ethanolic extracts were prepared. Thus, prepared extracts were subjected to phytochemical investigation and evaluated for antioxidant effect using DPPH and anti-solar efficacy. Concentrations ranging from 7.8 to 1000 µg/mL (serial dilution) were used for the determination of radical scavenging effect and concentration of 2 mg/mL was used for screening of anti-solar potential using Shimadzu UV-1700 double beam UV-Visible spectrophotometer. Readings were recorded in triplicate and graphs were extrapolated. **Results:** Extracts revealed the presence of important phytoconstituents such as phenols, triterpenoids, flavonoids, tannins, saponins, phytosterols, alkaloids and glycosides. Further, it was observed that *Cassia auriculata* possesses remarkable antioxidant activity. Significant results of the anti-solar screening study confirm its use as a natural sunscreen. It showed a protective effect against carcinogenic UV radiation. Ethanolic extract was found to be more effective in comparison with aqueous extract. **Conclusion:** From the data, it can be concluded that aqueous and ethanolic extracts showed promising antioxidant and anti-solar activity which could be considered as a rational alternative to chemically prepared synthetic sunscreen formulations available in the market.

Key words: Anti-solar, Carcinogenic, *Cassia auriculata* Linn, DPPH, SPF, UV.

INTRODUCTION

Skin: the largest organ comprising almost about 16% of body mass, formed by bi-layers epidermis and dermis. The epidermis is the outermost layer gets exposed to environmental hazards. It plays a major role in the protection and management of the harmful effects of environmental factors such as chemicals, UV radiation and infectious pathogens.¹ These solar UV radiations are classified as UVC (200-280 nm), UVB (280-320 nm) and UVA (320-400 nm).² UVC being most harmful could not penetrate the ozone layer. UVB is found to be almost 30-40 times more potent compared to UVA and is mainly responsible for biological damage as well as anomalies in DNA.³ Skin is continuously exposed

to UVB radiation. It suffers from various damaging effects, ranging from acute damages like erythema, pain, edema followed by chronic damages like premature aging, skin wrinkling, cataracts and cancer. Also, oxidation of protein and MMPs (metalloproteinases) was observed due to UVB.⁴ Skin protects itself from this radiation by producing melanin which darkens the skin due to pigmentation: a tendency of melanin. In this context, the use of topical formulations (with Sun Protection Factors-SPFs) to the skin may be a preferred choice to act as a sunscreen (Table 1). It may scatter, reflect or absorb the harmful radiation falling on the skin. These sunscreens are grouped as chemical and physical sunscreens.⁵ There is

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a substantial growth in cosmetics and sunscreen market, which is an indication of consciousness among the peoples about hazardous effects of sunlight as well as about their looks, if not taken proper precautions. These sunscreens are associated with some side effects and contraindicated in patients using some other medications. Some adverse effects reported are contact dermatitis, burning, phototoxic, Vitamin D deficiency and estrogenicity,⁶ Damaging DNA, mutation, hormonal imbalance and allergic manifestations particularly in the patients allergic to benzocaine, procaine etc.⁷ This situation has alarmed to search for new safe and efficacious anti-solar agents from natural origin. Plants or herbs are used for their therapeutic potentials since ancient times. Polyphenols, Vitamin C, Vitamin E is reported to have a significant protective role in UV radiation-induced skin damage.⁸ Natural products are still practiced for their ability to treat and cure dermatological diseases and enhance skin appearance also. Earlier studies have already proved the anti-solar potential of many herbal products. Plants consist of many photolabile compounds that may get degraded as plants are continuously exposed to sunlight. Plants consisting of ascorbic acid, tocopherol, flavonoids and polyphenols exert antioxidant action and are observed to protect photosensitive components in plants. This results in inhibition of photo degradation of photo-labile compounds.⁹ *Cassia auriculata* L. (Family *Ceasalpinaceae*) is a commonly available plant in India. Traditional and reported literature about this plant confirms the presence of secondary metabolites such as phenolics, alkaloids, flavonoids, anthraquinone compounds, saponins, proteins, tannins, etc. which are thought to be responsible for efficacious therapeutic effect.¹⁰ In the present study effect of aqueous and ethanolic extract of *Cassia auriculata* L. is evaluated for its anti-solar potentials using a UV spectrophotometer.

MATERIALS AND METHODS

Preparation of Extract

The fresh flowers of *Cassia auriculata* L. were collected from surrounding areas of Gadhinglaj, District Kolhapur, Maharashtra, India. Authentication (Voucher Specimen No GSK-1) of the plant was done by the Department of Botany, Shivaji University, Kolhapur, Maharashtra, India. The flowers collected and authenticated were shade dried and powdered for extraction purposes. The powdered flowers were subjected to the cold maceration method using water and ethanol (95%) as solvents. For extraction, 100g dry powder and 500 mL each of water and ethanol was taken; macerated for

two days with occasional shaking. Further, the macerates (aqueous and ethanolic) were filtered to remove any solid particles (if any) and dried. The dried extracts were used for phytochemical investigation and anti-solar activity.¹¹

Preliminary Phytochemical Investigation

Preliminary Phytochemical investigation of aqueous and ethanolic extracts was carried out for the determination of the presence of secondary metabolites such as flavonoids, alkaloids, saponins, triterpenoids, steroids, tannins, glycosides and phenolics.¹²⁻¹⁴ (Table 2)

Screening for *in-vitro* antioxidant activity by DPPH method

DPPH (1, 1-diphenyl-2-picrylhydrazyl) solution was prepared with 5 mg DPPH and 2 mL methanol, stored at 4°C away from light. Dried aqueous and ethanolic extracts were dissolved in methanol (1000µg/mL) and stock solutions were prepared. Further, these stock solutions were diluted serially (two-fold dilution) to achieve

Table 1: Suitable SPF for Different sensitive skins.

Suitable SPF	Skin Type	UV Sensitivity	Effect of UV radiation
10 ≤	I	Very sensitive	Burns easily, never tans
10 ≤	II	Very sensitive	Burns easily, minimum tanning
8 to 10	III	Sensitive	Moderately burns, uniform and gradual tanning to light brown
6 to 8	IV	Moderately sensitive	Minimum burning, tans to moderate brown
4	V	Less sensitive	Rarely burns, tans to dark brown
Not specified	VI	Not sensitive	Never burns, black pigmentation

Table 2: Results of preliminary phytochemical investigation.

Sr. No.	Chemical component	<i>Cassia auriculata</i> L.	
		Aqueous Extract	Ethanolic Extract
1	Flavonoids	+	+
2	Triterpenoids	+	+
3	Phenols	+	+
4	Tannins	+	+
5	Saponins	+	+
6	Phytosterols	+	+
7	Alkaloids	+	+
8	Glycosides	+	+

a solution of 7.8µg/mL (lowest concentration) in a well plate (96 well micro titer plates). A row of the negative control (methanol only) and Vit-C and quercetin were used as the reference standard. To each well 5 µl DPPH was added and stored the reaction mixture for 30 min protected from light. Using an Elisa Reader absorbance at 517 nm was recorded in triplicate and percentage scavenging potential was determined using the following formula.¹⁵ (Figure 1)

$$\text{DPPH radical scavenging effect (\%)} = \frac{\text{OD (DPPH)} - \text{OD (DPPH + Sample)}}{\text{OD (DPPH)}} \times 100$$

Screening for *in-vitro* anti-solar activity

The anti-solar activity was determined by Shimadzu UV-1700 double beam UV spectrophotometer having a 1 cm quartz cell. Sample (1g) was taken in a 100 mL volumetric flask and made up the volume with water and ethanol (95%) individually. Shaken well and kept for 5 mins. Further, a 5 mL solution from each flask was diluted with 25 mL of water and ethanol (95%) respectively, to achieve a final solution of 2 mg/mL concentration. UV absorption spectra of both aqueous and ethanolic (95%) extracts were recorded in a range between 290-320 nm. Three readings were recorded and the mean was calculated.¹⁶ Normalized product function [Table 3 (EE_(λ) · I_(λ) (Constant)]¹⁷ and mean of absorbance was used to calculate SPF values. (Table 4)

$$\text{SPF} = \text{CF} \times \sum_{290}^{320} \text{EE}_{(\lambda)} \cdot \text{I}_{(\lambda)} \cdot \text{abs}_{(\lambda)}$$

Where,

CF – Correction factor = 10

EE – Erythral effect spectrum

I – Solar intensity spectrum

Abs – Absorbance of prepared extracts (Aqueous and ethanolic)

RESULTS

Preliminary Phytochemical investigation confirms the presence of various secondary metabolites such as flavanoids, triterpenoids, phenols, tannins, saponins, phytosterols, alkaloids and glycosides (Table 2). Further, both aqueous and ethanolic extracts were screened for their antioxidant potential by using the DPPH method where it exhibits promising free radical scavenging effect (Figure 1). Ethanolic extract showed a more radical scavenging effect as compared to aqueous extract. In the present study anti-solar activity of aqueous and ethanolic extracts of *Cassia auriculata* L. was determined using Shimadzu UV-1700 double beam UV-Visible spectrophotometer. A solution of 2 mg/mL concentration was prepared for measuring absorption. Three

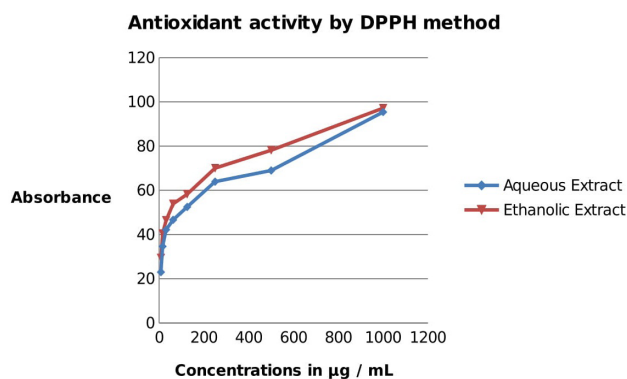


Figure 1: Antioxidant potential of aqueous and ethanolic extracts of *Cassia auriculata* L. using DPPH method.

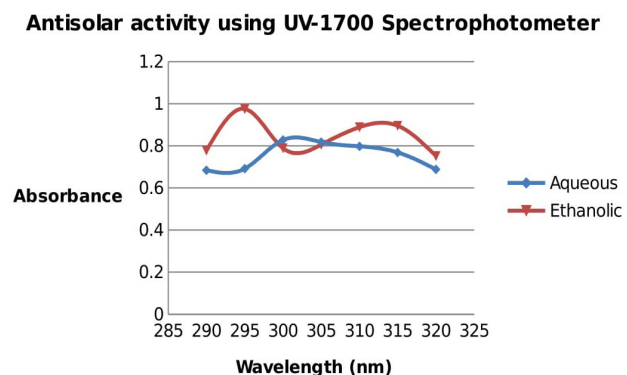


Figure 2: UV absorption of *Cassia auriculata* extracts.

Table 3: Standard values of EE _(λ) · I _(λ) used to calculate Sun Protection Factor (SPF).	
Wavelength (nm)	EE _(λ) · I _(λ) (Constant)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0837
320	0.0180
Total	1

readings were recorded and presented the data in the form of a scattered diagram. From the results obtained, it can be stated that both aqueous and ethanolic extracts exert significant anti-solar activity. Ethanolic extracts showed absorption maxima (λ_{max}) at two different wavelengths i.e. 295 nm (0.976 ± 0.003) and 315 nm (0.896 ± 0.0035) whereas aqueous extract showed absorption maxima (λ_{max}) at 300 nm (0.828 ± 0.0021) Using these λ_{max} values for both aqueous and ethanolic extracts, SPF value for aqueous and ethanolic extract was found to be 13.07 and 15.55 respectively (Table 4 and Figure 2).

Table 4: Absorption maxima of aqueous and ethanolic extracts of *Cassia auriculata* L. and calculated SPF Using mean value.

Sr. No.	λ nm	Absorbance (n=3) Mean of three	
		Aqueous Extract	Ethanolic Extract
1	290	0.684 \pm 0.0025	0.778 \pm 0.0035
2	295	0.692 \pm 0.0040	0.976 \pm 0.003
3	300	0.828 \pm 0.0021	0.789 \pm 0.0045
4	305	0.818 \pm 0.0025	0.806 \pm 0.003
5	310	0.798 \pm 0.0025	0.889 \pm 0.002
6	315	0.769 \pm 0.0031	0.896 \pm 0.0035
7	320	0.688 \pm 0.0035	0.752 \pm 0.0026
	SPF	13.07	15.55

DISCUSSION

White or fair skin complexion is highly susceptible to cutaneous melanoma due to the lesser quantum of dark pigments—eumelanin responsible for blocking UV radiation falling on the skin. Various harmful and undesirable effects on the skin such as inflammation, sunburns, tanning and peeling are observed due to exposure to UV radiation.¹ UV rays are also termed as “Complete Carcinogens” as in absence of any initiator and enhancer they can cause cancer. The effect is due to damage occurring to DNA, followed by the inability to regenerate.¹⁸ Human skin is frequently exposed to ultraviolet rays resulting in cell damage and inducing skin cancer. Melanin plays a vital role in skin pigmentation and serves as the most significant Solar Protection Factor. This activity is attributed to its UV absorbent and free radical scavenging effect.¹⁹ Also, proteins, lipids and nucleotides are natural UV radiation blockers of skin. Further, antioxidants are essential for the stimulation and regeneration of damaged skin. Antioxidant potential of aqueous and ethanolic extracts helps to speed up the recovery of damaged skin which contributes significantly to the anti-solar activity of the plant. Natural products are in medicinal practice since ancient days. They possess potential efficacy in treating many human ailments. Medicinal plants ability to treat skin infections or dermatological disorders and improvising the skin appearance is well established.⁶ Natural products are believed to be safe and economic with more acceptances among people. They act by different mechanism of actions such as detoxification of cancer causing factors, triggering immune response, gene suppression effect, protecting DNA from damage due to reactive oxygen species.⁴ Secondary metabolites from natural resources such as flavonoids, polyphenols, Vitamin E, Vitamin C are found responsible for anti-solar potential acting

either by chemical or physical method.⁶ In the present study, aqueous and ethanolic extracts of *Cassia auriculata* L. were screened for its anti-solar potential. From the results obtained, it is revealed that both the extracts are absorbing UV radiation between the range 290-320 nm, which are believed to be most harmful to the skin. This absorptive capacity may be attributed to the presence of flavonoids and polyphenols in both the extracts. It was evident from the previous studies that polyphenols exert remarkable antioxidant activity and also inhibit the damaging effect on DNA occurring due to harmful UV radiation. Also, it substantially nullifies various adverse biochemical events occurring due to solar UV radiation.²⁰ Ethanolic extract comparatively showed a more promising result as compared to aqueous extract, because of more solubility of flavonoids and polyphenols in ethanol.

CONCLUSION

Both the aqueous and ethanolic extracts of dry flowers of *Cassia auriculata* L. can absorb UV radiation thought to be responsible for skin cancer, especially; ethanolic extracts. The synergistic effect of free radical scavenging potential and SPF values more than 10 assures significant protection from UV radiation. The proved anti-solar activity of the plant shows its importance and prophylactic utility in anti-solar formulations. This will be a safe, better and economic option to harmful chemical sunscreens that are used nowadays in the market.

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CONFLICT OF INTEREST

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

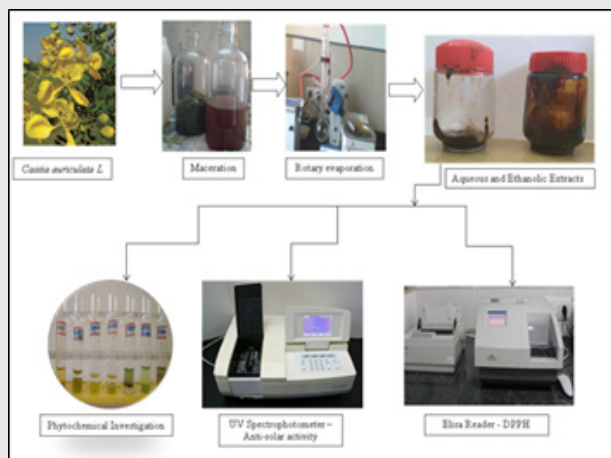
ABBREVIATIONS

UV: Ultraviolet; **DPPH:** 1, 1-diphenyl-2-picrylhydrazyl; **FDA:** Food and Drug Administration; **EWG:** Environmental working group.

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PICTORIAL ABSTRACT



SUMMARY

Current study; deals with the extraction and evaluation of aqueous and ethanolic extracts of flowers of *Cassia auriculata* L for their anti-solar and antioxidant potential by UV and DPPH method respectively. Phytochemical investigation of both extracts revealed the presence of flavonoids, triterpenoids, phenols, tannins, saponins, phytosterols, alkaloids and glycosides. Further, the anti-solar potential was evaluated by measuring the UV absorption capacity of both extracts. It was observed that aqueous and ethanolic extracts absorbed UV radiation to a significant level. Also, both the extracts showed promising free radical scavenging activity. Ethanolic extract showed a comparatively more potent antioxidant effect than aqueous extract. It can be summarized that significant UV absorptive properties with potent free radical scavenging effect are complementary to each other in exhibiting the promising anti-solar effect of *Cassia auriculata* L.

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