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GC-MS Analysis, Antioxidant and Genotoxic Assessment of Medicinally Important Plant *Paeonia emodi* Wall Ex. Hook

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Abstract
Paeonia emodi Wall Ex. Hook., a member of the Paeoniaceae family, is an herbaceous perennial plant. It survives in the winter through underground buds and is known for its large white flower and deeply divided leaves. Traditionally, it has been used to treat various ailments. The current study was carried out to find the bioactive compounds by GC-MS, antioxidant activity through DPPH, genotoxic activity through comet assay, GC-MS Chromatogram represents different compounds detected in the crude extract. Major pharmacological bioactive compounds are 4H-Pyran- 4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- 4-Methyl, 10E,12Z-Octadecadienoic acid, 1,2-Cyclohexanedicarboxylic acid 1,4-Benzenedicarboxylic acid, bi..., 2-Pentanone, 4-hydroxy-4-methyl-, Eugenol; 9,12-Octadecadienoic acid (Z,Z); 1,4-Benzenedicarboxylic acid, bi...; 1-Piperidinecarboxaldehyde, 2- After 90 minutes at a concentration of 300 (g/ml), the methanolic extract showed the highest antioxidant activity (67.7±1.54), closely followed by the ethanolic extract (72.4±2.06). The methanolic and ethanolic extract show genotoxic activity (114.6±63.03), (169.7±71), and (173±87.38), (205.9±85.62) at 75, and 100 mg/100 ml, respectively at high dose. This study emphasizes that the plant is rich both micro and macro nutrients, has significant nutritional value, and shows notable pharmacological effects. These characteristics make it a promising candidate for developing treatments for various health conditions.

Key words: GC-MS, antioxidant, genotoxic, *Paeonia emodi*

Introduction

Throughout history people have turned to plants for medicinal purposes drawing on their cultural heritage and traditional beliefs. Local communities, guided by personal experiences have employed medicinal plants to address various health remedies [1] Medicinal plants play a significant role as a varied reservoir of remedies in both traditional and contemporary medicine. They are utilized in a range of application, including nutraceutical, supplements, folk medicine, and pharmaceutical compounds [2]. The natural derivatives of plants are harnessed for the development of medicines, supplements, and a variety of healthcare products. These plants are essential in the exploration of novel medicinal compound with phytochemical constituents like antioxidants, hypoglycemic and hypolipidemic agents [3]. Utilizing natural, plant based products contributes to human well-being by reducing side effects and providing a cost effective alternative. This has led to an increasing demand for therapeutic products derived from plants [4]. According to the (WHO) World Health Organization, 80% of the global population in emerging economies depends on traditional medicine for the therapeutic needs [5]. Approximately 70,000 plant species are recognized for their potential in treating various diseases, yet only about 15% of the world's plant species have been subject to medicinal investigation. Despite this relatively low exploration rate, 25% of the pharmaceuticals employed in modern medicines trace their origins back to plants [6]. Pakistan, boasting a rich reserve of medicinal, harbors around 6,000 plant species, out of which 400-600 of which are utilized for medicinal purposes. This extensive biodiversity positions the country uniquely in the realm of ethno-pharmacology [7]. Medicinal plants contain a wide range of biological active compounds, including minerals and phytochemicals, which have diverse effects on human health [8, 9]. Within plants, there are diverse phytochemicals, also known as secondary metabolites. These compounds have the capability to address particular disorders through individual, additive, or synergistic mechanisms, ultimately contributing to the enhancement of overall well-being [10]. Phytochemicals play a vital role in the pharmaceutical industry, contributing to the development of new medications and the formulation of therapeutic agents [11]. The utilization of gas chromatography coupled with mass spectrometer (GC–MS) has emerged as a pivotal method for identifying and quantifying medically valuable compounds present in medicinal plants. This method provides a relatively quick, accurate and efficient way to detect a diverse range

of bio active substances, including alkaloids, long-chain hydrocarbons, steroids, sugars, amino acids, and nitro compounds. Importantly, this technique requires minimal extract volumes [12]. Reactive oxygen species (ROS), generated during aerobic metabolism, play crucial roles, but their excessive production can harm bio-molecules. Involvement of ROS is observed in conditions like Alzheimer's, atherosclerosis, diabetes, inflammation, and neurodegenerative diseases, and they also impact cancer and aging. Antioxidants counteract ROS by hindering oxidative reactions, reducing lipid peroxidation, minimizing free radicals, and chelating metal ions [13]. The impacts of oxidative stress on human health and growing concerns about synthetic antioxidants have determined the scientific community to explore secure and viable natural antioxidant alternatives [14–15]. Plant-based foods serve as abundant sources of naturally occurring antioxidants, including vitamin C, tocopherol (vitamin E), carotenoids, phenolic compounds, and polyphenolic compounds. Furthermore, research indicates that bioactive peptides derived from protein-rich foods of both plant and animal origin can also functions as antioxidants through similar mechanisms [16]. The term genotoxicity is frequently used interchangeably with mutagenicity, but it's important to note that while all mutagens are genotoxic, not all genotoxic substances are mutagenic. Several assays have been created to evaluate genotoxic effects and their correlation with alterations in plant growth and development [17]. Genotoxic agents can induce harm to genetic material, resulting in to gene mutation, chromosomal alterations, and DNA damage. These genetic changes may lead to cell death or malignancies, potentially impairing the organisms function and diminishing its chances of survival [18].

Paeonia emodi Wall Ex. Hook is an upright, leafy perennial herb with a height of 50 cm and a smooth, hairless surface. Its leaves are either bi-ternate or ternate, featuring a pale lamina. The flowers of *P. emodi* are solitary and found in the axils. The bracts are leafy, and it has eight petals, with seeds ranging from three to five [19]. Paeoniaceae is a flowering plants family with a single genus, encompassing 34 species. These species include shrubs and perennial herbs that are predominantly distributed across temperate, tropical and alpine zone in Asia, Eurasia, northwest Africa, and western North America [20, 21]. Tree peonies, classified under Section Moutan DC., are the shrub-type varieties whereas herbaceous peonies fall under section *Paeonia* or *Onaepia* Lindley [22].

Results

Gas Chromatography-Mass Spectroscopy ethanolic sample of *Paeonia emodi* Wall Ex. Hook

The ethanolic sample of *Paeonia emodi* Wall Ex. Hook plant was checked for the phytochemical study. In the present study the result showed in many peaks, which showed various bioactive compounds in the present plant sample. These peaks were compared to a database containing spectra of known components found in the GC-MS library. The GC-MS analysis of *Paeonia emodi* Wall Ex. Hook plant revealed the presence of several bioactive compounds at varying retention times per minute (RT/minutes). About 12 compounds were identified. These compounds are Benzoic acid (12.93), 1,2,3-Benzenetriol (0.45), n-Hexadecanoic acid (4.37), 10E,12Z-Octadecadienoic acid (1.32), 9-Octadecenoic acid (3.47), Oleic acid (5.12), Octadecanoic acid (2.14), Benzoic acid, 4-acetylbenzyl ester (3.43), Bis(2-ethylhexyl) phthalate (1.04), 1,2-Cyclohexanedicarboxylic-acid... (5.05), Cyclotrisiloxane, hexamethyl-(1.82), 1,4-Benzenedicarboxylic acid, bi... (59.78), (Table 2.1).

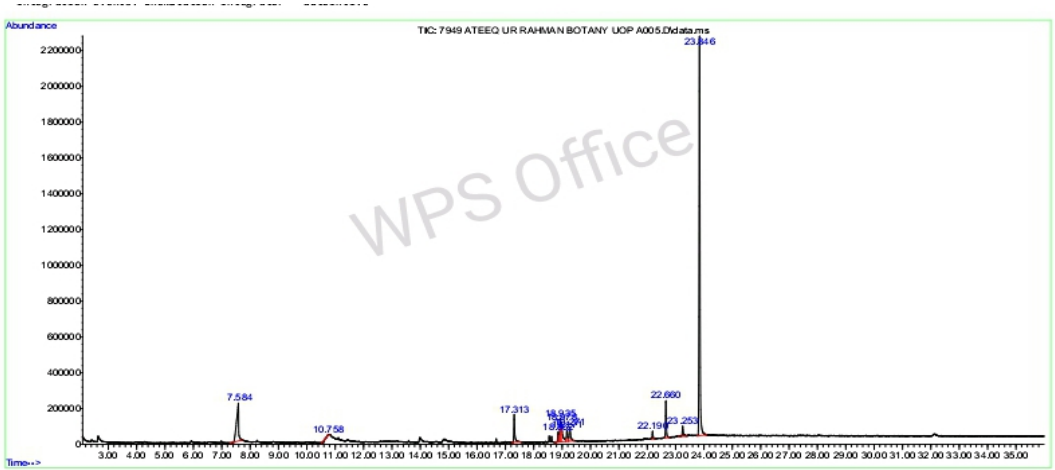

Figure 1. GC-MS Chromatogram of the ethanolic sample of *Paeonia emodi* Wall. Ex. Hook. The numbers showed the retention times of various compounds.

2.1. Bioactive compound identified plant ethanolic sample of *Paeonia emodi* Wall. Ex. Hook. Through GC-MS.

S.No	RT/(min)	SI	Area%	Compound name	Formula	MW	Prob
1.	7.584	11261	12.93	Benzoic acid	C_6H_5COOH	122.12	97
2.	10.758	12695	0.45	1,2,3-Benzenetriol	$C_6H_6O_3$	126.11	81
3.	17.313	143511	4.37	n-Hexadecanoic acid	$C_{16}H_{32}O$	256.42	99
4.	18.862	173577	1.32	10E,12Z-Octadecadienoic acid	$C_{18}H_{32}O_2$	280.4	98
5.	18.935	176210	3.47	9-Octadecenoic acid	$C_{18}H_{34}O_2$	282.46	99
6.	18.978	176208	5.12	Oleic Acid	$C_{18}H_{34}O_2$	282.5	99
7.	19.171	179093	2.14	Octadecanoic acid	$C_{18}H_{34}O_2$	284.5	98
8.	19.271	140676	3.43	Benzoic acid, 4-acetylbenzyl ester	$C_{16}H_{14}O_3$	254.28	93
9.	22.190	295608	1.04	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.6	70
10.	22.660	299628	5.05	1,2-Cyclohexanedicarboxylic acid...	$C_8H_{12}O_4$	172.18	62
11.	23.253	102257	1.82	Cyclotrisiloxane, hexamethyl-	$C_6H_{18}O_3Si_3$	222.461	18
12.	23.846	295779	59.78	1,4-Benzenedicarboxylic acid, bi...	$C_{20}H_{26}O_6$	362.4	95

Methanolic sample of *Paeonia emodi* Wall. Ex. Hook

The methanolic extract of the *Paeonia emodi* Wall Ex. Hook plant revealed numerous peaks in figure which represents various bioactive compounds found within the fraction. As demonstrated in figur the detected peaks were cross-referenced with a database comprising spectra of known components accessible within GC-MS library. The analysis of the methanolic sample from the *Paeonia emodi* Wall Ex. Hook plant unveiled a range of bioactive compounds at distinct retention times/minutes, as outlined (table 2**)** these compound are 2-Pentanone, 4-hydroxy-4-methyl-(3.60), 1,3-Dihydroxyacetone dimer(3.33), Glycerin(0.44), Benzaldehyde, 2-hydroxy-(0.84), 1,3,5-Triazine-2,4,6-triamine(1.49), Diglycerol(1.78),4H-Pyran-4-one,2,3-dihydro-3,5-...(1.09), Benzoic acid(25.99), 5-Hydroxymethylfurfural(1.76), 1,2,3-Propanetriol, 1-acetate(1.17),Salicyl alcohol (0.95), 4-Nonano (0.54), Eugenol(0.47),1,2,3-Benzenetriol(17.54), 1,2,3-Benzenetriol(7.39),1,2,4-Benzenetriol(1.41), 4, 5 Dihydroxytropolone (1.32), beta.-D-Glucopyranose, 1,6-anhy...(0.49), Acetamide, N-(4-hydroxycyclohexy...(0.46), 3-Deoxy-d-mannoic lactone(1.71), Phenol, TMS derivative(0.48), Daphnetin(3.22), 1-Piperidinecarboxaldehyde, 2-(1..(4.02), 1-Oxaspiro[2.5]octane, 2,4,4-tri...(0.18), n-Hexadecanoic acid(1.57), 9,12-Octadecadienoic acid (Z,Z)-(2.12), Oleic Acid(0.83), 9-Octadecenoic acid, (E)-(0.74), Benzoic acid, 4-acetylbenzyl ester(2.63), Bis(2-ethylhexyl) phthalate(0.54), 1,4-Benzenedicarboxylic acid, bi...(9.90), (Table 2.2).

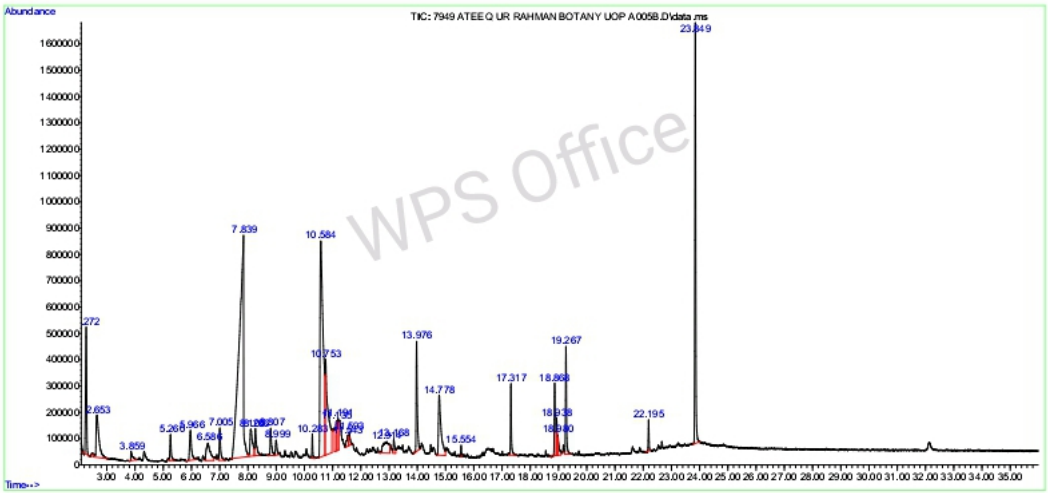

Figure 2. GC-MS chromatogram of the methanolic sample of *Paeonia emodi* Wall Ex. Hook the numbers showed the retention times of various compounds.

Table.2.2. for bio active compound identified methanolic plant sample of *Paeonia emodi* Wall. Ex. Hook through GC-MS

S.no	RT/(min)	SI	Area%	Compound name	Formula	MW	Prob
1.	2.272	9288	3.60	2-Pentanone, 4-hydroxy-4-methyl-	$C_6H_{12}O_2$	116.1583	50
2.	2.653	55207	3.33	1,3-Dihydroxyacetone dimer	$C_6H_{12}O_6$	180.16	50
3.	3.859	2720	0.44	Glycerin	$C_3H_8O_3$	92.093	83
4.	5.260	11277	0.84	Benzaldehyde, 2-hydroxy-	$C_7H_6O_2$	122.1213	97
5.	5.966	12521	1.49	1,3,5-Triazine-2,4,6-triamine	$C_3H_6N_6$	126.1199	78
6.	6.586	41740	1.78	Diglycerol	$C_6H_{14}O_5$	166.17	45
7.	7.005	23823	1.09	4H-Pyran-4-one, 2,3-dihydro-3,5-...	$C_6H_8O_4$	144.12	96
8.	7.839	11264	25.99	Benzoic acid	C_6H_5COOH	122.12	95
9.	8.105	12708	1.76	5-Hydroxymethylfurfural	$C_6H_6O_3$	126.11	96
10.	8.266	17231	1.17	1,2,3-Propanetriol, 1-acetate	$C_5H_{10}O_4$	134.13	53
11.	8.807	11907	0.95	Salicylic alcohol	$C_7H_8O_2$	124.14	93
12.	8.999	24370	0.54	4-Nonano	C_9H_{18}	126.24	27
13.	10.283	39164	0.47	Eugenol	$C_{10}H_{12}O_2$	164.2	98
14.	10.584	12692	17.54	1,2,3-Benzenetriol	$C_6H_6O_3$	126.11	95
15.	10.753	12692	7.39	1,2,3-Benzenetriol	$C_6H_6O_3$	126.11	93
16.	11.135	12698	1.41	1,2,4-Benzenetriol	$C_6H_6O_3$	126.11	73
17.	11.191	31803	1.32	4,5-Dihydroxytropolone	$C_7H_6O_4$	154.12	64
18.	11.543	38155	0.49	beta. -D-Glucopyranose, 1,6-anhy...	$C_6H_{10}O$	162.14	30
19.	11.593	34392	0.46	Acetamide, N-(4-hydroxycyclohexy...	$C_8H_{15}NO_2$	157.21	38
20.	12.914	38147	1.71	3-Deoxy-d-mannoic lactone	$C_6H_{10}O_5$	162.14	43
21.	13.168	42353	0.48	Phenol, TMS derivative	$C_9H_{14}OSi$	166.29	70
22.	13.976	53570	3.22	Daphnetin	$C_9H_6O_4$	178.14	53
23.	14.778	52430	4.02	1-Piperidinecarboxaldehyde, 2-(1.	$C_6H_{11}NO$	113.16	62
24.	15.554	41475	0.18	1-Oxaspiro [2.5] octane, 2,4,4-tri...	$C_7H_{12}O$	112.17	53
25.	17.317	143511	1.57	n-Hexadecanoic acid	$C_{16}H_{32}O$	256.42	99
26.	18.868	173583	2.12	9,12-Octadecadienoic acid (Z, Z)-	$C_{18}H_{32}O$	280.44	99
27.	18.938	176208	0.83	Oleic Acid	$C_{18}H_{34}O_2$	282.47	98
28.	18.980	176223	0.74	9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	282.46	99
29.	19.267	140676	2.63	Benzoic acid, 4-acetylbenzyl ester	$C_{16}H_{14}O_3$	254.28	91
30.	22.195	295612	0.54	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.6	83
31.	23.849	295779	9.90	1,4-Benzenedicarboxylic acid, bi...	$C_8H_6O_4$	166.31	95

Antioxidant Activity

During metabolism, living cells produce free radicals, which can contribute to the development of various harmful conditions such as diabetes mellitus, atherosclerosis, ageing, ischemic heart disease and cancer. Antioxidant help protect cells by neutralizing these free radicals and preventing oxidative damage. Compared to synthetic antioxidants, natural antioxidant is generally less toxic and safer to use [23]. The antioxidant activity of methanolic and ethanolic extracts of *Paeonia emodi* Wall Ex. Hook was evaluated using the DPPH method. The findings showed that the ethanolic extract exhibited higher antioxidant activity (72.4±2.06%) than the methanolic extract (67.7±1.54%) at a concentration of 300 mg/ml after 90 minutes. However, both extracts were less effective compared to the standard antioxidant, ascorbic acid, which showed an activity of (88.2±2.08%). Interestingly, the methanolic extract had the lowest (IC_{50} = 130.51µg/ml), indicating higher potency at lower concentrations. Overall, the scavenging activity of both extracts was found to be dependent on both does and time. Table 2.3). These results agree with [24] who reported similar findings for the methanolic extract of *Datura metel,* where the scavenging activity of the extracts was found to be both dose- and time-dependent. The antioxidant properties of the plant extracts are likely attributed to the presence of phenolic compounds and flavonoid content assays, it was evident that the methanolic and ethanolic extracts of *P. emodi* Wall. Ex. Hook contained the highest levels of these bioactive compounds. (Table 2.3). the phenolic and flavonoid compounds in *Paeonia emodi* Wall. Ex. Hook are likely responsible for its antioxidant properties. [25] Phenolic compounds exhibit strong free radical- scavenging abilities because their hydroxyl groups can donate electrons, thereby neutralizing harmful radicals. [26]. Similarly, flavonoids contribute to antioxidant activity through their phenolic hydroxyl groups, which enable them to efficiently neutralize (ROS) reactive oxygen species [27].

Table 2.3. Antioxidant Activity of methanolic and ethanolic extract of *Paeonia emodi* Wall. Ex. Hook.

Sample	Conc. (µg/ml)	% DPPH radical scavenging activity	IC_{50} (µg/ml) 90 min
Ascorbic acid	100	53.3±0.36	57.64
	200	67.4±0.45	
	300	88.2±2.08	
Methanolic Extract	100	40.3±0.04	189.48
	200	52.5±0.02	
	300	67.7±1.54	
Ethanolic Extract	100	51.2±1.03	93.62
	200	63.6±0.65	
	300	72.4±2.06	

Genotoxic Activity

Genotoxicity is a critical property of toxic substance, often arising from changes in genetic material or damage to DNA strands. Such alterations can lead to apoptosis, cancer development, or change in cellular phenotype. [28]. The genotoxic effects of methanolic and ethanolic extracts of *Paeonia emodi* Wall. Ex. Hook were evaluated on lymphocytes DNA using the comet assay. The findings revealed that the highest level of DNA damage occurred in lymphocytes treated with both hydrogen peroxide (as the standard) and methanolic extract of *Paeonia emodi* (Table 2.4).

After 72 hours, the methanolic extract showed dose-dependent genotoxicity at concentrations of 75 mg/100ml and 100 mg/100ml, with value of (114.6±63.03) and (169.7±71), respectively. However, the total comet scoring decreased sequentially, yielding insignificant results. (Table 2.4). Lymphocytes treated with standard hydrogen peroxide and ethanolic extract of *Paeonia emodi* Wall. Ex. Hook exhibited significant DNA damage, as shown in table **(**Table 2.5). after 72 hours, the ethanolic extract induced dose-dependent genotoxicity, with value of (173±87.38), (205.9±85.62) at concentrations of 75 mg/100ml and 100 mg/100ml, respectively. However, the total comet scoring decreased consecutively, resulting in statistically insignificant findings as shown in table (Table 2.5). The genotoxic effects observed in the comet assay after exposure to the methanolic and ethanolic extracts of *Paeonia emodi* Wall. Ex. Hook may be attributed to the presence of various bioactive compound, including flavonoids, saponins, tannins, steroids, phenols, fibers and fatty acids these findings are consistent with previous studies on medicinal plants, such as (*E. triaculeata* [29]. *Solanum lycocarpum* [30] *Acacia aroma* [31], which also reported genotoxic effect. Tannins have a reductive chemical structure that enables them to neutralize free radicals. [32] Flavonoids, with hydroxyl groups can act as phytochelatins, chelating exogenous compounds [33] and detoxifying reactive oxygen species (ROS), particularly in the presence of metals. [34]

Table 2.4. comet assay of genomic DNA of human lymphocytes exposed to methanolic extract of *Paeonia emodi* Wall. Ex. Hook.

Comet assay of genomic DNA of human lymphocytes exposed to methanol extract				
Class	**Negative Control (Only Lymphocytes)**	**Positive Control (Lymphocytes + H_2O_2)**	**75mg/100ml**	**100mg/100ml**
Class 0	76.5 ± 14.6	7.4 ± 4.4	83.0 ± 13.06	76.0 ± 16.5
Class 1	7.4 ± 3.6	10.5 ± 6.04	22.6 ± 10.6	34.5 ± 15.06
Class 2	5.0 ± 3.2	46.6 ± 14.5	18.4 ± 11.5	42.5 ± 13.6
Class 3	2.6 ± 1.06	52.0 ± 12.5	10.0 ± 7.05	15.0 ± 9.5
Class 4	0.5 ± 0.03	38.6± 6.4	6.3 ± 2.07	1.3 ± 0.06
TCS	27.2±13.3	414.1±98.14	114.6±63.03	169.7±71
TCS: Total comet score. Values are expressed as mean ± standard deviation (S.D). Difference significant relative to positive control at **P*<0.002, ***P*<0.0001 (One-way ANOVA, Tukey Test).				

The total comet score was calculated by formula; TCS=0(n) + 1(n) + 2(n) + 3(n) + 4(n), where (n) shows number of cells in each class.

Table 2.5. Comet assay of genomic DNA of human lymphocytes exposed to ethanolic extract of *Paeonia emodi* Wall. Ex. Hook.

Comet assay of genomic DNA of human lymphocytes exposed to Ethanolic extract				
Class	**Negative Control (Only Lymphocytes)**	**Positive Control (Lymphocytes + H_2O_2)**	**75mg/100ml**	**100mg/100ml**
Class 0	72.3 ± 10.7	5.6 ± 3.06	80.5 ± 12.6	76.0 ± 16.7
Class 1	8.2± 4.6	14.3 ± 8.07	32.4 ± 13.04	26.7 ±12.08
Class 2	6.5 ± 4.2	48.0 ± 12.5	28.0± 9.5	18.0 ± 9.06
Class 3	4.0 ± 2.06	57.5 ± 11.3	17.0 ± 11.7	28.4 ± 9.06
Class 4	2.7 ± 1.05	36.3± 8.5	8.4 ± 5.06	14.5 ± 7.06
TCS	44±23.38	428±87.21	173±87.38	205.9±85.62

TCS: Total comet score. Values are expressed as mean ± standard deviation (S.D). Difference significant relative to positive control at **P*<0.002, ***P*<0.0001 (One-way ANOVA, Tukey Test).

The total comet score was calculated by formula; TCS=0(n) + 1(n) + 2(n) + 3(n) + 4(n), where (n) shows number of cells in each class.

Discussion

Throughout history, medicinal plants have served as remedies for a variety of human ailments. In our modern industrialized society, the utilization of medicinal plants can be attributed to the extraction and development of numerous drugs, building upon their traditional applications in folk medicine [35]. Medicinal flora contains powerful phytoconstituents that serve as a significant reservoir of antibiotic compounds, contributing to their therapeutic characteristics. These phytoconstituents confer upon them their valuable medicinal attributes [36,37]. About 12 and 31compounds were identified in the ethanolic and methanolic fraction of *Paeonia emodi* Wall. Ex. Hook. through GC-MS. From the identified compounds some were found to have significant pharmacological activities. For example, 4H-Pyran- 4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- has Anti-oxidant, anti-microbial, laxative and anticancer activities [38].

It additionally restrains growth and triggers cell death in tumor cells via a mechanism that includes the suppression of NF-κB activity [39]. 1, 2, 3-Propanetriol, 1-acetate has antibacterial activity [40]. 1, 2, 3-Benzenetriol have antibacterial, antifungal, anti-inflammatory, analgesic, antidepressant, anticonvulsant, anti-tumor, imaging, Anti-HIV, anti-diabetic, anti-tubercular, antioxidant and miscellaneous activities [41]. N-Hexadecanoic acid possess Anti-inflammatory, antispasmodic, anticancer and antiviral activities [38]. 9, 12-Octadecadienoic acid (Z, Z) - are used as Urine acidifier and increase zinc bio-availability [42]. N-Hexadecanoic acid have antioxidant, anti-tumor, immune stimulant, Anti-inflammatory, cytotoxic, anti-arthritic, nematicide, antihistaminic and cancer preventive potential [43, 44] Previously Riaz et al. [45] investigated *Mazus goodenifolius* for GC-MS analysis which showed that the essential oil has some phytochemicals which may have antimicrobial and antioxidant activity. The connection between mineral levels in the body and specific health disorders suggests that plants containing these minerals could help prevent illness. Therefore, the elemental composition of plants plays a vital role in enhancing their effectiveness against various diseases [46]. Minerals play a crucial role in nutrition, with key essential elements being provided through well-balanced diets. These minerals serve important functions in the body, encompassing structural, physiological, and metabolic processes [47]. Calcium is crucial for various bodily functions, including neuromuscular activity, blood clotting, metabolic processes, and bone health. It also plays a key role in bone formation and acts as a mediator for hormonal effects on target organs.

Plants are rich in antioxidants that protect against diseases caused by free radicals. These antioxidant, often produced as secondary metabolites, can neutralize free radicals and prevent oxidative damage by sacrificing themselves in the process [48]. The DPPH radical scavenging assay revealed that the ethanolic extracts of *Paeonia emodi* Wall Ex. Hook shows higher antioxidant activity (72.4±2.06), compared to the methanolic (67.7±1.54), at 300 mg/ml after 90 minutes. However, both extract showed lower antioxidant activity than the standard ascorbic acid (88.2±2.08). The ethanolic extract had a lower IC50 value (93.62 µg/ml), than the methanolic extract (189.48µg/ml), indicating greater potency. These results are consistent with previous findings reported by Jahanban-Esfahlan *et al.* [49] researchers studied the effects of methanolic and ethanolic extracts *Juglans regia* L. Medicinal plants like walnut are rich in natural antioxidants, including polyphenols (flavonoids, lignans, phenolic acids, anthocyanin, and stilbenes), carotenoids (carotenes and xanthophyll's), and vitamins, (C and E). these plants are valuable source of antioxidants [50,51].

Genotoxicity refers to the ability of certain substances to damage genetic material, potentially leading to changes in DNA, mutations, or break in DNA strand. This can result in harmful effects, including cell death (apoptosis), cancer development (carcinogenesis), or change in physical characteristics (phenotypic alterations). [52]. Oxidative stress can cause DNA damage, potentially leading to diseases like cancer, genetic mutations, and accelerated aging. Understanding the impact of compounds on DNA and their biological effects is essential for identifying potential risks and benefits [53]. The comet assay is a popular technique for detecting DNA damage. Hydrogen peroxide (H2O2) is commonly used as a model genotoxic agent to test the protective effects of various compounds against DNA damage [54]. The genotoxic activity of the plant indicates that the methanolic fraction of the plant possess genotoxic activity at high concentration (100 mg/100 ml) whereas the ethanolic fraction show dose-dependent genotoxicity at 75, and 100 mg/100 ml, respectively Mattana *et al*. [55]. Researchers like Andrade *et al*. [56], Al-Faifi *et al*. [100] have investigated the genotoxic effects of medicinal plants and reports similar findings. The genotoxic potential of selected plant is due to the presence of several phytochemicals such as 4HPyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- and Hexadecanoic acid, methyl ester [43].

Materials and methods

Collection and Identification of Plant

Paeonia emodi was collected from their natural habitats in Dir Kohistan latitude.35.51485° or 35° 30'54' north; Longitude. 72.29547° 72° 17' 44''east. KPK Pakistan Later than collection the plant was identified with the help of the Flora of Pakistan. The plants identity was confirmed by experts at the Department of Chemical and Life Sciences, Qurtuba University of Science and Information Technology, Peshawar, Pakistan. The collected plant was washed with tap water to remove any kind of adulterants and applied onto blotting papers and allowed to dry at ambient temperature. The dried plant was finally chopped into small pieces and further processed into powdered form using an electric blender.

Extract preparation

The grinded plant material was utilized to prepare extracts. To do this, 500 g of the powder were combined separately with 95 % pure methanol and ethanol at room temperature. And placed them for 72 hours and this process was repeated three times. After the extraction process the resulting mixture were filtered using whatman filter paper one to remove solid particles. The filtered liquids from each extraction round were then combined or pooled together. Next, the pooled filtrate was subjected to concentration using a rotary evaporator "Laborota 4000, Germany" at a temperature of 40 Celsius under the lowered pressure. This process aimed to remove the solvent and concentrate extract. The final dried and concentrated extract was used for results

GC-MS Analysis

For the purpose of identifying the various phytochemical compounds present in the Ethanolic and methanol sample of the selected plants. The samples underwent Gas Chromatography-Mass Spectrometry. The analysis was performed using a model (DSQ-II) Thermo Scientific GC instrument, equipped with a 30-meter long model(TR-5MS) capillary column that had a width of 0.25 micrometer(μm) and an inside diameter of 0.25 mili meter(mm). Helium gas served as the carrier gas, flowing at a rate of 1ml/minutes. The injection method employed a split mode with an initial temperature within the oven of 70°C which was kept for three min. Afterword; temperature was gradually increased at a rate of 10°C/minutes until it reached 50°C. Subsequently the temperature was raised further at a rate of 15°C per minute until it reached 270°C and was held at that temperature for 13 minutes. During this process a 1 μl sample volume was injected for analysis. This analytical approach allowed for the identification of the chemical constituent's presents in the plant extracts [57]

Antioxidant Activity

The antioxidant activity of methanolic and ethanolic extracts of the medicinal plant was evaluated using the DPPH assay, following a modified protocol [58]. A 0.135 mm DPPH solution was prepared, and 1 ml of this solution was mixed with 1 ml of plant extract at various concentrations (100- 300 μg/ml). the mixture was incubated in the dark at room temperature, and absorbance's was measured at 517nm after 30, 60, and 90 minutes using a UV-Vis spectrophotometer. Ascorbic acid served as a reference standard, and a mixture of methanol and DPPH solution was used as a control. The percentage inhibition was calculated using the formula.

% Inhibition = (Ac-As) /Ac) ×100, where 'Ac' is the absorbance of the control and 'As' is the absorbance of the sample.

Genotoxic Activity

Genotoxic activity assessment is conducted through the comet assay, following the methodology outlined by Singh *et al*., [59] with some modification. Initially, cell undergoes cultivation and exposure to the test compound, after which they are harvested and suspended in an appropriate buffer. The cells are then embedded in a layer of

low-melting-point agarose on a microscope slide, lysed to eliminate cellular components, and subjected to electrophoresis to induce DNA migration. Subsequently, the slide is stained using a DNA-specific fluorescent dye and examined through fluorescence microscopy and image analysis software to quantify parameters such as tail length, tail intensity, and tail moment. Comparing these parameters between treatment groups and controls enables the determination of genotoxic activity.

Statistical Analysis

The data analysis was carried out using SPSS Version 20.0. The results were expressed as the Mean ± standard error (SE) based on three repeated determinations.

Conclusion

The present study underscores the abundant presence of phytochemicals as well as micro and macro nutrients in the examined plant. This indicates that the plant serves as a remarkable natural reservoir of essential mineral elements at moderate concentrations, playing an essential role in the treatment of various diseases. The plants pharmacological and pharmaceutical value stems from the presences of the nutritional components. Additionally, the plant displays pharmacological activities, including genotoxic and antioxidant properties. These characteristics make the plant a promising candidate for the development of remedies aimed at treating a diverse range of the therapeutic diseases**.**

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