In Vitro Evaluation Of Sun Screen Activity And Phytochemical Screening Of Methanolic Leaf Extract Of *Magnolia Figo*

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Abstract: *Magnolia figo* (Local name: *"Madana-kama"*) is a native Chinese plant, which belongs to Family Magnoliaceae. Although, different parts of the plant have been used as a treatment for several types of disease conditions such as malaria, cardiovascular diseases and as a strengthening agent for sexual virility in traditional medicine. The aims of the present study were to evaluate the sunscreen activity and to qualitatively analyze the phytochemicals present in methanolic leaf extract of *Magnolia figo.* Collected leaves were air-dried, powdered and macerated in methanol. The filtrate was evaporated to dryness and subjected to freeze-drying process. The SPF values of methanolic extract of *Magnolia figo* and the reference agent; Dermatone® were analyzed *in vitro* by using spectrophotometric method. The results exhibited that 2.00 mg/mL methanolic extract of *Magnolia figo* has high sun protection activity (29.94). The dose response relationship of *Magnolia figo* and Dermatone® was analyzed according to concentration series of each, respectively. In the current study EC 50 was calculated to methanolic leaf extract of *Magnolia figo* (0.9135) which is lower than Dermatone® (1.7820) suggesting that the potency of methanolic leaf extract of *Magnolia figo* is higher than the reference agent. According to the phytochemical screening, methanolic leaf extract of *Magnolia figo* was rich in alkaloids, phenols, tannins, terpenoids, flavonoids, steroidal glycosides and saponins. The results concluded that leaf extract of *Magnolia figo* possesses marked sunscreen activity which exhibits its potential use for development of sunscreen formulations.

Keywords: *Magnolia figo*, sun screen activity, phytochemical screening

Introduction:

In the present day, most practical and popular strategy to protect from the UV radiation is the application of topical broad-spectrum sunscreen formulations on the skin, because they have the ability to reduce the penetration of UV-A and UV-B radiation through the skin. Sun screen formulations act by either absorbing or reflecting the sun's ultraviolet radiation (Napagoda *et al.*, 2016). The Sun Protection factor (SPF), which is expressed as numeric measurement on sunscreen products is the recognized universal indicator for the photo protective performance of the product (M.S. Latha, 2013**).** SPF is defined as the UV energy required to produce a minimal erythema dose (MED) on protected skin, divided by the UV energy required to produce a MED on unprotected skin (Saraf and Kaur, 2010). The SPF values of a sunscreen product can be determined using *in-vitro* methods. (Mansur J.S., 1986, Sayre *et al*., 1979). There are many factors that should be considered when formulating a new sun screen to the public because it has to be safe, chemically inert, non-irritating, non-toxic and photo-stable and should provide complete protection to the skin (Mbanga *et al.*, 2014). Synthetic products are known to cause photo

allergic reactions and potential to develop skin melanoma due to complete absorption of UV-B radiation allowing transmission of large quantities of UV-A in to the deeper layers of the skin, exacerbation of acne and rosacea because they contain zinc oxide and titanium dioxide which can block skin pores (Latha *et al*., 2013). Due to associated adverse effects synthetic sunscreen products are rapidly being replaced by the herbal sunscreen products because most of the materials which are used in sunscreen products have not been established as safe for long term human use (Korać and Khambholja, 2011). There is strong evidence that UV light induces the accumulation of UV light absorbing flavonoids and other phenols in dermal tissues of plant body. This is a protective mechanism in plants (Bissonnette, Nigen and Bolduc, 2012). Naturally occurring phytochemicals such as phenolic acids, flavonoids and high molecular weight poly phenols are very useful for prevention of harmful effects generated by UV radiation (Saraf and Kaur, 2010).In order to fulfill this great demand of novel plant based clinical herbal sunscreens, many pharmaceutical companies try to invest lots of money for their research & development to get a product outcome which can compete with other products, with higher efficacy, relatively cheap and user friendly. Species of genus *Magnolia* have many traditional uses. In Ayurvedic medicine, diseases such as fever, colic, leprosy, eye disorder, gonorrhea, and gout can be treated with *Magnolia champaca. Magnolia alba* is used to treat bronchitis, prostatitis, and leucorrhoea. *Magnolia hypoleuca* and *Magnolia officinalis* have been used to treat carcinomatous sores in leukemia. Flowers of *Magnolia figo* are used as a cardiac tonic and roots and bark are used as antidote for fish poison (Kumar, 2012). There is no scientific evidence available about *Magnolia figo* leaves that correspond to *in vitro* evaluation of sunscreen activity. Phytochemical analysis studies proved that the leaves of *Magnolia figo* have several phyto constituents such as polyphenols, flavonoids, alkaloids, aporphines and sesquiterpene lactones which possess exquisite biological activities (Kumar *et al.*, 2012). The presence of magnolamine, magnoline and tetrandrine in the leaves also has been reported (Jayaweera and Senaratna, 2006). Accordingly, the current study was conducted to evaluate the sun screen activity and carry out a qualitative analysis of the phytochemicals present in methanolic leaf extract of *Magnolia figo.*

Methodology:

Matured leaves of plant *Magnolia figo* ("*Madana-kama*") were collected,air-dried, powdered and macerated in methanol. The filtrate was evaporated to dryness and subjected to freeze-drying process. The freeze-dried sample from the plant extract (2 mg) was weighed using an analytical balance and put into 1.5 mL eppendorf tube. Eppendorf with extract was filled with 1000 µl ethanol and it was properly mixed using vortex mixer. In addition, the reference agent; Dermatone® was dissolved in ethanol to obtain a solution of 2.0 mg/mL and absorbance of UV radiation by the methanolic plant extract and Dermatone® were determined in triplicate using SPECTRA max PLUS 384 microplate spectrophotometer from 290 to 320

$$SPF_{in\ vitro} = CF \times \sum_{320}^{290} EE \times (\lambda) \times I(\lambda) \times abs(\lambda)$$

nm, at 5 nm intervals taking ethanol as the blank and data were recorded using the software-Softmax Pro. SPF values were then determined using the Mansur equation (Mansur, *et al*., 1986).

EE = erythemal effect spectrum

I = solar intensity spectrum

abs = absorbance of sunscreen product;

CF = correction factor (= 10)

λ = wavelength

For Determination of the dose – response relationship, the freeze-dried sample which obtained from *Magnolia figo* was redissolved to obtain 2.0 mg/mL, 1.0 mg/mL, 0.5 mg/mL, 0.25

mg/mL, 0.125 mg/mL, 0.0625 mg/mL, 0.03125 mg/mL samples. In addition, Dermatone® was dissolved in ethanol to obtain a solution of 2.0 mg/mL, 1.0 mg/mL, 0.80 mg/mL, 0.40 mg/mL, 0.20 mg/mL, 0.10 mg/mL, 0.05 mg/mL. The Absorbance of UV radiation from samples prepared were measured in micro plates, in triplicate using SPECTRA max. PLUS384"

Microplate spectrophotometer from 290 to 320 nm at 5 nm intervals using methanol as the blank. The SPF values were calculated using Mansur equation. Two graphs were plotted to determine the EC_{50} values separately. Statistical analysis was performed using GraphPad Prism 8.0.1 (244) software. The significance level was set at $p < 0.05$.

UV Absorbance values of 2.0 mg/mL methanolic leaf extract of *Magnolia figo* was measured within the 190 nm-690 nm wavelength range using a microplate spectrophotometer to show the spectral analysis of methanolic leaf extract.

Qualitative analysis of methanolic extract of *Magnolia figo* for alkaloids, saponins, flavonoids, tannins, phenols, sterols, glycosides was done using standard procedures as described in Evans (2000) and Harbone (1998).

Results and Discussion

Table 1 - Comparison of SPF values of plant extract and the Dermatone® sample at 2.0 mg/mL concentration

Tested Sample	SPF
Magnolia figo	29.94
Dermatone®	33.77

In SPF rating, the values 2-12, 12-30 and ≥30 are considered as having respectively minimum, moderate and high sun protective activity respectively. The results showed that 2.00mg/mL methanolic extract of *Magnolia figo* exhibited high sun protection activity (29.94) which is a novel finding of this study. Further, SPF value of 29.94 suggests that this plant extract can protect the skin against 97% of harmful UV-B rays and the 1.00mg/mL extract showed moderate sun protective activity which is 8.19% lower than the SPF of highest concentration. Positive control, Dermatone® showed SPF value of 33.77 in 2.00mg/mL concentration. The sun protective activity of methanolic extract of *Magnolia figo* is 3.83% lower than the reference agent. According to the guidelines of international regulatory agencies, only SPF value equal or greater than 6 is preferred in cosmetic products (Costa *et al*.,

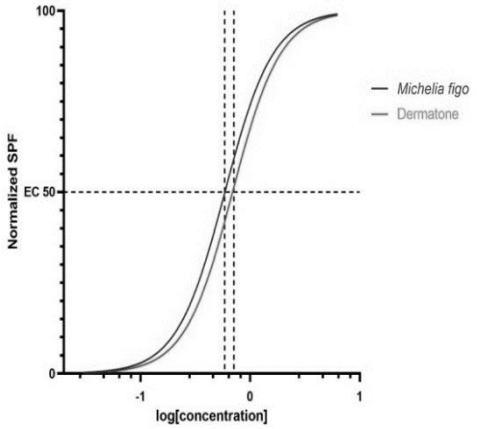

Figure 1 - Comparison of sun protection factor of methanolic leaf extract of Magnolia figo with the Dermatone® sample using normalized SPF Vs Log[concentration] graph.

2015). Hence the results suggested that *Magnolia figo* extract can be considered as a promising active ingredient for sunscreen formulation.

Potency refers to the concentration or dose of a drug required to produce the drugs maximum effect. The lower the dose of the drug required for a given response the more potent the drug and usually describe as half maximal effective concentration (is the dose at which 50% of the maximum effect is produced or the concentration of drug at which the drug is half maximally effective) known as EC_{50}. Smaller the EC_{50} the more potent the drug (Lambert, 2004) as shown in the dose response relationship. In the current study EC_{50} was calculated to methanolic leaf extract of *Magnolia figo* (0.9135) which is lower than the reference agent Dermatone® (1.7820) suggesting that the potency of methanolic leaf extract of *Magnolia*

figo is higher than the reference agent. The potency difference is illustrated clearly shown in Figure 1.

If higher SPF value is desired, it can be achieved by reducing the dilution factor when preparing the extract because SPF is found to be concentration dependent (Costa *et al*., 2015).

It is clearly depicted by the strong positive statistically significant correlation between concentration of the methanolic extract of *Magnolia figo* and the *in vitro* SPF value as shown in (Table 2) ($r = 0.9745$, $p < 0.05$). Furthermore, UV absorption spectrum of methanolic extract of *Magnolia figo*, the highest absorbance peak (~3.9) was observed in UV-C region (150 nm - 270 nm) and another high absorbance peak (~3.5) was observed in UV-B region (270 nm - 350 nm) which exhibit effective sun protection activity against harmful UV-B and UV-C rays.

Test	Result
Alkaloids	
Mayer's Test	+++
Wagner's Test	++
Phenols & Tannins	
Ferric Chloride Test	+++
Terpenoids	
Salkowski's Test	++
Flavonoids	
Zn/HCl Reduction Test	+++
Steroidal Glycosides	
Libermann Burchards Test	+
Saponin	
Foam Test	+

Table 2 - Correlation between concentration of the methanolic leaf extract and in vitro SPF

	Pearson Correlation Coefficient (r)	p	r^2
Concentration Vs SPF	0.9745	0.0002	0.9497

Table 3.

Table 3- Results of phytochemical screening for Magnolia figo

The phytochemical analysis for leaf extract *Magnolia figo* revealed the presence of alkaloids, tannins, terpenoids, phenols, flavonoids,

steroidal glycosides and saponins. The results are exhibited as the presence of bioactive compound (+) and the results are shown in

[Mild presence: (+), Moderate presence: (++), High presence: (+++)]

Conclusion

The methanolic extract of *Magnolia figo* exhibits promising sun protection activity (SPF = 29.94) and there is correlation between concentration and the sun protection factor, display a huge potential to be developed as a safe, cheap and effective topical sunscreen formulation. From the results obtained in the present study, it is concluded that methanolic leaf extract of *Magnolia figo* contains alkaloids, phenolics, flavonoids, terpenoids, steroidal glycosides and saponin. Final results clearly indicate that potential use of *Magnolia figo* leaves for development of cosmetic formulations.

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