



International Journal of Allied Medical Sciences and Clinical Research (IJAMSCR)

ISSN:2347-6567

IJAMSCR /Volume 6 / Issue 2 / Apr-Jun - 2018
www.ijamscr.com

Research article

Medical research

Isolation and Characterisation of Lutein from *Commelina benghalensis* L. Leaves and its Sun Protection Factor activity

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ABSTRACT

Back ground

Objective

To isolate the lutein from *Commelinabenghalensis* L. and its sun protection factor determination

Methods

Lutein was isolated by diethyl ether and methanol followed by saponification process. Isolated compound was identified by using UV, NMR and GC-MS spectral analysis. Sun Protection Factor determined by using spectrophotometer. Readings were taken in wavelength ranging from 290 to 320 at 5nm interval and readings were noted down. SPF for isolated lutein can be calculated by applying the formula known as Mansur equation.

Results

The isolated compound shows absorbance maxima at 470, 444 nm it indicate the presence of lutein. UV, NMR, GC-MS studies confirmed the isolated compound is lutein. The SPF number of lutein 50µg/ml is 1.15 ± 0.43 . The SPF number of lutein 100µg/ml is 2.38 ± 0.90 . The SPF number of lutein 200µg/ml is 4.75 ± 1.79 . SPF value for sunscreen above 2 is considered as having good sunscreen activity. Marketed sunscreen lotion having concentration 200 µg/ml shows SPF value about 0.66 ± 0.006 whereas lutein showed high sun protection factor.

Conclusion

Isolated compound lutein possessing good sun screen activity.

Recommendation

Herbal formulations can be prepared and evaluated further with other sensitivity tests

Keywords: *Commelina benghalensis* L., Lutein, Isolation, Sun Protection Factor

INTRODUCTION

Lutein

Carotenoids are fat soluble nutrients and categorized as either xanthophylls or carotenes

according to their chemical structure. They are very important natural antioxidants that have wide application on human health benefits. The pigment properties of carotenoids have granted to some extensive application in the food and feed

industries (Alves-Rodrigues & Shao, 2004). Carotenoids are naturally found in edible leaves, flowers and fruits. Currently attention is being drawn towards exploring plant sources for substances that provide nutritional and pharmaceutical advantages to humans. Green leafy vegetables (GLVs) are good sources of minerals and vitamins and also have health benefits. GLVs are rich sources of carotenoids especially lutein. Lutein a member of the xanthophylls family of carotenoids, decreases the risk for eye diseases such as Age-related Macular Degeneration (Seddon et al., 1994), protects the skin (Alves-Rodrigues & Shao, 2004), reduces cardiovascular problems, aging etc., and is being recommended for human, veterinary and poultry uses.

Lutein and zeaxanthin belong to the xanthophyll family of carotenoids and are the two major components of the macular pigment of the retina. Lutein and zeaxanthin differ from other carotenoids in that they each have two hydroxyl groups, one on each side of the molecule. Zeaxanthin is a stereoisomer of lutein, differing only in the location of a double bond in one of the hydroxyl groups. The hydroxyl groups appear to control the biological function of these two xanthophylls. The macula lutea or “yellow spot” in the retina is responsible for central vision and visual acuity. Lutein and zeaxanthin are the only carotenoids found in both the macula and lens of the human eye, and have dual functions in both tissues – to act as powerful antioxidants and to filter high-energy blue light. Lutein is found in high amounts in human serum. In the diet it is found in highest concentrations in dark green, leafy vegetables (spinach, kale, collard greens, and others), corn, and egg yolks. Zeaxanthin is the major carotenoid found in corn, orange peppers, oranges, and tangerines. In addition to playing pivotal roles in ocular health, lutein and zeaxanthin are important nutrients for the prevention of cardiovascular disease, stroke, and lung cancer. They may also be protective in skin conditions attributed to excessive ultraviolet (UV) light exposure.

SUN PROTECTION FACTOR

Solar ultraviolet radiation (UVR) is divided into three categories UV-C (200-280 nm), UV-B (280-320) and UV-A (320- 400 nm). UV light has been classified by WHO as carcinogenic and produces several adverse effects including mutagenicity, immune depression of the skin, accelerated skin ageing and photo dermatoses. The most biologically damaging radiation UV-C has been filtered out by the ozone layer and it is mainly UV-B that is responsible for causing the adverse effects of the UV radiation (Kaur and Saraf, 2010; Mishra *et al.*, 2011) [14]. Application of sunscreen to the skin changes the way the body reacts to the sun rays (Mishra *et al.*, 2012). Sunscreens and sun blocks are chemicals that absorb or block UV rays and show a variety of immunosuppressive effects of sunlight. There are several agents available from both synthetic and natural sources with UV-filtering properties. Given their potential to produce considerable human local and systemic exposure, UV filters have to be safe (Nohynek *et al.*, 2010). Synthetic UV filters are known to have potential toxicity in humans and also showed ability to interfere only in selected pathways of multistage process of carcinogenesis (Chanchal and Saraf, 2009). In contrast, herbal botanical sunscreens are safe, widely accepted by consumers and also work in various ways, playing multiple roles in ameliorating the process of carcinogenesis.

SPF is a number given to sunscreen formulations to determine its effectiveness and it is also useful when applied about 2mg/cm. SPF numbers indicates the time period for the product up to which it protects the person while stay in the sun before burning. In order to protect the skin against ultraviolet radiation, the formulation should have good SPF number and also the formulation should have wide range of absorbance between 290 and 400nm range. In the present research work, isolated lutein from *Commelinabenghalensis L.* were subjected for SPF evaluation by ultraviolet spectroscopic method. SPF value for sunscreen above 2 is considered as having good sunscreen activity.

The effectiveness of a sunscreen is usually expressed by sun protection factor (SPF) which is

the ratio of UV energy required to produce a minimal erythral dose (MED) in protected skin to unprotected skin. A simple, rapid and reliable in vitro method of calculating the SPF is to screen the absorbance of the product between 290-320 nm at every 5 nm intervals.

The commercially available sun protecting agents are not safe and causes change in coloration of the skin. It is immense and immediate to search of alternate sun protecting preparations from herbal source.

An attempt has been made to study the sun protection potential property of *Commelinabenghalensis* was carried out.

Commelinabenghalensis L. commonly known as **Benghaldayflower**, belongs to the family Commelinaceae. *Commelinabenghalensis* L. is a perennial herb native to tropical Asia and Africa. Valaiyans of Piranmalai hills, Tamilnadu used the leaves for the treatment of rabies and wounds (Sandhya et al., 2006 & Gupta et al., 2010). Bangladesh the kavirajestribals used the young leaves for external poisoning (Mahabub Nawaz AH Md et al., 2009).

The phytochemical screening of previous studies of *Commelinabenghalensis* L revealed the presence of tannins, phlobatannins, saponins, flavonoids, alkaloids, steroids and flavonoids, carbohydrates, phytosterol, terpenoids, quinon, volatile oil, anthraquinone (UdayaPrakash NK et al., 2011, Bodke et al., 2012, Bibin Baby Augustine et al., 2013, PrayagaMurty et al., 2014, KharadeAmit et al., 2013, UdayaPrakash NK et al., 2013, Chichioco-Hernandez et al., 2014, Krishna Satya et al., 2016, Sumithra and Sumithra Purushothaman, 2017). GC-MS analysis of *Commelinabenghalensis* L. revealed the presence of bioactive compounds such as 3-dodecene, 1-hexadecanol, 9-eicosene and tetratriacontane, Phenol 2,4 bis(1,1 dimethyl ethyl), hexadecanol, trans-9, 9,10 anthracenedione, tetracosane, 1,4 benzene-dicarboxylic acid, bis (2ethylhexyl) ester, 13 docosenamide, tetracosane 11 decyl (Sumithra and SumithraPurushothaman, 2017)

The plant exhibited various pharmacological activities such as anti inflammatory activity, 15-lipoxygenase inhibition, anticoagulant activity, antibacterial activity, antimicrobial activity, antiplasmodial activity, thrombolytic and cytotoxic

activity and antidiarrhoeal & anthelmintic activity (Bibin Baby Augustine et al., 2013, Chichioco-Hernandez et al., 2014, Krishna Satya et al., 2016, Sumithra and Sumithra Purushothaman, 2017, Mukesh Chandra Sharma and Smita Sharma, 2010, Gothandam et al., 2010, Rajesh F Udgirkar et al., 2012, Joy Prabu and Johnson, 2015, NjanNloga, Ngo Yebga & Ngo Bum, 2014, AbulHasanat et al., 2015, Mohammad Mamun Ur Rashid, 2016).

MATERIALS AND METHODS

Isolation of Lutein (Vatsala & Rekha, 2013)

50 G of crude powder extracted with Diethyl ether : Methanol (2:1). DE/Met (300 ml) solvents added and incubated for 30 minutes with agitation. Filtered and extraction was repeated upto 600 ml solvents. The crude extract after removal of solvents were re-constituted with known volume of DE/Met (2:1 v/v). An aliquot of crude extract was analyzed on UV-Vis spectrophotometer from 300-700 nm to check the presence of chlorophyll and lutein. Crude extracts partitioned with saturated NaCl 1:1 (v/v) at $(26 \pm 2^\circ\text{C})$ for 5 min followed by overnight saponification with methanolic KOH 10% at $(26 \pm 2^\circ\text{C})$. Saponified mixtures were transferred to separating funnel and equal volume of DE was added for further carotenoid separation from chlorophyll and incubated at $(26 \pm 2^\circ\text{C})$ for 5 min, followed by addition of equal volume of distilled water to the saponified mixture. Shaken vigorously and allowed to stand for 15 – 20 min at $26 \pm 2^\circ\text{C}$. Bottom layer was removed. Top layer washed repeatedly with distilled water for complete removal of alkali. Top layer was analysed by spectrophotometer from 300 – 700 nm. **Determination of Sun Protection Factor** (Manoj A. Suva, 2014)

Materials

Sample Preparation

Lutein isolated from *Commelina benghalensis* L. 50 mg was dissolved in 50ml of ethanol (1000 µg/ml). From this stock solution three different concentration (50, 100, 200 µg/ml) was prepared

Methods

50, 100, 200 µg/ml of isolated lutein were subjected for SPF evaluation by ultraviolet spectroscopic method. Then spectrophotometer readings (Shimadzu 1800 UV-VIS Spectrophotometer) of these solutions were taken in wavelength ranging from 290 to 320 at 5nm interval and readings were noted down. SPF for

isolated lutein can be calculated by applying the following formula known as Mansur equation (Kaur and Saraf, 2010; Mishra *et al.*, 2012):

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

Where CF = correction factor (10), EE (λ) = erythemogenic effect of radiation with wavelength λ, Abs (λ) = Spectrophotometric absorbance values at wavelength λ. The values of EE x λ are constants.

Table 1: Values of EE * I used in the calculation of SPF

Wavelength (λ nm)	EE * I (Normalized)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	1

EE – Erythema effect spectrum , I – solar intensity spectrum

Spectral Analysis of Isolated Compound
(Vatsala&Rekha, 2013 &Omayma A. Eldahshan et al., 2013 &Finar, 1975)

RESULTS

UV Spectral Studies of Lutein and Chlorophyll

Aliquot of crude extract was transferred to cuvette (quartz) and scanned under range from 300

– 700 nm in the UV – Visible spectrophotometer to check the presence of chlorophyll and lutein. It shows the absorbance maxima at 665, 466, 412 nm. It indicates the presence of chlorophyll (665 nm) and lutein (466, 412 nm)

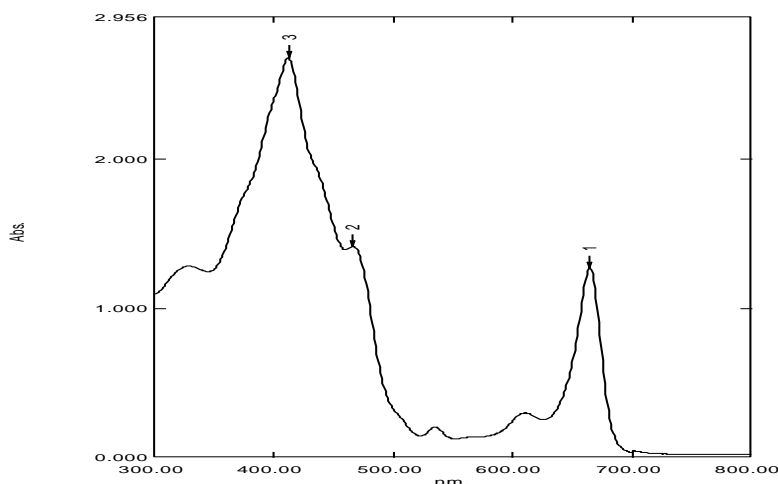


Figure 1: UV Spectrum Lutein And Chlorophyll

Table 2: UV spectrum of lutein and chlorophyll

Sl.No	Wavelength (nm)	Maximum Absorbance Maxima
1	665	1.274
2	466	1.417
3	412	2.689

UV spectral studies (Shimadzu 1800)

LUTEIN

The isolated compound lutein was dissolved in diethyl ether and transferred to cuvette (quartz)

and was scanned under UV range from 300 – 700 nm in the UV – Visible spectrophotometer. The isolated compound lutein shows absorbance maxima at 470, 444 nm.

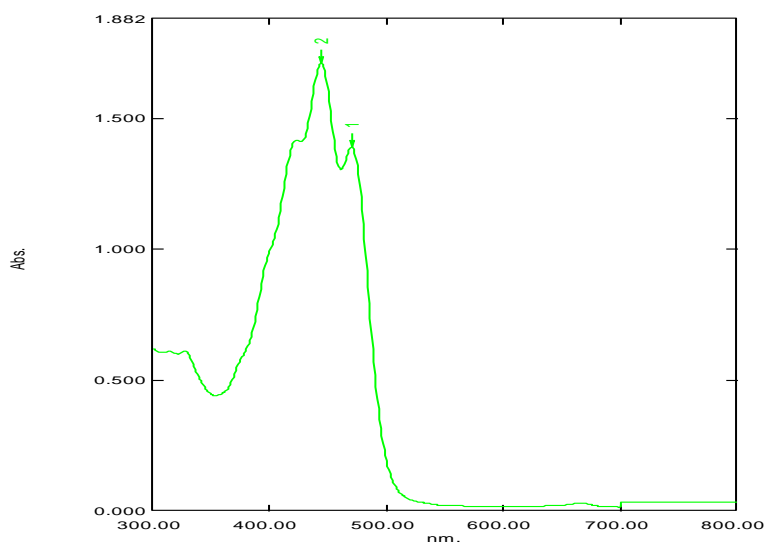


Figure 2: UV Spectrum of Lutein

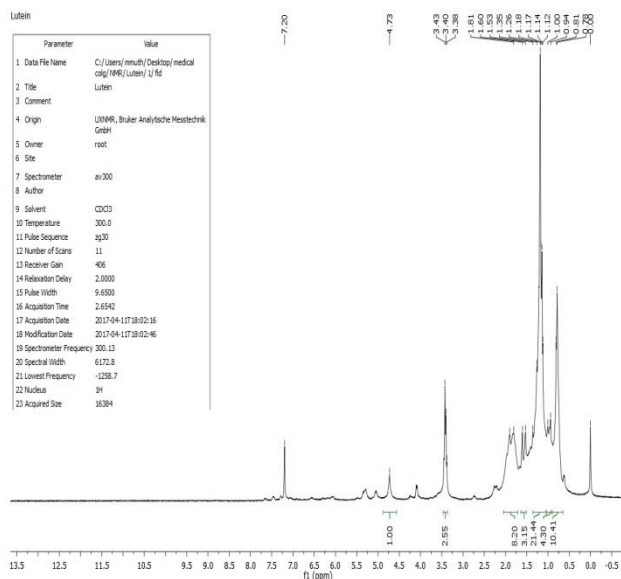
Table 3: UV spectrum of lutein

Sl.No	Wavelength (nm)	Maximum Absorbance Maxima
1	470	1.392
2	444	1.712

¹H NMR

The Isolated compound was dissolved in Deuterated chloroform (CDCl₃) and the NMR

spectrum was obtained by using UXNMR Bruker Analytische Messtechnik GmbH.

¹H NMR**Figure 3: ¹H NMR Spectrum of Lutein****Chemical shift values**

¹H NMR: (300 MHz, CDCl₃) δ_H 0.78 (s, 10H), 0.94 - 1.00 (bd, 2H), 1.12 - 1.35 (bm, 21H), 1.53-1.60 (bd,

3H), 1.81-1.90 (bd, 8H), 3.38-3.45 (m, 2H), 4.73 (s, 1H).

Table 4: Chemical shift values of ¹H NMR

Sl.No	δvalue	Band name	Position of hydrogen atom
1	0.78	s	10H
2	0.94 - 1.00	bd	2H
3	1.12 - 1.35	bm	21H
4	1.53-1.60	bd	3H
5	1.81-1.90	bd	8H
6	3.38-3.45	m	2H
7	4.73	s	1H

^{13}C NMR

The Isolated compound was dissolved in Duterated chloroform (CDCl_3) and the NMR

spectrum was obtained by using UXNMR Bruker Analytische Messtechnik GmbH.

^{13}C NMR

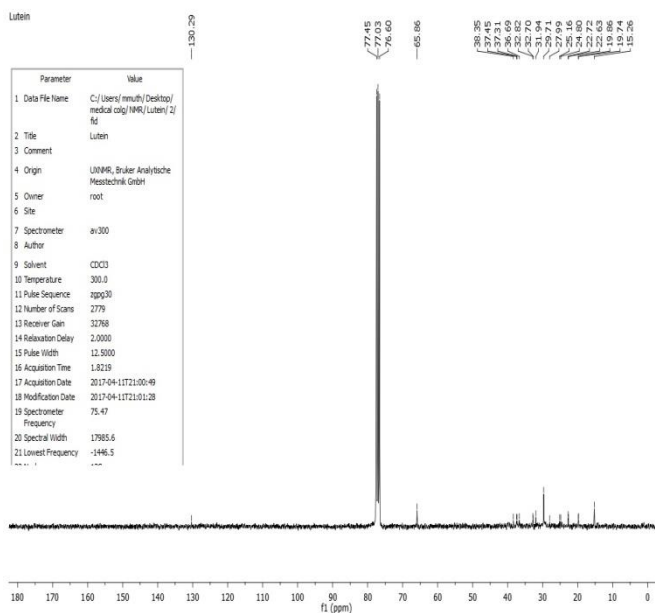


Figure 4: ^{13}C NMR Spectrum of Lutein

Chemical shift values

^{13}C NMR: (75 MHz, CDCl_3); δ_{C} 15.3, 19.7, 19.9, 22.6, 22.7, 24.8, 25.2, 28.0, 29.7, 31.9, 32.7, 32.8, 36.7, 37.3, 37.5, 38.4, 65.9, 130.3.

Table 5: Chemical shift values of ^{13}C NMR

Sl.No	Chemical Shift Value of ^{13}C NMR	Chemical Shift Value of ^{13}C NMR
1	15.3	13.2
2	19.7	21.6
3	19.9	22.8
4	22.6	24.3
5	22.7	28.7
6	24.8	29.5
7	25.2	30.2
8	28.0	34.0
9	29.7	37.1
10	31.9	65.9
11	32.7	130.0
12	32.8	
13	36.7	
14	37.3	
15	37.5	
16	38.4	
17	65.9	
18	130.3	

GC-MS Studies

The isolated compound was subjected to GC-MS studies and the spectrum was obtained by using bruker instrument.

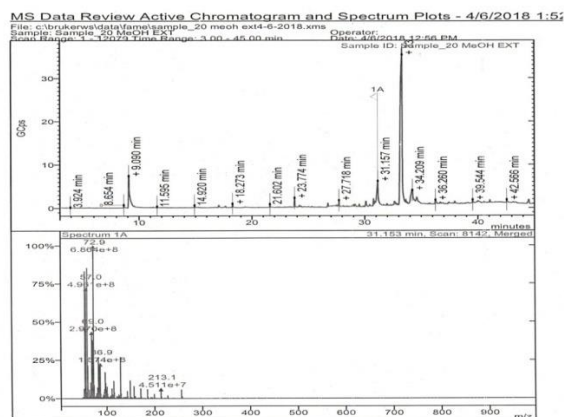


Figure 5: GC-MS Profile of Proposed Lutein

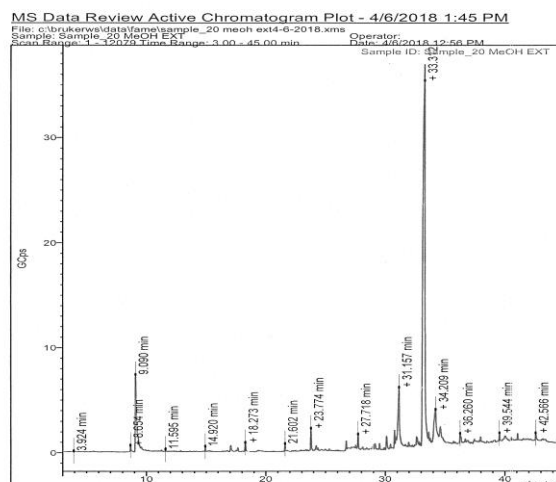


Figure 6: GC-MS Profile of Proposed Lutein

GC-MS Profile

Lock Peak Width: Yes
 Parameters: Local
 Peak Width (sec): 50.0
 Slope Sensitivity (SN): 25
 Tangent %: 20
 Peak Size Reject (counts): 2000
 Smoothing: Mean 7 Point Smooth
 Spike Threshold Factor: 10
 Noise: Peak to Peak

	Retention Time	Area	% of Total	Signal/Noise	Scan Description
1.	7.249	110051536	0.013	18.35	Merged
2.	8.899	7.040e+10	8.312	-2.707	Merged
3.	16.608	4.086e+9	0.482	10.27	Merged
4.	17.939	7.482e+9	0.883	0.6828	Merged
5.	21.356	2.855e+9	0.337	14.7	Merged
6.	21.850	2.215e+9	0.262	22.79	Merged
7.	23.567	1.174e+10	1.386	4.836	Merged
8.	23.847	5.956e+9	0.703	25.08	Merged
9.	24.403	5.003e+9	0.591	9.307	Merged
10.	27.230	1.814e+10	2.142	20.16	Merged
11.	29.167	5.118e+9	0.604	38.93	Merged
12.	30.787	1.895e+10	2.237	83.71	Merged
13.	31.304	5.861e+10	6.921	44.45	Merged
14.	32.947	4.271e+11	50.426	9.485	Merged
15.	33.952	1.204e+11	14.213	10.42	Merged
16.	36.079	2.218e+10	2.619	12.66	Merged
17.	37.549	1.880e+10	2.219	20.7	Merged
18.	39.767	2.110e+10	2.492	16.46	Merged
19.	40.209	1.193e+10	1.409	28.24	Merged
20.	42.491	1.481e+10	1.749	16.15	Merged

Table 6: GC-MS Analysis of Proposed Lutein

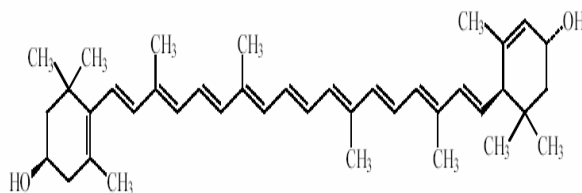
Sl.No	Peak No	Retention Time (minutes)	Molecular Weight	Name of the compound
1	1	8.654	124	3-Octyne, 7-methyl-
2	2	9.091	128	Naphthalene
3	3	11.645	212	Pentadecane

4	4	14.919	184	Tridecane
5	5	18.275	220	ButylatedHydroxy Toluene
6	6	21.683	212	Pentadecane
7	7	23.775	262	Sulfurous acid, cyclohexylmethyl hexyl ester
8	8	24.212	296	Heptadecane, 2,6,10,15-tetramethyl-
9	9	25.388	272	Methoxyacetic acid, 3-tridecyl ester
10	10	26.731	296	1-Iodo-2-methylundecane
11	11	27.717	268	2-Pentadecanone, 6,10,14-trimethyl-
12	12	29.507	284	Hexadecanoic acid, 15-methyl-, methyl ester
13	13	30.106	278	Dibutyl phthalate
14	14	30.872	340	Eicosanoic acid, ethyl ester
15	15	31.153	256	n-Hexadecanoic acid
16	16	32.672	326	Hexadecanoic acid, 3,7,11,15-tetramethyl-, methyl ester
17	17	33.307	296	Phytol
18	18	33.630	294	1-Hexadecyn-3-ol, 3,7,11,15-tetramethyl-
19	19	34.155	266	9-Octadecenal
20	20	34.589	394	Cyclopropanetetradecanoic acid, 2-octyl-, methyl ester
21	21	36.260	242	1-Hexadecanol
22	22	39.542	414	Heptacosane, 1-chloro-

Some of the peaks were relevant with molecular weight in the GC-MS profile (Figure 5&6.) identified the presence of certain linkages of lutein. Hence the derived Ultraviolet spectrum (Figure 1&2.), Nuclear Magnetic Resonance (^1H NMR, ^{13}C

NMR) spectrum (Figure 3&4.) and GC-MS profile (Table 6.) confirmed the molecular weight of lutein and also supports the additional research work indicating the chemical structure of lutein from the leaf powder.

Structure of Lutein



IUPAC NAME: β,ϵ -carotene-3,3'-diol

Other name

4-[18-(4-Hydroxy-2,6,6-trimethyl-1-cyclohexenyl)-3,7,12,16-tetramethyloctadeca-1,3,5,7,9,11,13,15,17-nonaenyl]-3,5,5-trimethyl-cyclohex-2-en-1-olXanthophyll

Chemical formula : $\text{C}_{40}\text{H}_{56}\text{O}_2$

Appearance : Red – orange crystalline solid

Solubility in water : Insoluble

Solubility in fats : Soluble

Determination of Sun Protection Factor of Isolated Lutein From *Commelinabenghalensis* L.

Isolated compound lutein was subjected to sun protection factor determination and the results are showed in Table 7-9.

Sun Protection Factor

Determination of Sun Protection Factor of Isolated Lutein From *Commelinabenghalensis* L. (LUTEIN 50,100,200 $\mu\text{g/ml}$)

Table 7: Determination of Sun Protection Factor of Isolated Lutein 50 $\mu\text{g/ml}$				
Sl.no	Wavelength (λ nm)	EE * I (Normalized)	Absorbance (Lutein 50 $\mu\text{g/ml}$) Mean \pm SEM	SPF value
1	290	0.0150	0.119 \pm 0.0017	0.01785
2	295	0.0817	0.115 \pm 0.0003	0.093955
3	300	0.2874	0.116 \pm 0.0003	0.333384
4	305	0.3278	0.113 \pm 0.0003	0.370414
5	310	0.1864	0.114 \pm 0.0006	0.212496
6	315	0.0839	0.119 \pm 0.0003	0.099841
7	320	0.0180	0.122 \pm 0.0003	0.02196
SPF				1.1499 \pm 0.43

Table 8: Determination of Sun Protection Factor of Isolated Lutein 100 µg/ml

Sl.no	Wavelength (λ nm)	EE * I (Normalized)	Absorbance Lutein (100 µg/ml) Mean ± SEM	SPF value
1	290	0.0150	0.241 ± 0.0003	0.03615
2	295	0.0817	0.241 ± 0.0003	0.196897
3	300	0.2874	0.238 ± 0.0003	0.684012
4	305	0.3278	0.234 ± 0.0003	0.767052
5	310	0.1864	0.234 ± 0.0003	0.436176
6	315	0.0839	0.252 ± 0.0006	0.211428
7	320	0.0180	0.262 ± 0.0006	0.04716
			SPF	2.378875 ± 0.90

Table 9: Determination of Sun Protection Factor of Isolated Lutein 200 µg/ml

Sl.no	Wavelength (nm)	EE * I (Normalized)	Absorbance Lutein (200 µg/ml) Mean ± SEM	SPF value
1	290	0.0150	0.495 ± 0.0006	0.07425
2	295	0.0817	0.484 ± 0.0003	0.395428
3	300	0.2874	0.475 ± 0.0006	1.36515
4	305	0.3278	0.463 ± 0.0003	1.517714
5	310	0.1864	0.475 ± 0.0003	0.8854
6	315	0.0839	0.495 ± 0.0006	0.415305
7	320	0.0180	0.511 ± 0.0006	0.09198
			SPF	4.745227 ± 1.79

The SPF number of lutein 50µg/ml is 1.15 ± 0.43. The SPF number of lutein 100µg/ml is 2.38 ± 0.90. The SPF number of lutein 200µg/ml is 4.75 ± 1.79.

SPF value for sunscreen above 2 is considered as having good sunscreen activity. Marketed sunscreen lotion having concentration 200 µg/ml shows SPF value about 0.66±0.006. (Manoj A. Suva., 2014) [8]

CONCLUSION

The above preparations can be formulated with suitable water soluble bases and further studies are envisaged to improve the scientific and clinical information. Therefore these formulations may be improved so as to reduce the usage of synthetic sun protection preparations.

Acknowledgement

We thank our respected dean **Dr.MarudhuPandian, M.S., FICS., FAIS.,** Madurai Medical College, Madurai, for carrying out this research work.

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How to cite this article: A. Krishnaveni, A. Iyappan, B. Ezhilarasan, A. Abdul HasanSathali. Isolation and Characterisation of Lutein from *Commelina benghalensis* L. Leaves and its Sun Protection Factor activity. *Int J of Allied Med Sci and Clin Res* 2018; 6(2): 462-475.

Source of Support: Nil. **Conflict of Interest:** None declared.