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Research article

# Antioxidant properties of *Moringa oleifera* and *Withania somnifera* extracts and their use in cosmetics for men

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***Abstract:*** *Nowadays, men are paying more and more attention to their appearance, and thus try to provide their skin with proper care so that it looks healthy and without signs of aging. In the present study, ashwagandha (Withania somnifera) and moringa oleifera (Moringa oleifera) were selected from the most popular adaptogens used in cosmetics, which are currently not commonly used in cosmetic preparations for men. The selected adaptogens were compared for their phytochemical and antioxidant properties to determine their skin care effects in cosmetics. The phytochemical content was evaluated through the determination of total phenolic content and in vitro antioxidant capacity was determined by DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS{2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)} free radical scavenging tests. The total phenolic contents 1.81±0.13 and 0.14±0.02 mg GAE/g extract were found to be present in Moringa oleifera and Withania somnifera glycol-water extracts, respectively. Among the two chosen extracts, Moringa oleifera exhibited significant free radical scavenging activity, ABTS (0.964 ± 0.021 mg TE/g extract) and DPPH (0.822 ± 0.004 mg TE/g extract). It can be concluded that Moringa oleifera extract has strong antioxidant properties than Withania somnifera extract.*

***Key words:*** *extracts; antioxidants; Moringa oleifera; Withania somnifera; cosmetics for men; polyphenol;*

## Introduction

As of 2018, a thriving industry sector is the men's cosmetics market. The reason for this phenomenon is the increased interest in grooming and following the current media-created model of a well-groomed and strong man. Men are reaching for increasingly high-end cosmetics guided by quality and desired performance. It is estimated that from 2018 to the second quarter of 2021, interest in cosmetics increased by additional 21%. The men's cosmetics market is nothing to compare with the women's cosmetics market. Nevertheless, despite its

small size, it is possible to see continuous growth in it. The only decline was observed in 2020, sales of men's grooming products fell by 3.3% during this period. The losses were significant for shaving products and fragrances, due to the pandemic and the quarantine occurring at the time [1].

Despite many physiological and hormonal differences, men's skin, like women's, requires basic care to keep it in good condition. It is assumed that the male cosmetics market still accounts for only 5 % of the total beauty industry. However, this cosmetic department is showing steady growth, which is probably due to following the current trend of "well-being". The modern man strives to maintain a balance between good mental and physical health, which allows him to achieve his goals. Nowadays, appearance is becoming an important element, and maintaining a good appearance is provided by cosmetic products [2,3].

Men most often reach for personal hygiene cosmetics. The most popular among them are shower gels, especially multifunctional or 3-in-1 cosmetics that moisturise and care for the skin, as well as deodorants and antiperspirants. In the case of shower gels, men most often reach for preparations that additionally function as a shampoo and shaving gel. Multifunctional, or "all in one" cosmetics are useful for athletes and active people who want to take care of themselves and at the same time not spend a lot of time on it [4].

*Moringa oleifera* is an Indian tree of the *Moringaceae* family found in Thailand, Taiwan, Africa and the Philippines, valued for its wealth of nutrients and micronutrients, which are found in the greatest quantities in the plant's leaves. The Moringa family consists of 14 species, the best known and most widely used of which is Moringa oleifera. Depending on the part of the plant, it has various application, however it is mostly used in cosmetics and food industries as supplements, due to high content of active compounds such as phenols, saponins, tannins and flavonoids [5-7]

The raw material of *Moringa oleifera* is most often introduced in preparations in the form of powdered leaves or oils. Due to high content of polyphenols, phenolic acids, flavonoids, vitamin C, beta-carotene, quercetin and chlorogenic acid, the plant is credited with anti-inflammatory and antioxidant effects. In addition, the presence of fatty acids, niazimicins, glucomorginins in the plant gives it anticancer activity. The iron which is present in the leaves of the plant is responsible for antiseptic properties, which regenerate minor skin conditions such as bruises, insect bites, cuts. *Moringa oleifera* extracts are attributed to other medicinal activities such as antihypertensive, diuretic, antioxidant, antidiabetic, antipyretic, anti-ulcer and hepatoprotective [8,9]. The aforementioned properties make this adaptogen used in rejuvenating and antioxidant cosmetics. To date, *Moringa oleifera* leaf extracts have been used as body mists, in shampoos, conditioners to strengthen and regenerate hair. Seed extracts, on the other hand, have been used in foams and cleansers, micellar liquids with antipollution properties, and biphasic micellar liquids for washing off waterproof makeup. For this, seed oil has a regenerative effect, and its high content of antioxidants slows

down the aging process. These properties are perfect for use in facial scrubs and biosoaps [5].

*Moringa oleifera* extract is popular in face masks as a nourishing ingredient, shampoos and conditioners in the form of natural proteins. However, it is difficult to find it in typically male cosmetics, and according to research [10-12], it is a potent antioxidant ingredient in skin care products.

*Withania somnifera* functions under the names Ashwagandha, Indian winter cherry, Indian ginseng. The plant is included in the Solanaceae family of dry-herbs, and its habitat is Southeast Asia, Mediterranean areas and India. The distinctive parts of the *Withania* semi-bush are the short hairs found all over its surface and the round orange-red fruits [13,14]. Depending on the occurrence of *Withania somnifera*, it may contain different components. Two groups of active compounds have now been identified: alkaloids and steroidal lactones called vitanolides. Flavonoids such as quercetin, saponins, coumarins, chlorogenic acid and -sitosterol are also present in the plant [14,15].

*Withania somnifera's* uses are varied due to the wide range of health-promoting properties it possesses. The root is used as a supplement, mostly with adaptogenic effects, resulting in stress reduction. In addition, this part of *Withania somnifera* has a high content of micro- and macronutrients such as iron, magnesium, phosphorus, copper and zinc, which further explains the occurrence of this compound in dietary supplements [16].

*Withania somnifera* extracts are commonly used in the cosmetic industry as skin conditioners, shampoos and anti-aging agents. *Withania somnifera* has many benefits in cosmetic formulations, these include protection against UV radiation, pollution and other environmental factors, regeneration and restoration of the skin. In addition, it is credited with healing wounds and swelling, improving pigmentation, rejuvenating properties, moisturising due to the presence of amino acids, and supporting dermatological therapies to combat common skin diseases like acne and psoriasis [17].

*Withania somnifera* extract in men's cosmetics is an ingredient in creams with strong anti-aging and moisturising properties. An example here is a natural day and night cream from Orientana®. *Withania somnifera* extract is also found in preparations for men in anti-dandruff shampoos as an ingredient that improves the condition of the scalp (ECOLAB anti-dandruff shampoo).

In the present study, ashwagandha and *Moringa oleifera* were selected from the most popular adaptogens, which are currently not commonly used, especially, in cosmetic preparations for men. The chosen adaptogens were analysed for their phytochemical and antioxidant properties to determine their skin care effects in cosmetics.

## Experimental

### Materials

Plant extracts and reagents used during the study: glycol-water extract of *Moringa oleifera*, (Naturex, Poland), glycol-water extract from *Withania somnifera*, (Naturex, Poland), propylene glycol (Avantor Performance Materials, Poland). For the determination of total polyphenols, the following were used: 20% sodium carbonate (Chempur, Poland), Folin-Ciocalteau reagent (Chempur, Poland), distilled water. For the determination of antioxidant activity using the DPPH radical, the following were used: 99.8% methanol (PochBasic, Poland). - DPPH (2,2-Diphenyl- 1- picrylhydrazyl), Sigma-Aldrich (Germany). For the determination of antioxidant activity using the ABTS radical: ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (Sigma-Aldrich, Germany), 99.9% anhydrous ethanol (WITKO, Poland), potassium persulfate p.d.a. (Chempur, Poland). The standard compounds in the studies conducted were: 1% gallic acid anhydrous (Aktyn, Poland), Trolox (Sigma-Aldrich, Germany).

### Apparatus

Absorbance studies were carried out using a single-beam UV-Vis spectrophotometer (HP 8453, Shimadzu UV-1900, Germany). Measurements were made in quartz cuvettes with an optical path length of 1 cm.

### Methods

*Determination of total polyphenols using Folin-Ciocalteau reagent*

The total phenolic content of the extracts were determined by the Folin-Ciocalteau method with some modifications [18]. To each sample were added 0.1 ml of glycol (control sample), *Moringa oleifera* extract (triplicate MO1, MO2, MO3) and *Withania somniefera* extract (triplicate WS1, WS2, WS3), 5 ml of distilled water, 2 ml of previously prepared 20% sodium carbonate, 0.5 ml of Folin - Ciocalteu reagent. The samples prepared this way were topped up with distilled water to the mark, mixed and stored in a dark place for 30 minutes. After the time, the samples were taken out and the absorbance was measured at 815 nm. Calibration of the method was carried out using gallic acid at a concentration of 1 mg/ml (0.1%). From the base mixture, ten working samples were prepared with gallic acid concentrations ranging from 0.02 to 0.2 mg/ml. Thus prepared solutions of specific concentrations were added to the samples with the same value of distilled water, 20% sodium carbonate and Folin - Ciocalteu reagent as for the determination of total polyphenols in the extracts in question. Absorbance measurements were made for a given concentration of gallic acid in triplicate. This made it possible to obtain a standard curve from which the polyphenol content of the samples, expressed as mg gallic acid/g, was determined. Based on this, the mean value of the three repetitions was also determined, as well as the standard deviation and relative error. After calculating the mean values and

standard deviation, the relative error was calculated, which indicates the accuracy of the measuring instruments used during the test.

*Free radical scavenging ability by the use of a stable ABTS radical cation*

Free radical scavenging activity was determined by ABTS radical cation decolorization assay [19]. ABTS was dissolved in water to a 2.5 mM concentration and radical cation ($ABTS^{+\bullet}$) was produced by reacting ABTS solution with 1 mM potassium persulphate at room temperature in dark (18 h) before use. For assay, $ABTS^{+\bullet}$ solution was diluted with ethanol to an absorbance value of 0.700 ± 0.02 at 745 nm. After addition of 2.0 ml of diluted $ABTS^{+\bullet}$ solution to 100 µl of extracts solutions, absorbance was recorded after 5 and 10 min. Trolox was used as standard and the results were calculated as milligrams of Trolox equivalents per ml of extract (mg TE/g extract). The experiment was done in triplicate .

*Radical scavenging activity using DPPH method*

The *in vitro* DPPH radical scavenging activity was performed following the method of Brand-Williams and coworkers [20]. 10 µL of each sample extracts, 90 µL of methanol, and 3.9 mL DPPH (57 µM) methanol solution were mixed. After spinning, samples were allowed to stand at the dark place for 10 and 20 min. The absorbance of the mixture was read against a blank at 517 nm using a spectrophotometer, whereas Trolox was used as standard and the results were calculated as milligrams of Trolox equivalents per ml of extract (mg TE/g extract). The experiment was done in triplicate.

## Results and Discussion

*In vitro* antioxidant activity of two extracts (glycol-water) of *Moringa oleifera* and *Withania somnifera* was determined through the determination of total polyphenols content, and through the determination of DPPH and ABTS.

Table 1 expresses the amount of total polyphenols content of the extracts studied. The total phenolic content in the *Moringa oleifera* extract was found to be 1.81±0.13 mg GAE/g extract, whereas that of the *Withania somnifera* extract was 0.14±0.02 mg GAE/g extract. The total polyphenols content was found to be higher in *Moringa oleifera* extract than *Withania somnifera* extract. The highest relative error value was observed for WS extract, it may be due to human error created during the determination.

**Table 1.** Total polyphenols content (TPC) of *Moringa oleifera* (MO) and *Withania somnifera* (WS) glycol-water extracts

| Compound | Total polyphenols content [mg GAE/g extract] | Relative error [%] |
|---|---|---|
| **MO** | 1.81±0.13 | 7.23 |
| **WS** | 0.14±0.02 | 16.46 |

Table 2 represents the ABTS radical scavenging activity of the glycol-water extracts of *Moringa oleifera* and *Withania somnifera*. At 4.76 μg/mL concentration after 5 and 10 min of *Moringa oleifera* extract percent inhibitions were 98.69 ± 0.0033 and 98.48 ± 0.0022 respectively. At the same concentration after 5 and 10 min of *Withania somnifera* extract percent inhibitions were 26.22 ± 0.0069 and 33.06 ± 0.074 respectively. Free radical scavenging activities of the extracts studied are compared to their respective bioactive contents (Table 1). Polyphenols compounds present in the extracts are responsible for DPPH and ABTS free radical scavenging activities, since phenolic compounds capture free radicals by transfer of a hydrogen atom, from its hydroxyl group. It is also an established fact that phenolic containing plant extracts can be good candidates against oxidative stress [21].

**Table 2.** ABTS radical scavenging activity of *Moringa oleifera* (MO) and *Withania somnifera* (WS) glycol-water extracts after 5 and 10 min

| Compound | % inhibition after 5 min | % inhibition after 10 min | ABTS after 5 min [mg TE/g extract] | Relative error [%] | ABTS after 10 min [mg TE/g extract] | Relative error [%] |
|---|---|---|---|---|---|---|
| **MO** | 98.69±0.0033 | 98.48±0.0022 | 0.964±0.021 | 37.48 | 0.962±0.015 | 21.84 |
| **WS** | 26.22±0.069 | 33.03±0.074 | 0.233±0.033 | 13.83 | 0.302±0.048 | 16.51 |

**Table 3.** DPPH radical scavenging activity of *Moringa oleifera* (MO) and *Withania somnifera* (WS) glycol-water extracts after 10 and 20 min

| Compound | % inhibition after 10 min | % inhibition after 20 min | DPPH after 10 min [mg TE/g extract] | Relative error [%] | DPPH after 20 min [mg TE/g extract] | Relative error [%] |
|---|---|---|---|---|---|---|
| **MO** | 90.57±0.77 | 91.06±0.17 | 0.822±0.004 | 8.14 | 0.886±0.001 | 1.92 |
| **WS** | 8.13±2.49 | 9.44±2.94 | 0.051±0.051 | 2.71 | 0.049±0.017 | 3.24 |

Table 3 indicates the DPPH radical scavenging activity of glycol-water extracts of *Moringa oleifera* and *Withania somnifera*. At 4.76 μg/mL concentration after 10 and 20 min *Moringa oleifera* extract percent inhibitions were 90.57±0.77 and 91.06 ± 0.17, respectively. At the same concentration after 10 and 20 min *Withania somnifera* extract percent inhibitions were 8.13±2.49 and 9.44±2.94, respectively. Among the two extracts *Moringa oleifera* extract exhibits the highest free radical scavenging.

The above data show a good correlation between the content of polyphenols and their antioxidant activity, suggesting the likely contribution of phenols and flavonoids to the radical scavenging activity of the extracts of these plants. By comparing the results of the two radical scavenging tests, a strong correlation was noticed. This is explained by the fact that both tests are based on a similar mechanism, i.e. the ability to donate electrons.

In comparison to the literature reports testing antioxidant activity of the two analyzed extracts [21,22], the results obtained for total polyphenols content, DPPH and ABTS assays in the present study are significantly lower. Tousif *et al*. [21] performed the content of phenolic compounds and ABTS and DPPH free radical scavenging assays in *Withania somnifera* extracts. In their work the *Withania somnifera* fraction of methanolic extract (WS-M) was found rich in phenolics at the level 39.96 ± 0.90 mg GAE/g extract, and it exhibited significant DPPH (30.69 ± 0.78 mg TE/g extract) and ABTS (113.60 ± 2.41 mg TE/g extract) free radical inhibitory activities. In the article published by Rodrigez-Pérez *et al*. [23] on extraction studies from *Moringa oleifera* leaves, the parameter values for total phenolic content, ABTS and DPPH were also significantly higher than those obtained in the present study.

A reason for obtaining lower parameters in the tested extracts may be the viscosity of the solvent, and thus the dependence of its penetration deep into the matrix and the resulting deterioration of the extraction process of both polar and less polar compounds [23]. The extracts tested were made with a glycol-water mixture. It is possible that extraction with such a system was not efficient as with water, methanol, ethanol, or water-alcohol mixture [24,25].

## Conclusions

The present study led to conclude that *Moringa oleifera* and *Withania somnifera* glycol-water extracts possess potential antioxidant properties.

Among the two studied extracts, *Moringa oleifera* extract was found to be rich in polyphenols (1.81± 0.78 mg GAE/g extract) and exhibited significant DPPH (0.822 ± 0.004 mg TE/g extract) and ABTS (0.964 ± 0.021 mg TE/g extract) free radical inhibitory activities, while *Withania somnifera* showed the lower values in each performed assays: total polyphenols content (0.14± 0.02 mg GAE/g extract), DPPH (0.051 ± 0.0051 mg TE/g extract) and ABTS (0.302 ± 0.048 mg TE/g extract). These results suggest that *Moringa oleifera* extract might be suitable for further applications beauty industry and may become source of polyphenols in cosmetics for men.

## References

1. https://www.mordorintelligence.com/industry-reports/men-grooming-products-market (online available 04.09.2022)
2. https://www.wiadomoscikosmetyczne.pl/artykuly/rynek-kosmetykow-i-uslug-beauty-dla-mezczyzn-rosni,69030 (online available 04.09.2022)
3. https://www.cnbc.com/2016/01/28/new-standards-of-male-beauty-in-mens-cosmetics-sector.html (online available 04.09.2022)
4. https://www.tiege.com/blogs/news/mens-skin-care-statistics-2020 (online available 04.09.2022)
5. Sawicka B, Krochmal-Marczak B, Bienia B. Rośliny lecznicze występujące w południowej części Indii. HERB **2018**, 1:86-100. https://apcz.umk.pl/HERB/article/view/35802/30669

6. Peñalver R, Martínez-Zamora L, Lorenzo JM, Ros G, Nieto G. Nutritional and Antioxidant Properties of Moringa oleifera Leaves in Functional Foods. Foods **2022**, 11:1107-1119. https:// doi.org/10.3390/foods11081107
7. Gopalakrishnan L, Doriya K, Kumar DS, *Moringa oleifera*: A review on nutritive importance and its medicinal application, Food Sci Hum Wellness **2016**, 5:49–56.
8. Meireles D, Gomes J, Lopes L, Hinzmann M, Machado J, A review of properties, nutritional and pharmaceutical applications of *Moringa oleifera*: integrative approach on conventional and traditional Asian medicine, Adv Tradit Med **2020**, 20:495–515. https://doi.org/10.1007/s13596-020-00468-0;
9. Priyatama VP. *Moringa Oleifera*: Panoramic View on Nutritional, Therapeutic Activity and Patent Landscape. Int J Pharm Sci Dev Res **2017**, 3:024-028. DOI: https://dx.doi.org/10.17352/ijpsdr.000012
10. Xu YB, Chen GL, Guo MQ. Antioxidant and Anti-Inflammatory Activities of the Crude Extracts of Moringa oleifera from Kenya and Their Correlations with Flavonoids, Antioxidants **2019**, 8:296-308. doi:10.3390/antiox8080296
11. Jahan IA, Hossain MH, Ahmed KS, Sultana Z, Biswas PK, Nada K. Antioxidant activity of *Moringa oleifera* seed extracts, Orient Pharm Exp Med **2018**, 18:299–307. https://doi.org/10.1007/s13596-018-0333-y;
12. Athikomkulchai S, Tunit P, Tadtong S, Jantrawut P, Sommano SR, Chittasupho C. *Moringa oleifera* Seed Oil Formulation Physical Stability and Chemical Constituents for Enhancing Skin Hydration and Antioxidant Activity. Cosmetics **2021**, 8:2-20. https://doi.org/ 10.3390/cosmetics8010002
13. Palliyaguru DL, Singh SV, Kensler TW. *Withania somnifera*: From prevention to treatmentof cancer, Mol Nutr Food Res **2016**, 60:1342–1353.
14. Połumackanycz M, Forencewicz A, Wesołowski M, Viapiana A. Ashwagandha (*Withania somnifera L.*) – roślina o udokumentowanych właściwościach prozdrowotnych. Farm Pol **2020**, 76 (8): 442–447.
15. Dar NJ, Hamid A, Ahmad M, Pharmacologic overview of *Withania somnifera*, the Indian Ginseng. Cell Mol Life Sci **2015**, 72:4445–4460. DOI 10.1007/s00018-015-2012-1
16. Afewerky HK, Ayodeji AE, Tiamiyu BB, Orege JI, Okeke ES, Oyejobi AO, Bate PNN, Adeyemi SB. Critical review of the *Withania somnifera (L.)* Dunal: ethnobotany, pharmacological efficacy, and commercialization significance in Africa. Bull Natl Res Cent **2021** 45:176-191. https://doi.org/10.1186/s42269-021-00635-6
17. Bungau S, Vesa CM, Abid A, Behl T, Tit DM, Purza AL, Pasca B, Todan LM, Endres L. Withaferin A. A Promising Phytochemical Compound with Multiple Results in Dermatological Diseases. Molecules **2021**, 26:2407-2420. https://doi.org/10.3390/ molecules26092407
18. Gungor N, Sengul M. Antioxidant Activity, Total Phenolic Content and Selected Physicochemical Properties of White Mulberry (*MorusAlba L.*) Fruits, Int J Food Prop 2008, 11:44-52. DOI: 10.1080/10942910701558652
19. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med **1999**, 26:1231–1237.
20. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity using the DPPH free radical method. LWT **1995**, 28:25-30.
21. Tousif MI, Nazir M, Saleem M, Tauseef S, Uddin R, Altaf M, Zengin G, Ak G, Ozturk RB, Mahomoodally MF. Exploring the industrial importance of a miracle herb

*Withania somnifera* (L.) Dunal: Authentication through chemical profiling, in vitro studies and computational analyses, Process Biochem **2022**, 121:514–528.
22. Nile SH, Park SW. HPTLC Analysis, Antioxidant and Antigout Activity of Indian Plants. Iran J Pharm Res **2014**, 13(2): 531-539.
23. Rodrìguez-Pérez C, Gilbert-López B, Mendiola JA, Quirantes-Piné R, Segura-Carretero A, Ibáñez E. Optimization of microwave-assisted extraction and pressurized liquid extraction of phenolic compounds from *Moringa oleifera* leaves by multiresponse surface methodology. Electrophoresis **2016**, 37:1938–1946.
24. Dhanani T, Shah S, Gajbhiye NA, Kumar S. Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of *Withania somnifera*. Arab J Chem 2017, 10:S1193–S1199.
25. Braham F, Carvalho DO, Almeida CMR, Zaidi F, Magalhães JMCS, Guido LF, Gonçalves MP. Online HPLC-DPPH screening method for evaluation of radical scavenging phenols extracted from Moringa oleifera leaves. S Afr J Bot **2020**, 129:146–154.