Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v30i19.3673

Exploring HPLC Analysis, Antioxidant Power, Hemolysis Inhibition, and Anti-Mutagenic Potential in Various Extract Fractions of *Sessuvium Sessuvoides*

Abdul Wadood Chishti[1]*, Muhammad Akram[2], Abid Rashid[3], Sabira Sultana,[4] Sultan Ayaz[5],

[1*, 2, 3, 4, 5]Department of Eastern Medicine, Government College University Faisalabad

***Corresponding Authors:** - Abdul Wadood Chishti
*Department of Eastern Medicine, Government College University Faisalabad

Abstract
Medicinal plants, rich in bioactive compounds, are extensively studied for their therapeutic potential. *Sessuvium sessuvoides*, a plant from the Cholistan desert, Pakistan, is used in traditional medicine. The aim of present study was to analyze the phytochemical analysis and its antioxidant, cytotoxic and antimutagenic potential of *Sessuvium sessuvoides*. The quantification of total phenolic and flavonoid content was carried out using the Folin-Ciocalteu and aluminum chloride colorimetric assay respectively. For the identification of phytocompounds, the HPLC was used. DPPH radical scavenging activity was used to determine the antioxidant potential. Cytotoxicity was assessed by RBCs hemolysis and antimutagenic potential was analyzed by Ames assay. Total phenolic compounds in fractions varied widely, ranging from 134.2±4.1 and 279.4±3.1 mg/g expressed as gallic acid equivalents (GAE). SSCE exhibited the highest total phenolic content. The content of flavonoid expressed as catechin equivalents, varied from 208.684±0.4 to 219.737.9±2.2 mg catechin equivalent/g extract. SSCE exhibited the highest total flavonoid content. The HP-LC analysis confirmed the presence of Salicylic acid, Chlorogenic acid, p-coumaric acid, Gallic acid, Caffeic acid, Ferulic acid, Quercetin, hydroxybenzoic acid and Rutin. It exhibits antioxidant activity, with the methanol fraction showing the highest potential. The plant fractions display cytotoxic effects on human erythrocytes, with the ethanol fraction being the least harmful. Notably, all fractions are non-mutagenic, suggesting protective properties against genetic mutations. In conclusion, the study provides compelling evidence of the phytochemical richness of *Sessuvium sessuvoides*, particularly in terms of phenolic and flavonoid content. This study not only adds to our understanding that *Sessuvium sessuvoides* has significant antioxidant, antihemolytic and antimutagenic potential. Future research should focus on the identification and isolation of specific bioactive compounds responsible for these health-promoting properties.

Keywords: *Sessuvium sessuvoides*, Phytochemicals, HPLC, antioxidant, hemolytic, anti-mutagenic

Exploring HPLC Analysis, Antioxidant Power, Hemolysis Inhibition, and Anti-Mutagenic Potential in Various Extract Fractions of *Sessuvium Sessuvoides*

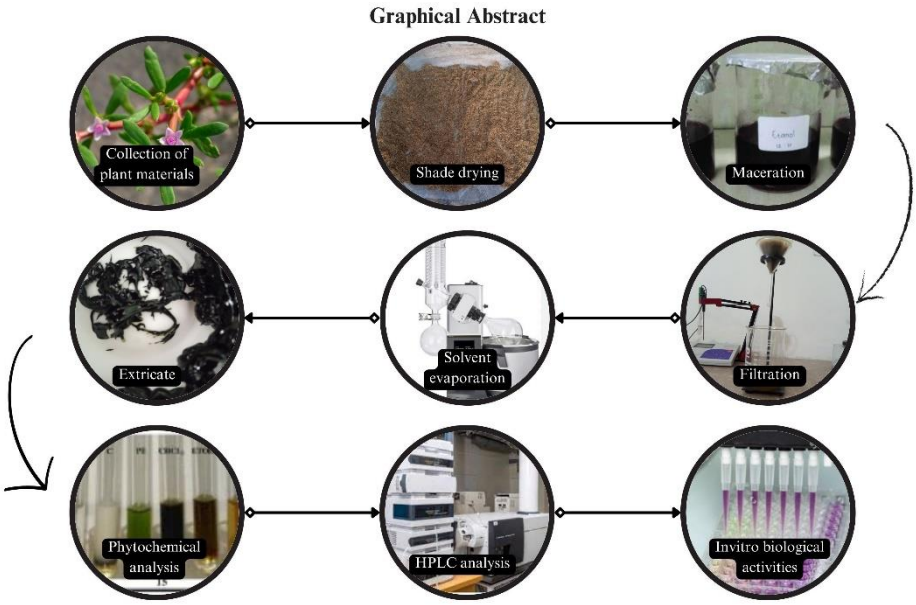

Figure 01: Graphical presentation of abstract

Introduction:

Medicinal plants and their bioactive constituents have been the subject of extensive research, revealing their potential for antioxidant and anti-diabetic properties in numerous scientific studies(Lin et al., 2016, Krishnaiah et al., 2011). These plants are rich sources of secondary metabolites like flavonoids, alkaloids, terpenoids, anthocyanins, glycosides, and phenolic compounds, which have demonstrated their ability to function as antioxidants and inhibitors of enzymes related to glucose metabolism(Kumar et al., 2011, Carocho et al., 2013). It's worth noting that these phytochemicals serve as natural defense mechanisms for plants, safeguarding them against various environmental challenges, including microorganisms, pests, and insects (Fürstenberg-Hägg et al., 2013, Bartwal et al., 2013, Asif, 2015e, Asif, 2015d, Asif, 2015c, Asif, 2015b, Asif, 2015a). In many developing countries, herbal medicines derived from plants are widely utilized due to their accessibility and affordability. They are often perceived as safer alternatives to synthetic drugs, believed to be associated with fewer side effects, and are known for their versatility in various therapeutic applications(Adaramola and Onigbinde, 2017, Organization, 2013, Adaramola et al., 2016, Hamid et al., 2016).Furthermore, there's a prevailing belief that bioactive compounds from plants are less likely to produce side effects compared to synthetic drugs, and they exhibit a broad spectrum of therapeutic applications(Hamid et al., 2016, Gupta, 2003).*Sessuvium sessuvoides* whole plant is used in different traditional therapeutic practices by local practitioner (Hakeem). The Cholistan desert, situated in Pakistan, hosts a diverse array of salt-tolerant plants known as halophytes. This unique ecosystem covers a significant portion of the Bahawalpur region. Halophytes are known for their special adaptations, including succulence, which allows them to thrive in high-salt environments without needing to excrete excess salts. These plants accumulate both salts and water as they grow, leading to their succulent nature. Halophytes have the remarkable ability to extract and incorporate various compounds from their challenging soil environment. This adaptation results in the production of biologically active substances that hold significant pharmacological potential(Flowers and Colmer, 2015). As a result, halophytes present promising opportunities across a wide range of applications, including food production, medicinal uses, chemical compounds, forage for livestock, and biomass for the sustainable generation of renewable energy(El Shaer et al., 2015).

Exploring HPLC Analysis, Antioxidant Power, Hemolysis Inhibition, and Anti-Mutagenic Potential in Various Extract Fractions of *Sessuvium Sessuvoides*

The main objective of our current study is to analyze the phytoconstituents of *Sessuvium sessovoides*to determine the total flavonoids and phenolic content in this plant and whereas, HPLC was performed for tentative identifications of bioactive components. assess its antioxidant, anti-mutagenic, and cytotoxic properties. The results obtained from these analyses was provide valuable insights into the medicinal properties of *Sessuvium sessuvoides*. Furthermore, this research may pave the way for the sustainable development of natural antioxidant and anti-diabetic medications, using plants as a valuable resource.

Material and methods

Collection of plant materials

In December 2022, the entire *Sessuvium sessuvoides* plant, comprising its leaves, stems, roots, and flowers, was meticulously gathered from the Rohi area Dingarh, Bahawalpur, Punjab, Pakistan (28.942045, 71.848193). To ensure accurate identification, the plant was authenticated by a taxonomist affiliated with the Department of Botany at Government College University, Faisalabad. Following collection, the plant material was subjected to a shade-drying process on filter paper for a period of forty days. Subsequently, it was finely ground using an electric grinder and sieved through a No. 60 mesh. The resulting dried powder was then securely stored in an airtight container for further use and reference.

Preparation of extracts fractions

The dried plant sample was initially subjected to a grinding process to prepare it for extraction. A sequential extraction protocol was meticulously designed, involving five steps, each employing solvent of varying polarities, commencing with the least polar solvent (n-hexane, chloroform, methanol, and ethanol, in that order).

Step 1: A total of 0.750 kg of finely ground plant material was macerated with 2.5 liters of n-hexane for 24 hours, twice, within a dedicated extraction tank.
Step 2: The residual material left after Step 1 underwent further shade-drying, and subsequently, a second round of extraction was conducted using 2.5 liters of chloroform for 24 hours, repeated twice.
Step 3: Following Step 2, the residue was subjected to another round of shade-drying, and then, 2.5 liters of ethanol were used for extraction, with the process repeated twice for 24 hours each time.
Step 4: Building on the preceding steps, 2.5 liters of ethanol were introduced to the dried plant residue from Step 3, resulting in the extraction of methanol extract. This step was also conducted over 24-hour periods and repeated twice.
The obtained extracts were subsequently filtered to remove impurities and subsequently concentrated through evaporation using a rotary evaporator under reduced pressure at a temperature of 40°C.

Determination of total phenolic contents (TPC)

The quantification of total phenolic content in the sample was carried out using the Folin-Ciocalteu method, following the procedure outlined by Naz et al. (2016)(Naz et al., 2016). To establish the calibration curve, solutions of gallic acid at different concentrations were prepared. Specifically, 1 mL aliquots of gallic acid solutions at concentrations of 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, and 0.10 mg/mL in methanol were combined with 5 mL of Folin-Ciocalteu reagent (diluted tenfold) and 4 mL of 20% sodium carbonate solution. Subsequently, the absorbance was measured after 1 hour at a wavelength of 765 nm, and a calibration curve was constructed by plotting absorbance as a function of concentration. For the sample extract, 1 mL of the extract at a concentration of 0.001 g/mL was mixed with the same reagent composition mentioned above. After

an incubation period of 1 hour, the absorbance of the resulting blue color complex was recorded at 765 nm. All measurements were performed in triplicate. Quantitative analysis was conducted relative to the gallic acid standard, as described by Sharif et al. (2018(Sharif et al., 2018). Total content of phenolic compounds in plant extracts in gallic acid equivalents (GAE) were calculated by the following formula.

$$T = C \times V / M$$

Where,
T = total contents of phenolic compound in mg GAE/g plant extract.
C = the concentration of gallic acid calculated from calibration curve in mg/mL.
V = the volume of extract in mL.
M = the weight of plants extracts in grams.

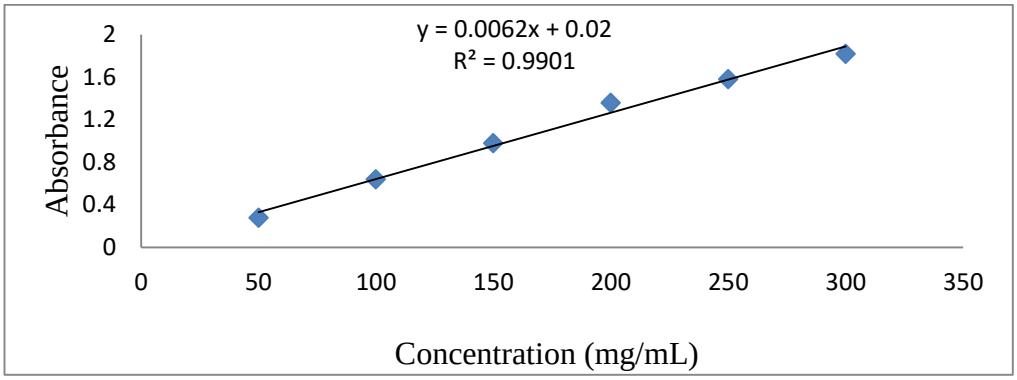

Figure 02 Standard curve of Gallic acid

Total flavonoids contents

The determination of total flavonoid content in the sample followed the method outlined by Rehman et al. (2013)(Rehman et al., 2013). In brief, 0.5 mL of the sample was mixed with 2 mL of distilled water, to which 0.15 mL of a 5% NaNO2 solution was added. This mixture was incubated for 6 minutes. Subsequently, 0.15 mL of a 10% AlCl3 solution was introduced, followed by another 6-minute incubation. To this, 4% NaOH solution was added, and the reaction mixture was adjusted to a final volume of 5 mL by the addition of methanol, ensuring thorough mixing. The absorbance of the resulting reaction mixture was measured at 510 nm after a 15-minute incubation period, following the procedure described by Ayub et al. (2017)(Ayub et al., 2017). The total flavonoid content (TFC) of the extracts was expressed in micrograms of catechin equivalents per milliliter (µg catechin equivalents/mL) of the plant extract, as determined from the linear regression curve of catechin.

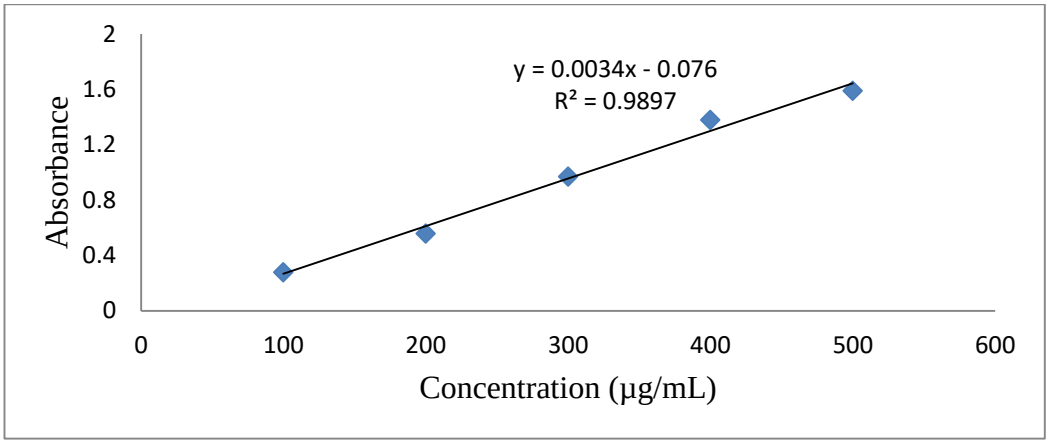

Figure 03: Standard curve of Catechin.

High performance liquid chromatography (HPLC)

HPLC was used to examine the medicinal plant selected. Before analysis, the plant extract was be filtered via a 0.45 µm syringe filter. Surveyor plus HPLC System with Surveyor auto was used for separation. The analytical column is placed to the pump. The mobile phases A and B in the solvent elution was be LCMS grade methanol and acidified water (0.5 percent formic acid v/v). A gradient system with a flow rate of 0.3 mL/min was be used for solvent elution. The gradient elution was be programmed as follows: from 10% to 30% A in 5 minutes; from 10% to 50% A in 20 minutes; and this was be maintained until the end of the analysis. After each analysis, a 20-minute re-equilibration period was be used. The injection volume was be 5.0 L and the column was be maintained at 25 °C. The HPLC column effluent was be sent to an electron spray ionization mass spectrometer. An ESI ionization source was be installed in the mass spectrometer. Negative ion mode was be used to set the parameters for the study, with spectra obtained spanning a mass range of m/z 260 to 800. Spray voltage was be +4.0 kV; sheath gas and auxiliary gas was be 45 and 5 units/min, respectively; capillary temperature was be 320 °C; capillary voltage was be -20.0 V; and tube lens was be -66.51 V(Dewanto et al., 2002). The X-caliber Software (Thermo Fisher Scientific Inc, Waltham, MA, USA) was process the precise mass spectra data of the molecular ions (Mradu et al., 2012).

DPPH Scavenging Activity

The assessment of the antioxidant potential of the sample was conducted through the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, following the procedure elucidated by Shahid et al. (2014)(Chatha et al., 2014). In accordance with this method, 1 mL of a freshly prepared 0.004% DPPH solution in methanol was combined with 3 mL of the sample. The resulting mixture was then incubated for 30 minutes in the absence of light. Subsequently, the absorbance of the solution was recorded at 517 nm. A lower absorbance value in the reaction mixture indicates a higher radical scavenging activity. As a reference, the antioxidant activity of ascorbic acid was also assessed, and a solution devoid of plant extract served as the control, as specified by Naseem et al. (2020)(Naseem et al., 2020). All the tests were performed in triplicate. The percentage inhibition of DPPH radical samples was calculated as follows.

$$\text{DPPH Inhibition (\%)} = \frac{\text{Blank absorbance } (A_0) - \text{Sample absorbance } (A_1)}{\text{Blank absorbance } (A_0)} \times 100$$

Where

A_1 = Absorbance of sample.

A_0 = Absorbance of blank.

The sample concentration providing 50% inhibition (IC50) was calculated from the graph of inhibition percentage against sample concentration. Tests were carried out in triplicate. Ascorbic acid was used as a positive control.

Cytotoxicity through Hemolytic activity:

Hemolytic activity.

Hemolytic activity of the compound was studied by the method used by Shahzadi*et al*. (Shahzadi et al., 2019) and Rubab *et al*. (Rubab et al., 2017). Three mL freshly obtained heparinized bovine blood was collected from Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan. Blood was centrifuged for 5 min at 1000x*g* plasma was discarded and cells were washed with three times with 5 mL of chilled (4^{o}C) sterile isotonic Phosphate-buffered saline (PBS) pH 7.4. Erythrocytes were maintained 10^8 cells per mL for each assay. Hundred µL of each compound was mixed with human (10^8cells/mL) separately. Samples were incubated for 35 min at 37^{o}C and agitated after 10 min. Immediately after incubation the samples were placed on ice for 5 min then centrifuged for 5 min at 1000x*g*. Supernatant 100 µL were taken from each tube and diluted 10 time with chilled (4^{o}C) PBS. Triton X-100 (0.1% v/v) was taken as positive control and phosphate buffer saline (PBS) was taken as negative control and pass through the same process. The absorbance was noted at 576 nm using uquant (Biotech, USA). The % RBCs lysis for each sample was calculated.

%age hemolysis = Absorbance of sample – Absorbance of negative control/ Absorbance of positive control × 100

Anti-mutagenic activity

Two mutant strains *Salmonella typhimurium* TA98 and TA100 were used. 200 µL of the prepared contents was dispensed into each well of a 96-well microtitration plate. The plate was placed in an air tight plastic bag to prevent evaporation and incubated at 37 ºC for 4 days.

The blank plate was observed first and the rest of plates were read only when all wells in the blank plate were colored purple indicating the assay was not contaminated. The background, standard, and test plates were scored visually and all yellow, partial yellow or turbid wells were scored as positive wells while purple wells were scored as negative. The extract was considered toxic to the test strain if all wells in the test plate showed purple coloration. For an extract to be mutagenic, the number of positive wells had to be more than twice the number of positive well in the background plate (Iqbal et al., 2017).

Mutagenic study by Ames bacterial reverse-mutation test (fluctuation test)

Methodology:

Reagent mixture comprising of Davis-Mingioli salt, D-glucose, bromocresol purple, D-Biotin and L-Histidine. These were mixed aseptically in a sterile bottle. Reagent mixture, extract, sterile deionised water, strains and standard mutagens were mixed in several bottles at the amount indicated in table:

Treatment	Volume added (mL)				
	Mutagen standard	**Extract**	**Reagent mixture**	**Deionised water**	***Salmonella* test strain**
Blank	-	-	2.5	17.5	-
Background	-	-	2.5	17.5	0.005
Standard mutagen	0.1	-	2.5	17.4	0.005
Test sample	-	0.005	2.5	17.5	0.005

Table 01: Mutagenic study by Ames bacterial reverse-mutation test (fluctuation test)

Statistical analysis

Result values were represented in mean ± SEM. One -way ANOVA was performed using GraphPad Prism (San Diego, CA, USA) software. A p value of less than 0.05 was considered statistically significant.

RESULTS

Total phenolic and flavonoid contents

Figure 3below summarizes that total phenolic compounds in fractions varied widely, ranging from 134.2±4.1 and 279.4±3.1 mg/g expressed as gallic acid equivalents (GAE). SSCE exhibited the highest total phenolic content. The content of flavonoid expressed as catechin equivalents, varied from 208.684±0.4 to 219.737.9±2.2 mg catechin equivalent/g extract. SSCE exhibited the highest total flavonoid content.

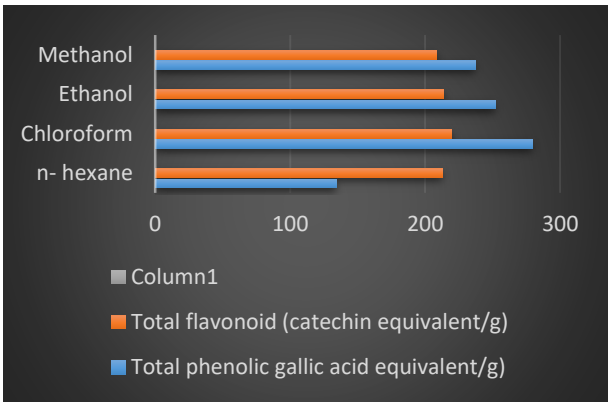

Figure 04: Total phenolic and flavonoid contents of different extract fractions of *Sessuvium sessovoides*

Identification of phytoconstituents by HPLC

HPLC analysis of SsCr crude four extracts fractions (EMCH) strengthened the phytochemical screening result and showed the presence of phytoconstituents as shown in Table 2 and 3.

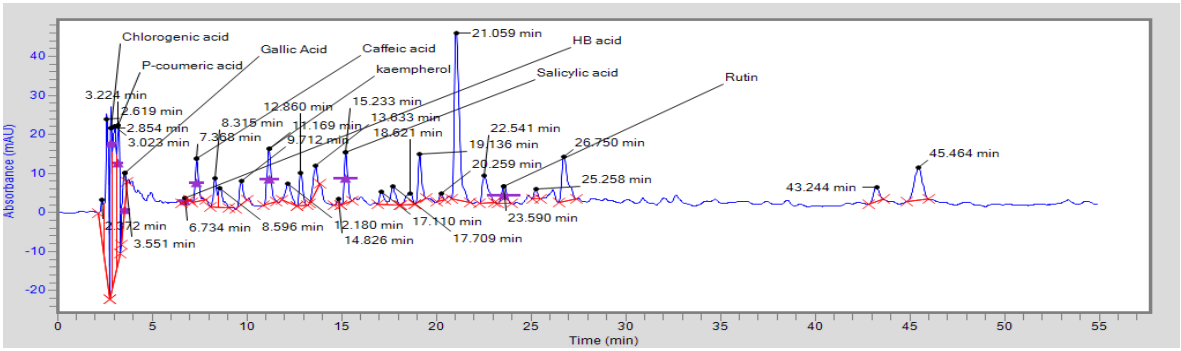

Figure 05: HPLC spectra elucidating the chemical composition of the chloroform fraction isolated from Sessuvium sessuvoides

Exploring HPLC Analysis, Antioxidant Power, Hemolysis Inhibition, and Anti-Mutagenic Potential in Various Extract Fractions of *Sessuvium Sessuvoides*

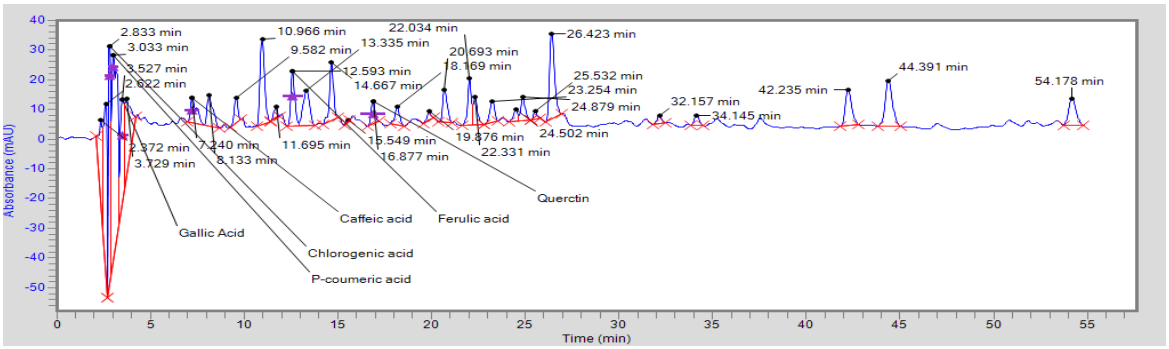

Figure 06: HPLC spectra elucidating the chemical composition of the ethanol fraction isolated from *Sessuvium sessuvoides*

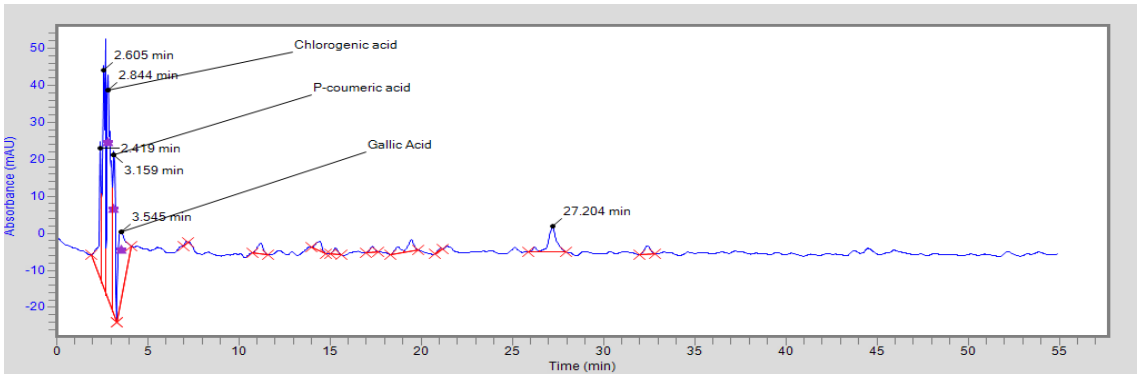

Figure 07: HPLC spectra elucidating the chemical composition of the n-hexane fraction isolated from *Sessuvium sessuvoides*

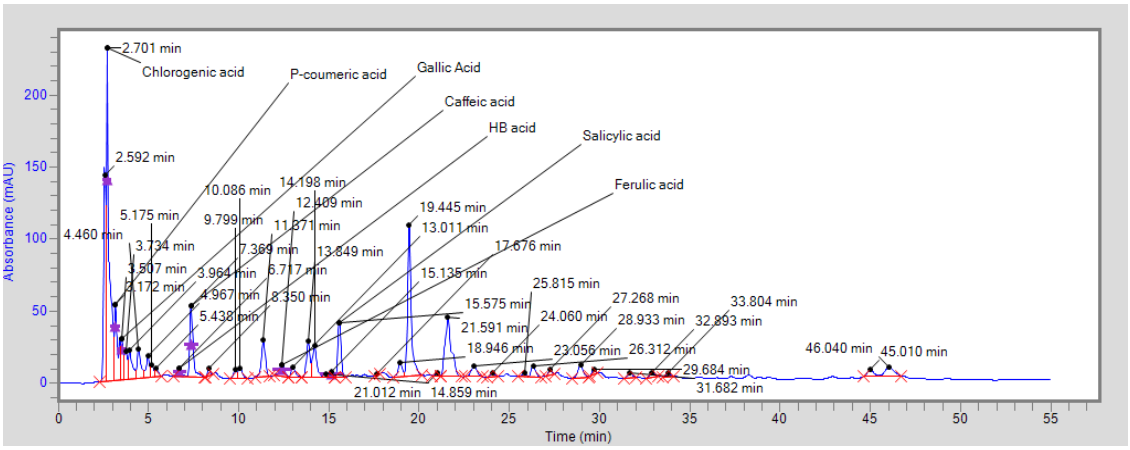

Figure 08: HPLC spectra elucidating the chemical composition of the methanol fraction isolated from *Sessuvium sessuvoides*

Plant fraction *(Sessuvium sessuvoides)*	Salicylic acid	Chlorogenic acid	p-coumaric acid	Gallic acid	Caffeic acid	Ferulic acid	Quercetin	HB acid	Rutin
Ethanol	ND	2.833	3.033	ND	7.240	12.593	16.877	ND	ND
Methanol	15.575	2701	3.172	3.507	7.369	12.409	ND	6.717	ND
Chloroform	15.233	2854	3.224	3.551	7.368	ND	ND	ND	23.59
n-hexane	ND	2844	3.159	3.545	ND	ND	ND	ND	ND

Table 02: Each value is the mean (mg/100 g of dry sample) ND, not detected. HB, Hydroxybenzoic acid

Table 03: HPLC reports phytochemicals present in different four fractions of *Sesuvium sesuvioides* (EMCH)

RT (min)	Compound name	Chemical name of compound	Compound class	Molecular formula	Molecular mass
2808	Chlorogenic acid	3-(3,4-Dihydroxycinnamoyl) quinic acid	Polyphenol	$C_{16}H_{18}O_9$	354.311 g/mol
3.14	p-coumaric acid	(2E)-3-(4-Hydroxyphenyl) prop-2-enoic acid	hydroxycinnamic acids	$C_9H_8O_3$	s164.160 g/mol
3.53	Gallic acid	3,4,5-Trihydroxybenzoic acid	phenolic acid	$C_7H_6O_5$	170.12 g/mol
7.32	Caffeic acid	3-(3,4-Dihydroxyphenyl) -2-propenoic acid	Phenolic acid	$C_9H_8O_4$	180.16 g/mol
12.501	Ferulic acid	2-propenoic acid, 3-(4-hydroxy-3-methoxyphenyl)-ferulic acid	Aromatic acid	$C_{10}H_{10}O_4$	194.18 g/mol
16.877	Quercetin	3,3′,4′,5,7-Pentahydroxyflavone	Flavonoid	$C_{15}H_{10}O_7$	302.236 g/mol
6.717	HB acid	4-Hydroxybenzoic acid	Phenol	$C_7H_6O_3$	138.122 g/mol
15.404	Salicylic acid	2-Hydroxybenzoic acid	Phenolic acid	$C_7H_6O_3$	138.122 g/mol

11.169	Kaempferol	3,4′,5,7-Tetrahydroxyflavone	Flavonoid	$C_{15}H_{10}O_6$	286.23 g/mol

Biological activities

DPPH radical scavenging activity

Fig:04below shows that the scavenging effects of samples on DPPH radical and were in the following order: SSn-H > SSCE > SSEE > SSME. Though the antioxidant potential of fractions was found to be low ($P < 0.05$) than those of ascorbic acid. The study revealed that SSME has prominent antioxidant activity. The presence of phenolic compounds (containing phenolic hydroxyls) are mainly found in these two fractions and could be attributable to the observed high antiradical properties of these fractions.

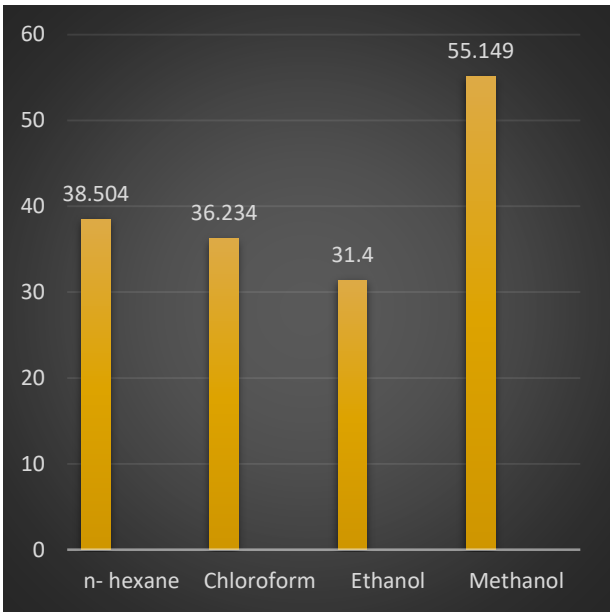

Figure 09: Anti-oxidant capacity of n-hexane, chloroform, ethanol and methanol fractions of *Sesuvium sesuvioides*.

Cytotoxic activity:

Hemolysis method:

Hemolytic activity of different fraction of *Sessuvium sessuvoides* was screened against normal human erythrocytes. Different fractions exhibited differential pattern hemolytic effect towards human erythrocytes. Result indicated that the ethanol fraction exhibited minimum hemolytic activity, whereas aqueous fraction showed the highest hemolytic activity. Lysis of erythrocytes was found to be increased with an increase of extract or fraction concentration.

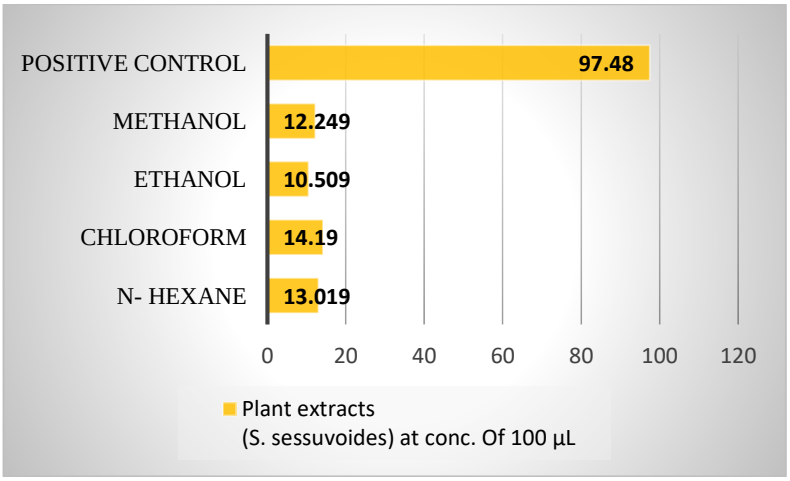

Figure 10: Hemolytic activity of methanol, ethanol, chloroform and n-hexane fractions of *Sesuvium sesuvioides*.as compared to positive control

Anti-mutagenic activity

Below figure 11-17 and Table 04 elucidate the study of anti-mutagenic potential of *Sessuvium sessuvoides* different fractions. The blank plate was observed first and the rest of plates were read only when all wells in the blank plate were colored blue/ purple indicating the assay was not contaminated. The background, standard, and test plates were scored visually and all yellow, partial yellow or turbid wells were scored as positive wells while blue/purple wells were scored as negative. The extract was considered toxic to the test strain if all wells in the test plate showed purple coloration. For an extract to be mutagenic, the number of positive wells had to be more than twice the number of positive well in the background plate as shown in the figures below.

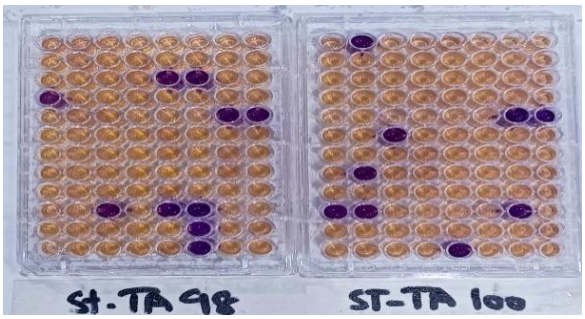

Figure 11: Standard (K2Cr2O7) TA-98 and Standard (NaN3**) TA-100**

Figure 12: Sterility Plate

Exploring HPLC Analysis, Antioxidant Power, Hemolysis Inhibition, and Anti-Mutagenic Potential in Various Extract Fractions of *Sessuvium Sessuvoides*

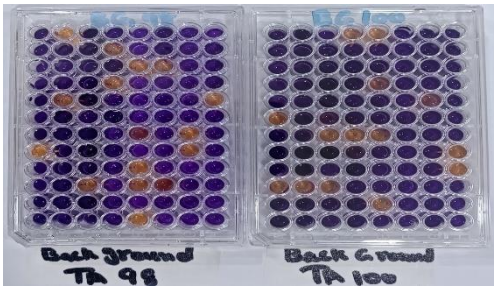

Figure 13 Ethanolic fraction against TA-98 and Ethanolic fraction against TA-100

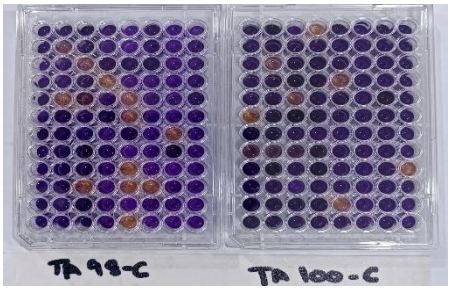

Figure 14 Chloroform fraction against TA-98 and Chloroform fraction

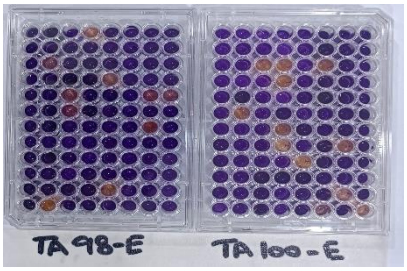

Figure 15 Background plate TA-98 and Background Plate TA-100

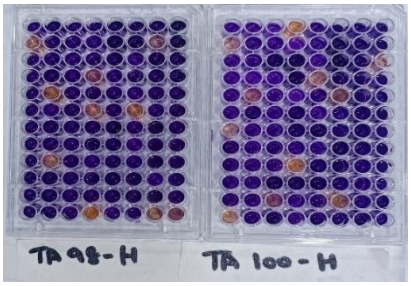

Figure 16 n-hexane fraction against TA-98 and n-hexane fraction against TA-100

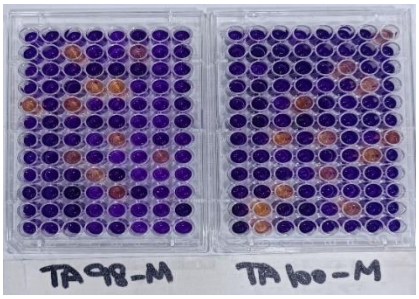

Figure 17 Methanolic fraction against TA-98 and methanolic fraction against TA-100

Plant extracts (*S. sessuvoides*)	TA 98 No of positive Wells/Total number of Wells	TA 100 No of positive Wells/No of total wells	Outcome
C (Chloroform)	12/96	9/96	Non-mutagenic
E (Ethanol)	10/96	12/96	Non-mutagenic
H (n Hexane)	10/96	11/96	Non-mutagenic
M (Methanol)	11/96	13/96	Non-mutagenic

DISCUSSION

The study investigated the phytochemical composition and bioactivity of *Sessuvium sessuvoides*, an indigenous plant from the Cholistan desert region of Pakistan. The results of various assays shed light on the plant's potential as a source of natural compounds with health-promoting properties.

Phytochemical Composition

First, the study assessed the phytochemical composition of *Sessuvium sessuvoides* by measuring the total phenolic and flavonoid content in different fractions of the plant. These compounds are well-known for their antioxidant properties and potential health benefits.

Total phenolic content, expressed as gallic acid equivalents (GAE), showed considerable variation among the fractions. The n-hexane fraction exhibited the lowest phenolic content at 134.2 mg/g, while the chloroform and ethanol fractions had higher phenolic content at 279.4 mg/g and 251.8 mg/g, respectively. Notably, the methanol fraction demonstrated a substantial phenolic content of 237.2 mg/g.In parallel, the assessment of total flavonoid content, expressed as catechin equivalents, revealed that this plant is a rich source of flavonoids. The content ranged from 208.684 mg/g in the methanol fraction to 219.737.9 mg/g in the chloroform fraction. The highest total flavonoid content was observed in the SSCE (*Sessuvium sessuvoides* chloroform extract) fraction.

These findings highlight the remarkable phytochemical diversity within *Sessuvium sessuvoides*, suggesting its potential for therapeutic applications, particularly in the realm of antioxidant and anti-inflammatory therapies.

Identification of phytoconstituent by HPLC

The presence of components by HPLC analysis also encouraged the potential of the plant for its vast therapeutic activities.

The compound 3-(3,4-Dihydroxycinnamoyl) quinic acidis a dietary polyphenol with numerous therapeutic and essential functions. It acts as an antioxidant, antibacterial, and anti-inflammatory, and is also a central nervous system stimulator. Moreover it plays a crucial role in lipid metabolism and glucose levels, potentially offering therapeutic benefits for metabolic-related disorders(Naveed et al., 2018).

(2E)-3-(4-Hydroxyphenyl) prop-2-enoic acidhas various biological functions such as anti-oxidant, anti-inflammatory, anti-diabetic, anti-ulcer, anti-platelet, anti-cancer activities, and antimicrobial properties. Furthermore, it prevents atherosclerosis, oxidative cardiac damage, UV-induced damage to ocular tissues, neural injury and gout(Zang et al., 2000, Abdel-Wahab et al., 2003, Hudson et al., 2000, Lodovici et al., 2009, Vauzour et al., 2010, Cho et al., 1998, Pragasam et al., 2013).

3,4,5-Trihydroxybenzoic acid has been reported to have therapeutic activities in gastrointestinal, neuropsychological, metabolic, and cardiovascular disorders(Kahkeshani et al., 2019).

3-(3,4-Dihydroxyphenyl)-2-propenoic acidis a natural phenylpropanoid compound with many important medicinal properties. It functions as an antioxidant and it attenuates multidrug resistance in cancer cells(Sato et al., 2011, Jiménez-Bonilla et al., 2020). Caffeic acid, which inhibits TMEM16A (a calcium-activated chloride channel), is also regarded as an active agent in the treatment of lung cancer (Bai et al., 2022).

Studies have demonstrated3-(4-hydroxy-3-methoxyphenyl)-ferulic acidexhibits various pharmacological activities, including antimicrobial, antioxidant, anti-inflammatory, antifungal,

anticancer, antiallergic, hepatoprotective, and antidiabetic activities, as well as neuroprotective effects including against Alzheimer's disease(Rezaeiroshan et al., 2022, Trombino et al., 2009, Trombino et al., 2013, Wang et al., 2017, Helmy et al., 2022, Hassanein et al., 2021, Shayan-Nasr et al., 2021, Chotimarkorn and Ushio, 2008, Singh and Patil, 2022, Zafeer et al., 2019).

A total of 345 articles about 3,3′,4′,5,7-Pentahydroxyflavonewere reviewed, and it was observed that more than 40% of articles were about quercetin's use as an antioxidant agent, more than 25% of studies were about its use as an anticancer agent, and articles on antimicrobial activity were more than 15%. 10% of the articles showed anti-inflammatory effects of quercetin(Azeem et al., 2023).

4-hydroxybenzoic acid is an important class of preservatives extensively used in the cosmetic and pharmaceutical industries for preparing shampoos, commercial moisturizers, shaving gels, personal lubricants, topical/parenteral pharmaceuticals, spray tanning solutions, and toothpaste. Although effective as antibacterial and antifungal agents, these compounds are supposed to possess hepatotoxic effects(Shivashankara et al., 2013).

2-Hydroxybenzoic acid as a medication is used to help remove the outer layer of the skin. As such it is used to treat warts, calluses, psoriasis, dandruff, acne, ringworm, and ichthyosis(Formulary, 2009).

3,4′,5,7-Tetrahydroxyflavone is as anti-inflammatory activity, anti-cancer activity, antioxidant activity, and anti-diabetic activity(Yang et al., 2023).

Antioxidant Activity

Next, the study explored the antioxidant potential of the plant fractions using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Antioxidants play a critical role in mitigating oxidative stress and preventing cellular damage. The results showed that *Sessuvium sessuvoides* fractions possessed antioxidant activity, albeit lower than that of ascorbic acid. The SSME (*Sessuvium sessuvoides* methanol extract) fraction stood out as it exhibited the highest antioxidant activity. Previously the antioxidant capacity of *Sessuvium sessuvoides*crude extract showed significant DPPH, ABTS, CUPRAC, FRAP, PBD and metal chelating results(Sajid-Ur-Rehman et al., 2021). The correlation between the presence of phenolic compounds and antioxidant potential was evident, emphasizing the role of these phytochemicals in the observed antiradical properties. This suggests that *Sessuvium sessuvoides*, particularly the methanol fraction, could be a valuable source of natural antioxidants. These antioxidants may help protect cells from oxidative damage, potentially contributing to the prevention of various chronic diseases and the promotion of overall health.

Cytotoxicity Assessment

The study also delved into the cytotoxicity of the different *Sessuvium sessuvoides* fractions. The hemolytic activity assay was used to evaluate the impact of the fractions on human erythrocytes. The results showed significant hemolytic activity i.e. $P<0.05$. The results demonstrated that the SSEF displayed the lowest hemolytic activity, while the aqueous fraction exhibited the highest hemolytic activity. This is a crucial finding as it suggests that the ethanol fraction may be safer for potential therapeutic applications, indicating a lower risk of causing damage to red blood cells. The observed dose-dependent increase in erythrocyte lysis with higher concentrations of the extract emphasizes the importance of appropriate dosage and safety considerations when utilizing *Sessuvium sessuvoides* extracts in medicinal or clinical applications.

Anti-Mutagenic Potential

Finally, the study assessed the anti-mutagenic potential of *Sessuvium sessuvoides* fractions. Mutagens are agents that can cause genetic mutations, potentially leading to various diseases, including cancer. The results indicated that all fractions at the concentration of 100 µLi.e. n-hexane,

chloroform, ethanol, and methanol were "non-mutagenic." This is a significant finding, suggesting that these fractions may have protective properties against mutagenic agents. It appears that the antimutagenic properties of *Sessuvium sessuvoides* extracts are attributed to the presence of flavonoids, as suggested by previous research on other plants(Abad and Nadaf, 2023, Whaley et al., 2023).However, the statement also mentions contradictory findings in the literature. Some medicinal plant extracts and essential oils, when administered at high doses, have been reported to exhibit pro-mutagenic or direct mutagenic effects(Merrouni). This suggests that the biological effects of plant extracts can be dose-dependent, with a potential shift from beneficial to harmful at higher concentrations. The implications of this result are profound, as it highlights the potential of *Sessuvium sessuvoides* in preventing genetic mutations that could lead to serious health conditions.

Conclusion and Future Prospects

In conclusion, the study provides compelling evidence of the phytochemical richness of *Sessuvium sessuvoides*, particularly in terms of phenolic and flavonoid content. These compounds are renowned for their antioxidant and anti-inflammatory properties, making *Sessuvium sessuvoides* a promising candidate for the development of natural antioxidant therapies. Moreover, the study's findings on cytotoxicity and anti-mutagenic activity underscore the importance of safety and potential protective effects when considering the application of *Sessuvium sessuvoides* extracts in medicine or biopharmaceuticals. Future research should focus on the identification and isolation of specific bioactive compounds responsible for these health-promoting properties. Additionally, clinical trials and further investigations into the specific therapeutic applications of *Sessuvium sessuvoides* are warranted. This study not only adds to our understanding of the therapeutic potential of natural plant extracts but also underscores the value of indigenous knowledge and traditional medicine in the search for new sources of bioactive compounds with health benefits.

References:

1. ABAD, M. H. K. & NADAF, M. J. J. O. E. O. R. 2023. The ethnobotanical properties and medicinal application of essential oils of Ziziphora persica Bunge from different habitats: A review. 35**,** 177-196.
2. ABDEL-WAHAB, M., EL-MAHDY, M., ABD-ELLAH, M., HELAL, G., KHALIFA, F. & HAMADA, F. J. P. R. 2003. Influence of p-coumaric acid on doxorubicin-induced oxidative stress in rat's heart. 48**,** 461-465.
3. ADARAMOLA, B., ONIGBINDE, A. & SHOKUNBI, O. J. C. I. 2016. Physiochemical properties and antioxidant potential of Persea Americana seed oil. 2**,** 168-175.
4. ADARAMOLA, B. & ONIGBINDE, A. J. C. I. 2017. Influence of extraction technique on the mineral content and antioxidant capacity of edible oil extracted from ginger rhizome. 3**,** 1-7.
5. ASIF, M. J. C. I. 2015a. Anti-neuropathic and anticonvulsant activities of various substituted triazoles analogues. 1**,** 174-183.
6. ASIF, M. J. C. I. 2015b. Anti-tubercular activity of some six membered heterocycle compounds. 1**,** 134-163.
7. ASIF, M. J. C. I. 2015c. Antiviral and antiparasitic activities of various substituted triazole derivatives: A mini review. 1**,** 71-80.
8. ASIF, M. J. C. I. 2015d. Chemistry and antioxidant activity of plants containing some phenolic compounds. 1**,** 35-52.
9. ASIF, M. J. C. I. 2015e. Pharmacologically potentials of different substituted coumarin derivatives. 1**,** 1-11.
10. AYUB, M. A., HUSSAIN, A. I., HANIF, M. A., CHATHA, S. A. S., KAMAL, G. M., SHAHID, M., JANNEH, O. J. C. & BIODIVERSITY 2017. Variation in phenolic profile,

β-Carotene and flavonoid contents, biological activities of two Tagetes species from Pakistani flora. 14**,** e1600463.
11. AZEEM, M., HANIF, M., MAHMOOD, K., AMEER, N., CHUGHTAI, F. R. S. & ABID, U. J. P. B. 2023. An insight into anticancer, antioxidant, antimicrobial, antidiabetic and anti-inflammatory effects of quercetin: A review. 80**,** 241-262.
12. BAI, X., LI, S., LIU, X., AN, H., KANG, X., GUO, S. J. J. O. A. & CHEMISTRY, F. 2022. Caffeic acid, an active ingredient in coffee, combines with DOX for multitarget combination therapy of lung cancer. 70**,** 8326-8337.
13. BARTWAL, A., MALL, R., LOHANI, P., GURU, S. & ARORA, S. J. J. O. P. G. R. 2013. Role of secondary metabolites and brassinosteroids in plant defense against environmental stresses. 32**,** 216-232.
14. CAROCHO, M., FERREIRA, I. C. J. F. & TOXICOLOGY, C. 2013. A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. 51**,** 15-25.
15. CHATHA, S. A. S., HUSSAIN, A. I., ASAD, R., MAJEED, M., ASLAM, N. J. J. O. F. P. & TECHNOLOGY 2014. Bioactive components and antioxidant properties of Terminalia arjuna L. extracts. 5**,** 1.
16. CHO, J.-Y., MOON, J.-H., SEONG, K.-Y., PARK, K.-H. J. B., BIOTECHNOLOGY, & BIOCHEMISTRY 1998. Antimicrobial activity of 4-hydroxybenzoic acid and trans 4-hydroxycinnamic acid isolated and identified from rice hull. 62**,** 2273-2276.
17. CHOTIMARKORN, C. & USHIO, H. J. P. 2008. The effect of trans-ferulic acid and gamma-oryzanol on ethanol-induced liver injury in C57BL mouse. 15**,** 951-958.
18. DEWANTO, V., WU, X., ADOM, K. K., LIU, R. H. J. J. O. A. & CHEMISTRY, F. 2002. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. 50**,** 3010-3014.
19. EL SHAER, H., ATTIA-ISMAIL, S. J. H., SALT-TOLERANT FEEDSTUFFS IMPACT ON NUTRITION, P., TAYLOR, R. O. L. B. R. C. P. & GROUP, F. 2015. Halophytic and salt tolerant feedstuffs in the Mediterranean basin and Arab region: an overview. 21-36.
20. FLOWERS, T. J. & COLMER, T. D. J. A. O. B. 2015. Plant salt tolerance: adaptations in halophytes. 115**,** 327-331.
21. FORMULARY, W. W. M. J. W. H. O. G., SWITZERLAND 2009. Stuart, MC, Kouimtzi, M., Hill, SR, Eds.
22. FÜRSTENBERG-HÄGG, J., ZAGROBELNY, M. & BAK, S. J. I. J. O. M. S. 2013. Plant defense against insect herbivores. 14**,** 10242-10297.
23. GUPTA, A. K. J. Q. S. O. I. M. P. V. 2003. Quality standards of Indian medicinal plants. Volume 1.
24. HAMID, A. A., OGUNTOYE, S. O., ALLI, S. O., AKOMOLAFE, G. A., ADERINTO, A., OTITIGBE, A., OGUNDARE, A. M., ESINNIOBIWA, Q. M. & AMINU, R. O. J. C. I. 2016. Chemical composition, antimicrobial and free radical scavenging activities of Grewia pubescens. 2**,** 254-261.
25. HASSANEIN, E. H., ABDEL-WAHAB, B. A., ALI, F. E., ABD EL-GHAFAR, O. A., KOZMAN, M. R., SHARKAWI, S. M. J. E. S. & RESEARCH, P. 2021. Trans-ferulic acid ameliorates cisplatin-induced testicular damage via suppression of TLR4, P38-MAPK, and ERK1/2 signaling pathways. 28**,** 41948-41964.
26. HELMY, S. A., EL-MOFTY, S., EL GAYAR, A. M., EL-SHERBINY, I. M., YOUSRA, M. J. B. & PHARMACOTHERAPY 2022. Novel doxorubicin/folate-targeted trans-ferulic acid-loaded PLGA nanoparticles combination: In-vivo superiority over standard chemotherapeutic regimen for breast cancer treatment. 145**,** 112376.

27. HUDSON, E. A., DINH, P. A., KOKUBUN, T., SIMMONDS, M. S., GESCHER, A. J. C. E. B. & PREVENTION 2000. Characterization of potentially chemopreventive phenols in extracts of brown rice that inhibit the growth of human breast and colon cancer cells. 9**,** 1163-1170.
28. IQBAL, M., NISAR, J., ADIL, M., ABBAS, M., RIAZ, M., TAHIR, M. A., YOUNUS, M. & SHAHID, M. J. C. 2017. Mutagenicity and cytotoxicity evaluation of photo-catalytically treated petroleum refinery wastewater using an array of bioassays. 168**,** 590-598.
29. JIMÉNEZ-BONILLA, P., ZHANG, J., WANG, Y., BLERSCH, D., DE-BASHAN, L.-E., GUO, L. & WANG, Y. J. B. T. 2020. Enhancing the tolerance of Clostridium saccharoperbutylacetonicum to lignocellulosic-biomass-derived inhibitors for efficient biobutanol production by overexpressing efflux pumps genes from Pseudomonas putida. 312**,** 123532.
30. KAHKESHANI, N., FARZAEI, F., FOTOUHI, M., ALAVI, S. S., BAHRAMSOLTANI, R., NASERI, R., MOMTAZ, S., ABBASABADI, Z., RAHIMI, R. & FARZAEI, M. H. J. I. J. O. B. M. S. 2019. Pharmacological effects of gallic acid in health and diseases: A mechanistic review. 22**,** 225.
31. KRISHNAIAH, D., SARBATLY, R., NITHYANANDAM, R. J. F. & PROCESSING, B. 2011. A review of the antioxidant potential of medicinal plant species. 89**,** 217-233.
32. KUMAR, S., NARWAL, S., KUMAR, V. & PRAKASH, O. J. P. R. 2011. α-glucosidase inhibitors from plants: A natural approach to treat diabetes. 5**,** 19.
33. LIN, D., XIAO, M., ZHAO, J., LI, Z., XING, B., LI, X., KONG, M., LI, L., ZHANG, Q. & LIU, Y. J. M. 2016. An overview of plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes. 21**,** 1374.
34. LODOVICI, M., CALDINI, S., MORBIDELLI, L., AKPAN, V., ZICHE, M. & DOLARA, P. J. T. 2009. Protective effect of 4-coumaric acid from UVB ray damage in the rabbit eye. 255**,** 1-5.
35. MERROUNI, A. J. J. M. E. S., 14 , 153 I., Elachouri. M.(2023) NEMMAP Database: A platform for exploring the Ethnobotanical, Pharmacological, and Phytochemical profile of North-Eastern Morocco Medicinal plants. 160.
36. MRADU, G., SAUMYAKANTI, S., SOHINI, M., ARUP, M. J. I. J. O. P. & RESEARCH, P. 2012. HPLC profiles of standard phenolic compounds present in medicinal plants. 4**,** 162-167.
37. NASEEM, Z., ZAHID, M., HANIF, M. A. & SHAHID, M. J. P. J. O. E. S. 2020. Environmentally friendly extraction of bioactive compounds from Mentha arvensis using deep eutectic solvent as green extraction media. 29**,** 3749-3757.
38. NAVEED, M., HEJAZI, V., ABBAS, M., KAMBOH, A. A., KHAN, G. J., SHUMZAID, M., AHMAD, F., BABAZADEH, D., FANGFANG, X., MODARRESI-GHAZANI, F. J. B. & PHARMACOTHERAPY 2018. Chlorogenic acid (CGA): A pharmacological review and call for further research. 97**,** 67-74.
39. NAZ, S., HANIF, M., BHATTI, H. & SHAHID, M. J. O. C. 2016. Partition, fractionation, antioxidant potential and phenolics profiling of Cannabis sativa growing in Pakistan. 39**,** 2946-2960.
40. ORGANIZATION, W. H. 2013. *WHO traditional medicine strategy: 2014-2023,* World Health Organization.
41. PRAGASAM, S. J., MURUNIKKARA, V., SABINA, E. P. & RASOOL, M. J. R. I. 2013. Ameliorative effect of p-coumaric acid, a common dietary phenol, on adjuvant-induced arthritis in rats. 33**,** 325-334.
42. REHMAN, F.-U., ADEEL, S., SHAHID, M., BHATTI, I. A., NASIR, F., AKHTAR, N., AHMAD, Z. J. R. P. & CHEMISTRY 2013. Dyeing of γ-irradiated cotton with natural flavonoid dye extracted from irradiated onion shells (Allium cepa) powder. 92**,** 71-75.

43. REZAEIROSHAN, A., SAEEDI, M., MORTEZA-SEMNANI, K., AKBARI, J., HEDAYATIZADEH-OMRAN, A., GOLI, H. & NOKHODCHI, A. J. J. O. P. I. 2022. Vesicular formation of trans-ferulic acid: an efficient approach to improve the radical scavenging and antimicrobial properties. 1-10.
44. RUBAB, K., ABBASI, M. A., SIDDIQUI, S. Z., SHAH, S. A. A., ASHRAF, M., AHMAD, I., LODHI, M. A., GHUFRAN, M., SHAHID, M. & FATIMA, H. J. P. J. O. P. S. 2017. Synthesis, pharmacological screening and computational analysis of some 2-(1H-Indol-3-yl)-N'-[(un) substituted phenylmethylidene] acetohydrazides and 2-(1H-Indol-3-yl)-N'-[(un) substituted benzoyl/2-thienylcarbonyl] acetohydrazides. 30**,** 1263-1275.
45. SAJID-UR-REHMAN, M., ISHTIAQ, S., KHAN, M. A., ALSHAMRANI, M., YOUNUS, M., SHAHEEN, G., ABDULLAH, M., SARWAR, G., KHAN, M. S. & JAVED, F. J. I. 2021. Phytochemical profiling, in vitro and in vivo anti-inflammatory, analgesic and antipyretic potential of Sesuvium sesuvioides (Fenzl) Verdc.(Aizoaceae). 29**,** 789-800.
46. SATO, Y., ITAGAKI, S., KUROKAWA, T., OGURA, J., KOBAYASHI, M., HIRANO, T., SUGAWARA, M. & ISEKI, K. J. I. J. O. P. 2011. In vitro and in vivo antioxidant properties of chlorogenic acid and caffeic acid. 403**,** 136-138.
47. SHAHZADI, T., ZAIB, M., RIAZ, T., SHEHZADI, S., ABBASI, M. A., SHAHID, M. J. A. J. F. S. & ENGINEERING 2019. Synthesis of eco-friendly cobalt nanoparticles using Celosia argentea plant extract and their efficacy studies as antioxidant, antibacterial, hemolytic and catalytical agent. 44**,** 6435-6444.
48. SHARIF, S., RASHID, S., ATTA, A., IRSHAD, A., RIAZ, M., SHAHID, M., MUSTAFA, G. J. J. O. B. R. & AGENTS, H. 2018. Phenolics, tocopherols and fatty acid profiling of wild and commercial mushrooms from Pakistan. 32**,** 863-867.
49. SHAYAN-NASR, M., GHANIEI, A., ESLAMI, M. & ZADEH-HASHEM, E. J. P. S. 2021. Ameliorative role of trans-ferulic acid on induced oxidative toxicity of rooster semen by β-cyfluthrin during low temperature liquid storage. 100**,** 101308.
50. SHIVASHANKARA, R., HANIADKA, R., FAYAD, P., PALATTY, R., ARORA, M., BALIGA, A. J. W., RR, PREEDY, VRBT-BF AS DI FOR L. & GD , A. P., SAN DIEGO 2013. Chapter 42—Hepatoprotective Effects of Zingiber officinale Roscoe (Ginger): A Review. 657-671.
51. SINGH, S. S. & PATIL, K. N. J. J. O. F. B. 2022. trans-ferulic acid attenuates hyperglycemia-induced oxidative stress and modulates glucose metabolism by activating AMPK signaling pathway in vitro. 46**,** e14038.
52. TROMBINO, S., CASSANO, R., BLOISE, E., MUZZALUPO, R., TAVANO, L. & PICCI, N. J. C. P. 2009. Synthesis and antioxidant activity evaluation of a novel cellulose hydrogel containing trans-ferulic acid. 75**,** 184-188.
53. TROMBINO, S., CASSANO, R., FERRARELLI, T., BARONE, E., PICCI, N., MANCUSO, C. J. C. & BIOINTERFACES, S. B. 2013. Trans-ferulic acid-based solid lipid nanoparticles and their antioxidant effect in rat brain microsomes. 109**,** 273-279.
54. VAUZOUR, D., CORONA, G., SPENCER, J. P. J. A. O. B. & BIOPHYSICS 2010. Caffeic acid, tyrosol and p-coumaric acid are potent inhibitors of 5-S-cysteinyl-dopamine induced neurotoxicity. 501**,** 106-111.
55. WANG, S., SUH, J. H., ZHENG, X., WANG, Y., HO, C.-T. J. J. O. A. & CHEMISTRY, F. 2017. Identification and quantification of potential anti-inflammatory hydroxycinnamic acid amides from wolfberry. 65**,** 364-372.
56. WHALEY, A., IVKIN, D., ZHAPARKULOVA, K., OLUSHEVA, I., SEREBRYAKOV, E., SMIRNOV, S., SEMIVELICHENKO, E., GRISHINA, A. Y., KARPOV, A. & ELETCKAYA, E. J. J. O. E. 2023. Chemical composition and cardiotropic activity of Ziziphora clinopodioides subsp. bungeana (Juz.) Rech. f. 116660.

57. YANG, L., GAO, Y., BAJPAI, V. K., EL-KAMMAR, H. A., SIMAL-GANDARA, J., CAO, H., CHENG, K.-W., WANG, M., ARROO, R. R., ZOU, L. J. C. R. I. F. S. & NUTRITION 2023. Advance toward isolation, extraction, metabolism and health benefits of kaempferol, a major dietary flavonoid with future perspectives. 63**,** 2773-2789.
58. ZAFEER, M. F., FIRDAUS, F., ANIS, E. & HOSSAIN, M. M. J. N. 2019. Prolong treatment with Trans-ferulic acid mitigates bioenergetics loss and restores mitochondrial dynamics in streptozotocin-induced sporadic dementia of Alzheimer's type. 73**,** 246-257.
59. ZANG, L.-Y., COSMA, G., GARDNER, H., SHI, X., CASTRANOVA, V. & VALLYATHAN, V. J. A. J. O. P.-C. P. 2000. Effect of antioxidant protection by p-coumaric acid on low-density lipoprotein cholesterol oxidation. 279**,** C954-C960.