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Evaluation of Sun Protection Factor of Northern Iraqi Propolis

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Abstract: One of the main risk factors for skin cancer and photoaging is excessive sun exposure combined with the lack of sun protection. Incorporating natural antioxidant and anti-inflammatory agents into sunscreens and taking natural antioxidant extracts orally are two recent photoprotection approaches. Propolis and its plant precursors have the potential to be used as active components in pharmaceutical and skin care products that mitigate the effects of ultraviolet radiation from the sun. To date, no research has been carried out on the photoprotective effects of Northern Iraqi propolis. Ethanol and water extracts of propolis were used in the current research due to its potential sun protection factor. This investigation aimed to assess the sun protection factor (SPF) of Northern Iraqi propolis collected from Erbil, Mosul and Sulaymaniyah regions. The SPF of propolis extracts were assessed using the methods of Mansur. According to the findings, the extracts' SPF varied from 5.1 to 17.9. The Musol propolis ethanol extract had the greatest SPF at a concentration of 20 ppm (17.9). At 0.5 ppm (5.1), the Erbil water extract had the lowest SPF. Overall, it was noticed that the SPF value of the propolis ethanolic extract was higher than the water extract for each concentration studied.

Kuzey Irak Propolisinin Güneş Koruma Faktörünün Değerlendirilmesi

Anahtar Kelimeler
Propolis,
Güneş koruma faktörü,
Etanol ekstraktı,
Fotoyaşlanma,
Antioksidan

Öz: Cilt kanseri ve fotoyaşlanma için ana risk faktörlerinden biri güneşe aşırı maruz kalma ve güneşten korunma eksikliğidir. Güneş kremlerine doğal antioksidan ve antiinflamatuar ajanların dahil edilmesi ve doğal antioksidan ekstraktlarının ağızdan alınması fotokorunmaya yönelik yeni iki yaklaşımdır. Propolis güneşten gelen ultraviyole radyasyonun etkilerini azaltan farmasötik ve cilt bakım ürünlerinde aktif bileşenler olarak kullanılma potansiyeline sahiptir. Şu ana kadar Kuzey Irak propolisinin fotokoruyucu etkileri üzerine herhangi bir araştırma yapılmamıştır. Potansiyel güneş koruma faktörü nedeniyle bu çalışmada propolisin etanol ve su ekstraktları kullanılmıştır. Bu araştırma, Erbil, Musul ve Süleymaniye'den toplanan Kuzey Irak propolisinin güneş koruma faktörünü değerlendirmeyi amaçlamıştır. Propolis ekstraktlarının güneş koruma faktörü Mansur'un geliştirmis olduğu yöntem kullanılarak hesaplanmıştır. Bulgulara göre ekstraktların güneş koruma faktörü 5,1 ile 17,9 arasında değişmektedir. Musol propolis etanol ekstraktının 20 ppm (17.9) konsantrasyonda en yüksek güneş koruma faktörüne sahip olduğu gözlemlenmiştir. 0,5 ppm (5,1) ile Erbil propolisinin su ekstraktının en düşük güneş koruma faktörüne sahip olduğu sonuçlardan anlaşılmaktadır. Genel olarak, incelenen her konsantrasyon için propolis etanolik ekstraktının güneş koruma faktörü değerinin su ekstraktından daha yüksek olduğu sonucuna varılmıştır.

1. INTRODUCTION

Over the years, propolis has acquired reputation all over the world as a unique naturally derived resinous medicine with a wide range of medical applications. Significant clinical trials are underway to highlight the therapeutic value of propolis and advance its use in the nutraceutical and pharmaceutical industries. In addition to providing beeswax, propolis, royal jelly, venom and apitherapy as dietary and therapeutic resources for human health, honey bees also serve as pollinators in natural environments [1-5]. Propolis has a wide range of components and these components change according to the type of honeybee species, the plant that bees use to produce propolis, the environment and the time of year when it is harvested [6-8].

Propolis's bioactive profile is correlated with habitats, particularly with regard to the wide range of floral resources that are essential to bee survival and biodiversity [9]. Propolis contains more than 300 chemical components that have biological activities, such as chalcones, phenolic acids, benzofuranes, benzopyranes and flavonoids [10]. Numerous large-scale experimental studies have documented the biomedical benefits of propolis, particularly that sourced from Brazil, China, Taiwan, and Iran, as well as its constituents' anti-inflammatory, antioxidant, anticancer, immunomodulatory, antibacterial, antiviral, antifungal and antidiabetic properties [11].

Numerous studies have demonstrated the protective effects of polyphenols against UV radiation exposure. Overexposure to UV light can harm a cell's DNA and result in negative reactions [12]. Natural polyphenols are often UV-absorbing pigments that are yellow, red, or purple in color. Several in vitro and clinical investigations have demonstrated that skin disorders, melanoma, dry skin, ageing skin, vasodilation and skin cancers may all be directly caused by exposure to UV radiation [13]. Phenolic chemicals are distinguished by their absorption spectrum, which filters out UV radiation to lessen oxidative stress, DNA damage and the penetration of harmful UV rays into the skin [14]. Using natural polyphenol compounds, which have photoprotective as well as antioxidant and anti-inflammatory qualities, can help to protect against the harmful effects of UV radiation from the sun [14].

Natural polyphenol compounds, which possess both antioxidant and anti-inflammatory qualities in addition to photoprotective effects, can be used to protect against the harmful effects of UV radiation from the sun [15]. As an active component in skin care products and pharmaceutical formulations for the prevention of sun UV radiation, propolis and its botanical antecedents are promising options [16]. There hasn't been any research done on the photoprotective qualities of Northern Iraqi propolis or its plant precursors. The aim of this study was to evaluate the possible photoprotective effects of Northern Iraqi propolis collected from Erbil, Musol and Sulaymaniyah.

2. MATERIAL AND METHOD

2.1. Chemicals Used and Material Collection

All chemicals were provided by Sigma Chemical Co. (USA) and were of the reagent grade. Deionized water that has been purified with the Milli-Q® water purification system (Millipore, Burlington, MA, USA). Spectrophotometric measurements were taken by using a Shimadzu UV-1800 double-beam UV-Vis instrument. Samples of propolis were gathered from several areas in the northern Iraqi regions of Erbil, Musol and Sulaymaniyah. The propolis samples were obtained directly from beekeepers and were not purchased from marketplaces. Table 1 displays the geographical locations of the samples. The samples were collected on July, 2021.

Table 1. Coordinates of the locations of the propolis samples

Locations		Coordinate	Altitude
1	Erbil	N 36°23'18.6792" E 44°12'07.6356"	1063.0m
2	Musol	N 36°48'53.6796" E 42°17'07.6956"	406.0m
3	Sulaymaniyah	N 35°43'12.3888" E 45°34'12.5724"	1170.0m

2.2. Hexane Extraction

Using a grinding tool, the raw propolis was crushed and ground into a fine grain in order to maximize the amount of surface area that could be extracted using hexane solution. Subsequently, 10 g of propolis were weighed, put into appropriate containers, and put in a vacuum machine to reduce the possibility of poisoning. 100 ml of hexane was then added, and the propolis was tightly sealed with aluminum paper and paraffin. After that, the containers were maintained at 40°C and 150 rpm for a whole day in an orbital shaker. The solutions were vacuum-filtered the next day using paper filters, and the resulting mixtures were then put in an oven set at 37°C to evaporate the leftover hexane together with the ingredients.

2.3. Ethanol Extraction

The propolis sample was kept at room temperature while it was extracted using ethanol by shaking and extraction. Following that, Whatman No. 1 filter paper was used to filter the propolis extracts. After the filtrates were evaporated using a vacuum incubator, the produced propolis was kept dry for storage.

2.4. Water Extraction

The remaining propolis from the ethanol extraction were collected and 100 ml of distilled water was then added. Afterwards, the propolis were put in an orbital shaker system at 150 rpm for 24 hours at 37°C. The following day, the extracts were filtered via Whatman 1 paper filters, and heated at 37°C to evaporate any remaining water.

2.5. Photoprotective Effects of Propolis

The sun protection factor (SPF) of water and ethanol extracts of Northern Iraqi propolis (Erbil, Musol and Sulaymaniyah) was assessed according to Mansur et al. with minor modifications [17]. Through the investigation, each extract was diluted with 96% ethanol (v/v) to a concentration of 0.5, 1, 5, 10 and 20 ppm (µg/mL). The absorption spectrum of the test samples was obtained in the range of 290–320 nm. A 1 cm quartz element was used for the study. Absorbance data were obtained from 290 to 320 nm in 5 nm increments. 96% ethanol (v/v) was used as a blank.

SPF values were calculated according to Mansur et al. equation [17]:

$$CF \times \Sigma EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

EE (λ)—erythemal effect spectrum; I (λ)—solar intensity spectrum; Abs (λ)—absorbance of extract; CF—correction factor (= 10).

2.6. Statistical Analysis

Results were expressed standard deviation of three measurements. These data were calculated using Microsoft Excel software.

3. RESULTS

The SPF of the ethanolic and water extracts of Erbil, Musol and Sulaymaniyah propolis were assessed spectrophotometrically (Table 2-4). The results showed that the SPF of extracts ranged from 5.1 to 17.9. The highest SPF was found in the ethanol extract of Musol propolis at 20 ppm concentration (17.9). The lowest SPF was found in the water extract of Erbil at 0.5 ppm (5.1). In general, it could be observed that the SPF value of the ethanolic extract of propolis for each concentration is greater than that of water extract.

The SPF analysis of ethanolic and water extracts of Erbil propolis demonstrated that greatest SPF value was obtained at 20 ppm ethanol extract (9.9) while this value was 35% was lesser (6.7) for the same concentration of water extract. On the other hand, the lowest SPF was obtained at 0.5 ppm concentration of water extract (5.1).

Table 2. The SPF analysis of ethanolic and water extracts of Erbil propolis. E denoted ethanol extract, W denotes water extract

Concentration (ppm)	SPF (E, Erbil)	SPF (W, Erbil)
20	9.9±0.2	6.7±0.16
10	6.6±0.12	6±0.14
5	5.9±0.14	5.7±0.14
1	5.5±0.12	5.6±0.1
0.5	5.4±0.1	5.1±0.12

The SPF analysis of ethanolic and water extracts of Musol propolis demonstrated that greatest SPF value was obtained at 20 ppm ethanol extract (17.9) while this value was almost 3 fold lesser (6.6) for the same concentration of water extract. On the other hand, the lowest SPF was obtained at 0.5 ppm concentration of water extract (5.4). The analysis also revealed that SPF of ethanolic extract of Musol propolis was twofold greater than that of Erbil propolis.

Table 3. The SPF analysis of ethanolic and water extracts of Musol propolis. E denoted ethanol extract, W denotes water extract

Concentration (ppm)	SPF (E, Musol)	SPF (W, Musol)
20	17.9±0.45	6.6±0.16
10	10±0.24	5.9±0.1
5	7.5±0.22	5.7±0.15
1	5.7±0.12	5.4±0.12
0.5	5.5±0.15	5.4±0.1

The results of the SPF study of the ethanolic and water extracts of Sulaymaniyah propolis showed that the highest SPF value was achieved at 20 ppm ethanol extract (11.9), whereas the value for the same concentration of water extract was nearly 2 fold lesser (6.5). Conversely, the water extract concentration of 0.5 ppm produced the lowest SPF (5.5).

Table 4. The SPF analysis of ethanolic and water extracts of Sulaymaniyah propolis. E denoted ethanol extract, W denotes water extract

Concentration (ppm)	SPF (E, Sulaymaniyah)	SPF (W, Sulaymaniyah)
20	11.9±0.32	6.5±0.21
10	8.7±0.23	5.9±0.22
5	6.9±0.20	5.7±0.14
1	5.9±0.14	5.5±0.16
0.5	5.5±0.11	5.5±0.11

4. DISCUSSION AND CONCLUSION

The ultraviolet (UV) radiation emitted by the sun are classified into three groups based on their wavelengths: UVA (320–400 nm), UVB (290–320 nm) and UVC (100–280 nm) [18]. Since UVA and UVB may reach the earth's surface, unlike UVC, which is absorbed by the atmosphere's ozone layer, they have the potential to cause a wide range of skin conditions, from moderate sunburns and minor skin inflammations to serious cancers [19,20]. UVB rays can reach a depth of 160–180 µm in the skin, which increases the risk of melanoma and skin cancer as well as causing wrinkles, scaling, dryness and blood vessel dilatation. UVA rays can cause reactive oxygen species, which can harm proteins, lipids, and DNA structures by penetrating deeper into the epidermis and dermis [21]. As a result, efforts to produce a sun protection formulation have accelerated in recent decades. The sun protection benefits of sunscreens are attributed to either of two processes: chemical (i.e., radiation absorption) or physical (i.e., radiation blocking) [22]. SPF unit explains how well they protect the skin [23]. This phrase describes the level of skin protection against the sun's piercing rays. Common synthetic sunscreen ingredients like oxybenzone, avobenzone, ecamsule and octocrylene, however, can penetrate the skin and cause major adverse effects, such as allergic reactions, DNA damage, antiandrogenic effects, the development of cancer and other health problems [24]. Consequently, a lot of research is being done to create a natural sunscreen that should have less adverse consequences.

Attributable to the protective effects of propolis on the skin, it has been deliberated as a decent candidate for integration in skin-care cosmeceuticals such as sunscreens [25]. Propolis also has strong anti-inflammatory properties, making it a good treatment for sunburns and other skin conditions that are influenced by radiation [26]. Concurrently, preserving the collage contents of the skin, it can act as an anti-aging product [27]. Furthermore, since propolis contains various flavonoids and antioxidants, it can protect the skin from UV rays.

Spectrophotometric analysis of SPF of Lithuanian propolis revealed that SPF of the ethanolic extracts 10 µg/mL had SPFs ranging from 2.010 to 4.851. The propolis extract has the highest SPF of 4.851. The pine buds extract had the lowest SPF [28], all which are lower than Northern Iraqi propolis. Using topical formulations based on a Brazilian red propolis hydroalcoholic extract was shown to provide photoprotective effects in a mouse model [29]. Furthermore, these findings imply that the biological impact may have something to do with anti-inflammatory and antioxidant processes. These results suggest that the Hydroalcoholic Extract of Red Propolis is a natural substance with promise for photoprotection applications in cosmetic formulations. The study showed how crucial it is to incorporate green propolis extracts into Gel Permulen TR-1 sunscreen preparations since significant antioxidant and SPF activity was noted, which may enhance the phytopharmaceutical product's ability to protect skin. The HET-CAM test indicated that the formulation made with Gel Permulen TR-1 and enhanced with EEP was safe to apply directly to the skin. In their study, all EEPs of 70% and 75% (at room temperature and high temperature) increased sun protection at the tested concentrations in the experimental settings of this investigation. This composition can therefore be applied to photoprotective materials [29].

As seen in the experiments deposited in the literature, when the extract was added to the Polawax cream sunscreen, the outcomes show that the ethanolic red propolis extracts had photoprotective qualities [30]. This study was reported as the first investigation into the photoprotective properties of red propolis ethanolic extracts added to a cream sunscreen formulation like Polawax. Their research comes to a different conclusion, despite the fact that many writers demonstrate a correlation between antioxidant activity, flavonoid concentration, and phenol content in the importance of UV protection. The antioxidant activity was strong, however the extraction procedure was insufficient to remove numerous phenols and flavonoids. As a result, they linked antioxidant activity to the high benefit of sun protection that was only discovered. Furthermore, the findings demonstrated that the addition of propolis ethanol extract to photoprotective formulations not only enhanced the sun protection values but also guaranteed the preservation of propolis's other qualities, including antimicrobial, anti-inflammatory, antioxidant and healing. As a result, their study indicates that it is a potential source of natural chemicals for the creation of novel formulations that offer photoprotection [31].

The purpose of this study was to evaluate the level of sun protection provided by Northern Iraqi propolis that was gathered in Sulaymaniyah, Mosul and Erbil. Mansur's method was used to evaluate the propolis extracts' sun protection factor. The results showed that the SPF of the extracts ranged from 5.1 to 17.9. At a dosage of 20 ppm, the Musol propolis ethanol extract exhibited the highest level of sun protection (17.9). The Erbil water extract had the lowest SPF at 0.5 ppm (5.1). Overall, it was shown that at every concentration under investigation, the propolis ethanolic extract's SPF value was higher than the water extract's. Conducting in vivo experiments to evaluate the effectiveness of propolis or investigating other extraction techniques, would offer a good guide for future investigations.

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