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IN VITRO SPF DETERMINATION OF *EMBILICA OFFICINALIS* AND *NELUMBU NUCIFERA* SEED OILS

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ABSTRACT: Medicinal plants are natural gift to human lives to promote disease free healthy life. The phytochemicals present in leaf, fruits and vegetables could reduce various risks of diseases owing to prevent the oxidative damage produced by free radicals. The plant seed also contain many active molecules responsible for various medicinal properties. *N.nucifera*, *E,officinalis*, *M. oleifera*, *T.chebula*, *T.bellerica* and flax seed oils are tested for SPF determination by in vitro method. The seed oils had shown very good SPF values. *E.officinalis* seed showed 25.77 as Sun Protection Factor (SPF) value and *N.nucifera* seed Cotyledon had 21.45 SPF values which was quite high when comparing other seed oils. This study supports the usage of seed oil as a UVB sun screen protector, an alternative source of nutrition and as well as renewable resources.

Key words: UV rays, Sunscreen, Seed Oils, *E.officinalis*, *N.nucifera*

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INTRODUCTION

Sun is the vital energy source for all living forms on earth. This energy of sunlight supports almost all life on earth by photosynthesis and drives earth's climate and weather (Afaq[a], 2002). Eventhough the light from sun is the source of survival energy; it has its own negative impacts upon overexposure (Afaq[b], 2002). Ultraviolet (UV) radiation is a type of radiation that is produced by the sun and some artificial sources, such as solariums. The sun's UV radiation is the major cause of sunburn, premature ageing, eye damage and skin damage leading to skin cancer (John D, 2013). UV radiation is divided into three distinct bands in order of decreasing wavelength and increasing energy: UVA (320-400 nm), UVB (290-320 nm), and UVC (200-290 nm). Different wavelengths and energy associated with UV subdivision correspond to distinctly different effects on living tissue (Tuchina, 2006). UVA is considered as harmless for many years as it cannot induce dimmers but actually it is a tumor promoter and is one of the main causative factors for wrinkle formation and developing signs of aging. High exposure of UVB is potential carcinogen (Nadim, 2005) and more efficient in inducing erythema (Moyal, 2004).

Skin is the outermost organ of the body which is affected by UV radiations. Among the UV radiations, UVB radiation is the most active constituent of solar light. It acts mainly in the epidermal basal cell layer of the skin (Katiyar,2001 : Mittal, 2000). The UVB radiation imparts its ill effects to the skin through activation of inflammatory signals (Villalobos, 2006). Skin care products for UV protection should be used to reduce harmful effects of UV radiation and/or suppress them totally.

Therefore, UV protection becomes a major function of more types of cosmetic formulations. Hence, the finding of the active molecules that protects the skin from UV radiations becomes the need for the hour. The sunscreens protect the sun burn in two ways. They are chemical and physical sunscreens. A chemical sunscreen absorbs the UV rays, while the physical sunscreen reflects and scatters the rays like a temporary coat of armor (Freund, 2010).

The concept of complementary or alternative medicine is becoming popular and widely accepted. There is an increasing interest in remedies interns of herbal medicines. Recently, the role of herbal drugs, herbal products and certain phytochemicals in the control of ageing has been shown (Kapoor.2009). Traditionally life threatening diseases have been treated with herbal medicines which are taken as food not as medicine or drug. In the same way, the following study is conducted in plant seed oils for their activity on sun protection factor.

Embilica Officinalis (amla), one of the most common medicinal herbs has been widely used in ayurvedic medicines (Zhang, 2000). Numerous experimental evidences have shown that amla fruit possess antioxidant (Bhattacharya, 1999), hepatoprotective (Jeena, 1999), hypocholesterolemic (Mishra, 1981) and anti-inflammatory activities (Asmawi, 1993). The study of Arul *et al* (2011) showed that *Embilica officinalis* had good sun protection and anti-inflammatory activity.

Nelumbo nucifera is the common aquatic plant comes under the family Nelumbonaceae, which has various medicinal properties. Because of the high nutritional value of fresh lotus, its consumption in recent years has increased rapidly, showing that lotus is a valuable food (Yen, 2006) that have been used for medicinal properties that include improving learning and memory (Duan, 2013), hepatoprotective (Sohn,2003), anti-obesity, anti-HIV activity, anti-tumor effect (Duan,2010), diuretic activity, antipyretic activity, antidepressant and anti-inflammatory activity (Sugimoto,2010). *N.nucifera* seeds have also been widely used as food and medicine in Asia because they have high nutritional values and much functionality. One particular effect of lotus seeds is their protective effect on skin. Liu *et al* (Liu,2004) reported that ethanol extracts of *Nelumbo nucifera* seeds are effective in the treatment of tissue inflammation and cancer. Huang *et al.*(Huang,2013) reported that lotus leaves extract have protective effects on skin against UVB irradiation.

Moringa oleifera L (Moringaceae) has good sunscreen activity and can be considered as active sunscreen agent. *M. oleifera* is an important food commodity which has enormous attention as the 'natural nutrition of the tropics (Anwar[b], 2007). A number of medicinal properties have been ascribed to various parts of this plant. Most parts of this plant like root, bark, gum, leaf, fruit (pods) flowers, seed and seed oil have been used in folk medicine in Africa and South Asia (Fahey,2005). Furthermore, *M. oleifera* is found to be a potential new source of oil especially with the advent of the need for oleo-chemicals and oils/fats derived fuels (Biodiesel) all over the world (Anwar[a], 2007).

T. chebula (haritaki) and *T.Bellerica* enjoys the prime place among medicinal plants not only in India but also in like Asia and Africa. It is extensively used in ayurveda, siddha, unani and homeopathic medicines in India. *T.chebula* used in Ayurvedic Materia medica for treatment of asthma, bleeding piles, sore throat, vomiting and gout (Aneja,2009). The extract of *T. bellerica* is reported to exhibit a variety of biological activities and pharmacological effects including anti-malarial, antibacterial, anti-HIV, anti-fungal, anti-mutagenic, and antioxidant effects (Aqil,2007). Flaxseed or linseed (Linum usitatissimum L.) has been used as food and medicines in many countries. Flaxseed and flaxseed oil is considered as healthy due to the presence of various bioactive compounds in it (Sunita, 2013). The seed is a healthy source of oil containing poly-unsaturated fatty acids, digestible proteins, and lignans. Major nutritional components of flaxseed include Alpha linolenic acid rich oil, protein, minerals and a greater proportion of non-nutritional lignan-rich dietary fiber (Madhusudan, 2009).

The efficacy of UVB sunscreen is characterized by the sun protection factor (SPF). The SPF is a numerical rating system to indicate the degree of protection provided by a sun care products like sunscreen. SPF is defined as the ratio of the minimal erythema dose (MED) of solar radiation measured in the presence and in the absence of a sunscreen agent (Colipa,2007)]. Many regulatory agencies, such as the US Food and Drug Administration (USFDA) and The European Cosmetic Toiletry and Perfumery Association (COLIPA), mandate in-vivo testing on human subjects, using an erythemal end point to determine the SPF of a topical sunscreen (Mukund,2016)

For the in vitro SPF test, the source of UV energy should be identical to that used in the human SPF test in terms of spectrum, total power, beam uniformity and collimation, and temporal stability. Ideally, the UV source will have a feedback system for regulating output. Accurate and reliable in vitro measurement of sunscreen SPF is difficult due to the large number of variables. These variables can be managed to produce acceptable results. The *in vitro* SPF test should incorporate the best available substrate for matching the properties of skin, and the application technique. UV source and beam geometry should mimic those used in the *in vivo* test[5]. Initially solar light single port solar simulator, used for sunscreen SPF testing, consists of a 150 W ozone-free xenon arc which is filtered with a WG-320 filter of appropriate thickness and generally a UG-11 to remove excess visible.

The other type of solar simulator commonly used for sunscreen SPF testing in North America is the Solar Light multiport. It has six complete set of optics, configured with WG-320 and UG-11 filters, and six waveguides[5]. In-vitro SPF determination by the COLIPA standard and other regulatory agencies involve measurement of the percent transmission of a sunscreen lotion sample across the UV spectrum weighted by the erythemal weighting factors at different wavelengths (Allen, 2007). The *in vitro* SPFs are determined according to the method described (Mansur,1986). The observed absorbance values at 5 nm intervals (290-320 nm) are calculated by using the formula,

$$\text{SPF spectrophotometric} = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

where CF = correction factor , EE (λ) = erythmogenic effect of radiation with wavelength λ, Abs (λ) = spectro photometric absorbance values at wavelength λ. The values of EE × I are constants.

MATERIALS AND METHODS

Reagents: Isopropyl Alcohol (Merck) Analytical Grade

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

Sourcing:

N.nucifera seed, *E.officinalis* seed, *M.oleifera* Seed,*T.chebula* and *T.bellerica* purchased from ASP Herbs ,Chennai and it is cleaned and it's seed coat and cotyledons are separated physically. Flax seed oil is purchased from Prano Flax (India) Pvt, Ltd- Jaipure, Brand name – Health First.

Extraction of Seed oils:

150g of each seed was weighed and hexane was used solvent. The extraction was carried out by Soxhlet apparatus.

Estimation of UV Absorbance:

1.0 g of all samples were weighed, transferred to a 100 ml volumetric flask, made up to 100 ml using isopropyl alcohol, 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 and 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 are taken for SPF Calculation.

Determination of Fatty Acids Pattern by GLC:

The oil was converted into methyl esters via transesterification according to Chrisite (Chrisite, 1973). The identification of the components of fatty acids methyl esters was done using gas liquid chromatography (Trace GC 700) under the following conditions: Capillary column size was about 30.0 m x 530 µm x 1.0 µm. Column temperature was 240° C with temperature programming: Initial temperature 100°C to 240°C maximum with 10°C rising for each minute and then hold at 240°C for ten minutes. Injection temperature was around 280°C with carrier gas as nitrogen (flow rate 15 ml / min). Flame ionization detector temperature was 280°C. Hydrogen flow rates were 30 and 300 ml/ min, respectively.

RESULTS & DISCUSSION

From the obtained results, it showed that *E.officinalis* seed (25.77) and *N.nucifera* seed cotyledon (21.45) have high SPF values when comparing other seed oils. *E.officinalis* has shown high SPF value and it might be attributed to its many active metabolites and it is mentioned as one of the precious gift from nature and natural wonder. The Seeds of *E.officinalis* contain high antibacterial and antioxidant property (Gupta priya, 2012). The presence of phytochemicals like tannins, flavanoids, terpenes, saponins, pregnanes and phenolic compounds are major constituents in *E.officinalis* which may acknowledge the medicinal as well as antioxidant property of this plant (Olukoya, 1993). The major components in *E.officinalis* seed coat oil are 9,12,15 octadecatrienoic acid, tetradecanoic acid and linoleates whereas benzoic acid, 6 tetradecansulfonic acid, hydroquinone, dodecane 1-fluoro, phthalic acid 2- cyclohexyl ethyl iso butyl ester were the minor components (Shodhganga,2002).

N.nucifera seed has high moisture retaining capacity. Moisture content is crucial in maintaining the beauty and health of the skin. The skin with enough amounts of moisture looks smooth and glossy, due to UV radiation the moisture content falls below a threshold, the skin loses its elasticity and glossi ness.

The study of Su-Yeon Kim (2015) revealed that the *N.nucifera* seed tea given group of mice showed significantly higher moisture content with 32.6±6.95%, compared to 22.67± 4.68% of the water-UV group. The yield percentage of each seed oils are given in Table 1.

The study by Moustafa *et al*..,(2015) revealed that they have indentified thirty eight phytochemicals by GC-MS from *N.nucifera* seed which have potential medicinal activities. The most predominant components are lupeol (22.95%), ctadecatrienoic acid-2- [(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy] methyl]ethyl ester (Z,Z,Z) (7.86%), 2,3-dihydroxypropyl-cis-13-docosenoate (5.35%), oleanolic acid(5.19%), tristrimethylsilyl ether derivative of 1,25-dihydroxyvitamin D2 (3.36%), trimethylsilyl derivative of 2-monoolein (3.26%), lucenin 2 (3.02%) and lupanol (2.95%), tetraneurin-A-diol (2.78%), carotene-1,1',2,2'-tetrahydro-1,1'- dimethoxy (2.32%),1-oxo-forskolin (1.99%), oleic acid-3- hydroxypropyl ester (1.78%), oleic acid trimethylsilyl ester (1.69%), betulin (1.61%), à-amyrin-trimethylsilyl ether (1.49%), stigmast-5-en-3-ol-(3á,24S)(1.43%), flavone-4'-OH,5-OH,7-di-Oglucoside (1.11%) (Moustafa, 2015). Embryo of lotus seeds is used in traditional Chinese medicine as Lian Zi Xin, which primarily helps to overcome nervous disorders, insomnia, and cardiovascular diseases (Keshav, 2015). Thus, lotus can be regarded as a potential nutraceutical source.

The most prevailing component in the extract, lupeol (22.95%), is a triterpene, reported to possess several pharmacological activities including anticancer, antiprotozoal, anti-inflammatory, antimicrobial and chemopreventive properties (Gallo, 2009). Theoretically presence of these active compounds might be responsible for effective sun protection (Table 2).Previous study also mentioned that *Moringa oleifera L* (Moringaceae) has good sunscreen activity and can be considered as active sunscreen agent or can be incorporated into other sunscreen formulations as an additive to enhance the activity (Megha Gaikward, 2011). *M.oleifera* seed coat also have comparatively good SPF value, that is 6.21 followed by *T.bellerica* seed 4.6, *M.oleifera* seed cotyledon 4.37, *T.Chebula* 1.68 and flax seed 1.18 (Table: II). The Seed oils are tested for their fatty acid profile. The fatty acid profile is determined by converting the triglycerides to their corresponding FAME (fatty acid methyl ester) and analyzed by Gas Chromatography. The data is presented in Table 3.

Table 1: Yield Percentage of Seed Oils.

Plant	Oil yield (%)
Nelumbo nucifera Seed Cotyledon	1.64±0.1
Embilica officinalis Seed	1.17±0.3
Moringa oleifera Seed coat	1.92±0.11
Moringa oleifera Cotyledon	28±1.0
Terminalia chebula Seed	30.33±0.5
Terminalia bellerica Seed	17.67±0.5

Values represent mean ± SD, n=3

Table 2: Spectrophotometrically calculated sun protection factor values of Seed oils

SPF Values at different Concentration				
Seed Oil	**1%**	**0.50%**	**0.10%**	**0.05%**
Nelumbo nucifera seed (Cotyledon)	21.5±0.4	9.45±0.01	1.09±0.01	0.75±0.01
Embilica officinalis seed	25.76±0.01	12.68±0.01	2.21±0.02	1.20±0.01
Moringa oleifera seed (Outer part)	6.21±0.01	3.18±0.01	0.60±0.01	0.32±0.01
Moringa oleifera seed (Cotyledon)	4.36±0.01	2.12±0.01	0.26±0.01	0.32±0.01
Moringa oleifera seed	1.68±0.06	0.89±0.01	0.15±0.01	0.04±0.01
Terminalia bellerica seed	4.53±0.02	2.04±0.01	0.29±0.01	0.31±0.01
Linum usitatissimum Seed	1.16±0.02	0.68±0.01	0.55±0.01	0.50±0.01

Values represent mean ± SD, n=3

Table 3: Fatty acid composition of seed Oils by Gas Chromatography.

Fatty acids	*Nelumbo nucifera* seed (Cotyledon)	*Embilica officinalis* seed	*Moringa oleifera* Seed (Outer part)	*Moringa oleifera* Seed (Cotyledon)	*Terminalia chebula* seed	*Terminalia bellerica* seed	Linum usitatissimum Seed
Caproic acid (C6:0)	NIL	NIL	NIL	NIL	NIL	NIL	NIL
Caprylic acid (C8:0)	NIL	NIL	NIL	NIL	NIL	NIL	NIL
Capric acid (C10:0)	NIL	NIL	NIL	NIL	NIL	NIL	NIL
Lauric acid (C12:0)	0.047	0.952	0.066	NIL	NIL	NIL	NIL
Myristic acid (C14:0)	0.338	3.526	0.379	0.101	0.047	0.137	0.035
Palmitic acid (C16:0)	14.725	18.534	6.764	7.317	23.420	17.284	5.081
Palmitoleic acid (C16:1)	0.169	0.155	1.248	1.361	0.365	0.139	0.075
Stearic acid (C18:0)	1.399	9.145	4.972	4.977	10.345	5.917	5.880
Oleic acid (C18:1)	11.914	30.881	67.452	73.010	37.976	37.561	20.085
Linoleic acid (C18:2)	51.195	24.899	1.498	0.619	26.365	36.732	13.549
Linolenic acid (C18:3)	5.894	0.165	0.198	0.128	0.073	0.147	0.199
Arachidic acid (C20:0)	2.378	1.222	3.759	3.197	0.831	0.917	54.388
Gadoleic acid (C20:1)	0.459	1.601	2.460	2.390	0.100	0.258	0.259
Behenic acid (C22:0)	9.178	6.966	7.959	5.451	0.208	0.467	0.199
Erucic acid (C22:1)	NIL	NIL	NIL	0.135	NIL	NIL	NIL
Lignoceric acid (C24:0)	2.104	0.993	2.571	1.316	0.090	0.116	0.104
Cerotic acid (C26:0)	0.200	0.962	0.675	NIL	NIL	NIL	0.026

Abbreviation: *N.nucifera - Nelumbo nucifera* , *E,officinalis - Embilica officinalis* , *M. oleifera - Moringa oleifera* , *T.chebula- Moringa oleifera* , *T.bellerica - Terminalia bellerica.*

CONCLUSION

There is a great market potential for sunscreen chemicals either synthetic or natural or in combination due to awareness of environmental pollution thereby protection from hazardous UVA as well as UVB rays. Thus it can be concluded that these herbs could be employed in the preparation of herbal formulations which could prevent the skin from harmful effects of UV radiation. Further study is needed in order to elucidate active metabolite exerting the sun protection efficacy and anti-inflammatory efficacy.

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

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