International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
DOI: 10.22192/ijarbs Coden: IJARQG(USA) Volume 3, Issue 10 - 2016

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2016.03.10.027

# Sun Screen Efficacy of *Punica granatum* (Pomegranate) and *Citrullus colocynthis* (Indrayani) Seed Oils

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## Abstract

With increasing awareness about skin cancer and its relation to UV radiation, there is much focus on sunscreen agents and products. Sunscreens provide protection by absorbing, reflecting and / or scattering the radiation. Sunscreens are rated according to their effectiveness by sun protection factor (SPF). The present study evaluates ultraviolet (UV) absorption ability of *Citrullus colocynthis* (Bitter Apple, Indrayani) seed oil, *Punica granatum* (Pomegranate) seed oil and few other vegetable fixed oils. The *in-vitro* SPF is determined according to the spectrophotometric method of Mansur *et al*. The UV absorbances of alcoholic solutions of the oils were studied by UV spectrophotometer in the range of 290 nm to 320 nm at 5 nm increments. The *in-vitro* test results show that Pomegranate seed oil shows an SPF at par with synthetic sunscreens at 1.0 % level, with Indrayani seed oil following closely.

**Keywords:** Sun protection Factor (SPF), Indrayani (*Citrullus colocynthis*) seed oil and Pomegranate (*Punica granatum*) seed oil.

## Introduction

Sun is the source of energy. It is vital for life. Exposure to UV light has number of different effects on the human body. UV radiation is absorbed by photoreceptors in the skin, after which substances that suppress cell-mediated immunity may be released. As a consequence, both local and systemic immune functions may be modulated. Recent evidence suggests a beneficial effect of UV light on the severity of some autoimmune diseases, such as multiple sclerosis (David S, Strayer, 2015). According to its physiological activity, UV radiation is subdivided into UV A (400 nm to 320 nm), UV B (320 nm to 280 nm) and UV-C (280 nm to 100 nm) (De Polo,1998). UV C, the high energetic electromagnetic radiation, lethal to all living organism, is absorbed by the ozone layer located in the stratosphere. It also blocks most, but not all, of the UV B rays. With depleting ozone layer, protection from UV B rays is gaining great importance (Shantanu Kale et al., 2010).

The UV A and UV B radiations reaching earth's crust are of lower energy. UV A radiation's photo-biological effects are cumulative (long term) effects. UV A radiation penetrates deep into the dermis and beyond, i.e. 20 % to 30 % reaches the dermis. UV A has been shown to cause cell damage (Wondrak et al., 2003, Scharffetter et al., 1997), connective tissue damage (Lorraine et al., 1985) and induce premature ageing. UV B radiation is energy rich and produces intense short range and long range patho-physiological photo-damage to skin. About 70 % is reflected by the horny layer, 20 % penetrates into deeper layers of the epidermis and 10 % reaches the dermis (De Polo, 1998). The short terms effects of UVB radiation are immediate tanning or immediate pigment darkening (IPD) (Rainer et al., 2009) and solar erythema (sun burn). The sun burning effective energy curve has a maximum at about 305 nm. UV B sunscreen agents must possess an absorption maximum at or near this wave length (Documentation of Solar Light, Sayre R.M, 1992). Exposure to UV radiation on the skin results in mutagenic effects (Ouhtit et al., 1998). It can damage DNA and is a leading cause of skin cancer (David S, Strayer, 2015).

Photo-protection is therefore focused on protecting the skin from the damage that occurs due to UV exposure. Increasing awareness of the damaging effects of sunlight has led to increased need for adequate photo-protection. Primary prevention includes a regimen consisting of effective sunscreen and protective clothing. Clothing is the best way to get protection from these UV A and UV B rays. However, depending on circumstances, sunscreen products need to be used. Sunscreens work primarily through two mechanisms: (i) absorption and (ii) scattering and reflection of UV energy. Many current sunscreen products contain sunscreen agents that work through both mechanisms in terms of UV protection. Chemical Sunscreens and Physical Sunscreens are the two types of sunscreens used in Sunscreen products (Brummitte et al., 2012). Physical sunscreens, the barrier substances, are particles that mainly scatter and reflect UV energy back into the environment. Only fifteen chemical sunscreens are approved by US FDA for use in Sunscreen products, with use level restrictions (Steven Q Wang and Henry W, 2011).

Since no single agent effectively provides adequate protection from both UVA and UVB radiation, nearly all commercially available sunscreen products contain agents from both groups. Two or more sunscreen active ingredients may be combined with each other in a single product when used in the concentrations approved by the US FDA for each agent. Each individual active ingredient must contribute a minimum SPF of at least 2 to the finished product. The finished product should have a maximum SPF of not less than the number of sunscreen active ingredients used in the combination multiplied by two (Nathalie and Darell, 2006). Exposure to UV radiation stimulates endogenous production of vitamin D in the skin. UV wavelengths between 270 nm and 300 nm, the UV B region, result in production of a precursor for vitamin D from a cholesterol derivative (David S, Strayer, 2015).

The efficacy of a sunscreen agent or product is expressed by Sun Protection Factor (SPF) for UV B. SPF is defined as the UV energy required to produce a Minimal Erythemal Dose (MED) in protected skin divided by the UV energy required to produce the same MED in unprotected skin (Nadim A, 2005).

$$SPF = \frac{\text{Minimal erythema dose in sunscreen-protected skin}}{\text{Minimal erythema dose in non-sunscreen-protected skin}}$$

MED is defined as the lowest time interval or dosage of UV light irradiation sufficient to produce minimal perceptible erythema (redness of skin) on unprotected skin. The higher the SPF, the more effective is the product in preventing sun burn.

There are various methods available for the determination of SPF. It can be determined by *in-vivo* or *in-vitro* and it is ideally determined by photo-testing on human volunteers. Both the *in-vitro* and *in-vivo* methods are approved by US FDA and COLIPA (Allen MW , 2007). Though photo-testing on humans, an *in-vivo* method, is useful and precise, it is time consuming, complex and expensive. So, effort has been devoted to the development of *in-vitro* techniques.

Mansur et al., 1986 developed a very simple mathematical equation which substitutes the *in- vitro* method proposed by Sayre et al., 1979 utilizing UV spectrometry and the following equation:

$$SPF_{spectrophootometric} = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

Where

EE – Erythemal Effect spectrum
I – Solar Intensity spectrum
Abs – Absorbance of sunscreen product
CF – Correction Factor
The value of EE x I are constants and were determined by Sayre *et al* and the normalized values are presented in Table 1 (Sayre et al., 1979 )

**Table 1 –** Normalized product function used in calculation of SPF

| Wavelength [a] nm | EE x I (normalized) |
|---|---|
| 290 | 0.0150 |
| 295 | 0.0817 |
| 300 | 0.2874 |
| 305 | 0.3274 |
| 310 | 0.1864 |
| 315 | 0.0839 |
| 320 | 0.0180 |
| TOTAL | 1 |

EE – Erythemal Effect Spectrum; I – Solar Intensity Spectrum

Mansur equation has been used for the determination of SPF of various sunscreen agents, synthetic molecules, natural extracts, oils, emulsions, etc. For example, SPF value of *Usnea rocellina* (Jose et al., 2015) *Musa accuminata*, *Psidium guaja* & *Pyrus communis* (S. Imam and Mahmood , 2015) *Zingiber officinale* (Manoj ,2014) , *Murraya koenigii* (Rekha et al., 2010) *Psidium guaja* (Vandana et al., 2016) *Portulaca* oleracia (Vandana et al., 2015) Opuntia ficus-indica (Cinthya et al, 2014) Corn cob (Zea mays et al., 2013), Garcinia mangostana (Liandhajani et al., 2013), Calendula officinalis (Mishra et al., 2012) were evaluated using Mansur equation.

Jyostna A S Suryavanshi , 2016 published the SPF value of few herbal oils using Mansur equation. However, the concentration of the herbal oils tested was not mentioned. Chanchal Deep Kaur and Swarnalata Saraf (Chanchal and Swarnlata, 2010) determined the SPF of Olive, Coconut, Castor, Almond, Mustard, Sesame and a list of essential oils in aqueous ethanolic solutions. In this, due to the presence of water, the aqueous alcoholic solution of the fixed oils clarity could not be obtained. This restricts the free evaluation of various concentrations. They have not tested in pure ethanolic solution.

The UV absorbance of compounds with conjugated and extended conjugated double bonds could be predicted by Woodward Fieser rule. The wavelength will have bathochromic or hypsochromic shift, depending on the extension of conjugation, presence of electron donating and withdrawing groups. Woodward–Fieser rule is empirically derived, which helps to predict the absorption maxima ($\lambda_{max}$) in an ultraviolet–visible spectrum of a given compound (Valdas and Gerald, 2015).

On the basis of Structure Activity Relationship, dienes and conjugated dienes should absorb UV radiation. 1,3 - Butadiene and 1,3 – Hexadiene are absorbing UV radiation and also as predicted by Woodward–Fieser rule (Wagemaker et al., 2011).

| | |
|---|---|
| CH2=CH-CH=CH2 | CH2=CH-CH=CH-CH2-CH3 |
| 1,3-Butadiene | 1,3-Hexadiene |
| λmax 290 nm | λmax 350 nm |

As per Woodward–Fieser rule, and based on Structure Activity Relationship, conjugated fatty acids present in the triglyceride form also should exhibit absorbance in the UV range (Wagemaker et al., 2011). If they possess UV radiation capabilities, they will be of use in sunscreen preparations.

We identified Indrayani (Bitter Apple, *Citrullus colocynthis*) seed oil and Pomegranate (*Punica granatum*) seed oil as having potential for high SPF value, based on their fatty acid profile.

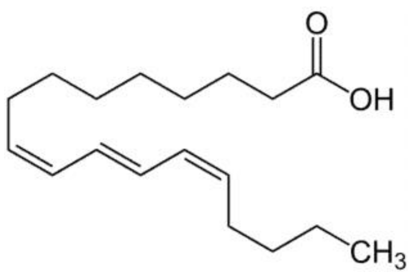
*Citrullus colocynthus* (of the family *Cucurbitaceae*), also known as bitter apple, Indrayani, is a fruit-bearing plant. It is traditionally known both as a medicinal and toxic plant (Jouad , 2001). In Folklore, it is used for the treatment of rheumatism, stimulating the immune system, tuberculosis, diabetes, and analgesic (Daoudi , 2013). Oral ingestion of the fruit prescribed for diabetes (Huseini , 2009). Petroleum ether extract of the fruits of C*itrullus colocynthis* shown to induce hair follicle density (Dhanotia , 2011).

*Punica granatum L.*, commonly known as Pomegranate, belongs to Punicaceae family, is one of the most ancient edible fruits, widely grown in India and Mediterranean regions. Traditionally it has been esteemed as a food and medicine in India (Nadkarni, 2007). Pomegranate, known for its high antioxidant potential, has been used for medicinal properties for centuries (Syed et al., 2007).

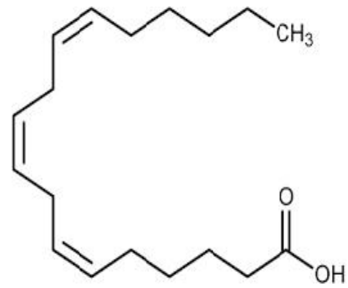
Pomegranate seed oil (PSO) represents between 12 to 20 % of the seed. The seed oil is a triglyceride. The predominant fatty acid is Punicic Acid (Mukherjee and Bhattacharyya , 2002) a conjugated fatty acid with three double bonds. PSO has been reported to exhibit *in-vivo* antioxidant and anti-inflammatory activities, rheumatoid arthritis and coronary heart disease (Boussetta et al., 2009).

These two oils are fixed oils, meaning triglycerides, along with unsaponifiables in minor quantity, like any other vegetable derived fixed oil.

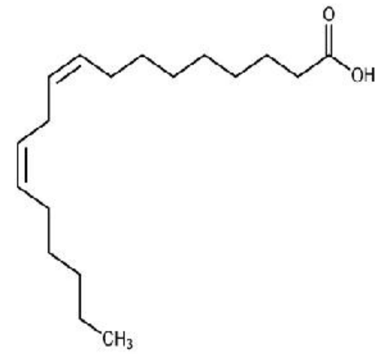
**Figure 1:** The structure of Punicic acid is given below along with Linolenic and Linoleic acids



Punicic Acid



Linolenic Acid



Linoleic Acid

Triglycerides are glycerol molecule esterified with three molecules of fatty acids. The fatty acids present in the triglyceride vary from oil to oil. They may be saturated, monounsaturated or polyunsaturated, with their chain length normally varying from C4 to C22, unsaturation usually vary from 1 to 6. In the polyunsaturated molecules, the double bonds may be conjugated or unconjugated. The conjugation is responsible for the UV absorption property. This conjugation allows the molecule to absorb high-energy ultraviolet rays and release the energy as lower-energy rays (Tentative Final Monograph, 2009).

*Punica granatum* seed oil and *Citrullus colocynthis* seed oil, along with few more vegetable oils, were screened for UV absorbance. For comparison, three US FDA approved synthetic sunscreens (Benzophenone 3, Octocrylene and Ethylhexyl Salicylate) were also included in the study.

## Materials and Methods

**Reagents:** Ethanol (Merck) Analytical Grade

**Apparatus:** UV Double Beam Spectrophotometer, equipped with quartz cell.

**Sample Preparation / Sourcing:**

**Sourcing:**

Indrayani (*Citrullus colocynthis*) seed were obtained from ASP Herbals, (Parrys, Chennai, Tamil Nadu, India).

Pomegranate (*Punica granatum*) Seed Oil was obtained from Cyrus Enterprises, (Mugappair East, Chennai, Tamil Nadu, India).

All the other vegetable oils were bought from the market:

(1) Sesame Oil (Kaleesuwari Refinery)
(2) Coconut Oil (Eldia Pure™)
(3) Sunflower Oil (Gold Winner™)
(4) Extra Virgin Olive Oil (Cardia™)
(5) Refined Olive Oil (Kaleesuwari Refinery)
(6) Groundnut Oil (Gold Winner™)
(7) Canola Oil (Borges™)
(8) Corn Oil (Kaleesuwari Refinery)
(9) Avocado Oil (free from Textron)
(10) Sweet Almond Oil (Dabur Badam Ki Tail)

Neo Heliopan BB® (Benzophenone 3), Neo Heliopan 303® (Octocrylene) and Neo Heliopan OS® (Ethylhexyl Salicylate) were received free from M/s Symrise.

**Extraction of Oil:** The dried seeds were powdered, filled in thimbles and extracted with Diethyl ether as a solvent using soxhlet. The filtered extracts were dried over anhydrous sodium sulphate. The dried extracts were distilled off using thin film evaporator. The final traces of solvents were removed using dry nitrogen. They were kept at -15° C in hermetically sealed amber coloured culture tubes.

### UV Absorbance:

1.0 g of all samples was weighed, transferred to a 100 ml volumetric flask, made up to 100 ml using ethanol, followed by mixing in sonicator for 8 minutes. The clear solution was further diluted to 0.1 %, 0.5 % and 0.05 % solutions in volumetric flasks. The aliquots absorbance was measured between 290 nm and 320 nm at 5 nm increments. The obtained values were multiplied with the respective EE (λ) values. All experimental absorbance values were collected in triplicate and the average values were taken for SPF calculation.

## Results and Discussion

All the oils were tested for their physiochemical properties and fatty acid profile. The fatty acid profile was determined by converting the triglycerides to their corresponding FAME (fatty acid methyl ester) and was analyzed by Gas Chromatography. The data is presented in Table 2. The *in vitro* test results (Table :3) show that Pomegranate Seed Oil shows an SPF equivalent to Synthetic Sunscreens at 1.0 % level, with Indrayani Seed Oil following closely. Their results were good even at 0.50 % level. Corn, Canola, Extra Virgin Olive, Refined Olive, Ground Nut and Sesame oils, while having respectable SPF at 1.0 % level, they fail at 0.50 % level (Figure 2).

**Figure 2:** Bar Graph of SPF of vegetable oils

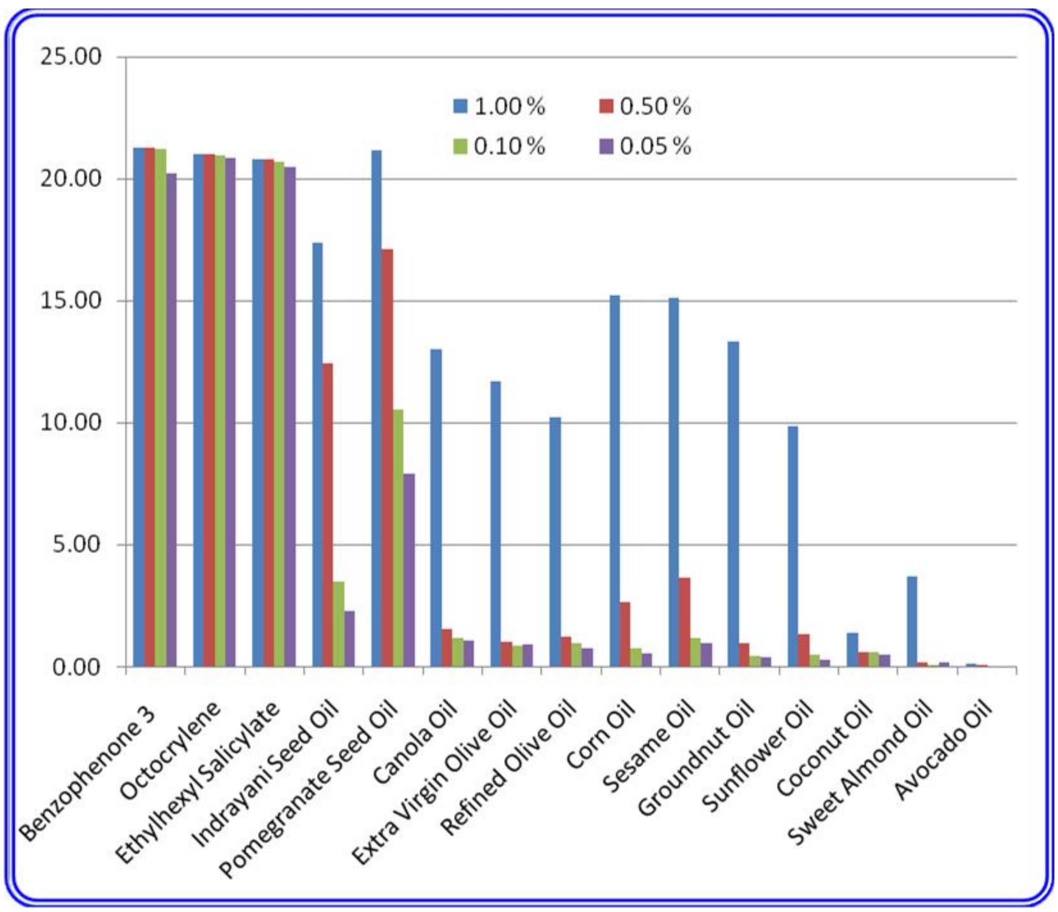


**Table 2 -** Physicochemical and Fatty acid profile of vegetable oil

| | PSO | CCSO | RBO | Coconut | Sunflower | EVOO | Groundnut | Canola | Corn | ROO | SSO |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Physiochemical Properties** | | | | | | | | | | | |
| Colour (Lovibond, Y+5R) | 12 | 31 | 15 | 2 | 8 | 35 | 8.5 | 2.5 | 5.0 | 3 | 11.5 |
| Acid Value (mg KOH / g) | 3.867 | 6.36 | 0.2 | 3.53 | 0.2 | 0.46 | 0.08 | 4.22 | 0.1 | 0.2 | 6.3 |
| Saponification Value (mg KOH / g) | 193.23 | 201.23 | 192 | 251.69 | 190.8 | 190.16 | 192.16 | 190.75 | 191.08 | 190 | 190.83 |
| Free Fatty Acid | 3.60 | 4.75 | 0.10 | 1.26 | 0.10 | 0.23 | 0.04 | 0.03 | 0.05 | 0.1 | 3.15 |
| Iodine Value (g Iodine / 100 g) | 160 | 118.64 | 97 | 8.59 | 128 | 81.98 | 92.13 | 87.2 | 114.65 | 83 | 107 |
| Peroxide Value (meq oxygen / Kg) | 3.60 | 13.3 | 1.00 | 1.26 | 0.65 | 6.26 | 2.14 | 4.50 | 0.89 | 1.25 | 16.2 |
| Specific Gravity at 20° C | 0.940 | 0.9168(30° C) | 0.915 (20° C) | 0.9115 (30° C) | 0.918 (20° C) | 0.912 (20° C) | 0.916(20° C) | 0.910(20° C) | 0.912(20° C) | 0.910 | 0.915 (20 C) |
| Refractive Index at 20° C | 1.5160 | 1.4650(40° C) | 1.4608 (40° C) | 1.4610 (40° C) | 1.4670 (40° C) | 1.4606 | 1.4626 | 1.4621(40° C) | 1.4652(40° C) | 1.4679(40° C) | 1.4656(40° C) |
| Moisture | negligible | 0.01 | 0.01 | 0.02 | 0.01 | 0.04 | 0.01 | 0.001 | 0.12 | 0.01 | 0.06 |
| **Fatty Acid profile by Gas Chromatograph** | | | | | | | | | | | |
| Caproic Acid (C6:0) | NIL | NIL | NIL | 0.694 | NIL | NIL | NIL | NIL | NIL | NIL | NIL |
| Caprylic Acid (C8:0) | NIL | NIL | NIL | 7.986 | NIL | NIL | NIL | NIL | NIL | NIL | NIL |
| Capric Acid (C10:0) | NIL | NIL | NIL | 5.424 | NIL | NIL | NIL | NIL | NIL | NIL | NIL |
| Lauric Acid (C12:0) | NIL | NIL | NIL | 46.519 | NIL | NIL | NIL | NIL | NIL | NIL | NIL |
| Myristic Acid (C14:0) | 0.010 | | 0.319 | 19.752 | 0.048 | 0.011 | 0.056 | 0.013 | 0.040 | 0.009 | 0.015 |
| Palmitic Acid (C16:0) | 2.708 | 11.723 | 19.991 | 8.417 | 5.730 | 10.724 | 13.326 | 4.421 | 13.574 | 10.150 | 9.353 |
| Stearic Acid (C18:0) | 2.282 | 7.823 | 1.941 | 2.728 | 3.703 | 3.994 | 4.149 | 1.52 | 2.135 | 3.587 | 6.511 |
| Oleic Acid (C18:1) | 5.739 | 20.538 | 41.363 | 6.607 | 27.873 | 76.660 | 41.033 | 60.97 | 0.207 | 78.463 | 43.198 |
| Linoleic Acid (C18:2) | 5.808 | 58.884 | 30.834 | 1.680 | 60.890 | 5.924 | 32.784 | 19.557 | 47.334 | 5.153 | 39.359 |
| Linolenic Acid (C18:3) | 0.009 | 0.080 | 0.666 | 0.12 | 0.033 | 0.628 | 0.097 | 9.532 | 0.807 | 0.420 | 0.258 |
| Arachidic Acid (C20:0) | 0.433 | 0.380 | 0.843 | 0.077 | 0.308 | 0.425 | 4.802 | 0.592 | 0.622 | 0.506 | 0.700 |
| Gadoleic Acid (C20:1) | 0.486 | NIL | 0.521 | 0.042 | 0.152 | 0.237 | 0.909 | 1.442 | 0.355 | 0.235 | 0.184 |
| Punicic Acid (C18:3, conjugated) | 81.879 | NIL | NIL | NIL | NIL | NIL | NIL | NIL | NIL | NIL | NIL |
| Eicosenoic acid (C20:1) | | NIL | NIL | NIL | NIL | NIL | NIL | NIL | NIL | NIL | NIL |
| Behenic Acid (C22:0) | NIL | 0.136 | 0.284 | 0.015 | 0.817 | 0.111 | 3.949 | 0.347 | 0.178 | 0.113 | 0.152 |
| Erucic Acid (C22:1) | NIL | NIL | NIL | NIL | NIL | NIL | 0.084 | 0.715 | NIL | NIL | NIL |
| Lignoceric Acid (C24:0) | 0.066 | 0.138 | 0.496 | 0.034 | 0.300 | 0.051 | 1.471 | 0.11 | 0.245 | 0.049 | 0.108 |

PSO – Cold Pressed Pomegranate Seed Oil, CCSO – Bitter Apple Seed Oil, RBO – Refined Rice Bran Oil (Orysa), CNO - Coconut Oi (Eldia Pure), SFO –Sunflower Oil (Gold Winner), EVOO – Extra Virgin Olive Oil (Cardia), GNO – Groundnut Oil (Gold Winner), Canola – Canola Oil (Borges), Corn – Corn Oil, ROO – Refined Olive Oil (Cardia), SSO – Sesame Oil

**Table 3 -** SPF of Vegetable Oils determined using Mansoor's equation

**SPF as determined using Mansoor Equation**

| Ethanolic solution (in %) | 1.00 % | 0.50 % | 0.10 % | 0.05 % |
|---|---|---|---|---|
| Benzophenone 3 | 21.28 | 21.27 | 21.23 | 20.23 |
| Octocrylene | 21.00 | 20.98 | 20.94 | 20.87 |
| Ethylhexyl Salicylate | 20.79 | 20.79 | 20.70 | 20.48 |
| Indrayani Seed Oil | 17.37 | 12.43 | 3.49 | 2.28 |
| Pomegranate Seed Oil | 21.16 | 17.08 | 10.54 | 7.93 |
| Canola Oil | 13.02 | 1.51 | 1.19 | 1.08 |
| Extra Virgin Olive Oil | 11.71 | 0.99 | 0.90 | 0.91 |
| Refined Olive Oil | 10.21 | 1.23 | 0.97 | 0.76 |
| Corn Oil | 15.22 | 2.61 | 0.77 | 0.53 |
| Sesame Oil | 15.12 | 3.65 | 1.19 | 0.95 |
| Groundnut Oil | 13.30 | 0.93 | 0.48 | 0.39 |
| Sunflower Oil | 9.82 | 1.31 | 0.52 | 0.28 |
| Coconut Oil | 1.36 | 0.60 | 0.62 | 0.48 |
| Sweet Almond Oil | 3.70 | 0.14 | 0.08 | 0.17 |
| Avocado Oil | 0.08 | 0.07 | 0.04 | 0.03 |

Following the numbering from the methyl end, the double bonds are at carbon atoms 5, 7 and 9 positions (Figure 1). As per configuration, Punicic Acid has 5-cis, 7-trans and 9-cis conformation. It has a 1,3,5-hexatriene backbone with two alkyl groups at the two ends (i.e. at C5 and C10). As per Woodward Fisher rule, UV absorbance maxima of 247 nm (base wave length) + (2 x 5nm) [for the two alkyl groups] = 257 nm could be predicted for this molecule.

The result of Pomegranate seed oil obtained is as expected and also as calculated by Woodward Fieser rule. With respect to Indrayani seed oil, the results were more than expected. Indrayani Seed contains neither any conjugated fatty acids nor any molecules in the unsaponifiable part bearing chromophoric group possessing UV absorbance properties. More research will be continued with Indrayani seed oil to find out the reason for its high UV absorbance properties. All the other oils, since comprised of mainly of saturated (CNO), or monounsaturated (olive oils) or polyunsaturated (without any conjugation, Sunflower, Canola, Corn, Ground nut, Sesame oils) fatty acids, are not expected to show any reasonable UV absorbance. So, they are not expected to display good SPF values too. As per US FDA, any sunscreen agent used in sunscreen products should display a minimum of SPF 2 (Nathalie and Darell , 2006). Even at 0.05 %, Pomegranate seed oil shows an in vitro SPF of 8 and Indrayani seed oil SPF of 2.3, they shall be evaluated in sunscreen preparations, intended for skin and hair care. In addition to their sun protection properties, being triglycerides, they also act as good emollients / spreading agents for other sunscreens.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Acknowledgement:** The authors are thankful to the Directors - Mr. Arun, M., and Mr. Ashok, K., and to Chief Operating Officer Mr. Anurag Lodha of Kaleesuwari Refinery Pvt. Ltd. for providing constant inspiration for this work.

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**DOI:**10.22192/ijarbs.2016.03.10.027

**How to cite this article:**
Janaky Ranjithkumar, Akhila Sameesh, Hari Ramakrishnan, K. (2016). Sun Screen Efficacy of *Punica granatum* (Pomegranate) and *Citrullus colocynthis* (Indrayani) Seed Oils. Int. J. Adv. Res. Biol. Sci. 3(10): 198-206.
**DOI:** http://dx.doi.org/10.22192/ijarbs.2016.03.10.027