In Vitro Evaluation of Sun Protection Factor of Plant Extracts By Ultraviolet Spectroscopy Method

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Abstract-Acute and chronic exposure to non-physiological doses of ultraviolet radiation leads to variety of changes of skin ranging from sun-burn, erythema to skin cancer. For skin protection from deleterious effects of sunlight, sunscreen products are used in various forms having chemical and physical filters. However, synthetic sunscreen formulations are reported to cause adverse effects. Thus the present study was planned to evaluate the sun protection factor (SPF) of leaves extract of *Murraya koenigii* for their application as herbal sunscreens. The results suggested that the occurrence of active compounds responsible for ultraviolet absorption can be extracted for use in sunscreens preparations as better, cheaper and safe alternative to harmful chemical sunscreens.

Keywords: UV radiations, SPF, *Murraya koenigii*

Introduction

The harmful effects of solar radiations are caused predominantly by the ultraviolet (UV) region of the electromagnetic spectrum, which consists of UV-A (320400 nm), UV-B (290320 nm), UV-C (100290 nm), and vacuo UV (10100 nm). It has been reported that adverse effects by UV-B radiation on the human skin include erythema (or sunburn), accelerated skin aging, cutaneous degeneration, photosensensitivity, phototoxicity, ectinic elastisis and induction of skin cancer (Maske et al. 2013; Mbanga et al. 2014). UV-A radiations produce immediate tanning effect and darkening of melanin in the epidermis. It also causes premature photoaging, suppression of immunological functions and necrosis of endothelial cells. UV-A radiations reaches the deeper layers of the epidermis, dermis, and generates free radicals. The main destroying factors of UV radiations for skin are oxygenated molecules which are often called free radicals such as; superoxide anions (O^-_2), hydroxyl radical (OH), singlet oxygen, hydrogen peroxide (H_2O_2), ferric ion, nitric oxide (NO) etc. It is well documented that ultraviolet (UV) light indices immune suppression and oxidative stress, which play an important role in the induction of skin cancers (Mishra, *et al*. 2012).

Sunscreens are chemicals that provide protection against the adverse effects of solar and, in particular, UV radiation. There are several agents available from both synthetic and natural sources with UV-filtering properties. Due to their capability to impact considerable human local and systemic exposure, the UV filters should be risk-free (Nohynek *et al*., 2010). Synthetic UV filters have been found to impose toxic effects on humans. 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. Phytoconstituents extracted from plants have been recently considered as potential sunscreen resources because of their UV ray absorption capacity in the UV regions and their antioxidant property. Effective botanical antioxidant compounds are widely used in traditional medicine including tocopherols, flavonoids, phenolic acids, nitrogen containing compounds (indoles, alkaloids, amines, and amino acids), and monoterpenes. Green tea polyphenols, *Aloe barbadensis* extract, and aromatic compounds isolated from lichens are examples of natural substances evaluated for their sunscreen properties (Bonina *et al*. 1996). Antioxidants from natural sources may provide new possibilities for the treatment and prevention of UV-mediated diseases. 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 erythemal dose (MED) in protected skin to unprotected skin (Sutar and Chaudhari, 2020).There is an extreme potential in various herbals and thus can be explored for the sun protective characteristics along with being highly rich source of phytoconstituents and antioxidants. Thus considering above factors, the current study was planned to evaluate the sun protection capability of *Murraya koenigii.*

Material and Methods

Sample Preparation

Murraya koenigii leaves were washed with distilled water twice and dried. The samples were then ground separately in a mixer grinder. 20 gm from each were taken separately in a beaker. This powder was weighed and loaded to the Soxhlet apparatus and 200 ml of methanol/water was used as solvent for preparing the alcoholic and aqueous extract respectively. The extraction process was carried out overnight and the extract was then filtered .The filtered extract was suitably diluted with methanol/water, dried and kept at 4˚C for measuring SPF and phytochemical analysis.

Phytochemical Analysis of Plant Extract

Flavonoid Test: 5ml of diluted ammonia solution was added to aqueous filtrate of the plant extract followed by the addition of concentrated H_2SO_4. Formation of yellow color indicated the presence of flavonoids.

Saponins Test: One ml of plant extract was diluted with 20 ml distilled water and the tube was shaken. Formation of foam indicates the presence of saponins.

Alkaloids Test: To one ml of plant extract, 3ml of ammonium solution was added and incubated at 37˚C for few minutes. The tubes were then placed in water bath and then mayer's reagent was added. Formation of cream color precipitation showed the presence of alkaloids.

Terpenoids Test: 2ml of chloroform was added to 5ml of plant extract. Conc. H_2SO_4 (3ml) was then carefully added to form a layer. Reddish brown coloration of the interface indicated the presence of terpenoids.

Carbohydrate Test: 1ml of Fehling's A and Fehling's B were heated in a boiling water bath for 5-10 min with the plant extract. Appearance of reddish orange precipitate shows the carbohydrate presence.

Tannins Test: About 2ml of the plant extract was stirred with 2ml of distilled water and few drops of $FeCl_3$ solution (5%w/v) were added. The formation of a green precipitate was an indication for the presence of tannins.

Phenol Test: Plant extracts were treated with 3-4 drops of $FeCl_3$ solution. Formation of bluish black colour indicates the presence of phenols.

Steroid Test: A red colour produced in the lower chloroform layer on addition of 2 ml plant extract to 2 ml of chloroform and 2 ml conc. H_2S0_4 indicates the presence of steroids.

Determination of SPF

100 mg of aqueous and methanolic extract were dissolved in 100ml of distilled water. From this 2 ml and 4ml of the extract was withdrawn and diluted to 10 ml with distilled water so as to prepare extract with the final concentration of 200µg/ml and 400µg/ml. Thereafter, the absorbance of these extracts was taken by spectrophotometer from wavelength ranging from 290 to 320 at 5nm. SPF for aqueous and methanolic extract was calculated by the formula given by Mansur Methametical equation below and by utilizing values given by Sutar and Chaudhari, 2020. SPF was calculated three times and then mean value was taken in consideration.

In vitro SPF is calculated by following equation:

$$SPF = CF \times \sum EE \times I \times Abs$$

Where;

(I) - the solar irradiance spectrum,

EE (I) - the erythemal action spectrum,

Abs- absorbance of sunscreen product,

CF-correction factor (=10)

The value of EE x I are constant and predetermined as shown in Table -1

Table -1 Values of EE×I used in the calculation of SPF

Wavelength (nm)	EE*I (Normalised)
290nm	0.0150
295nm	0.0817
300 nm	0.2874
305nm	0.3278
310nm	1.864
315nm	0.0839
320nm	0.0180
TOTAL	1

Results and Discussion

Phytochemical Analysis of Plant Extracts

Phytochemical examination revealed the presence of constituents such as carbohydrates, alkaloids, glycosides, saponins, tannins, flavinoids, phenol and terpenoids (Table -2).

Table -2 Phytochemical Analysis

Constituents	*Murraya koenigii*
Carbohydrates	+
Saponins	++
Alkaloids	+
Terpenoids	-
Flavonoids	++
Phenols	-
Tannin	-
Steroids	++

In a similar study conducted by Sivanantham and Thangaraj (2015) on phytochemical composition of carrot (Daucus carota , the preliminary qualitative analysis of phytochemical investigation revealed the presence of alkaloids, carbohydrate, phenol, flavonoids, coumarin and chlorogenic acid in carrot. This study also confirmed that terpenoid is an active component in carrot. Rashmi et al. (2020) have reported the presence of phytochemicals i.e. alkaloids, carbohydrates, cardiac glycosides, phenol, phylobatannins, tannins, terpinoids, in ethanolic extracts and alkaloids, carbohydrate, cardiac glycosides, phylobatannins, tannins, terpinoids, in methanolic extracts and alkaloids, carbohydrate, phenols, terpinoids, tannins, quinons in aqueous extracts of *Murraya koenigii*.

Determination of SPF

SPF numbers have become a worldwide standard for measuring the effectiveness of photoprotective products. The in vitro SPF determination is one of the useful tools for screening tests during product development, as a supplement base for in vivo studies. Whereas the in vivo test is time consuming and includes various degrees of variability. In order to protect against UV radiations, the formulation should have good SPF number and also the formulation should have wide range of absorbance between 290-400 nm ranges. The SPF numbers of extracts were calculated by applying Mansur mathematical equation in the UV-B region, which is considered to be the region of highest incidence during the day and people are exposed for a longer time (Lefahal *et al.* 2018).

In the present research work aqueous and methanolic extract of Murraya koenigii leaves was subjected for SPF evaluation by UV spectroscopic method. SPF value for sunscreen above 2 is considered as having good sunscreen activity. The calculated values of SPF of aqueous and methanoic extract of *Murraya koenigii* are presented in the Table -3 and 4. SPF value of aqueous extract of Murraya koenigii was 0.221 and 0.466 at concentration of 200µg/ml and 400µg/ml respectively. Methanolic extract of *Murraya koenigii* have SPF value 3.469 and 1.704 at a concentration of 200µg/ml and 400µg/ml respectively.

Table -3 Absorbance and SPF value of methanolic extract of *Murraya koenigii*

S.No	Wave length	EE*I	Absorbance 200 µg/ml	EE*I*abs. (SPF)	Absorbance 400 µg/ml	EE*I*abs. (SPF)
1	**290**	0.015	2.244	0.03366	1.101	0.016515
2	**295**	0.817	2.119	1.731223	1.041	0.850497
3	**300**	0.287	1.983	0.569121	0.976	0.280112
4	**305**	0.327	1.889	0.617703	0.927	0.303129
5	**310**	0.186	1.805	0.33573	0.924	0.171864
6	**315**	0.083	1.803	0.149649	0.832	0.069056
7	**320**	0.018	1.801	0.032418	0.722	0.012996
	Total			**3.469504**		**1.704169**

Table -4 Absorbance and SPF value of aqueous extract of *Murraya koenigii*

S.No	λ	EE*I	Absorbance 200 µg/ml	EE*I*abs. (SPF)	Absorbance 400 µg/ml	EE*I*abs. (SPF)
1	**290**	0.015	0.123	0.001845	0.321	0.004815
2	**295**	0.817	0.122	0.099674	0.3	0.2451
3	**300**	0.2874	0.143	0.0410982	0.273	0.0784602
4	**305**	0.3278	0.132	0.0432696	0.256	0.033792
5	**310**	0.1864	0.124	0.0231136	0.252	0.0469728
6	**315**	0.0837	0.121	0.0101277	0.233	0.0195021
7	**320**	0.018	0.115	0.00207	0.244	0.004392
	Total			**0.2211981**		**0.4668261**

Methanolic extract of *Murraya koenigii* showed the higher SPF value in comparison to aqueous extract which falls near the range of good sunscreen activity. Thus it can proposed that this plant extract can absorb the ultraviolet radiation since they possesses good sun protection activity against ultraviolet radiations.

Mishra *et al*. (2012) reported the SPF values of Calendula oil in cream formulation to be in the range of 14.84 ± 0.16. Several authors have conducted similar studies and reported SPF values for *Boerhavia diffusa* (3.5397.174), fresh *Aloe vera* gel (0.0995), the aqueous and methanolic extracts of *Zingiber officinale* (1.441.82 and 1.481.99, respectively) (Ashawat and Saraf, 2006;2008; Suva, 2014).

Conclusion

The SPF values of the leave extracts of *Murraya koenigii* were assessed and UV protection capabilities were reported. Further, it has been suggested that active components responsible for ultraviolet absorption can be isolated from these plant extracts. Along with their many beneficial effects and safety, this botanical could thus become good, cheap and easily available formulation ingredients in sunscreen products.

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