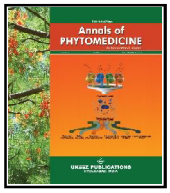
Annals of Phytomedicine 11(2): 516-524, 2022

DOI: http://dx.doi.org/10.54085/ap.2022.11.2.63

Annals of Phytomedicine: An International Journal

http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Original Article : Open Access

# Study on aloe processed by traditional and modern methods to use as an antioxidant and UV protective ingredient

**Vishal Prakashrao Nalamwar◆ and Somashekhar Sanganabasappa Khadabadi**

*Government College of Pharmacy, Kathora Naka, Amravati-444604, Maharashtra, India*

**Article Info**

**Article history**

Received 19 September 2022

Revised 12 November 2022

Accepted 13 November 2022

Published Online 30 December-2022

**Keywords**

*Aloe vera* (L.) Burm. f.

Traditional process

Aloe material

Antioxidant

UV protective

Ingredient

**Abstract**

Antioxidant activity and UV opacity potential of 'aloe gel' has been documented prior to our study, but it was not compared with other forms of aloe processed by traditional methods like drying over flame (*agnitapi*) and drying under sun (*suryatapi*). Lower DPPH scavenging activity and lower UV opacity potential with lyophilized crude aloe gel was observed in previous study. Prior study did not give an idea about how much concentration of the aloe material can block more than 93.3 % of UVB radiation. Processing of aloe leaf is labour intensive and time consuming operation, hence herbal product manufacturers use different ready to use 'aloe materials' to manufacture herbal products, hence there is need of such materials. This study was aimed to find out suitable aloe material that can be used as an ingredient in topical antioxidant UV protective formulation. Leaves of *Aloe vera* (L.) Burm.f. Syn. *Aloe barbadensis* Mill. were processed by different methods to obtain dry solid aloe materials which are named as *Agnitapi kanyasara*, *Suryatapi kanyasara* and *Vacuum dried aloe gel*. *Agnitapi kanyasara* (AK) was prepared by drying aloe leaf juice including yellow sap by heating overmild flame. *Suryatapi kanyasara* (SK) was prepared by drying aloe leaf juice without yellow sap under sunlight. *Vacuum dried aloe gel* (VG) was prepared from aloe leaf juice (mucilaginous liquid gel) without yellow sap by applying ethanol precipitation and vacuum drying technique. Suitability of the aloe material out of AK, SK and VG was decided on the basis of presence of desirable constituents (phenol and polysaccharide), absence of undesirable constituents (aloe anthraquinone), possessing desirable activities that is DPPH radical scavenging activity (DRSA) and UV radiation blocking activity (UVBA). AK was found to contain aloe anthraquinones which are known to cause allergic skin reaction on topical use. It was found that SK does not contains aloe anthraquinones, it contains phenol and polysaccharide. It was found that VG does not contains aloe anthraquinones and phenols, it contai polysaccharide. SK and VG have shown positive test for protein also. At equal concentrations AK, SK and VG shown DRSA and UVBA in the order AK > SK > VG. AK shown highest DRSA and UVBA, but it was found to contain aloe anthraquinone which are undesirable in topical formulation. VG have shown lowest DRSA and UVBA. SK shown 46.02% and 65.19% DRSA when 20 mg in 5 ml and 30 mg in 5 ml quantity was used, respectively. SK has shown more than 97% UVBA when 20 mg/ml and 30 mg/ml quantity was used to prepare sample solutions. Out of the studied, aloe materials, AK, SK and VG, *Suryatapi kanyasara* (SK) was found as suitable material to use as an ingredient in topical antioxidant UV protective formulation. *Suryatapi kanyasara* (SK) was found to contain more than one constituent from aloe leaf, hence it is a mixture. At the outset, it is noted that AK is a suitable aloe material for unani or ayurvedic oral formulation intended to treat constipation and digestion problems where purgative action due to antharquinone is required. Established analytical methods, materials and instruments feasible to individual researcher and small scale entrepreneur having low financial budget were effectively used in this study, so that it can be helpful to them.To the best of our knowledge, this is the first study of its kind.

## 1. Introduction

*Aloe vera* (L.) Burm. f. is the naturalized and commonly found species of aloe in India. The term 'aloe' is commercially used for aloe plant as well as for dried juice obtained from the plant. Aloe is used as medicine in traditional system of medicines such as ayurveda and unani which play significant role in empowering national healthcare (Khan, 2020). Product research should be evidence based to increase acceptance and spread of phytomedicine to global markets (Sekeroglu, 2019). Processing of aloe leaf is labour intensive and time consuming operation, hence herbal product manufacturers use different ready to use 'aloe materials' that can be used as an ingredient to manufacture herbal products. The ready to use 'aloe materials' are mixtures containing different constituents from aloe mixed with pharmaceutical excipients. One such example is spray dried aloe in which maltodextrin is used as excipient (Vaidya *et al.*, 2021). Traditional processes include drying of aloe leaf juice in iron pan by heating over flame and drying under sun (Chunekar and Pandey, 2004; Mishra, 2006). Aloe processed by heating over flame

**Corresponding author:** Mr. Vishal Prakashrao Nalamwar

*Government College of Pharmacy, Kathora Naka, Amravati-444604, Maharashtra, India*

**E-mail:** vishalpn19@gmail.com

**Tel.:** +91-7057872535

is available in the market of traditional unani and ayurvrdic medicinal substances, it occurs as black solid or semisolid masses and known by the names *Eluva*, *Musabbar* and *Kanyasara*.

Literature review on aloe strongly support that phenolic constituents possess antioxidant property, aloe anthraquinone causes purgative action and allergic skin reaction and inner gel portion that contain polysaccharide possess emollient property (Reynolds, 2004). Oxidative stress can occur in the skin due to pollution and sunlight. Relationship between photocarcinogenic damage to skin from sunlight and oxidative stress is well known. Allergic and inflammatory skin diseases are mediated by oxidative stress (Okayama, 2005). Sun protection factor (SPF) 15 is correlated with 93.3% UVB radiation absorption (Brummitte *et al.*, 2012). Antioxidants provide protection when used topically (Sheldon, 2003).

Antioxidant activity (Taukoorah and Mahomoodally, 2016) and UV opacity potential (Kumar *et al.*, 2009) of 'aloe gel' has been documented prior to our study, but it was not compared with other forms of aloe processed by traditional methods like drying over flame (*agnitapi*) and drying under sun (*suryatapi*). Prior studies do not give an idea about how much concentration of the aloe material can block more than 93.3% of UVB radiation. Antioxidant and UV radiation blocking are important properties that are useful to treat skin ailments and to protect skin. It was needed to conduct the study to find out suitable aloe material that can be used as an ingredient in topical antioxidant UV protective formulation based on presence of desirable constituent (phenol and polysaccharide), absence of undesirable constituent (aloe anthraquinone) coupled with desirable activities DPPH radical scavenging activity (DRSA) and UV radiation blocking activity (UVBA).

## 2. Materials and Methods

### 2.1 Selection and authentication of aloe plants

More than two years old plants of aloe, naturally growing in campus of Government College of Pharmacy, Amravati, Maharashtra (India) were selected. One of the plant and its flowers are shown in Figure 1 and Figure 2, respectively. Voucher specimen was deposited in Government Vidarbha Institute of Science and Humanities, Amravati, Department of Botany herbaria with accession numbers Bot. GVISH 102. It was verified and authenticated as *Aloe vera* (L.) Burm.f. belonging to family Asphodelaceae. The authentication certificate was obtained. Synonym of the species is *Aloe barbadensis* Mill (Almeida, 2009).

**Figure 1: *Aloe vera* (L.) Burm.f. plant.**

**Figure 2: *Aloe vera* (L.) Burm.f. flower.**

### 2.2 Study of morphological characters

Naturally grown aloe plants in campus of Government College of Pharmacy, Amravati, Maharashtra (India) were observed for morphological characters of stem, scape, leaves, flowers, fruits and seeds. Observed morphological characters are given in Table 1.

**Table 1: Morphological characters**

| Part | Observed morphological characters |
|---|---|
| Stem | Short thick divided, scape |
| Leaf | Glaucous green, fleshy, sessile, crowded, lanceolate, erect spreading, plane-concave upper surface, convex beneath, yellowish toothed spines along margin, 1-3cm sheath at base |
| Scape | Longer than the leaves, scaly, branched, bearing long racemose inflorescence |
| Bract | Short, lanceolate, membranous, longer than the short pedicel |
| Flower | Pendulous, buds red, full grown flower with yellow-orange imbricated corolla, somewhat exerted anthers |
| Fruit | Capsules, ovoid-ellipsoid, obtusely trigonous |
| Seed | Black, many in each fruit, trigonous, winged |

Kirtikar and Basu (2007) in their book titled 'Indian Medicinal Plants' mentioned colour of *A. barbadensis*. flower as yellow and Naik (1998) in his book titled 'Flora of Marathwada' mentioned colour of *A. barbadensis* flower as oranged-red. We have observed during study of morphological characters that initially flower buds were red in color and as their maturation progress colour changes to yellow to orange, full grown flowers which we observed were partly yellow and partly orange, hence we noted the color of flowers as 'yellow-orange' as shown in Figure 2.

### 2.3 Selection of aloe leaves

Leaves towards bottom of plant lying on soil, diseased leaves and old yellowish leaves were skipped, 5-6 leaves towards top portion

of plant which included young leaves were skipped. Healthy leaves from middle portion of the plant having length 45-50 cm were selected, so that the plants were allowed to grow further and not sacrificed.

### 2.4 Preparation of *Agnitapi kanyasara* (AK) by traditional method

Selected aloe leaves were collected in the month of February-March by transversely cutting at distance 2-3 cm away from their attachment to stem. The leaves were washed under tap water and gently wiped with clean dry cotton cloth. 5 cm long apical portion and the spike bearing lateral edges of the leaves were cut. Then each leaf was cut longitudinally to obtain two halves, yellow sap coming out of leaf and inner gel layer was collected in beaker by scrapping with spatula, homogenized at 18000 rpm and filtered. Thus, juice containing yellow sap and inner gel from aloe leaf was obtained. The juice was boiled over mild flame until water was evaporated completely in an iron pan coated with soil layer at the bottom. Thus, *Agnitapi kanyasara* (AK) was obtained as black colored semisolid sticky masses, shown in Figure 3. It was weighed and kept in tightly closed container. Percent yield of AK is given in Table 2.

### 2.5 Preparation of *Suryatapi kanyasara* (SK) by traditional method

Selected aloe leaves were collected in the month of February-March by transversely cutting at distance 2-3 cm away from their attachment to stem. The leaves were washed under tap water and gently wiped with clean dry cotton cloth. The leaves were kept in vertical position in porcelain dish for 30 min, so that yellow sap from the leaves was drained out. 5 cm long apical portion and the spike bearing lateral edges of leaves were cut. All four sides of each leaf were wiped with tissue paper to soak and remove slight traces of yellow sap oozing out from it. Thus, care was taken to eliminate yellow sap. Then each leaf was cut longitudinally to obtain two halves and inner gel was collected in beaker by scrapping with spatula, homogenized at 18000 rpm and filtered. Thus, juice containing inner gel portion of aloe leaf without yellow sap was obtained. The juice was transferred into china clay saucersand kept under sunlight for drying. During this drying period, day after day the juice became more and more yellowish brown with reduction in volume and weight. Thus, *Suryatapi kanyasara* (SK) was obtained as brownish yellowdry brittle flakes, shown in Figure 4. It was weighed and kept in tightly closed container. Per cent yield of SK is given in Table 3.

### 2.6 Preparation of *Vacuum dried aloe gel* (VG)

Same procedure as given for 'SK', beginning from the words 'selected aloe leaves' to the words 'without yellow sap was obtained'.With some modifications, vacuum drying method used by Xing and Li (2009) was applied. The juice was transferred into petri dishes and kept in vacuum oven (Lab Star, India) at 30°C and evaporated to reduce volume to about 1/4$^{th}$of initial volume. Thus, concentrated thick juice was obtained. 3 volumes of the concentrated juice were stirred with 7 volumes of ethanol and kept at room temperature for overnight. The precipitated agglomerates were separated by filtration, stirred with 70% (v/v) ethanol, filtered and dried in vacuum oven at 30°C. Thus, *Vacuum dried aloe gel* (VG) was obtained as faint brownish grey dry flakes, shown in Figure 5. It was weighed and kept in tightly closed container. Per cent yield of VG is given in Table 4.

**Figure 3 : *Agnitapi kanyasara* (AK).**

**Figure 4: *Suryatapi kanyasara* (SK).**

**Figure 5: Vacuum dried aloe gel (VG).**

### 2.7 Detection of anthraquinone, phenol and polysaccharide in aloe materials

Substance under examination (200 mg) was mixed with 20 ml distilled water in test tube, kept for 1 h, stirred intermittently and filtered to obtain filtrate A.

#### 2.7.1 Test for anthraquinone

Filtrate A (5 ml) was taken into test tube, added 2 ml 10% w/v methanolic solution of ferric chloride hexahydrate and mixed, added 2 ml hydrochloric acid and mixed, heated on low flame for 10 min (care was taken to avoid boiling), allowed to cool to lukewarm, added 7 ml carbon tetrachloride and shaken. Organic layer was separated and taken into other test tube, to it added 3 ml ammonia solution and shaken (pink or cherry red coloured ammonical layer indicate presence of anthraquinone).

#### 2.7.2 Test for phenol

Filtrate A (5 ml) was taken in test tube and 5 ml of 10% (w/v) aqueous solution of lead acetate was added to it (formation of precipitate indicate presence of phenolic constituent).

### 2.7.3 Test for reducing sugar

Fehling's solution A (2 ml) and Fehling's solution B (2 ml) were mixed in a test tube and boiled for 1 min, added 20 mg substance under examination and stirred, heated for 2 min, care was taken to avoid vigorous boiling (formation of yellow to brick red precipitate indicate presence of reducing sugar).

### 2.7.4 Molisch's test

Substance under examination (1 g) was taken, mixed with 20 ml of 70% (v/v) ethanol, kept for 1h, filtered; residue was washed with 70% (v/v) ethanol and filtered. 10 mg of washed residue was taken into a test tube; added 1ml distilled water and mixed, added 2 drops of 10% (w/v) ethanolic solution of a-naphthol and mixed. The test tube was slightly inclined and 1 ml sulphuric acid was added slowly drop by drop from the side of test tube. A layer was formed by acid below the aqueous layer (formation ring at the junction of two layers and formation of dark solution on shaking indicate presence of polysaccharide).

### 2.7.5 Staining with congo red solution

Substance under examination (1 g) was taken, mixed with 20 ml of 70%(v/v) ethanol, kept for 1h, filtered; residue was washed with 70%(v/v) ethanol, filtered and dried in air. About 10 mg washed residue was taken in watch glass and 1 ml congo red solution was put over it, mixed and kept for 20 min. 1-2 drops were taken on microslide and observed under microscope (polysaccharide particles stain pink-red).

### 2.7.6 Test for protein

Substance under examination (50 mg) was stirred with 2 ml of distilled water, added 1ml of 4%(w/v) aqueous solution of sodium hydroxide and mixed, added 3 drops of 1%(w/v) aqueous solution of copper sulphate and mixed (solution becomes violet if protein is present). Observations of the phytochemical tests performed to find out presence or absence of anthraquinone, phenol, polysaccharide and protein in AK, SK and VG are given in Table 5. Inference drawn on the basis of observations is given in Table 6.

### 2.7.7 Thin layer chromatography of aloe materials

Thin layer chromatography to find out presence or absence of anthraquinone was performed by procedure given in Indian Pharmacopoeia (2007) with some modifications. Silica gel 60 $F_{254}$ aluminium sheet (Merck) was used as stationary phase. Mixture of ethyl acetate: methanol: distilled water in ratio 100:17:13 was used as mobile phase.

Reference solution (RS)-yellow sap obtained from aloe leaf was dried in air (atmospheric temperature 36°C, relative humidity 30 %). 50 mg dried yellow sap was mixed with 10 ml methanol in test tube and kept in boiling water bath for 1-2 min (care was taken to avoid vigorous boiling), removed from water bath and allowed to cool to room temperature, immediately filtered through sintered glass filter (Borosil 32060) into a 10 ml volumetric flask, sufficient methanol was added and mixed to make up the volume to 10 ml. This was used as reference solution (5 mg/ml).

Test solution-500 mg substance under examination was mixed with 10 ml methanol in test tube and kept in boiling water bath for 1-2 min (care was taken to avoid vigorous boiling), removed from water bath and allowed to cool to room temperature, immediately filtered through sintered glass filter (Borosil 32060) into a 10 ml volumetric flask, sufficient methanol was added and mixed to make up the volume to 10 ml. This was used as test solution (50 mg/ml). Separate test solutions containing substances under examination AK, SK and VG were prepared.

Spots of reference solution and test solution(s) were applied with the help of capillary tube. Chromatogram was developed. The plate was dried in current of air, sprayed with 10%(w/v) methanolic potassium hydroxide, again dried in air and observed under UV light at wavelength 365 nm. Aloin produce spot at Rf value 0.4-0.5. Observations are given in Table 7.

## 2.8 DPPH radical scavenging activity (DRSA) of aloe materials

Substances under examination (AK, SK and VG) were separately triturated in mortar and pestle to make fine powder, passed through bolt cloth (355 pores per inch, pore size 0.03 mm) and stored in desiccator at 25°C. Procedure was carried out at room temperature (25°C). 0.1 mM solution of 2,2-diphenyl-1-picryl hydrazyl (molecular weight 394.32) was prepared in methanol and λmax was determined by scanning on Shimadzu Pharmaspec UV-1700 UV-Visible spectrophotometer (absorbance mode, path length 1cm, wavelength range 400-600 nm, interval 1nm, slitwidth 1nm, medium speed). Quartz cuvettes having path length 1cm were used. Baseline clearance was done by using methanol. Three readings were taken for prepared 0.1 mM DPPH solution to confirm its λmax. It was found to be 517 nm (shown by blue solid line in Figure 9). To find out absorbance for control ($A_{control}$), 5 ml of prepared 0.1 mM DPPH solution was mixed with 5 ml methanol in a conical polypropylene tube. To find out absorbance for sample ($A_{Sample}$), 10 mg of fine powder of substance under examination was suspended in 5 ml methanol in a conical polypropylene tube, 5 ml of 0.1 mM DPPH solution was mixed into it. All the conical tubes were closed, wrapped in aluminium foil and kept for 25 min (shaken intermittently after every 2 min), centrifuged at 3500 rpm for 5 min. Absorbance at 517 nm was recorded for control and sample solutions. The procedure was carried out in triplicate, mean absorbance for control and sample was calculated and per cent DPPH radical scavenging activity (DRSA) was determined by using following equation:

$$\text{Per cent DRSA} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Same procedure was followed by taking 20 mg and 30 mg of SK in 5 ml of methanol to prepare sample solution. DPPH radical λmax (517 nm) is shown in Figure 9. Per cent DPPH radical scavenging activity of AK, SK and VG is given in Table 8 and represented in Figure 10.

## 2.9 UV radiation blocking activity (UVBA) of aloe materials

Quantity of AK, SK and VG corresponding to concentration 1 mg/ml were weighed separately and each of the aloe material was treated in following manner:

Weighed quantity of the aloe material was stirred with required quantity of distilled water for 1 h at 50 rpm and filtered through sintered glass filter (Borosil 32060). UV Transmittance spectrum

was recorded for each solution by using Shimadzu UV-1780 UV-VIS Spectrophotometer (transmittance mode, path length 1cm, wavelength range 200-400 nm, slitwidth 1nm, medium speed). Baseline clearance was done with distilled water. The procedure was performed in triplicate. Mean per cent transmittance at every 5 nm intervals in UVA (315-400 nm) and UVB (200-315 nm) region were calculated. Per cent UV radiation transmittance exhibited by 1 mg/ml solution of AK, SK and VG is shown in Figure 11. Per cent UVA and UVB transmittance exhibited by 10, 20 and 30 mg/ml solution of SK is shown in Figure 12.

Per cent blocking of UVA and UVB by the solution of substance under examination was calculated by using following equations given by Springteen *et al.* (1999):

$T(UVB)_{AV}$ represent average per cent transmittance in UVB region

$$T(UVB)_{AV} = \frac{\sum_{280\ nm}^{315\ nm} T\lambda x\ \Delta\lambda}{\sum_{280\ nm}^{315\ nm} \Delta\lambda}$$

Per cent blocking of UVB = $100\text{-}T(UVB)_{AV}$

$T(UVA)_{AV}$ represent average per cent transmittance in UVA region

$$T(UVA)_{AV} = \frac{\sum_{315\ nm}^{400\ nm} T\lambda x\ \Delta\lambda}{\sum_{315\ nm}^{400\ nm} \Delta\lambda}$$

Per cent blocking of UVA = $100\text{-}T(UVA)_{AV}$

where,

T I represent percent transmittance value at the particular wavelength

D I represent wavelength interval

100 is maximum transmittance (percent)

When average per cent transmittance value is deducted from 100we get per cent value for UV radiation which is not transmitted by the solution, means per cent value of UV radiation blocked.

Per cent blocking of UVA and UVB was determined for 10, 20 and 30mg/ml solutions of SK, results are given in Table 9, percent UVA blocking is represented in Figure 13 and per cent UVB blocking is represented in Figure 14.

### 2.10 Names and manufacturers of chemicals used

Ammonia solution, carbon tetrachloride, congo red solution, copper sulphate, 2,2-diphenyl-1-picryl hydrazyl, ethyl acetate, ferric chloride hexahydrate, hydrochloric acid and 2-naphthol were purchased from Central drug house (P) Ltd. Ethanol was purchased from Deccan sugar factory. Distilled water, Fehling's solution A, Fehling's solution B, methanol, potassium hydroxide and sodium hydroxide were purchased from Avantor (Rankem). Sulphuric acid was purchased from Pallav chemicals.

## 3. Results

**Table 2: Per cent yield of AK**

| Weight of leaves (g) | Weight of juice (g) | Weight of AK (g) | AK yield % (w/w) |
|---|---|---|---|
| 1727 | 823 | 8.67 | 0.50 |
| 3344 | 1695 | 21.20 | 0.60 |
| 2724 | 1450 | 15.88 | 0.58 |

**Table 3: Per cent yield of SK**

| Weight of leaves (g) | Weight of juice (g) | Weight of SK (g) | SK yield %(w/w) |
|---|---|---|---|
| 1744 | 940 | 11.07 | 0.63 |
| 1900 | 1150 | 12.65 | 0.66 |
| 1500 | 895 | 9.98 | 0.66 |

**Table 4: Per cent yield of VG**

| Weight of leaves (g) | Weight of juice (g) | Weight of VG (g) | VG yield %(w/w) |
|---|---|---|---|
| 1345 | 831 | 5.11 | 0.37 |
| 1124 | 711 | 4.60 | 0.41 |
| 1030 | 608 | 3.71 | 0.36 |

With respect to total weight of aloe leaves taken mean per cent yield of AK, SK and VG was found to be 0.57 %, 0.65 % and 0.38 % (w/w), respectively.

**Table 5: Observations of phytochemical tests**

| Test | AK | SK | VG |
|---|---|---|---|
| **Test for anthraquinone** | Faint pink ammonical layer | Colourless ammonical layer | Colourless ammonical layer |
| **Test for phenol** | Buff precipitate | White precipitate | No precipitate |
| **Test for reducing sugar** | Yellowish precipitate | Brick red precipitate | No precipitate |
| **Molisch's test** | Ring at the junction of two layers formed and solution became dark on shaking | Ring at the junction of two layers formed and solution became dark on shaking | Ring at the junction of two layers formed and solution became dark on shaking |
| **Staining with congo red** | Particles stained pink-red | Particles stained pink-red | Particles stained pink-red |
| **Test for protein** | No change in initial yellowish brown colour of solution | Solution became faint violet | Solution became faint violet |

**Table 6: Inference**

| Constituent | AK | SK | VG |
|---|---|---|---|
| Anthraquinone | P | A | A |
| Phenol | P | P | A |
| Reducing sugar | P | P | A |
| Polysaccharide | P | P | P |
| Protein | A | P | P |

'P' indicate that the constituent is present
'A' indicate that the constituent is absent

Phytochemical test indicated that aloe anthraquinone is present in AK and absent in SK and VG.

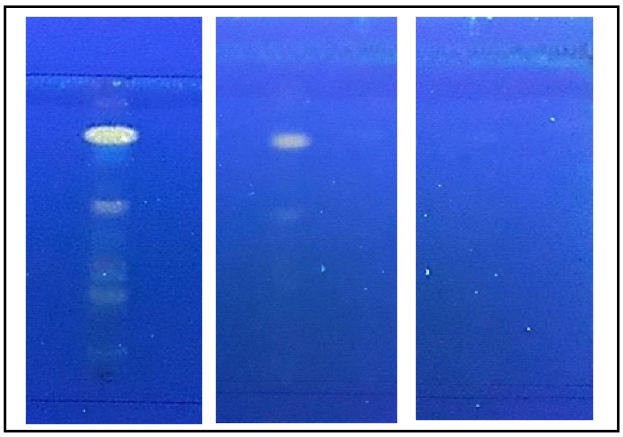


**Figure 6: TLC of RS, Figure 7: TLC of AK and Figure 8: TLC of SK.**

**Table 7: TLC observations**

| Aloe material | Spot at Rf 0.4-0.5 | Inference |
|---|---|---|
| Yellow sap (RS) | Faint yellow florescent spot | Aloin present |
| AK | Faint yellow florescent spot | Aloin present |
| SK | No spot | Aloin absent |
| VG | No spot | Aloin absent |

Indian Pharmacopoeia (2007) specified use of 5 mg/ml barbaloin solution as reference solution in TLC procedure for aloe. We have used reference solution containing 5 mg/ml dried yellow sap obtained from aloe leaves of same plants from which juice was prepared. Advantage of our reference solution was that it contained aloin along with other anthraquinones from aloe leaves of same plants from which juice was prepared.

On comparing developed chromatograms (TLC) against TLC plate images given by Reynolds (2004) in his book titled 'Aloes: The genus Aloe'. We found that chromatogram of reference solution (Figure 6: TLC of RS) showed spots corresponding to aloe emodin (bright yellow spot on top portion), aloin (faint yellow florescent spot in middle portion, Rf 0.4-0.5 as per IP 2007) and tetrahydroxy anthaquinone (faint yellow florescent spot below the spot of aloin). Chromatogram of AK 50 mg/ml solution (Figure 7: TLC of AK) showed similar spots as that of reference solution but all the spots were very faint. This has indicated that some quantity of anthraquinones is destroyed during heating process. Chromatogram of SK 50 mg/ml solution (Figure 8: TLC of SK) and VG 50 mg/ml solution did not show any of the spots corresponding to aloe anthraquinones. This indicated that aloe anthraquinones are absent in SK and VG.

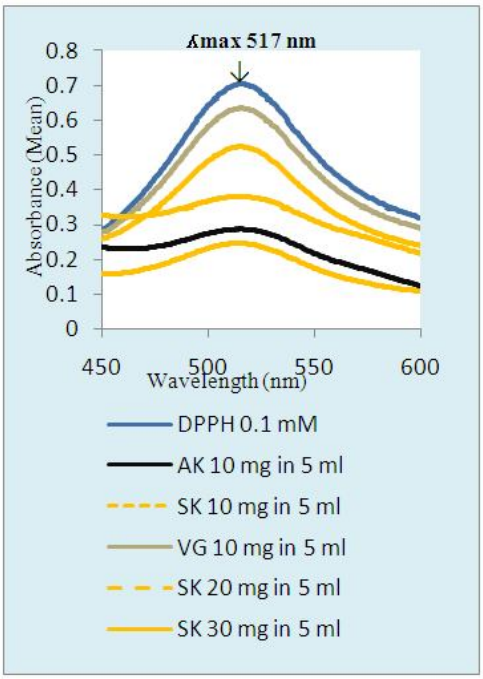



**Figure 9: DPPH radical scavenging.**

**Table 8: Per cent DRSA**

| Aloe material | Quantity taken in 5 ml methanol | DRSA (%) |
|---|---|---|
| AK | 10 mg | 59.51 |
| SK | 10 mg | 25.71 |
| VG | 10 mg | 9.94 |
| SK | 20 mg | 46.02 |
| SK | 30 mg | 65.19 |

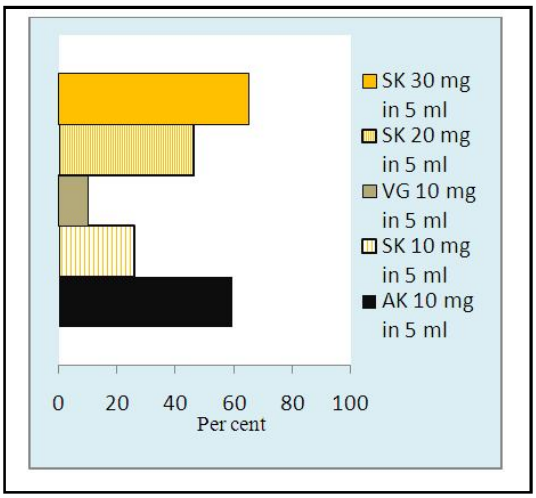



**Figure 10: Per cent DRSA of AK, SK and VG.**

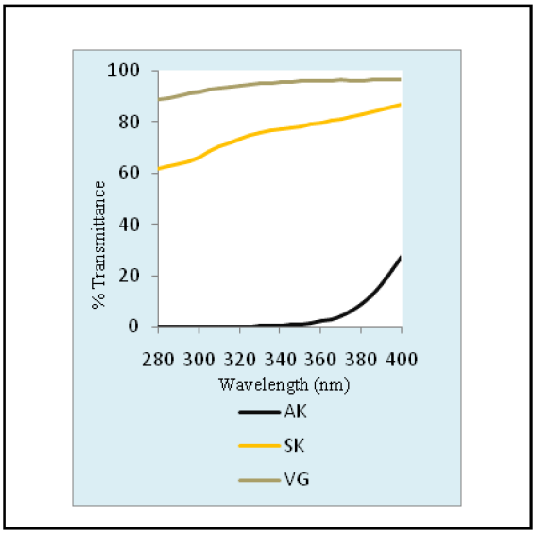



**Figure 11: UV radiation transmittance AK, SK and VG 1 mg/ml.SK 10, 20, 30 mg/ml.**

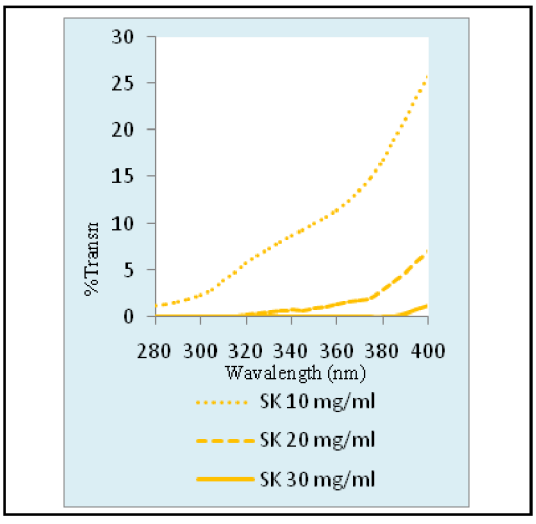



**Figure 12: UV radiation transmittance.**

**Table 9: Per cent UVBA**

| Aloe material Concentration | Per cent blocking of UVA | Per cent blocking of UVB |
|---|---|---|
| AK 1 mg/ml | 93.90 | 99.99 |
| SK 1 mg/ml | 20.28 | 26.02 |
| VG 1 mg/ml | 4.28 | 10.24 |
| SK 10 mg/ml | 87.21 | 97.50 |
| SK 20 mg/ml | 97.98 | 99.98 |
| SK 30 mg/ml | 99.86 | 100 |

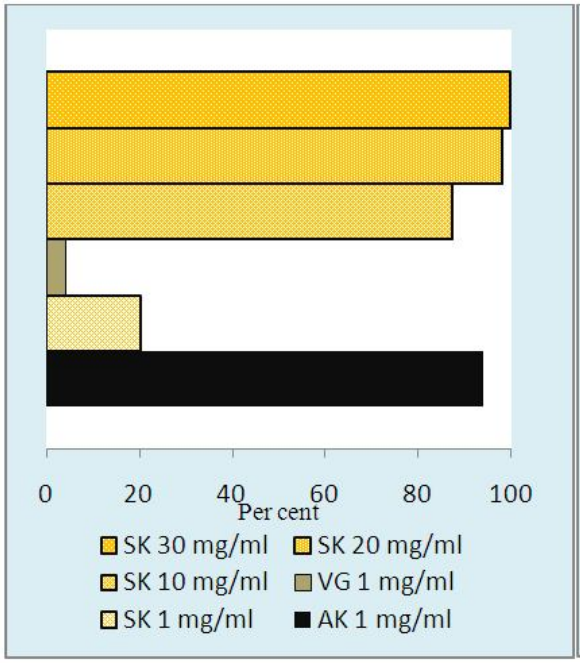



**Figure 13: Per cent UVA blocking by AK, SK and VG.**

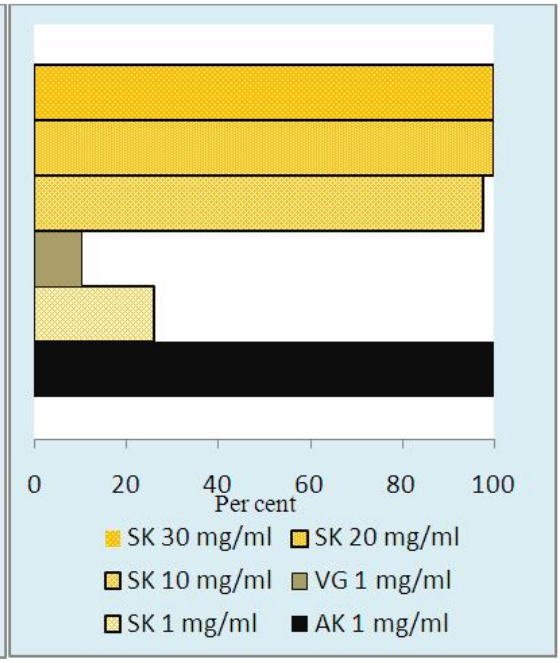



**Figure 14: Per cent UVB blocking by AK, SK and VG.**

## 4. Discussion

AK has shown presence of aloe anthraquinones which are undesirable in topical formulation. SK has shown presence of phenols and polysaccharides, VG has shown presence of polysaccharide and absence of phenol. It is well known that naturally occurring phenols possess antioxidant property and absorb UV radiation. Thus, SK carries advantage over AK and VG.

In case of DPPH method, there is difference of opinion about estimation of parameter called $EC_{50}$, that is, the concentration of antioxidant species able to scavenge 50% of the initial DPPH (Foti, 2015). Instead of $EC_{50}$, we have used per cent DPPH radical scavenging activity (DRSA) as a measure of antioxidant capacity of aloe materials (AK, SK and VG). This is because DPPH provide some unique advantages like the DPPH radical is remarkably stable, it is coloured, hence one can determine initial absorbance of its 0.1 mM solution and take this as number that represent total quantity (100%) of DPPH in the solution, reduction in the absorbance on addition of substance under examination can be used to calculated per cent DRSA which indicate antioxidant activity of the substance under examination. Due to this reason, DPPH radical method still remains method of choice to determine per cent antioxidant activity of polyherbal formulations and extracts (Sethumathi *et al.*, 2021; Baskaran and Subash, 2021).

At equal concentrations, AK, SK and VG (10 mg/5 ml) have shown DRSA in order AK > SK > VG. At equal concentrations AK, SK and VG (1 mg/ml) have shown UVBA in order AK > SK > VG. AK has shown highest DRSA and highest UVBA, but it was found to contain aloe anthraquinones which are undesirable in topical formulation. SK (10 mg/5 ml) has shown 25.71% DRSA. At higher concentrations 20 mg per 5 ml and 30 mg per 5 ml, SK has shown 46.02% and 65.19% DRSA. 1 mg/ml solution of SK has shown 20.28% blocking of UVA and 26.04% blocking at of UVB. At higher concentrations (when 20 mg/ml and 30 mg/ml SK was used to prepare sample solutions), SK has shown more than 97% UVBA. It is known that sun protection factor 30 correlates with 96.7% UVB absorption (Brummitte *et al.*, 2012). DRSA and UVBA of VG were lowest. VG (10 mg/5 ml) has shown 9.94% DRSA. VG (1 mg/ml) has shown 4.28% blocking of UVA and 10.34% blocking of UVB. Phenols were found absent in VG, it was found to contain polysaccharide and protein. Taukoorah and Mahomoodally (2016) had reported lower per cent activity of aloe gel by using DPPH method, Kumar *et al.*

(2008) had reported lower UV opacity with lyophilized crude aloe gel than methanolic extract. In our study, aloe material 'VG' that contain polysaccharide without phenols have shown lower DRSA and UVBA than aloe material SK which was found to contain polysaccharide and phenols. Aloe anthraquinone were found absent in SK, this is an advantage of SK over AK. AK is not suitable to use as an ingredient in topical formulation but AK is suitable to use in unani and ayurvedic oral formulation intended to treat constipation and digestion problems where purgative action due to aloe antharquinone is required.

## 5. Conclusion

Out of the studied, aloe materials AK, SK and VG, *Suryatapi kanyasara* (SK) prepared by drying aloe leaf juice without yellow sap under sunlight, was found as suitable to use as an ingredient in topical antioxidant UV protective formulation. *Suryatapi kanyasara* (SK) was found to contain more than one constituent from aloe leaf, hence it is a mixture.

## Acknowledgements

We are thankful to Dr. Satish N. Malode and Department of Botany, Government Vidarbha Institute of Science and Humanities, Amravati, for verification and authentication of voucher specimen of aloe submitted by us.

## Conflict of interest

The authors declare no conflicts of interest relevant to this article.

## References

**Almeida, M.R. (2009).** Flora of Maharashtra State, Vol. V, Thomas Paul Almeida Shreeji Enterprises, Mumbai, India, pp.:161

**Baskaran, N. and Subash, A. (2021).** *In vitro* DPPH and AChE inhibitory activity of hydroethanolic extract from *Camellia sinensis*. Ann. Phytomed., **10**(2):208-212.

**Brummitte, D.W.; Summer, M. and Frank, A. (2012).** Comprehensive review of ultraviolet radiation and the current status on sunscreens. J. Clin. Aesth.Dermatol., **5**(9):18-23.

**Chunekar, K.C. and Pandey, G.S. (2004).** Bhavaprakasa Nighantu. Chaukhamba Bharati Academy, Varanasi, India, pp:420.

**(1996).** Flora of Maharashtra State Monocotyledons, Botanical Survey of India, Calcutta, India, pp.:115

**Foti, M.C. (2015).**The use and abuse of the dpph radical. J. Agric. Food Chem., **63**(40):8765-8776.

**(2007).** Indian Pharmacopoeia (Vol. II), Indian Pharmacopoiea Commission, Ghaziabad, India, pp:77.

**Khan, A.A. (2020).** Role of unani medicine in empowering national healthcare. Ann. Phytomed., **9**(2):1-5.

**Kirtikar, K.R. and Basu, B.D. (2007).**Indian medicinal plants (Vol. IV). International Book Distributors, Dehradun, India, pp.:2504.

**Kumar, M.S.; Datta, P.K. and Gupta, S.D. (2009).** *In vitro* evaluation of UV opacity potential of *Aloe vera* L. gel from different germplasm. J. Nat. Med., **63**:195-199.

**Mishra, S. (2006).** Abhinav Bheshajya kalpana - vigyan. Chaukhamba Surabharati Prakashan, Varanasi, India, pp:163.

**Naik, V.N. (1998).** Flora of Marathwada (Vol. II). Amrut Prakashan, Aurangabad, India, pp:858.

**Okayama, Y. (2005).** Oxidative stress in allergic and inflammatory skin diseases. Current Drug Targets-Inflammation and Allergy, **4**(4):517-519.

**Reynolds, T. (2004).**Aloe chemistry. In Aloes: The genus Aloe (ed. Reynolds, T.). CRC Press, New York, U.S.A., pp:53,191.

**Sekeroglu, N. (2019).** Some customary edible plants of incredible medicinal worth. Ann. Phytomed., **8**(2):1-6.

**Sethumathi, P.P.; Manjuparkavi, K.; Lalitha, V.; Sivakumar, T.; Menaka, M.; Jayanthi, A. and Kumar, B.A. (2021).** Evaluation of *in vitro* antioxidant and antimicrobial activity of polyherbal formulation of thirikaduguchooranam and parangipattaichooranam. Ann. Phytomed., **10**(2):169-174.

**Sheldon, R.P. (2003).** Cutaneous photodamage, oxidative stress and topical antioxidant protection. J. Am. Acad. Dermatol.,48:1-19.

**Springsteen, A.; Yurek, R.; Frazier, M. and Carr, K.F. (1999).** *In vitro* measurement of sun protection factor of sunscreens by diffuse transmittance. Analytica Chimica Acta, **380**:155-164.

**Taukoorah, U. and Mahomoodally, M.F. (2016).** Crude *Aloe vera* gel shows antioxidant propensities and inhibits pancreatic lipase and glucose movement *in vitro*. Adv. Pharmacol. Sci., 2016:3720850.

**Vaidya, D.; Pandit, A.;Sharma, A.; Kaushal, M.; Saini, H.; Anand, A.; Sharma, R. and Gupta, A. (2021).** Morphological, functional characterization and evaluation of biological value of microencapsulated *Aloe vera* (L.)Burm.f. Ann. Phytomed., **10**(2):137-144.

**Xing, J.M. and Li, F.F. (2009).**Purification of aloe polysaccharides by using aqueous two-phase extraction with desalination. Nat. Prod. Res., **23**(15):1424-1430.

**Citation** **Vishal Prakashrao Nalamwar and Somashekhar Sanganabasappa Khadabadi (2022). Study on aloe processed by traditional and modern methods to use as an antioxidant and UV protective ingredient. Ann. Phytomed., 11(2):516-524. http://dx.doi.org/10.54085/ap.2022.11.2.63.**