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SUN PROTECTIVE ACTIVITY OF WATER IMMISCIBLE PIGMENTS OF FRUIT EXTRACT OF *GARCINIA INDICA*

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ABSTRACT: In order to protect from UV radiations, naturally occurring safe and broad spectrum components are desired. The chemical sun blockers viz. ZnO and TiO₂ gives broad spectrum UV protection but, its safety is doubted. Aim of present communication was to study UV protective activity of water immiscible pigments of fruit rinds of *Garcinia indica*. Out of various extracts used for sun protective activity n-butanol, and ethyl acetate extracts of fruit rinds were shortlisted. Both the ethyl acetate and butanolic extracts were fractionated with water to remove the Hydroxy citric acid present in the extract. The ethyl acetate extract after water fractionation exhibited three-fold increase in UV absorbance in both UV A and UV B regions. Fractionated ethyl acetate extract was incorporated in sun protective cream formulation and the Sun protection factor was found to be 3.43 with boot star rating of five. The fractionated ethyl acetate extract of *G. indica* can be used as natural component for sunscreens.

INTRODUCTION: Ultra violet (UV) radiations emitted from sun are mostly absorbed in the ozone layer which acts as protecting barrier against the harmful effects of these radiations. It absorbs radiations from UV C (200 nm-280 nm) region completely, part of UV B (280nm- 320nm) region whereas, complete UV A (320nm-400nm) region reaches to the earth surface. UV A is the most intense radiation reaching the earth and can penetrate deepest into subcutaneous layer of skin tissue. UV A generates oxygen and hydroxyl-radicals, which can cause damage to cellular proteins, lipids and carbohydrates. It can also cause adverse effects on the DNA.

UV B region is biologically more hazardous as it can penetrate through the skin; but most of it is absorbed by ozone layer. Majority of sunscreen products in market can block mainly UV B rays. There are a very few sun blockers like titanium dioxide and zinc oxide acts as UV A blockers. But these chemical compounds are doubted about their safety and stability¹. Thus it is necessary to find suitable and safe additives with appreciable stability, and it is also imperative to prepare balanced formulation which can provide protection against UV B as well as UV A radiations.

Benzophenone is class of organic aromatic compounds which can absorb sun radiations. A variety of substituted benzophenones are used as common additives in commercial sunscreen products as they absorb and facilitate dissipation of UV radiations consequently; acting as photo stabilizers in cosmetic and personal care products. A variety of benzophenone derivatives are reported as sun blockers for example, Benzophenone 3

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(oxybenzophenone) and Benzophenone 4 (sulisobenzene) protect the skin from the harmful effects of the sun². It is beneficial to find natural source of such benzophenones in order to provide safe and reliable way to improve efficiency of cosmetics products. Various types of polyisoprenylated benzophenones are reported in *Garcinia* species viz. semisones A-C (1-3) in *Garcinia semsei*³, 7-epiclusianone in *Garcinia brasiliensis*⁴ and Garcinol and isogarcinol in *Garcinia indica*⁵. In the present communication attempts have been made to screen UV protective activity of benzophenones from *Garcinia indica*.

In earlier report, we screened the extracts of *G. indica* viz. water, ethyl acetate, methanol and ethanol for UV absorption activity and concluded that, among them ethyl acetate extract exhibits higher UV absorption activity particularly in UV B and UV A region and could be incorporated in UV protective formulations⁶. Water immiscible pigments like garcinol and isogarcinol are soluble in organic solvents such as ethyl acetate. Hence in present communication we mainly focused on UV protective ability of water immiscible pigments of *G. indica*.

MATERIALS AND MEETHODS:

Materials:

Fresh fruits of *Garcinia indica* were collected from Dr. Balasaheb Sawant Kokan Krishi Vidhyapith, Dapoli, Dist. Ratnagiri Maharashtra, India. Cosmetic scale benzophenone- 3 of Chemical international was used.

Reagents and Instruments:

Ethyl acetate, Ethanol, Methanol, Hexane, n-butanol of analytical grade (Merck) were used for experiments. Genesys 10S UV-VIS spectrophotometer (Applied Bio system) was used for measuring UV absorption at wavelength 200-400nm. UV-2000S Ultraviolet Transmittance Analyzer (Labsphere inc.USA) was used to analyze In vitro SPF or UV A protection factor analysis of various formulations.

Methods:

Preparation of fruit rind extracts of *Garcinia indica*: 20 gm fresh fruit rinds of *G. indica* were cut into small pieces and suspended in 100 ml

water for 24 hours at room temperature. Rinds were filtered and re-suspended in respective solvent. Flasks kept on rotary shaker for 4days.

Water Fractionation of extract:

The ethyl acetate extract and n-butanol extract was fractionated with water 4-5 times using separating funnel till its pH increases to 6. This extracts were used for screening UV protective activity.

1. Comparison between activities of Ethyl acetate extracts before water fractionation (BWT) and after water fractionation (AWT) of fruit rinds of *Garcinia indica*.
2. 0.1 gm of dried ethyl acetate extract was re-suspended in 10ml of ethyl acetate (10 mg/ml) and was used to screen for UV absorbance. From this stock, dilutions of various concentration ranging 0.01, 0.03, 0.05, 0.07, 0.09, 0.1mg/ml were prepared using ethyl acetate as diluents.
3. Benzophenone 3 was used as a standard and 1mg/ml stock was used to prepare concentrations ranging from 0.001, 0.003, 0.005, 0.007, 0.009 and 0.01mg/ml.

Measurement of UV absorption:

Incorporation of Ethyl acetate extracts of fruit rinds in sun-protective formulation.

The basic formulation of sunscreen having components shown in **Table 1**. The ethyl acetate extract before water fractionation (BWT) and after water fractionation were incorporated in 100 gm formulation at various amount. Dried ethyl acetate extract 0.5 gm and 5 gm were dissolved in double the amount of ethanol (w/w) and were incorporated in sun protective formula. 3 gm of benzophenone 3 was used as standard. The details of formulation are provided in **Table 1**.

SPF Measurement:

SPF study was done with the help of UV-2000S Ultraviolet Transmittance Analyzer (Labsphere inc.USA) at V.G. Vaze College, Mumbai and Kelkar Trust's Scientific Research Center, Mulund, Mumbai. Basic sunscreen formulation was prepared which contained dried ethyl acetate

extract dissolved in Ethanol and devoid of any other active sun protection factors.

TABLE 1: COMPONENTS OF SUN PROTECTION FORMULATIONS (100g⁻¹).

Ingredients	Control % (w/w)	0.5% extract (% w/w)	5% extract (% w/w)
Benzophenone			
Capryliccapryl triglyceride	2	2	2
Isopropyl myristate	4	4	4
Cetostearyl alcohol	2	2	2
Stearic acid	3	3	3
Propyl paraben	0.02	0.02	0.02
EDTA	0.02	0.02	0.02
Glycerin	4	4	4
Triethanolamine	0.42	0.42	0.42
Methyl paraben	0.25	0.25	0.25
Water	75.29	82.79	69.29
Dried ethyl acetate extract	3	0.5	5
Ethanol	6	1.0	10

RESULTS AND DISCUSSION: In our earlier communication different extracts of fruit rinds of *Garcinia indica* were screened for UV protective ability. Fruit rinds of *G. indica* contains attractive red colored anthocyanins viz. cyanidine 3glucoside and cyanidine 3 sambubisides⁷. It also contain water immiscible yellow colored pigments or Garcinol / isogarcinol or cambogiol⁸, and xanthochymol or isoxanthochymol⁹, Compared to hydroalcoholic or methanolic extract which contain more amount of anthocyanins. Ethyl acetate extracts exhibited more UV absorption both in UV A as well as UV B region⁶. The ethyl acetate extracts dissolve more of water immiscible pigments like Garcinol and isogarcinol. Hence for further experiments we concentrated on water immiscible pigments for UV protective activity.

Determination of UV protective activity of various extracts of *Garcinia indica* fruit rinds using spectrophotometric method:

Initially the fruit rinds were immersed in ethyl acetate, n-butanol, hexane, water and methanol. The absorption ability of the extracts was studied in **Fig 1**. The n- butanolic and ethyl acetate extract showed highest UV absorption both in UV A as well as UV B region. Both extracts showed similar absorption pattern. It showed a large hump in UV A region with absorption maxima at 360nm. At 360nm n-butanolic extract (0.25mg/ml) showed absorbance 2.2, while for same concentration ethyl acetate extract at 360 nm shows optical density, 1.7. The aqueous and methanolic extract also

showed a moderate UV absorption ability. For same concentration of aqueous extract, the absorbance was 0.9 at 360nm, while for methanolic extract it was 1.3. The hexane extract had comparatively poor UV absorption ability having absorbance 0.7 at 360 nm.

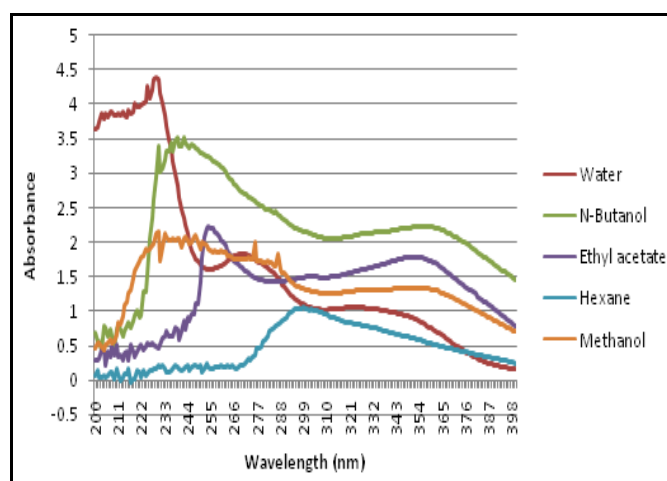


FIG. 1: COMPARISON OF UV ABSORPTION ACTIVITY OF HEXANE, METHANOL, n-BUTANOL, WATER AND ETHYL ACETATE EXTRACT (0.25mg/ml) OF FRUIT RINDS OF GARCINIA INDICA

Hence n-butanol and ethyl acetate extracts were shortlisted for further studies. These extracts [0.5 to 5gm/100gm (%)] were incorporated in cosmetic creams and further screened for sun protection factor. But when the concentration of extract exceeds 1.5gm, the pH of cream drastically dropped below 3, and this leads to phase separation of cream and it become watery. The acidity of the extract might be due to presence of Hydroxycitric acid in fruit extract of *G. indica*.

The presence of substantial amount of HCA along with xanthochymol and isoxanthochymol was also reported by Kumar and co-workers⁸ in butanolic and ethyl acetate extracts of fruit rinds of *G. indica*. Kaur co-workers⁹ proposed the large scale extraction method for Garcinol and isogarcinol from fruit rinds of *G. indica* where the butanolic and ethyl acetate extracts were fractionated with water. The hydroxycitric acid had more affinity to water and it remains in water fraction, Garcinol and isogarcinol remained in water immiscible solvents.

Removal of HCA from ethyl acetate or n-butanolic extract using fractionation method:

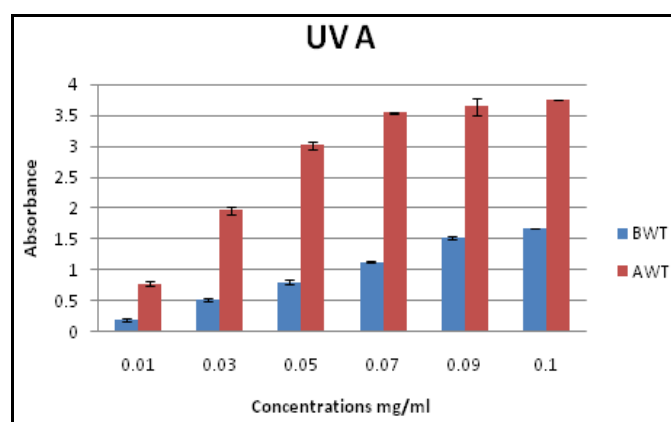
In present communication, to separate HCA, the same method was followed. The ethyl acetate and n-butanolic extracts were fractionated several times with water. After about 4 to 5 washings there was substantial improvement in pH of extract. It increased from pH 3 to pH 6. These fractionated extracts were then screened for UV absorption ability.

Comparison of ethyl acetate extract before water fractionation and after water fractionation (AWT):

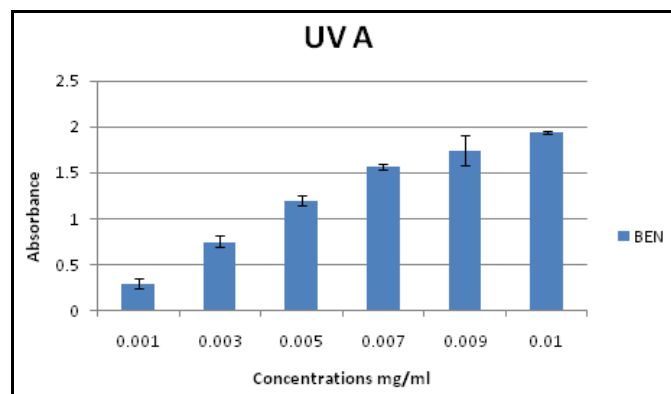
Ethyl acetate extract in various concentrations, ranging from 0.01 mg/ml to 0.1 mg/ml were used to screen UV protective activity. Absorbance of the sample solution was measured in UV A region at different wavelengths (400nm, 380nm, 360nm, 340 nm and 320nm) and UV B region (320nm, 300nm and 280nm). The absorbance in each region was calculated by taking average of absorbance for each concentration. **Fig. 2a** exhibits the comparison of UV protective ability of ethyl acetate extract of fruit rinds of *G. indica* in UV A region, before and after water treatment.

For lowest concentration 0.01 mg/ml, the ethyl acetate extract before water treatment shows average absorbance 0.24, when ethyl acetate extract was water fractionated, the absorbance increased to 0.7. This means, there was about three fold increase in activity of extract after water fractionation. For 0.05mg/ml concentration the non fractionated extract showed 0.81 absorbance, which substantially increased to relative absorbance of 3 after water treatment. That means there is more than three fold increase in UV absorbance, when the extract was

fractionated with water. The water fractionated extract showed steady increase in UV absorbance as the concentration increased from 0.01 to 0.1 mg/ml. At 0.01 mg/ml the UV absorbance is 0.7 which increased to optical density 2 at 0.03 mg/ml concentration. At 0.05 mg/ml it was as high as 3. At 0.07 mg/ml concentration the absorbance was 3.5, beyond this concentration absorbance increased but the increase was not considerable. **Fig.2b** gives UV absorption activity of benzophenone 3 in UV a region, which is used as standard. For 0.01 mg/ml of benzophenone the absorbance is 1.9. It is comparable to 0.03 mg/ml of fractionated ethyl acetate extract of *G. indica*.



(a)



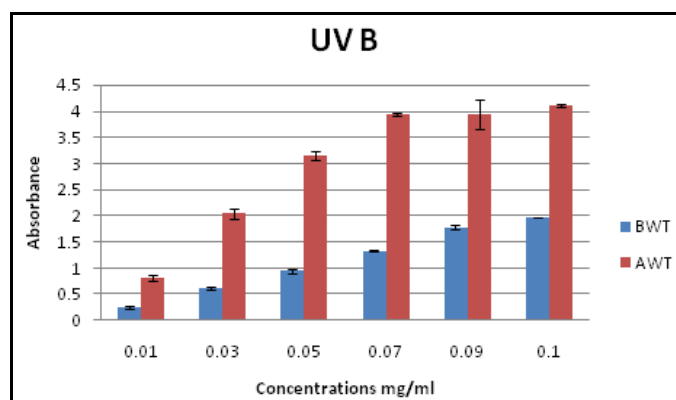
(b)

FIG.2: a) COMPARATIVE ACTIVITY OF ETHYL ACETATE EXTRACTS BEFORE WATER TREATMENT (BWT) AND AFTER WATER TREATMENT (AWT) OF *G. INDICA* FRUIT RINDS IN UV A REGION. b) ABSORBANCE OF BENZOPHENONE-3 IN UV A REGION.

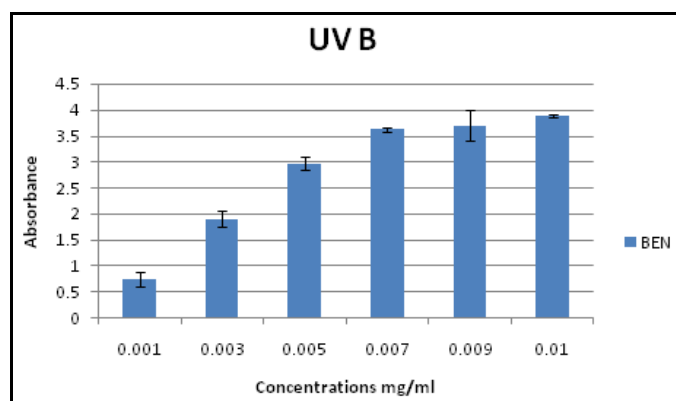
Similarly **Fig. 3a** illustrates the comparison of UV protective ability between non fractionated ethyl acetate extract (BWT) and fractionated extract (AWT) in UV B region. At 0.01 mg/ml concentration the ethyl acetate before water fractionation showed absorbance 0.25, but after water fractionation, it gives approximately three

fold increase in absorbance having optical density 0.7. For 0.05 mg/ml concentration the extract before water fractionation showed absorbance 0.9 whereas the extract after water fractionation shows absorbance 3.1. Hence in UV B region also ethyl acetate extract after water fractionation is more effective.

For water fractionated extract, as concentration increased, there was increase in absorbance of UV B region. For 0.01 mg/ml concentration, the absorbance is 0.81 which, increased to 2.04 for 0.03 mg/ml concentration. At 0.05 mg/ml absorbance increased to 3.1. For 0.07 mg/ml the OD was 3.9 but after this concentration there was no considerable increase in UV absorption activity. **Fig 3b** denotes UV absorption activity of benzophenone 3 at UV B region. At 0.09 mg/ml of fractionated ethyl acetate extract the UV absorption activity is 3.9 which are comparable to 0.01 mg/ml of benzophenone 3.



(a)



(b)

FIG.3: A) COMPARATIVE ACTIVITY OF ETHYL ACETATE EXTRACT BEFORE WATER TREATMENT (BWT) AND AFTER WATER TREATMENT (AWT) FROM *GARCINIA INDICA* FRUIT RIND IN UV B REGION. B) ABSORBANCE OF BENZOPHENONE-3 IN UV B REGION.

UV absorption ability of n-butanol extract of *G. indica*:

N-butanol extract of fruit rinds of *G. indica* was also showing good absorbance in UV A and UV B region (**Fig 4** and **5**). The n-butanol extract showed considerable increase in absorbance after fractionation with water. At 0.07 mg/ml n-butanol extract before water fractionation showed relative absorbance of 0.6 whereas after water treatment, the absorbance increased to 1.9 in UV a region.

The ethyl acetate extract at same concentration (0.07mg/ml, **Fig. 2a**) was appears to be more effective in UV absorption activity having optical density 3.5. The UV absorption activity of the ethyl acetate extract is more efficient than n-butanol extract in UV B (**Fig. 3a**) region also. For 0.07 mg/ml butanol extract after fractionation, the UV absorbance is 2.4 whereas for ethyl acetate extract fractionated with water, the OD was 3.9. Hence for formulation of sunscreen the ethyl acetate extract after fractionated with water was used.

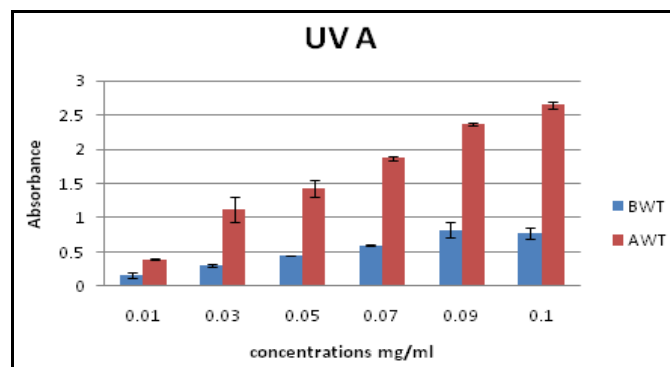


FIG. 4: COMPARATIVE ACTIVITY OF n-BUTANOL EXTRACT BEFORE WATER TREATMENT (BWT) AND AFTER WATER TREATMENT (AWT) FROM *GARCINIA INDICA* FRUIT RIND IN UV B REGION.

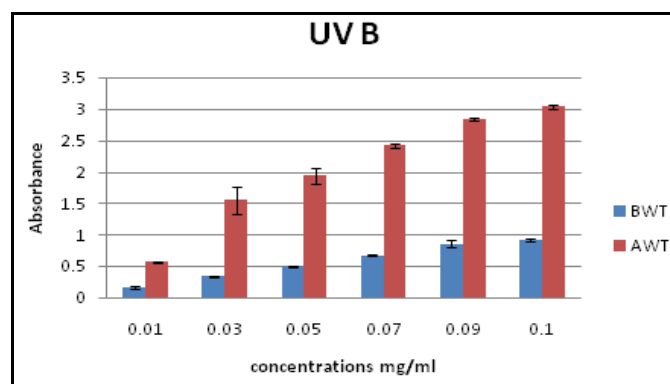


FIG. 5: COMPARATIVE ACTIVITY OF n-BUTANOL EXTRACT BEFORE WATER TREATMENT (BWT) AND AFTER WATER TREATMENT (AWT) FROM *G. INDICA* FRUIT RIND IN UV B REGION

Incorporation of ethyl acetate extract in cosmetic cream:

The ethyl acetate extract before water fractionation (BWT) and after water fractionation (AWT) were allowed to evaporate till dryness and then dissolved in ethanol and incorporated in sunscreen formulation as denoted in **Table 1**. When ethyl acetate extract (BWT) 0.5 gms was incorporated in 100 gm cream. The SPF was 1.25 with boot star rating 3, while for the same concentration of ethyl acetate extract after fractionation there is little increase in SPF which is

1.30. But boot star rating increased to four stars. Similarly when 5gm of ethyl acetate extract (BWT) was incorporated in cream, the SPF was 1.89. There was substantial boot star rating i.e. 5 stars. For water fractionated ethyl acetate extract (5%) the SPF was 3.43 with boot star rating 5. When benzophenone 3 (3%) was incorporated in cream the SPF was 5.04 but there was no boot star rating, this happened might be due to photodegradation of benzophenone **Table 2**.

TABLE 2: RESULTS OF SPF OF DRIED ETHYL ACETATE EXTRACT OF *G. INDICA* BEFORE WATER FRACTIONATION AND AFTER WATER FRACTIONATION

Sr. No	Test Sample	Parameters			Average Values	
		Scans	1	2		3
1.	BEN 3 % (STANDARD)	SPF	4.98	5.14	4.99	5.04
		Standard Deviation	0.19	0.14	0.27	0.20
		UVA/UVB Ratio	0.519	0.521	0.510	0.517
		Critical Wavelength	360.83	360.67	359.83	360.44
		Boots Star Rating	No Rating	No Rating	No Rating	No Rating
2.	BWT 0.5 %	SPF	1.13	1.33	1.29	1.25
		Standard Deviation	0.01	0.02	0.01	0.01
		UVA/UVB Ratio	0.767	0.786	0.799	0.784
		Critical Wavelength	383.83	384.17	384.00	384.00
		Boots Star Rating	***	***	***	***
3.	AWT 0.5 %	SPF	1.32	1.30	1.29	1.30
		Standard Deviation	0.01	0.06	0.03	0.03
		UVA/UVB Ratio	0.804	0.790	0.812	0.802
		Critical Wavelength	386.00	385.83	385.83	385.89
		Boots Star Rating	****	****	****	****
4.	BWT 5%	SPF	1.94	1.87	1.86	1.89
		Standard Deviation	0.02	0.01	0.02	0.02
		UVA/UVB Ratio	1.023	1.048	1.049	1.040
		Critical Wavelength	387.00	387.00	387.00	387.00
		Boots Star Rating	*****	*****	*****	*****
5.	AWT 5 %	SPF	3.37	3.45	3.48	3.43
		Standard Deviation	0.06	0.14	0.16	0.12
		UVA/UVB Ratio	1.008	0.999	0.998	1.002
		Critical Wavelength	388.00	388.00	388.00	388.00
		Boots Star Rating	*****	*****	*****	*****

CONCLUSION: Ethyl acetate extracts of *Garcinia indica* fruit rinds after water fractionation (AWT) gave three times better absorbance in both UV A and UV B region than the extract before water fractionation (BWT). In sun screen cream 5 % ethyl acetate extract after water treatment had SPF 3.43 with boot star rating 5. It can be used as natural component for sunscreen.

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