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Antioxidant and Anti-Tyrosinase Potentials of Extracts of *Nigella sativa* and *Senna alexandrina* from Sudan

Gareeballah Osman Adam[1,2*] , Yassir Adam Shuaib[1]

[1]Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, Sudan University of Science and Technology, P.O. Box No. 204, Hilat Kuku, Khartoum North, Sudan.
[2]R&D Division, HUVET Co., Ltd., Iksan-si 54531, Korea.
*Corresponding Authors: Gareeballah Osman Adam, E-Mail: gorba000@gmail.com

ABSTRACT

Exposure to sunlight causes melanin accretion, oxidative stress, and generations of free oxygen radicals. Therefore, using sun creams rich in natural antioxidants is common to protect the skin from direct sunlight contact. *Nigella sativa* (NS) and *Senna alexandrina* (SA) are medicinal plants with numerous health benefits and therapeutic effects due to their antioxidant and anti-inflammatory properties. This experiment was conducted to examine the antioxidant and whitening (antityrosinase) effects of NS and SA from Sudan *in vitro*. Hydro-methanol extracts (methanol: distilled water, 4:1 v/v) of NS and SA were made and partitioned into five fractions (n-hexane, chloroform, ethyl acetate, n-butanol, and water) to investigate the antioxidant effects, diphenylpicrylhydrazyl (DPPH) free radical scavenging assay and to explore the whitening effects, tyrosinase inhibition activity. The most potent antioxidant potential was shown by ethyl-acetate fractions of both plant extracts, which reduced the scavenging activity by 79.3 and 53.0%, respectively. In addition, the best whitening effect was revealed by chloroform and n-hexane fractions of SA and NS extracts, with 86.0 and 93.0% inhibitory activity of tyrosinase, respectively. The outcome is comparable to the existing theory that antioxidants of natural origin can reduce free radicals and potentiate the whitening effects. Nonetheless, more studies are recommended to confirm its efficacy using animal models and elucidate any side effects.

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INTRODUCTION

Medicinal plants have been extensively used to treat skin problems **(Bouasla and Bouasla, 2017; Madzinga *et al.*, 2018)**. *Nigella sativa* (NS) —an ubiquitous medicinal plant— has been confirmed to have several benefits, including anti-inflammatory, antioxidant or free radical scavenging properties, antifungal, and immunomodulatory activities **(Ahmad *et al.*, 2013, Kotb *et al.*, 2018)**. In addition, *Senna alexandrina* (SA), which grows in different continents, showed therapeutic activities, like antioxidant effects, and is widely used to treat respiratory distress and skin wounds **(Morales *et al.*, 2009; Moglad *et al.*, 2012)**.

Free radicals are defined as any molecular species that can exist independently and contain an unpaired electron in an atomic orbital, such as hydroxyl peroxide, superoxide anion, and odd-valence oxygen that are considered reactive oxygen species (ROS) **(Santa-Maria *et al.*, 2010; Wagemaker *et al.*, 2017)**. Accumulation of these ROS in the skin results in an inflammatory process that manifests as wrinkling, keratinization, and ageing of the skin's superficial layers **(Santa-Maria *et al.*, 2010; Adam *et al.*, 2016)**. Besides, hyperpigmentation due to exposure to ultraviolet (UV) is another major problem experienced by many people around the world, especially in tropical areas **(Ephrem *et al.*, 2017)**. Once the skin is exposed to UV, an enzyme named tyrosinase is activated and hydroxylates tyrosine to a substance called dihydroxyphenylalanine (DOPA), which is oxidized to quinine which in turn produce melanin **(Jin *et al.*, 2018)**. Suppression of tyrosine prevents melanin production, and hence, no black pigmentation or even whitening effects may develop **(Jin *et al.*, 2018)**.

1,1-diphenyl picrylhydrazyl (DPPH) radical is an odd-number valence influential free radical that is widely used to evaluate the antioxidant potential in

vitro assays for its simplicity and affordable price **(Eklund *et al.*, 2005; Hamlaoui *et al.*, 2018)**. The reaction is based on the ability of DPPH to accept an H molecule from the scavenger substance **(Mishra *et al.*, 2012)**. The violet DPPH is reduced into a yellowish DPPH-H **(Mishra *et al.*, 2012)**. The colour change can be read by a spectrophotometer **(Mishra *et al.*, 2012)**. Therefore, DPPH can be utilized to investigate medicinal plants fighting ROS in an attempt to find natural antioxidants **(Liu *et al.*, 2018; Vora *et al.*, 2018)**.

$$\% \text{ inhibition of tyrosinase} = \frac{\text{OD of Control} - \text{OD of the test}}{\text{OD of control}} * 100$$

The antioxidant and whitening effects of NS and SA from Sudan has not been investigated previously. Therefore, this experiment aimed to investigate the antioxidant and whitening effects of different organic fractions of hydro-methanol extracts of NS seeds and SA leaves from Sudan *in vitro*.

MATERIALS AND METHODS

Reagents:

$$\% \text{ inhibition of DPPH radical} = \frac{\text{OD of Control} - \text{OD of the test}}{\text{OD of control}} * 100$$

The experiment's ingredients included L-tyrosine, L-DOPA, mushroom tyrosinase, and DPPH and were obtained from Sigma (St. Louis, MO, USA).

Medicinal Plant:

NS seeds and SA leaves were obtained from Sudan and were confirmed at Woosuk University, South Korea. The plant parts were washed with running tap water, air-dried, homogenized to a fine powder, and stored in air-tight containers at 40°C until used.

Preparation of extracts:

Twenty-five grams of dried powder of the plants was extracted with 250 ml of aqueous methanol (methanol: distilled water, 4:1 v/v) for 48 h with occasional shaking and filtered through Whatman filter paper. The resulting residue was extracted twice with the same fresh solvent, and the filtrate was concentrated using a rotary vacuum evaporator at 30–40°C and pressure. Finally, the brown grease material was suspended in water and successfully partitioned with n-hexane, chloroform, ethyl acetate, n-butanol, and residual aqueous portions **(Hossain *et al.*, 2014)**, as shown in the workflow diagram in Figure 1.

Antioxidant assays:

To test the antioxidant effects is frequently tested *in vitro* **(Chaves *et al.*, 2020)**. DPPH radical scavenging activities of NS and SA extracts were determined using Mensor and Meneze **(Mensor *et al.*, 2001)**. Five microliters of NS and SA fractions were mixed with 195 microliters of 0.1 mM DPPH dissolved in methanol. The mixture was incubated at room temperature in the dark for 20 minutes until a purple colour was noticed. A mixture containing all the reagents except a test sample was used as a control. The DPPH radical scavenging capacity was determined by measuring the absorbance at 520 nm using a spectrophotometer. The percentage of inhibition was calculated as follows:

Whereas OD is optical density.

Mushroom Tyrosinase Inhibitor Assay:

The whitening effect is examined by reducing the activity of tyrosine *in vitro* **(Park *et al.*, 2020)**; tyrosinase activity was determined according to the method of Lee *et al.* **(Lee *et al.*, 2003)**. Briefly, a 50 mM potassium phosphate buffer (pH 6.8) containing 20 mM L-tyrosine and 125 U/mL mushroom tyrosinase was used. The reaction mixture with the corresponding solvents (without plant material) served as a control. The percentage of tyrosinase inhibition was measured at 492 nm by a spectrophotometer. The percentage of tyrosinase activity was calculated as follows:

Whereas OD is the optical density.

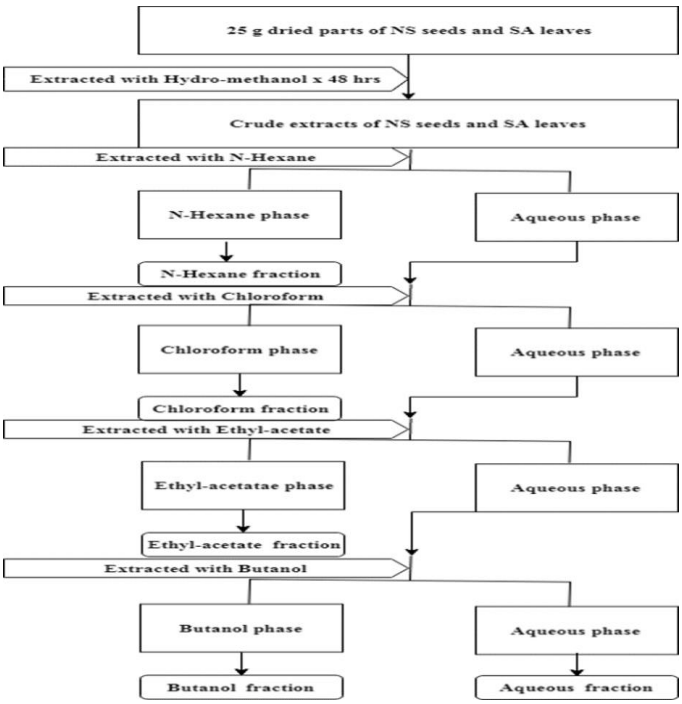

Fig.1: A flow diagram for the extraction of NS and SA fractions by decreasing the order of solvent polarity.

RESULTS

Antioxidant activity:

The DPPH radical scavenging potentials of the different fractions of NS and SA extracts are listed in

Table 1. All fractions showed an inhibitory potential against the DPPH free radical. The inhibitory percentages of the fractions of NS extract varied from 46.4± 0.053% to 79.3% ± 0.011% for butanol and ethyl acetate fractions, respectively, with the latter being the highest among other fractions. The fractions of SA extract showed different inhibitory percentages and ranged from 30.6±0.005% to 53±0.005% for butanol and ethyl acetate fractions. In general, fractions of NS extract showed higher levels of inhibition than fractions from SA extract. Butanol fractions showed the lowest level of inhibition in both extracts, whereas ethyl acetate showed the highest one. This result suggests that NS possesses antioxidant properties.

Table 1: Antioxidant activity of different fractions of NS and SA extracts determined as DPPH radical scavenging

Plant extract	Fraction	DPPH-scavenging activity %
NS extract	N-hexane	67.40±0.05
	Chloroform	50.60±0.07
	Ethyl acetate	79.30±0.01
	Butanol	46.40±0.05
	Water	74.00±0.06
SA extract	N-hexane	48.50±0.01
	Chloroform	34.00±0.05
	Ethyl acetate	53.00±0.05
	Butanol	30.60±0.05
	Water	51.00±0.14

Values are presented as mean ±standard deviation of samples analyzed in triplicate. NA, *Nigella sativa*; SA, *Senna alexandrina*; DPPH, diphenylpicrylhydrazyl.

Tyrosinase inhibitor:

Table 2 represents the percentage of tyrosinase inhibitory potential of fractions of NS and SA extracts. The results showed that all the fractions had a reductive activity. For fractions of NS extract, the inhibitory percentages ranged from 71.5±0.006% to 86±0.013% for ethyl acetate and n-hexane fractions. Similarly, fractions of SA extracts showed various inhibitory percentages which range from 16.4±0.073% to 92.8±0.117% for butanol and ethyl acetate fractions. Essentially, fractions of SA extract showed higher levels of inhibition than fractions of NA extract. The N-hexane fractions of both extracts showed the highest level of inhibition.

Table 2: Tyrosinase inhibition by different fractions of NS and SA extracts:

Plant extract	Fraction	Inhibition of tyrosinase %
NS extract	N-hexane	86.00±0.02
	Chloroform	81.50±0.05
	Ethyl acetate	71.50±0.06
	Butanol	80.20±0.02
	Water	76.80±0.14
SA extract	N-hexane	92.80±0.18
	Chloroform	93.00±0.14
	Ethyl acetate	92.50±0.05
	Butanol	16.40±0.08
	Water	89.50±0.17

Values are presented as mean ±standard deviation of samples analyzed in triplicate. NA, *Nigella sativa*; SA, *Senna alexandrina*.

DISCUSSION

The antioxidant activity of NS is due to thymoquinone (TQ), which is the main active component of NS [19]. The beneficial medicinal effects of TQ have been attributed to their radical scavenging (i.e., anti-oxidative) activity and their ability to inhibit the production of 5-lipoxygenase products during inflammation **(Alenzi 2013)**. In this study, it was found that the antioxidant properties of SA are almost similar to those of *S. velutina* and *S. mascranthera*, for which antioxidant and anti-inflammatory activities have been reported **(Campos *et al.*, 2016)**. Moreover, it has been shown that NS is widely used for its qualities, but SA is widely used as a therapy for constipation and skin wound **(Viswanathan and Nallamuthu 2012)**.

However, SA showed a higher percentage of inhibition than NS. The result is in good agreement with a former report where NS tyrosinase inhibitory activity was found to be 49.6±2.08% **(Subramanian and Sahithya 2016)**. This result was partially unexpected as hyperpigmentation is a sequel of skin inflammation, so NS, a potent antioxidant, was awaited to hold the whitening effects. People, probably, for this reason, use NS oil **(Siddiqui *et al.*, 2020)** rather than NS seed extract, *per se*. Importantly, SA was expected to show whitening effects since it is generally used to treat skin wounds.

No previous studies investigated the tyrosinase inhibition of SA to our knowledge. Nevertheless, a study on *Cassia fistula* showed no inhibitory activities against tyrosinase **(Sungthong and Phadungkit 2015)**. This inconsistency might be due to species dissimilarity or the source of the plant. Jeong *et al*. concluded that hydro-ethanol extract is the most effective solvent showing high antioxidant ability **(Seo *et al*., 2014)**. Limitations of the experiment include that NS and SA extracts were neither investigated pharmacognostically nor the active components were chromatographed. Despite this, our results can be a ground for future investigations.

CONCLUSION

The results revealed that, the ethyl acetate fraction of NS extract and chloroform fraction of SA extract exhibited the best antioxidant effect and whitening potential, respectively. The antioxidant effect is closely associated with reducing free radicals, making it a focus for anti-inflammatory studies. Similarly, the tyrosinase inhibitor is thought to have skin-lightening properties. As a result, AS is a promising candidate for anti-ageing research. Further studies employing animal models are recommended to confirm the efficacy of NS and SA in oxidative stress hyperpigmentation and figure out any adverse effects.

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