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Comparative chemical investigation and evaluation of antioxidant and tyrosinase inhibitory effects of *Withania somnifera* (L.) Dunal and *Solanum nigrum* (L.) berries

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In the present study, berries of two different species of Solanaceae family, *Withania somnifera* (WS) and *Solanum nigrum* (SN), were extracted in methanol and then fractionated with solvents, ranging from non-polar to polar, for their phytochemical profiling and investigation of antioxidant and tyrosinase enzyme inhibition capacity. The methanolic extract and *n*-hexane, ethyl acetate (WSEA, SNEA) and aqueous fractions were chemically analyzed and evaluated for biological activity. Total flavonoids and total phenolics were quantified in WSEA (96.91 ± 1.56 μg QE mg^{-1} sample and 178.45 ± 2.78 μg GAE mg^{-1} sample, resp.) and SNEA (89.58 ± 0.98 μg QE mg^{-1} sample and 120.15 ± 2.33 μg GAE mg^{-1} sample, resp.). HPLC-DAD analysis of ethyl acetate fractions of WS and SN measured 13.74 and 5.34 μg GAE mg^{-1} dry fraction and 3.72 and 3.41 μg QE mg^{-1} dry fraction, resp. WSEA and SNEA fractions showed the highest 2,2-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging, total antioxidant capacity and iron reducing power activity. The highest inhibition of tyrosinase enzyme was also exhibited by WSEA and SNEA (59.6 and 58.7 %) resp. This investigation justifies the medicinal value of *W. somnifera* and *S. nigrum* berry extracts as potential and readily available sources of natural antioxidants. Marked tyrosinase enzyme inhibition activity and antioxidant activity of both plant extracts might be due to polyphenols and flavonoids.

Keywords: *Withania somnifera*, *Solanum nigrum*, polyphenols, antioxidant, tyrosinase inhibition, HPLC-DAD

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Over the centuries, herbs have been used in medicine and regarded as excellent sources of nutrients and minerals for the human body (1). They may also act as natural antioxidant sources since they scavenge reactive oxygen species (ROS) (2). Oxygen free radicals (superoxide radicals, hydroxyl radicals) and oxygen non-free radicals (H_2O_2, O_2) are accountable

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for metabolic injury and aging (3). The human cell metabolic activity is affected by the actions of these free radicals, which enhance not only indigenous oxygen concentration but produce peroxides (4) and activate lipid peroxidation (5). Our body synthesizes various types of endogenous antioxidant enzymes (6) such as catalase, superoxide dismutase and glutathione peroxidase, *etc.* (7). Naturally occurring antioxidants in vegetables, spices and herbs show disease preventing abilities (8, 9). Among various phytochemicals, polyphenols are the most important group of compounds, which protect the plant against harmful UV radiation, pathogens and herbivores (10), and also exercise antioxidant activities that might influence enzymes and cell receptors (11).

The aim of the present study was chemical analysis of the extracts/fractions of *S. nigrum* and *W. somnifera* berries, along with evaluation of their antioxidant capacity and inhibition of tyrosinase activity. According to "QianJin Yi Fang" and "WaiTai MiYao" of ancient Chinese traditional medicine, crude drugs have been used to treat hyperpigmentation disorders such as melasma and ephelides. However, the exact biological mechanisms by which crude drugs exert their actions have not been well explained before (12). To the best of our knowledge, no scientific validation of berries of *W. somnifera* and *S. nigrum* as tyrosinase inhibitors has been reported to date.

EXPERIMENTAL

Chemicals and equipment

Methanol, ethyl acetate and *n*-hexane were obtained from Merck (Germany). 2,2-Diphenyl-1-picrylhydrazyl (DPPH), mushroom tyrosinase, L-DOPA, kojic acid, gallic acid, quercetin, rutin, catechin, apigenin, myricetin, kaempferol, ascorbic acid and caffeic acid were purchased from Sigma Aldrich (USA).

An electrical balance (Precisa BJ-210, Switzerland), a rotary evaporator (Heidolph, Co. Ltd., Japan), a microplate reader Synergy HT (BioTek Instrument, USA), a UV spectrophotometer (UV 4000 ORI, Germany) and a high performance liquid chromatograph with diode array detector (HPLC-DAD, Agilent, Germany) were used.

Plant samples

Ripe berries of *Solanum nigrum* (SN) and *Withania somnifera* (WS) were collected in the vicinity of Baghdad-ul-jadeed campus of the Islamia University of Bahawalpur, Pakistan, in March 2016. Certified specimens of dried plants were deposited in the herbarium of the Islamia University of Bahawalpur, Pakistan. Berries were selected based on uniformity in shape and color criteria, washed in running tap water, separated manually, shade dried and powdered using a blender. Moisture content was found to be 61.1 and 72.2 % for berries of *W. somnifera* and *S. nigrum*, resp.

Extraction

Dried powder (300 g) of berries of each plant was extracted for 72 h in a rotary shaker with 1500 mL methanol. The extract was filtered through Whatman No. 1 filter paper and evaporated to dryness using a rotary evaporator. Dried extract was subjected to fraction-

ation with several organic solvents in the increasing order of polarity. The obtained samples were designated WSH/SNH, WSEA/SNEA, WSM/SNM, WSW/SNW for *n*-hexane, ethyl acetate, methanol and water fractions of *W. somnifera* and *S. nigrum*, resp.

Phytochemical analysis

Extracts/fractions of *W. somnifera* and *S. nigrum* berries were checked with the aid of various phytochemical tests. The protocol of Hossain *et al.* (13) was exercised for detection of phenols, coumarin and saponins. The methodology described by Ramu *et al.* (14) was employed to detect flavonoids, tannins, terpenoids and alkaloids.

Total phenolics and total flavonoids. – Total flavonoid content (TFC) in *W. somnifera* and *S. nigrum* berries was determined by mixing 0.3 mL of each fraction/extract with 0.15 mL of 0.5 mol L^{-1} $NaNO_2$, followed by the addition of 0.1 mL of 0.3 mol L^{-1} $AlCl_3 \times 6H_2O$ and 3.4 mL of 30 % methanol (15). Absorbance was recorded at 506 nm. TFC was expressed as μg quercetin equivalents (QE) mg^{-1} of dry extract/fraction.

Total phenolic content (TPC) was evaluated by a slight modification of the reported method (16). To a mixture containing 1 mg of each fraction/extract in 9 mL water, 1 mL of Folin-Ciocalteu reagent was added and mixed thoroughly for 5 min. Then 10 mL of 7 % Na_2CO_3 were added, followed by the addition of water to a final volume of 25 mL. The mixture was then incubated for 90 min at room temperature and absorbance was read at 750 nm, and TPC was expressed as μg of gallic acid equivalents (GAE) mg^{-1} of dry extract/fraction.

HPLC analysis. – In view of the marked antioxidant activity of ethyl acetate fractions of *W. somnifera* and *S. nigrum* berries, these were selected for HPLC-DAD analysis with the Zorbax Rx-C8 (Agilent, USA) analytical column (250 × 4.6 mm, pore size 80 Å, particle size 5 μm) by the method of Majid *et al.* (17). Mobile phase was composed of eluent A (acetonitrile/methanol/water/acetic acid, 5:10:85:1) and eluent B (acetonitrile/methanol/acetic acid, 40:60:1). The gradient was as follows: 0–20 min (0 to 50 % B), 20–25 min (50 to 100 % B) and then isocratically 100 % B (25–40 min), at ambient temperature. Flow rate was 1.2 mL min^{-1} and the volume of injection was 20 μL. Each sample was filtered through a membrane filter of pore size 0.45 μm before injection. Among the standards, rutin was analyzed at 257 nm, gallic acid and catechin at 279 nm, caffeic acid and apigenin at 325 nm, quercetin, myricetin and kaempferol at 368 nm.

Antioxidant activity

1,1-Diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity. – To estimate the DPPH radical scavenging activity of berry fractions/extracts, we followed the methodology of Majid *et al.* (17). Stock solution was made by dissolving 24 mg of DPPH in 100 mL of methanol and was kept at 20 °C. About 3 mL of DPPH from the stock solution was added to samples (1 mg mL^{-1}) in test tubes and incubated at room temperature for 15 min. Absorbance was recorded at 517 nm with ascorbic acid as a standard.

Total antioxidant capacity (phosphomolybdenum assay). – The basic principle of the phosphomolybdate assay is the reduction of molybdenum(VI) to molybdenum(V) by antioxi-

dant species; the reduced form of molybdenum forms a complex with phosphate in acidic medium and at raised temperature to impart a dark green color to the final solution (18). To observe the total antioxidant activity of plant samples, the reported protocol of Umamaheswari and Chatterjee (19) was followed. Thus, 1 mL of the reagent solution (prepared by dissolving 28 mmol L^{-1} Na_3PO_4 in 0.6 mmol L^{-1} H_2SO_4 with 4 mmol L^{-1} ammonium molybdate) was blended with 0.1 mL of each plant sample. The samples were incubated for 90 min at 95 °C over a water bath. Absorbance was read at 760 nm with gallic acid as a standard.

Total reducing power assay. – The procedure of Siddhuraju *et al.* (20) was used. Briefly, 2 mL of the fraction/extract of plant berries (4 mg mL^{-1}) was added into 2 mL of 0.2 mol L^{-1} phosphate buffer (pH 6.6) and 2 mL of potassium ferricyanide (10 mg L^{-1}). After incubation for 20 min at 50 °C, the mixture was blended with 2 mL of trichloroacetic acid (100 mg L^{-1}), dilution was made with water (2 mL) and 0.1 % $FeCl_3$ (0.4 mL) in 2 mL of reaction mixture. Absorbance was measured after 10 min at 700 nm with gallic acid as a standard.

Mushroom tyrosinase enzyme inhibition activity

Tyrosinase enzyme inhibition assay was performed by exercising Kim's protocol (21). Briefly, an aqueous solution containing 1 mol L^{-1} L-DOPA and 50 mmol L^{-1} phosphate buffer (pH 6.5) mixed with 20 μL mushroom tyrosinase (1000 units) was incubated for 30 min at 25 °C; absorbance was taken using a microplate reader at 492 nm.

Statistical evaluation

One-way ANOVA was applied to analyze the variability among various parameters using Statistix 8.1. All data was presented as mean ± SD. Correlation studies between antioxidant assays and TFC and TPC were done with GraphPad Prim 5 applying Pearson's correlation coefficient. Tukey's multiple comparison test was used to confirm significant differences between the groups at a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Phytochemical grouping

In the current research, the presence of phenols, flavonoids, coumarins, saponins, tannins, terpenoids and alkaloids was confirmed (Table I). Qualitative analysis was performed using solvents of varying polarity. Phytochemical analysis showed the presence of phenols, tannins and flavonoids in all extracts/fractions of berries of both plants, except in WSH and SNH. Coumarins were present in WSM and WSEA while absent in other fractions of *W. somnifera*. However, coumarins were present in all samples of *S. nigrum* except in the *n*-hexane fraction. The presence of saponins was observed in WSM and WSEA only but not in other fractions of *W. somnifera*, and also in all fractions of *S. nigrum* except in the methanolic one. Terpenoids were present in all extracts/fractions of both plants except for SNEA and SNW. Alkaloids were only detected in WSM, SNM and WSEA.

Table I. Qualitative screening of W. somnifera *and* S. nigrum *berries*

Constituents	*W. somnifera*				*S. nigrum*			
	WSH	WSEA	WSM	WSW	SNH	SNEA	SNM	SNW
Phenols	–	+++	+++	+	–	+++	+++	+
Flavonoids	–	+++	+++	+	–	+++	+++	+
Coumarin	–	+	++	–	–	++	++	+
Saponins	–	+	+	–	–	–	+	–
Tanins	–	++	++	+	–	+++	++	+
Terpenoids	+	+	++	+	+	–	++	–
Alkaloids	–	+	+	–	–	–	+	–

WSH – *W. somnifera n*-hexane fraction, WSEA – *W. somnifera* ethyl acetate fraction, WSM – *W. somnifera* methanolic extract, WSW – *W. somnifera* water fraction, SNH – *S. nigrum n*-hexane fraction, SNEA – *S. nigrum* ethyl acetate fraction, SNM – *S. nigrum* methanolic extract, SNW – *S. nigrum* water fraction

Table II. Analysis of total phenolic and total flavonoid contents in W. somnifera *and* S. nigrum *berries using different non-polar and polar solvents*

Extract/fraction	TFC (μg QE mg^{-1} dry sample)	TPC (μg GAE mg^{-1} dry sample)
WSH	12.41 ± 0.30^{g}	27.14 ± 2.03^{h}
WSEA	96.91 ± 1.56^{a}	178.45 ± 2.78^{a}
WSM	63.75 ± 0.45^{d}	165.53 ± 2.03^{b}
WSW	19.75 ± 0.16^{e}	81.30 ± 1.23^{e}
SNH	13.92 ± 0.13^{f}	35.99 ± 0.78^{g}
SNEA	89.58 ± 0.98^{b}	120.15 ± 2.33^{c}
SNM	68.75 ± 0.21^{c}	88.76 ± 1.56^{d}
SNW	20.25 ± 0.17^{e}	67.45 ± 0.67^{f}

GAE – gallic acid equivalent, QE – quercetin equivalent, TPC – total phenolic content, TFC – total flavonoid content. For other acronyms see Table I. Each value is given as mean ± SD ($n = 3$). Means with different superscripts (a-h) in the column are significantly ($p < 0.05$) different from one another.

Total phenolic and flavonoid content

Total phenolic and flavonoid contents of berries were calculated on the basis of standard regression lines for gallic acid ($y = 0.013x + 0.061$, $R^2 = 0.999$) and quercetin ($y = 0.006x + 0.052$, $R^2 = 0.999$), respectivelly (Table II). Ethyl acetate fractions of the methanolic extract of berries had higher phenolic and flavonoid contents compared to the other studied fractions.

Maximum quantity of TPC was found in WSEA (178.45 ± 2.78 μg GAE mg^{-1} dry sample) and SNEA (120.15 ± 2.33 μg GAE mg^{-1} dry sample) while the lowest quantities were found in WSH (27.14 ± 2.03 μg GAE mg^{-1} dry sample) and SNH (35.99 ± 0.78 μg GAE mg^{-1} dry sample). Similarly, WSEA (96.91 ± 1.56 μg QE mg^{-1} dry sample) and SNEA (89.58 ± 0.98 μg QE mg^{-1} dry sample) were rich in flavonoids, while a minute flavonoid quantity was detected in WSH (12.41 ± 0.30 μg QE mg^{-1} dry sample) and SNH (13.92 ± 0.13 μg QE mg^{-1} dry sample). Our findings are analogous to those of Anagnostopoulou *et al.* (22), who reported that the ethyl acetate fraction of the methanolic extract of *Citrus sinensis* was richer in phenolic compounds than the other fractions analyzed.

HPLC-DAD analysis of ethyl acetate fractions

HPLC-DAD profiles of ethyl acetate fractions of *W. somnifera* and *S. nigrum* berries and of the standards are displayed in Figs. 1, 2 and 3, resp., and presented in Table III. HPLC analysis quantified high gallic acid contents (13.74 μg mg^{-1} dry fraction), followed by apigenin and kaempferol in WSEA, while high caffeic acid contents (14.09 μg mg^{-1} dry fraction) followed by gallic acid, kaempferol and quercetin were detected in SNEA. HPLC results correlated well with the antioxidant activity and TPC and TFC assays.

Antioxidative activity

DPPH radical scavenging activity. – Moderate to low DPPH radical-scavenging activity was exhibited by all the extracts/fractions studied (Table IV). Minimum concentrations exerting 50 % inhibition were observed for WSEA (104.3 mg mL^{-1}) and WSM and SNEA (149.4 mg mL^{-1}), while SNH showed the highest value (> 1000 μg mL^{-1}). Overall, the samples

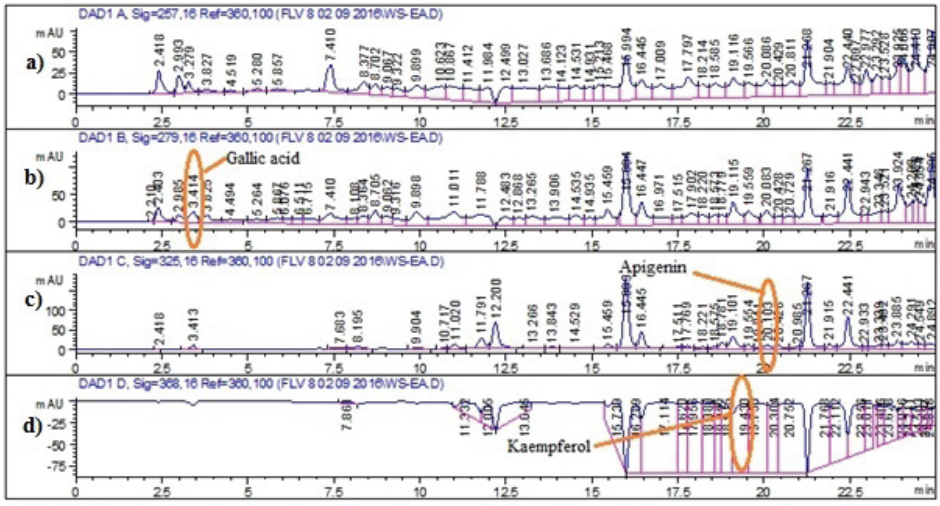

Fig. 1. HPLC-DAD profile of WSEA at different wavelengths: a) 257 nm, b) 279 nm, c) 325 nm, and d) 368 nm.

followed the following order of increasing 50 % inhibitory values: WSEA < SNEA < WSM < SNM < WSW < WSH < SNW < SNH. The DPPH radical scavenging activity of *W. somnifera* extract/fractions showed significant correlations with TPC (R^2 = 0.9903, p < 0.01) and TFC (R^2 = 0.9335, p < 0.01), as well as *S. nigrum* with TPC (R^2 = 0.9296, p < 0.01) and TFC (R^2 = 0.9851,

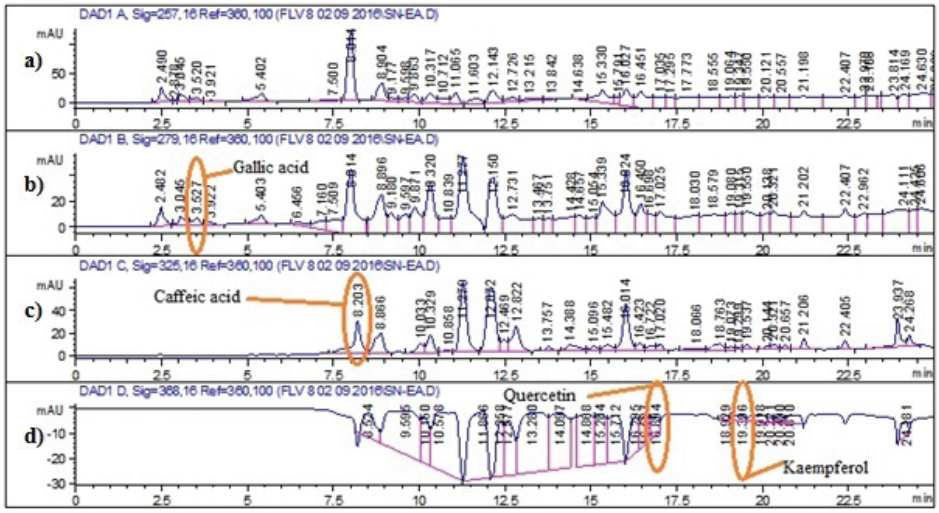

Fig. 2. HPLC-DAD profile of SNEA at different wavelengths: a) 257 nm, b) 279 nm, c) 325 nm, and d) 368 nm.

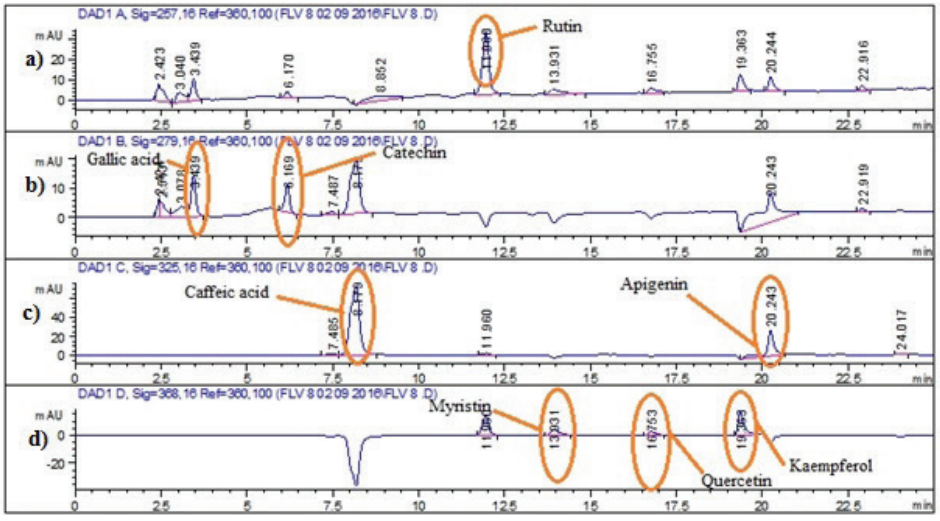

Fig. 3. HPLC-DAD profiles of standards at different wavelengths: a) 257 nm, b) 279 nm, c) 325 nm, and d) 368 nm.

Table III. HPLC-DAD analyses of SNEA and WSEA

Flavonoids/phenols	Detection wavelength (nm)	Content (μg mg^{-1} dry fraction)	
		WSEA	SNEA
Gallic acid	279	13.74 ± 1.91^{a}	5.34 ± 1.13^{b}
Caffeic acid	325	ND	14.09 ± 2.21^{a}
Quercetin	368	ND	2.77 ± 0.74^{c}
Kaempferol	368	3.72 ± 1.12^{c}	3.41 ± 0.93^{bc}
Apigenin	325	5.74 ± 1.21^{b}	ND
Rutin	257	ND	ND
Catechin	279	ND	ND
Myricetin	368	ND	ND

ND0 – not detected, SNEA, WSEA – *S. nigrum* and *W. somnifera* berry ethyl acetate fractions. Each value is presented as mean ± SD (n = 3). Means with different superscripts (a-c) in the column indicate a statistically significant ($p < 0.05$) difference from one another.

Table IV. DPPH radical scavenging activity of W. somnifera *and* S. nigrum *berries*

Sample	Concentration for 50 % DPPH inhibition (μg mL^{-1})
WSH	495.5 ± 5.7^{f}
WSEA	104.3 ± 4.9^{b}
WSM	149.4 ± 9.2^{c}
WSW	372.3 ± 11.3^{e}
SNH	> 1000
SNEA	149.4 ± 7.4^{c}
SNM	291.2 ± 8.2^{d}
SNW	924.9 ± 12.5^{g}
AA	59.93 ± 3.7^{a}

AA – ascorbic acid. Each value is presented as mean ± SD (n = 3). Means with different superscripts (a-g) in the column indicate significant ($p < 0.05$) differences from one another.

$p < 0.01$) (Table V). The strong antioxidant activity of gallic acid is attributable to its ability to scavenge hydroxyl radicals rather than to the hydrogen-donation mechanism (23). The high antioxidant activity of WSEA and SNEA is justified by the high concentration of polyphenolics in these fractions. In this research, the WSEA fraction exhibited stronger free radical scavenging activity than the SNEA fraction. This might be due to higher gallic acid content in WSEA whereas SNEA contains caffeic acid (Table III). Plant extracts exhibiting higher amounts of gallic acid scavenge more free radicals and protect human cells or tissues from their injurious effects (24). WSEA and SNEA fractions containing phenolics and flavonoids show a good scavenging ability to stabilize DPPH free radicals.

Table V. Correlations between antioxidant activities of W. somnifera *and* S. nigrum *and total phenolic and total flavonoid contents*

Antioxidant activity	R^2			
	W. somnifera		*S. nigrum*	
	TPC	TFC	TPC	TFC
DPPH radical scavenging activity	0.9903^{c}	0.9335^{a}	0.9296^{a}	0.9851^{b}
Total antioxidant activity	0.8891^{a}	0.8104^{NS}	0.9518^{b}	0.9576^{b}
Reducing power assay	0.9729^{b}	0.9258^{a}	0.9422^{b}	0.9265^{a}

TFC – total flavonoid content, TPC – total phenolic content. Significance of linear correlation: $^{a}p < 0.05$), $^{b}p < 0.01$, $^{c}p < 0.001$. NS – not significant.

Total antioxidant capacity. – Analysis of the total antioxidant capacity of various extracts/fractions by the phosphomolybdenum assay was expressed as GAE (mg g^{-1} dry sample), as shown in Fig. 6. Total antioxidant activity was found to increase in the following order: SNH < WSH < SNW < WSW < WSM < SNM < SNEA < WSEA and to have a significant correlation with TPC and TFC, as shown in Table V. Sahreen *et al.* (25) also reported that the ethyl acetate fraction of *Carissa opaca* fruit was the best fraction in the phosphomolybdenum assay.

Reducing power assay. – Potassium ferricyanide reduction method was exercised to determine the reducing power activity of *W. somnifera* and *S. nigrum* berries. In our study, ethyl acetate fractions of both plants showed the highest reducing power (in GAE) of 856.7 and 720.6 mg g^{-1} dry sample resp., as shown in Fig. 7. WSEA and SNEA fractions showed higher reducing capacity compared to other fractions. Duan *et al.* (26) reported that ethyl acetate fraction of *Polysiphonia urceolata* also showed higher reducing power than other

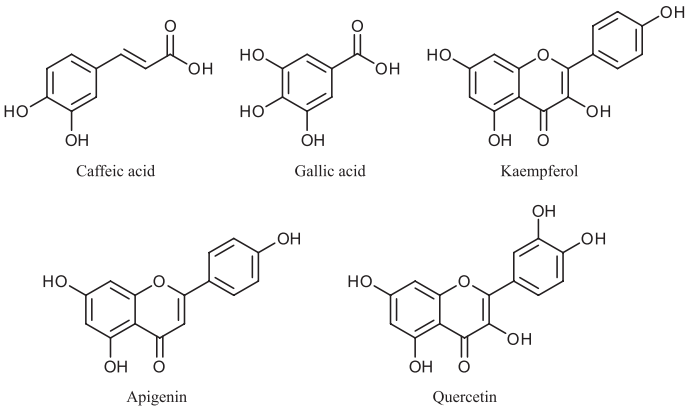

Fig. 4. Structures of caffeic acid, gallic acid, kaempferol, apigenin and quercetin.

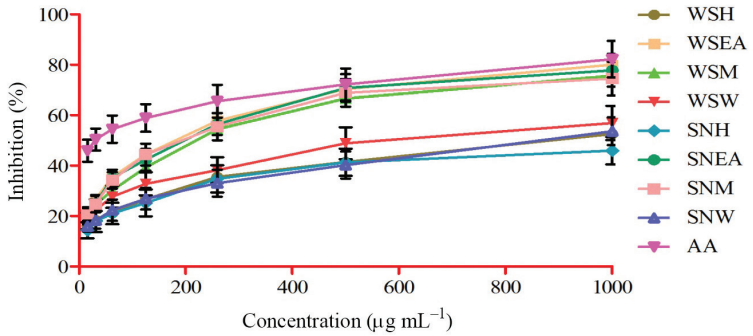

Fig. 5. Antioxidant activity (DPPH) of plant extracts/fractions and ascorbic acid (AA) at different concentrations. For other acronyms, see Experimental. Mean ± SD, $n = 3$.

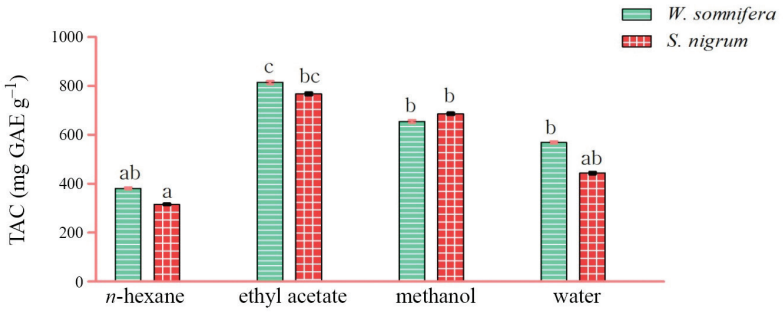

Fig. 6. Total antioxidant capacity (TAC) of *W. somnifera* and *S. nigrum* extracts/fractions (mg GAE g^{-1} extract). Mean ± SD, $n = 3$. Means with different superscripts (a-c) on the bars show significant ($p < 0.05$) differences between groups. GAE – gallic acid equivalent.

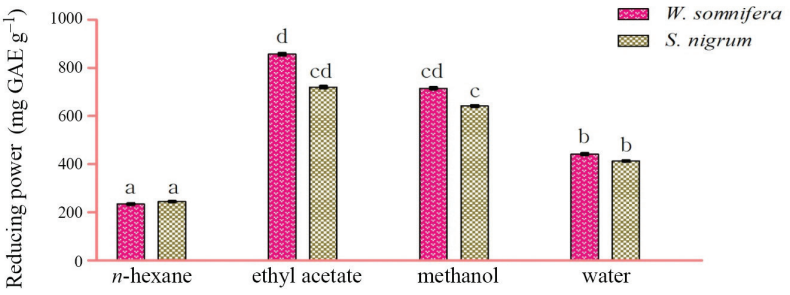

Fig. 7. Reducing power of *W. somnifera* and *S. nigrum* extracts/fractions (mg GAE g^{-1} extract). Mean ± SD, $n = 3$. Means with different superscripts (a-d) on the bars show significant ($p < 0.05$) differences between groups. GAE – gallic acid equivalent.

Table VI. Inhibition of free radicals by W. somnifera *and* S. nigrum *berries (1 mg mL^{-1})*

Sample	Inhibition (%)		
	DPPH radical scavenging	Antioxidant capacity	Reducing power
WSH	52.5 ± 3.4^{d}	38.1 ± 2.8^{g}	23.4 ± 2.1^{f}
WSEA	80.0 ± 4.5^{a}	81.4 ± 5.7^{a}	85.7 ± 4.5^{a}
WSM	75.6 ± 3.9^{b}	65.5 ± 4.8^{d}	71.6 ± 3.4^{b}
WSW	56.9 ± 2.6^{c}	56.9 ± 2.6^{c}	44.2 ± 4.3^{d}
SNH	45.9 ± 2.4^{e}	31.6 ± 2.9^{h}	24.6 ± 3.2^{f}
SNEA	77.8 ± 5.8^{a,b}	76.8 ± 5.3^{b}	72.1 ± 5.1^{b}
SNM	74.5 ± 4.1^{b}	68.6 ± 4.4^{c}	64.2 ± 4.8^{c}
SNW	53.6 ± 3.3^{d}	44.4 ± 3.6^{f}	41.3 ± 4.1^{e}

Each value is presented as mean ± SD (n = 3). Means with different superscripts (a-h) in the column are significantly ($p < 0.05$) different from one another.

fractions. The reducing activity showed a significant correlation with both TPC (R^2 = 0.9729, $p < 0.01$) and TFC (R^2 = 0.9258, $p < 0.01$) for *W. somnifera* and TPC (R^2 = 0.9422, $p < 0.01$) and TFC (R^2 = 0.9265, $p < 0.01$) for *S. nigrum* (Table V). The statement is supported by Chen and Ahn (27), who expressed the idea that phenolic compounds such as caffeic acid, quercetin, rutin and catechin have admirable reducing power for ferric ion.

In summary, ethyl acetate fractions of both *W. somnifera* and *S. nigrum* at a concentration of 1 mg mL^{-1} showed the highest DPPH radical scavenging of 80.0 ± 4.5 and 77.8 ± 5.8

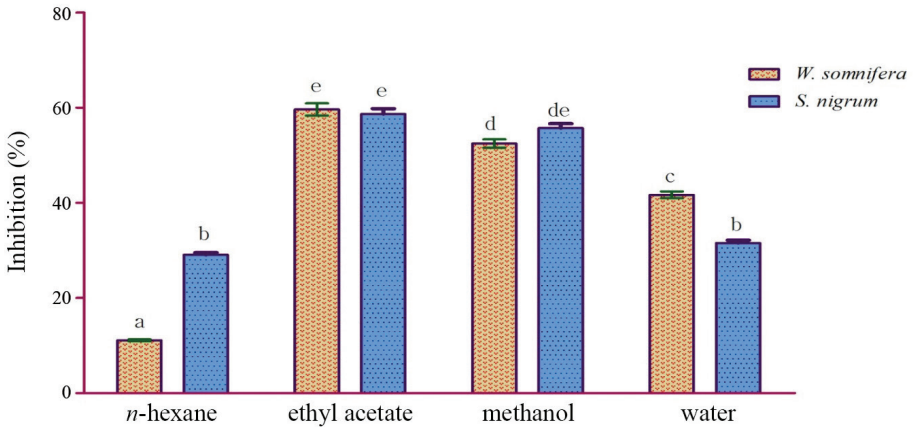

Fig. 8. Tyrosinase inhibition of berries of *W. somnifera* and *S. nigrum* extracts/fractions (1 mg mL^{-1}). Mean ± SD, n = 3. Means with different superscripts (a-e) on the bars show significant ($p < 0.05$) differences between groups.

%, total antioxidant capacity of 81.4 ± 5.7 and 76.8 ± 5.3 % and 85.7 ± 4.5 and 72.1 ± 5.1 % of ferric reducing antioxidant power, followed by the methanolic extract. *n*-hexane fraction showed the lowest antioxidant activity, as shown in Table VI.

Inhibitory activity of mushroom tyrosinase

Flavonoids showed a capability of tyrosinase inhibition by an active site chelation mechanism (28). In the current study, WSEA and SNEA fractions exhibited anti-tyrosinase activity greater than other fractions at a concentration of 1 mg mL^{-1}, *i.e.*, 59.6 and 58.7 % resp. (Fig. 8). HPLC profiling showed the presence of kaempferol and quercetin as major flavonoids in ethyl acetate fractions of herbal berries. These flavonoids, bearing a 3-hydroxy-4-keto group, may competitively inhibit tyrosinase activity by copper ion chelation at the enzyme active site (10).

CONCLUSIONS

In the current research, various polar and non-polar solvents were exercised to extract the different compounds from the berries of *W. somnifera* and *S. nigrum.* The study showed that the fractions with high phenolic and flavonoids contents ensured excellent antioxidant and anti-tyrosinase activity. Furthermore, this is the first report to identify phenolic constituents in ethyl acetate fractions: gallic acid, quercetin, kaempferol and caffeic acid in *S. nigrum* and gallic acid, kaempferol and apigenin in *W. somnifera***.**

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