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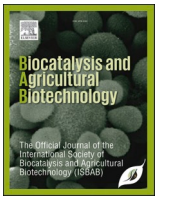

Hemolytic activity and protective potential of *Lamiaceae* plants seed extracts and their bioactive components profiling having potential for functional foods and nutraceutical formulations

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ABSTRACT

This study was designed to examine phenolic profile and bioactivities of two *Lamiaceae* species, *Salvia moorcroftiana* and *Ocimum sanctum*. Hydrolyzed and non-hydrolyzed seed extracts of both species were screened for their hemolytic and mutagenic effects. Both species were found to be nontoxic to red blood cells (cytotoxicity <4%) and selected bacterial strains (mutagenicity <9%), and were evaluated for their anti-hemolytic and anti-mutagenic potentials. The *S. moorcroftiana* presented better protection against standard cytotoxic and mutagenic compounds than *O. sanctum*. Moreover, hydrolyzed extracts of both species depicted superior anti-hemolytic (93.58 ± 2.04% for *S. moorcroftiana* and 90.78 ± 1.88% for *O. sanctum*) and anti-mutagenic potentials (70.24 ± 0.69% for *S. moorcroftiana* and 64.29 ± 0.69% for *O. sanctum*) than those of non-hydrolyzed samples. Chemical characterization by LC-ESI-MS/MS of hydrolyzed extracts of both species indicated the presence of various bioactives such as vanillic acid, quinic acid, gallic acid and myricetin. Hence, it is concluded that both of these species can find potential applications in functional food and nutraceutical formulations.

1. Introduction

Plants are the richest sources of bioactive compounds which have been the components of traditional medicines, nutraceuticals, functional foods and folk medicinal remedies. Nature has created a wide, diverse wealth of plants with different bioactive ingredients for protection of human health against different types of pathogens. Use of plant based natural products in human diet protects human body against several chronic diseases (Ashraf et al., 2022; uz Zaman et al., 2021; Salman et al., 2020; Ashraf et al., 2018).

The different biosynthetic pathways in plant cells lead to a wide variety of bioactive compounds, mainly as secondary metabolites, in response to environmental factors, pathogens and oxidative stress. The medicinal potential of a plant depends upon the combination of chemical compounds, it synthesizes; and their therapeutic potential. Phenolic compounds are the major contributors to potential health benefits as they impart several biological activities like antioxidant, antimicrobial, anti-allergenic, anti-thrombotic, anti-

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artherogenic and cardio-protective potential (Hassan et al., 2017; Kamran et al., 2018).

The application of phytochemicals in functional foods and pharmaceutical industries has been increased tremendously in the recent past. Plants are large reservoirs of natural bioactive compounds including phenolic, vitamins etc. However, sometimes their extraction becomes a problem. Plant tissue culture technique is very useful for production and concentration of secondary metabolites (Nazir et al., 2021a, 2022; Yaqoob et al., 2021).

Lamiaceae (mint family) is a large plant family of dicots, comprising of 252 genera and more than 7200 species. It comprises of many herbs, shrubs and sub-shrubs. Many species of this family have potent health benefits associated with their phenolic contents. Chemical characterization of *Lamiaceae* plants exposed the incidence of terpenoids, phenolic acids, flavonoids and other bioactive compounds. Their extracts and oils are used in formulation of food supplements and herbal products that exhibit strong antioxidant, anti-aging, anti-inflammatory and anti-bacterial properties. Essential oils of some species are also used in perfume industry (Iqbal et al., 2019; Abate, 2019; Nazir et al., 2021b).

Phytochemical characterization represents the presence of different diterpenoids, flavonoids like anthocyanin and chalkiness which are responsible for their anti-fungal, anti-viral, anti-bacterial and anti-microbial properties (Ukpaka and Neo, 2021; Muhammad et al., 2020; Talbaoui et al., 2020; Monajjemi et al., 2020; Gholamhoseinian et al., 2020). *Salvia moorcroftiana*, important medicinal specie of *Lamiaceae* family, is a member of Pakistani flora. It is a perennial, tall herb having sturdy stem which is branched at the base, widely distributed in Kashmir, Chitral, Quetta and Jhelum in Pakistan. Its leaves are applied in the form of paste to heal wounds, itch and guinea-worm. The seeds are prescribed to treat boils, also useful in dysentery, stomach pain and hemorrhoids. The roots are effective to relieve cough and other related diseases (Talbaoui et al., 2020; Gholamhoseinian et al., 2020; Balahbib et al., 2020; Abbas et al., 2022a; Naseer et al., 2020).

O. sanctum (Tulsi) is mostly grown in gardens. It is an aromatic, tall, soft hairy, biennial or triennial herb or under-shrub, sometimes regarded as 'Queen of Herbs' indigenous to South Asia. Its flowers are purplish, strongly accented, considered sacred wherever it found, hence known as 'Holy Basil'. Extracts and oil of *O. sanctum* have been widely used to treat several chronic diseases as they exhibit potential antioxidant, anti-bacterial, anti-cancer, chemo-protective and radio-protective activities (Balahbib et al., 2020). Its leaves are useful to cure bronchitis, hepatic disorders, gastric problems, malaise, dengue fever, cough and cold. Decoction is also a good mosquito repellant. Oil is useful during skin infection and ring worm infection. Roots in the form of decoction function as diaphoretic in malarial fever. Chemical characterization indicates the presence of various phytochemical constituents responsible for its therapeutic potential, which include β-carotene, vitamin C, eugenol, orientin, vivenin, linalool, chavicol, cineole, terpenoids, triterpenoids, tannins and camphor (Abbas et al., 2022a; Naseer et al., 2020; Fahim et al., 2019).

Few studies reported the chemical composition of both of selected species, but seeds of both species are yet to be analyzed. The main objective of this study is to investigate hemolytic, anti-hemolytic, mutagenic and anti-mutagenic potential of both species and their phenolic profile.

2. Materials and methods

Davis-Mingioli salt, hydrochloric acid, phosphate buffer saline (PBS), hydrogen peroxide, Folin–Ciocalteu reagent, *L*-histidine, D-glucose, bromocresol purple and methanol were procured from Sigma Aldrich (USA) while ascorbic acid and gallic acid were obtained from Merck (Germany). All chemicals and reagents used in this study were of highly pure grades.

S. moorcroftiana and *O. sanctum* seeds were obtained from a local herbal shop and samples were recognized and authenticated by Dr. Mansoor Hameed at the Taxonomic Laboratory, University of Agriculture, Faisalabad, Pakistan. Powdered samples were extracted with aqueous methanol (70%, 50% and 30% v/v) using orbital shaker (PA 250/25-H) to get non-hydrolyzed extracts and acidic (HCl) methanol (0.5M, 1.0M and 2.0M) to obtain hydrolyzed extracts (Zhu et al., 2011). Both types of extracts of both species were concentrated and dried using rotary evaporator (BUCHI EL 131).

Phenolics were evaluated following previously described procedure of Albano and Miguel (2011). Each plant extract (1 mg) was added to a mixture of aqueous Na_2CO_3 (7.5%, 2 mL) and Folin & Ciocalteu reagent (10%, 2.5 mL) and kept at ambient conditions for half an hour. After completion of reaction, the mixture was subjected to the measurement of UV/VIS absorbance using spectrophotometer (IRMECO, Germany) at 765 nm. Gallic acid was used as positive control to draw calibration curve and TPC for all extracts were calculated from calibration curve.

Hemolytic screening was carried out by modifying already reported procedure of Ebrahimzadeh et al. (2010). Fresh samples of human blood were centrifuged at 850*g* for 5 min to get erythrocytes as supernatant. The erythrocyte pellet was washed thoroughly with 10 mM phosphate buffer saline (PBS) and diluted to get 4% suspension. To this suspension (2 mL), 1 mL of different concentrations (0.5, 5 and 50 mg/mL) of optimized hydrolyzed and non-hydrolyzed plant extracts in PBS (100–500 μg/mL of PBS) was added and mixture was diluted to 5 mL with PBS. Then, the mixture was incubated for 5 min at ambient conditions. For 100% hemolysis, a parallel experiment was run with 100 μM H_2O_2, without plant extract. For hemolytic cytotoxicity, the reaction mixture was centrifuged at 800*g* for 10 min and lipoprotein membrane dissolution of erythrocytes was quantified in terms of release of hemoglobin into the reaction mixture which, in turn, calculated by measuring the absorbance at 540 nm for oxidized hemoglobin present in the supernatant.

The inhibitory action of plant extracts was measured by adding H_2O_2 solution (0.5 mL) to aliquots having erythrocytes and plant extracts, to carry out oxidative damage of lipid membranes of erythrocytes. The inhibition to hemolysis was measured by the following formula:

$$Inhibition\ (\%) to\ hemolysis = \left(1 - \frac{As}{Ac}\right) \times 100$$

where A_s is the absorbance of aliquots having erythrocytes, plant extract and H_2O_2 while A_c is the absorbance of aliquots having erythrocytes and H_2O_2 with no plant extract. Moreover, IC_{50} (concentration of plant extract required for 50% inhibition of hemolysis) values were also calculated applying linear regression analysis.

Mutagenic and anti-mutagenic potential was assessed following procedure of Razak and Aidoo (2011). Briefly, both types of extracts from each plant species, were mixed separately (in five different glass containers) with distilled water, any of two selected *S. typhimurium* strains (TA98 or TA100), any of two standard mutagens (potassium dichromate $K_2Cr_2O_7$ and sodium azide NaN_3) and reagent mixture (D-glucose, D-biotin, *L*-histidine, bromocresol purple and Davis-Mingioli salt) at concentrations given in Table 1. From each glass container, the mixture (200 μL) was shifted to five different micro titration plates, which then incubated for 3 day at 37 °C with air-tight packing's. All yellow/turbid or off white wells in the micro titration plate were regarded as positive (mutant), while purple wells as negative (no mutation). Number of mutant wells in the 'Background' plate corresponds to spontaneous mutation of selected bacterial strains. A sample can only be regarded as mutagenic when number of mutant wells in the 'Mutagenic test' plate is twice or more than twice the spontaneous mutants. The preventive effects of plant extracts against standard mutagens were evaluated by following the given formula:

$$\text{Antimutagenecity}\ (\%) = 1 - \frac{(\text{Number of mutants in plate 5} - \text{Spontaneous Mutants})}{(\text{Number of mutants in plate 3} - \text{Spontaneous Mutants})}\ \text{x}\ 100$$

Bioactive phenolic compounds of optimized plant extracts of both species were concentrated by solid phase extraction using C-18 cartridges before introducing into LC/MS instrumentation. Purified extract (5 μL) of each plant, was injected into HPLC system (Surveyor auto sampler plus) equipped with C-18 (Phenomenex 250 mm, 5 μm) column. Gradient elution (flow rate 5 mL/min) was carried using a mixture of two solvents A (90:10:0.1% v/v) and B (10:90:0.06% v/v) comprising of different concentrations of water, acetonitrile and trifluoroacetic acid, respectively. Gradients were performed as follows: 10–35% B (0–10 min), 35–42% B (10–20 min) and 42–100% B (20–30 min). Photodiode array (PDA) detector was used to detect separated phenolic which were subsequent analyzed by mass spectrometer (LTQ XL ThermoFisher Scientific) in negative ion mode using Electrospray Ionization (ESI) probe (Yasir et al., 2016). Most phenolic compounds eluted within 30min were found to exhibit their MS peaks in the range of 100–600 m/z. all these compounds were ascertained by their subsequent fragmentation pattern. Fragmentation (MS/MS analysis) was carried out at variable powers of Collision Induced Dissociation (CID). Mass spectrometric data was manipulated and interpreted using a software X-caliber 1.4 (Sadiq et al., 2014). All results were reported as mean ± S.D. of three concordant experiments. All statistical analysis was carried out on Minitab 2000 software (Minitab Inc. Pennsylvania, USA).

3. Results and discussion

3.1. Extract yield

The results for extract yield (g/100 g dry extract) are presented in Table 2. The differences in extraction yield may be due to different polarities and acidic strength of extracting solvents. Acidified methanol produced improved extract yield as compared to aqueous methanol which might be due to decomposition of plant cellular structures, liberating high concentrations of bound phytochemicals. Extraction with aqueous methanol also depicted variations in extract yield which is associated with varying concentrations of methanol.

Results indicated that, in case of hydrolyzed extraction, as the strength of acid was increased the extract yield was also increased (47.8 ± 0.1% for 0.5*M* acidified methanol and 50.2 ± 0.4% for 2.0*M* acidified methanol) that can be explained by the greater dissolution of plant cellular membranes to release more phytochemicals. These results are in line with the previous report of Usman et al. (2003) for recovery of albumin (1.008 + 0.02 mg/mL) and globulin (1.00 + 0.001 mg/mL) from *Thevetia peruviana* seeds cake residues by HCl induced hydrolysis. For non-hydrolyzed extraction, the extract yield was increased with increasing concentration of methanol. This might be explained by the fact that many of phytochemicals of both of selected plants have polarities compatible to methanol, hence become dissolved in greater amounts with increasing methanol concentration. Moreover, *S. moorcroftiana* exhibited greater extract yield than *O. sanctum* with both hydrolyzed and non-hydrolyzed extraction, which might be due to higher extractable constituents of *S. moorcroftiana.*

Table 1
Set-up of Ames test for mutagenic and anti-mutagenic assay.

Plate No.	Treatment	Volume added (mL)				
		Mutagen Standard	Herbal extract	Reagent mixture	Deionized Water	*Salmonella* test strain
1	Blank	__	__	2.5	17.5	__
2	Background	__	__	2.5	17.5	0.005
3	Standard Mutagens	0.1	__	2.5	17.4	0.005
4	Samples for Mutagenic Test	__	0.005	2.5	17.5	0.005
5	Samples for Anti-mutagenic Test	0.1	0.005	2.5	17.4	0.005

Table 2
Extract yields (%) and total phenolic contents (mg GAE/g dry extract) for different extracts of *S. moorcroftiana* and *O. sanctum*.

Extraction Solvent	*Salvia moorcroftiana*		*Ocimum sanctum*	
	Extract Yield[a]	TPC[b]	Extract Yield[a]	TPC[b]
70% Methanol	23.7 ± 0.1^{d}	202.5 ± 1.5^{d}	16.8 ± 0.1^{de}	165.3 ± 1.9^{d}
50% Methanol	17.7 ± 0.1^{e}	191.2 ± 1.6^{e}	11.9 ± 0.1^{e}	148.4 ± 1.2^{de}
30% Methanol	16.8 ± 0.1^{e}	189.4 ± 1.4^{e}	10.1 ± 0.1^{e}	138.5 ± 2.0^{e}
2.0M Acidified Methanol	50.2 ± 0.4^{a}	301.5 ± 2.8^{b}	40.8 ± 0.3^{a}	211.4 ± 1.5^{bc}
1.0M Acidified Methanol	48.5 ± 0.2^{a}	330.5 ± 3.2^{ab}	38.5 ± 0.4^{a}	231.4 ± 1.2^{b}
0.5M Acidified Methanol	47.8 ± 0.1^{a}	342.3 ± 3.1^{a}	37.1 ± 0.2^{a}	281.4 ± 2.0^{a}

Values are mean ± SD of three replicates.
Different letters in each column represent significant differences ($p \leq 0.05$) among solvents used.
[a] % (w/w dry biomass).
[b] Total phenolic contents expressed as mg Gallic acid equivalents (GAE)g^{-1}dry extract.

3.2. Total phenolic contents (TPC)

The results for TPC of both species are presented in Table 2. It was found that hydrolyzed extraction yielded much higher phenolic contents than that of non-hydrolyzed extraction that can be attributed to the efficiency of acidic methanol for the liberation of bound phenolic from plant internal cellular organelles. In case of non-hydrolyzed extraction, 70% methanol exhibited highest phenolic contents for both species (202.5 ± 1.5 mg Gallic acid equivalents/g of dry extract for *S. moorcroftiana* and 165.3 ± 1.9 mg Gallic acid equivalents/g of dry extract for *O. sanctum*), while 0.5 *M* acidified methanol showed maximum phenolic contents (342.3 ± 3.1 mg GAE/g dry extract for *S. moorcroftiana* and 281.4 ± 2.0 mg GAE/g dry extract for *O. sanctum*) in case of hydrolyzed extraction. Although 2.0*M* acid produced highest extract yield but such a high concentration might have degraded many of liberated phenolic. Higher the concentration of methanol in the extracting solvent, greater is the solubility of phenolic compounds, hence 70% methanol presented maximum phenolic contents. Among hydrolyzed extraction, 0.5*M* acidic strength was enough to squeeze and retain maximum phenolic compounds while higher acidic concentration may lead to decomposition of many of liberated phenolic.

3.3. Anti-hemolytic potential

The cytotoxicity of optimized hydrolyzed (0.5*M*) and non-hydrolyzed (70% aqueous methanol) extracts of *S. moorcroftiana* and *O. sanctum* was accomplished by a hemolytic *in vitro* assay. Sometimes, herbal plants may possess some harmful metabolite or reagent that proves to be toxic for red blood cells. Such toxic metabolites destroy the plasma membranes of red blood cells by denaturing different lipid and protein molecules of cell membrane bilayers called hemolysis. The extracts exhibiting hemolytic behavior cannot be effectively used in medicinal formulations. Thus, hemolytic assay is an important pathway to decide oral administration of a plant extract exhibiting high biological activity, thus determining its application in drug manufacturing (Nouren et al., 2019; Abbas et al., 2017). It was found that both types of extracts of *Lamiaceae* seeds showed very low cytotoxicity (less than 4%) as compared to H_2O_2 in hemolytic assay. Such results are in close approximation to literature reports of A Khursheed and V Jain (Khursheed and Jain, 2021) on

Table 3
Anti-hemolytic (%, IC_{50}) and anti-mutagenic activity (%) of optimized extracts of *S. moorcroftiana* and *O. sanctum*.

Plants	Extraction Solvent	Anti-hemolytic Activity			Anti-mutagenic Activity			
					TA 98		TA 100	
		% Inhibition	IC_{50} (μg/mL)		Mutants	% Anti-mutagenicity	Mutants	% Anti-mutagenicity
				Background	8	–	12	–
				$K_2Cr_2O_7$	92	–	–	–
				NaN_3	–	–	94	–
Salvia moorcroftiana	70% Methanol	88.21 ± 1.78^{b}	221.44 ± 2.24^{d}		37	65.48 ± 1.37^{bc}	43	62.20 ± 1.22^{c}
	0.5M Acidified Methanol	93.58 ± 2.04^{a}	168.46 ± 2.78^{a}		33	70.24 ± 0.69^{b}	38	68.29 ± 1.86^{b}
Ocimum sanctum	70% Methanol	86.59 ± 1.56^{c}	235.45 ± 3.2^{de}		42	59.52 ± 0.69^{de}	50	53.66 ± 1.41^{e}
	0.5M Acidified Methanol	90.78 ± 1.88^{ab}	198.65 ± 2.45^{bc}		38	64.29 ± 0.69^{bc}	44	60.98 ± 1.05^{cd}
Ascorbic Acid		92.92 ± 2.12^{a}	170.50 ± 2.56^{a}		28	76.19 ± 0.97^{a}	33	74.39 ± 0.66^{a}
Gallic Acid		94.22 ± 2.24^{a}	162.45 ± 1.46^{a}		30	73.81 ± 1.06^{a}	36	70.73 ± 0.61^{ab}

Values are mean ± SD of three replicates.
Different letters in each column represent significant differences ($p \leq 0.05$).
IC_{50} values were calculated by linear regression analysis.
The anti-mutagenic effect was considered as 'strong' when the percentage of mutagenic inhibition was more than 50%, moderate' when the percentage of inhibition was 25–50% and 'weak' when percentage of inhibition of mutagenicity was less than 25%.

proliferation inhibition potential of *S. moorcroftiana* against A549 lung cancer cell lines (90% inhibition at 12.5 μg/mL for 24 h exposure) and MDA-MB-231 breast cancer cell lines (76% inhibition at 12.5 μg/mL for 24 h exposure). Similar results were also reported by C Faujdar (2021) for cytoprotective effects of hydro-alcoholic extract of *O. Sanctum* leaves (79.16% at 40μ/mL) on calcium oxalate induced toxicity in renal epithelial cells and wound healing capacity (95.62% at EC 90).

The inhibitory action of optimized extracts of both plants against H_2O_2 generated hemolysis has been tabulated as Table 3. Extracts of both species, obtained by hydrolyzed extraction, presented anti-hemolytic potential quite comparable to those of positive standards i.e. ascorbic acid and Gallic acid; whereas much improved values compared to those of non-hydrolyzed extracts which might be attributed to the presence of higher concentration of bioactives in the hydrolyzed extracts as described by their phenolic contents (Table 2). Moreover, *S. moorcroftiana* exhibited greater protection to human RBCs (93.58 ± 2.04%) than *O. sanctum* (90.78 ± 1.88%). Literature also reported the strong anti-hemolytic potential of *Lamiaceae* species against AAPH (2,2′-azobis(2-amidino-propane) dihydrochloride)-induced erythrocyte hemolysis (*Origanum majorana* 269.55%; *Lavandula officinalis* 221.72% and *Ocimum basilicum* 182.70%) (Hmidani et al., 2021).

For all extracts of both species, the half maximal inhibitory concentration (IC_{50}) was also calculated. IC_{50} is the concentration (μg/mL) of plant extract required to inhibit the 50% hemolysis of RBCs. Hydrolyzed extracts exhibited lower IC_{50} (168.46 ± 2.78 μg/mL for *S. moorcroftiana* and 198.65 ± 2.45 μg/mL for *O. sanctum*) values than those of non-hydrolyzed extracts (221.44 ± 2.24 μg/mL for *S. moorcroftiana* and 235.45 ± 3.2 μg/mL for *O. sanctum*) showing higher anti-hemolytic potential of hydrolyzed extracts. Also, *S. moorcroftiana* exhibited IC_{50} (168.46 ± 2.78 μg/mL) value quite close to reference standards i.e. Ascorbic acid (170.50 ± 2.56 μg/mL) and Gallic acid (162.45 ± 1.46 μg/mL).

3.4. Anti-mutagenic potential

Mutations lead to multiple types of cancers in humans. Although not all types of cancers result from mutations only, but large percentage solely depends on some sort of malfunctioning in the genetic makeup of living organisms. Any change in the regular sequence of DNA nucleotides that may lead to the development of some tumor or malfunctioning in the living organisms is known as mutation. Such mutations may be caused by scission of nucleotide chain or incorporation of some irregular nucleotide during replication of DNA (Abbas et al., 2022b; Arshad et al., 2020a). It has been investigated that all types of DNA mutations finally lead to one or other type of cancer. Thus, cancers can be regarded as a mutating phenotype. Chromosomal aberrations are other causes of mutations in genetic makeup, that range from point mutations to alternations of millions of nucleotides. Numerous chromosomal aberrations have been identified to be culprit of different types of tumors and cancers. During clinical survey, some tumoral outgrowths have been diagnosed to be resulted from multiple chromosomal alternations. Sometimes, such mutations may proceed in a series of point mutations finally resulting in some severe tumoral appearance (Montalvo-Andía et al., 2022; Rauf and Ashraf, 2009).

Many epidemiological studies have shown that bioactive compounds of various herbal extracts can protect DNA from mutation (Nazir et al., 2021a; Chen et al., 2021). In this experiment, violet coloration in 'Blank plate' represented sterility of the reaction. Change of violet color to yellow/turbid yellow or off white color indicates some sort of mutation in the selected strains of bacteria. Number of mutants (yellow/turbid yellow or off white wells) in the 'Background plate' designates spontaneous mutation. 'Standard Mutagen' (third plate) having reagent mixture, bacterial strain and standard mutagens, shows higher mutation of added mutagens (92 mutants for $K_2Cr_2O_7$ and 94 mutants for NaN_3). Plate 4 was used for evaluation of mutagenicity of plant extracts. Lower number of mutants in this plate (lesser than twice the spontaneous mutation) indicated that both plants were non-mutagens. Plate 5 was designed to examine the anti-mutagenicity. In this plate, plant extracts were used to protect against the standard mutagens. Lesser number of mutants in this plate shows greater % anti-mutagenicity (Matiichuk et al., 2020).

Results of Ames test have also been presented in Table 3. Both (hydrolyzed and non-hydrolyzed) extracts of both species exhibited very low % mutagenic potential (less than 9%), hence regarded as non-mutagens. The aptitude for anti-mutagenic behavior of both species was also examined. It was found that both species presented considerable protection against the mutagenic action of both standard mutagens in both bacterial strains. *S. moorcroftiana* exhibited higher anti-mutagenic activity (70.24 ± 0.69% for TA98 and 68.29 ± 1.86% for TA100) than that of *O. sanctum* (64.29 ± 0.69% for TA98 and 60.98 ± 1.05% for TA100), but closer to standard antioxidants i.e. Gallic acid (73.81 ± 1.06% for TA98 and 70.73 ± 0.61% for TA100) and ascorbic acid (76.19 ± 0.97% for TA98 and 74.39 ± 0.66% for TA100). Such results are quite close to already published reports on antimutagenic potential of two *Lamiaceae* plants, *O. vulgare* (60%) and *S. officinalis* (51%) against methyl methanesulfonate (MMS) induced-chromosomal aberrations in barley seed root tips (Kolumbayeva et al., 2019). Both plants were regarded as strong anti-mutagenic as they had shown anti-mutagenic potential higher than 40%. Such a high anti-mutagenic potential can be associated with high phenolic contents of both plants (Abbas et al., 2022b; Bokhari et al., 2021; Arshad et al., 2020b; Khan et al., 2019).

3.5. LC-MS/MS profiling of S. moorcroftiana

LC/MS/MS profiling indicated the presence of nine phenolic compounds corresponding to relevant peaks in hydrolyzed extract of *S. moorcroftiana* belonging to different classes.

One hydroxybenzoic acid (vanillic acid) and its conjugate (vanillic acid glycoside) were identified as peak 2 and peak 4, respectively. These phenolic acids were further ascertained by MS/MS analysis. Peak 2 (RT 4.20 min) presented a strong parent ion peak (m/z 167.14) that produced two daughter ions; one presented a peak at m/z 152 by the loss of $–CH_3$ group, and other at m/z 123 by the removal of –COO group, assuring the existence of vanillic acid in the plant extract by matching the LC/MS/MS profile with that of reference standard. Peak 4 (RT 5.51 min) gave an MS peak at m/z 329.07 which produced a fragmented peak at m/z 167 by the loss of glucose residue, confirming vanillic acid glycoside.

Two hydroxycinnamic acids i.e. *p*-coumaric acid and ferulic acid were identified by peaks 1 and 5, respectively. Peak 1 (RT 3.34

min) yielded an $[M-H]^-$ peak at m/z 162.99 that split to give an MS/MS peak at m/z 119 by the removal of CO_2 group that authenticated the presence of a crucial member of hydroxycinnamic acid i.e. *p*-coumaric acid. Its fragmentation profile was found to be matched with that of standard *p*-coumaric acid. Peak 5 (RT 18.54 min) corresponded to ferulic acid, yielded an MS peak (m/z 193.09) that produced three daughter peaks at m/z 179, 149 and 134. One daughter ion peak at m/z 179 was achieved by dropping off $–CH_3$ group; other at m/z 149 by fragmenting –COO group; while third at m/z 134 by releasing both –COO and $–CH_3$ groups which clearly demonstrated the presence of ferulic acid as this fragmentation pattern matched with that of reference standard.

One conjugate of ferulic acid with quinic acid i.e. 5-O-feruloylquinic acid was identified by peak 6 in HPLC chromatogram. Peak 6 (RT 22.45min) produced a sharp $[M-H]-$peak at 367.03 mass value that, upon fragmentation, produced two fragment ions i.e. 1 at m/z 191 by exclusion of ferulic acid moiety; while second at m/z 193 due to loss of quinic acid residue.

Quinic acid, a polyhydroxy cyclohexane carboxylic acid, is a strong antioxidant mainly synthesized by hydrolysis of chlorogenic acid. Non-conjugated quinic acid was also identified by peak 3 in HPLC chromatogram. Peak 3 (RT 4.59 min) when subjected to MS analysis, produced a molecular ion peak at m/z 191.09 which fragmented to give two daughter ions at m/z 173 by dropping off H_2O molecule and m/z 127 by losing –CO group along with two H_2O molecules.

Peak 8 (RT 26.44 min) represented myricetin, an important flavonoid, by giving an intense peak at m/z 317.08 that was further authenticated by two fragment ion peaks at m/z 179 and 151 (Fig. 1) which are characteristics of this flavonol produced by cleavage of heterocyclic ring as shown by standard myricetin. One conjugate of myricetin with glucose i.e. Myricetin-3-gluoside was also identified by peak 10 (RT 28.39 min) in hydrolyzed extract of *S. moorcroftiana.* Its presence was confirmed by mass spectrometric fragmentation pattern that depicted two daughter ions i.e. one at 317 by removal of glucose residue and other at 179 by loss of myricetin residue (Khursheed and Jain, 2021). Peak 7(RT 22.76 min) showed the presence of Isorhamnetin-7-O-glucoside by presenting a parent ion peak at m/z 477.31 which was authenticated by a characteristic daughter ion at m/z 315 by cleaving of glucose moiety.

3.6. LC-MS/MS profiling of O. sanctum

Six bioactive compounds were identified in hydrolyzed extracts of *O. sanctum* by C-ESI-MS/MS profiling. Peak 1(RT 2.85 min) corresponds to ascorbic acid, also known as vitamin C, gave an MS peak at m/z 175 which produced a daughter ion peak at 115 mass number by dropping off $–COHCH_2OH$ group analogous to standard ascorbic acid. Peak 2 (RT 3.67 min) representing quinic acid, a potent antioxidant, that gave a sharp MS peak at m/z 191.09 which was further authenticated by two fragment ions i.e. one $[M-H-H_2O]^-$ at m/z 173 while other $[M-H–CO–2H_2O]^-$ at m/z 127 which are characteristic features of quinic acid (Fig. 2).

Peak 3 (RT 4.45 min) was related to the presence of a strong antioxidant hydroxybenzoic, i.e., Gallic acid in hydrolyzed extracts of *O. sanctum*. MS analysis created a sharp parent ion peak at m/z 169.08 that yielded two daughter ion peaks in MS/MS analysis which is in line with that of reference standard. One peak (m/z 125) corresponded to $[M-H-CO_2]^-[]$ ion while other (m/z 97) related to $[M-H-CO_2-CO]^-[]$ ion. Peak 7 (RT 22.30 min) indicated glycosyringic acid by presenting a parent ion peak at m/z 359.15 in MS analysis. It was further authenticated by one fragment ion at m/z 197 which was related to its aglycon. Peak 6 (RT 10.00 min) indicated a conjugate of hydroxycinnamic acid i.e. *p*-coumaric acid glycoside, giving a $[M-H]^-[]$ peak at m/z 324.96 in MS analysis. This parent

Fig. 1. Pictorial representation of plants and their seeds (A) *S. moorcroftiana* and (B) *O. sanctum* collected from local market, Faisalabad, Pakistan.

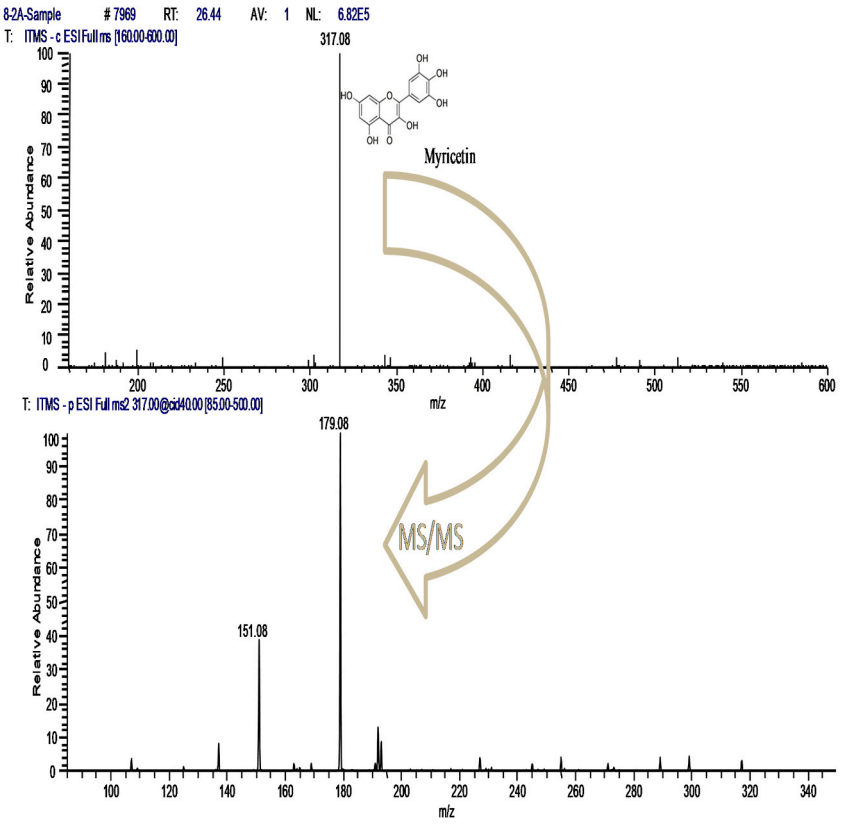

Fig. 2. Typical LC-ESI-MS/MS chromatogram of fragmentation pattern of myricetin.

ion peak when undergone fragmentation analysis, two sub-ionic peaks were obtained; 1 at m/z 163 by cleaving glucose moiety and other at m/z 119 for decarboxylated *p*-coumaric acid.

Peak 5 (RT 7.27 min) represented myricetin, an important flavonol, which was further authenticated by matching MS and MS/MS profiling with that of reference standard. Table 4 also presents the quantitative study of phenolic compounds of *S. moorcroftiana* and *O. sanctum.* It was found that myricetin (669.07 ± 8.45 ppm) and its glycon i.e. Myricetin-3-gluoside (338.91 ± 6.75 ppm) were the most abundant phenolic of *S. moorcroftiana*; while Gallic acid (397.86 ± 6.25 ppm) was found in highest concentration in *O. sanctum* extracts. Based on the study of the phenolic profile of *S. moorcroftiana* and *O. sanctum,* there is a possibility of their application in nutraceutical formulations and can also serve as functional food.

4. Conclusion

Current research project was designed to investigate the phenolic contents of hydrolyzed and non-hydrolyzed extracts of *S. moorcroftiana* and *O. sanctum*. Extracts of both species were screened for cytotoxic and mutagenic effects. Both species presented anti-hemolytic and anti-mutagenic potential. Hydrolyzed extracts of both species presented greater protection to standard cytotoxic and mutagenic agents than those of non-hydrolyzed extracts. Moreover, *S. moorcroftiana* presented higher biological activities than those of *O. sanctum*. Hydrolyzed extracts of both herbal species were subjected to LC-ESI-MS/MS analysis for chemical characterization of their bioactive constituents responsible for their bioactivities. Results of chemical characterization showed the presence of nine bioactive compounds in *S. moorcroftiana* and six in *O. sanctum*. From all these findings, it was concluded that both of selected species especially *S. moorcroftiana* can be effectively used in formulation of different medicines of degenerative diseases and in synthesis of functional foods and nutraceuticals.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Table 4
LC-MS/MS profile of hydrolyzed extract of *S. moorcroftiana* and *O. sanctum*.

Peak No.	RT (min)	MW	$[M-H]^-$	MS^2 Ions	Proposed Compounds	Molecular Formula	Concentration (ppm)	References
Salvia moorcroftiana								
1	3.34	164.16	162.99	119	*p*-Coumaric acid	$C_9H_8O_3$	270.93 ± 5.45	Standard
2	4.20	168.15	167.14	152, 123	Vanillic acid	$C_8H_8O_4$	293.06 ± 6.98	Standard
3	4.59	192.12	191.01	173, 127, 111	Quinic acid	$C_7H_{12}O_6$	289.27 ± 6.28	Khursheed and Jain (2021)
4	5.51	330.29	329.07	167	Vanillic acid glycoside	$C_{14}H_{18}O_9$	269.94 ± 5.20	Arshad et al. (2020b)
5	18.54	194.18	193.09	179, 149, 134	Ferulic acid	$C_{10}H_{10}O_4$	263.68 ± 4.88	Standard
6	22.45	368.34	367.03	191, 193	5-O-Feruloylquinic acid	$C_{17}H_{20}O_9$	264.76 ± 5.12	Khan et al. (2019)
7	22.76	478.40	477.31	315	Isorhamnetin-7-O-glucoside	$C_{22}H_{22}O_{12}$	264.41 ± 5.22	Carazzone et al. (2013)
8	26.44	318.24	317.08	179, 151	Myricetin	$C_{15}H_{10}O_8$	669.07 ± 8.45	Standard
9	27.82	____	414.03	396	Unknown	____	260.71 ± 3.45	____
10	28.39	480.38	479.05	317, 179	Myricetin-3-gluoside	$C_{21}H_{20}O_{13}$	338.91 ± 6.75	Hmidani et al. (2021)
Ocimum sanctum								
1	2.85	176.12	175.01	115	Ascorbic acid	$C_6H_8O_6$	258.76 ± 5.25	Standard
2	3.67	192.12	191.01	173, 127, 111	Quinic acid	$C_7H_{12}O_6$	251.95 ± 4.88	Khursheed and Jain (2021)
3	4.45	170.12	169.07	125, 97	Gallic acid	$C_7H_6O_5$	397.86 ± 6.25	Standard
4	5.67	____	303.11	285	Unknown	____	295.39 ± 5.65	____
5	7.27	318.24	317.08	179, 151	Myricetin	$C_{15}H_{10}O_8$	261.47 ± 5.45	Standard
6	10.00	326.29	325.16	163, 119	*p*-Coumaric acid glycoside	$C_{15}H_{18}O_8$	269.94 ± 5.32	Khursheed and Jain (2021)
7	22.30	360.31	359.15	197	Glycosyringic acid	$C_{15}H_{20}O_{10}$	270.15 ± 6.25	Hmidani et al. (2021)
8	27.13	____	327.26	309	Unknown	____	262.75 ± 4.58	____

Data availability

Data will be made available on request.

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