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GC-MS Analysis, *In Vitro* Antioxidant and Antidiabetic Activity of *Oxyria digyna* (L.) Hill: An Underexplored Trans-Himalayan Plant

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ABSTRACT

Oxyria digyna (mountain sorrel) is an edible and medicinal herb with a wide array of ethnopharmacological uses. A literature review revealed that this species is less explored for its pharmacological efficacy. Methanolic leaf extract of *O. digyna* was screened for antioxidant potential utilizing "2,2-diphenyl-1-picrylhydrazyl" DPPH and "Hydrogen peroxide" H_20_2 assay, in addition, the antidiabetic potential was evaluated using enzyme alpha-amylase. Further, the bioactive compounds were analyzed through GC/MS and FTIR techniques. The antioxidant results demonstrated that the extract showed strong reducing potential for both DPPH and Hydrogen peroxide assay, plant extract revealed scavenging activity with an IC_{50} value of 42.55±0.7311 µg/ml for DPPH and an IC_{50} value of 51.77±1.855 µg/ml for H_2O_2. Furthermore, *O. digyna* showed a moderate inhibitory outcome towards alpha-amylase with an IC_{50} = 131.02±1.90 µg/ml while the standard showed an IC_{50} = of 22.05±3.9 µg/ml. The extract exhibited an enormous amount of total flavonoid and phenolic. Moreover, the FTIR spectrum showed the presence of alcohol, alkanes, alkyne, aldehyde, etc. and the GC/MS study reveals the presence of sixty compounds. The most prevalent one is 9,12,15- Octadecatrienoic acid (Z, Z, Z) 38.33 %. According to our knowledge, this study is the first to validate its antidiabetic potential and identification of numerous phytoconstituents through GC/MS and identification of several functional groups employing FTIR analysis. The above finding suggests that *O. digyna* possess a high amount of phenols, and flavonoids, showing significant antioxidant properties, which makes it a promising source of natural antioxidant, also it can be used in food industries and for future drug synthesis. Further, the extract showed potential alpha-amylase inhibition but the potential was less, further, the active biochemical constituent can be isolated and utilized in therapeutic applications.

Keywords: *Oxyria digyna*; antioxidant; antidiabetic; GCMS; FTIR; Ladakh

1. INTRODUCTION

Ancient medicine practitioners and especially indigenous habitants from Trans-Himalayas had harnessed invaluable potentialities of medicinal plants to cure diseases and have been applying them since antiquity. Plants play a crucial and fundamental function in preventing and treatment of various ailments by producing an array of phytochemicals with pharmacological and biological potential. Owing to this, they are the source of a plethora of therapeutic drugs.

Free radicals are extremely reactive and toxic molecules produced in our body as by-products of normal cellular metabolism. If not quenched, they may react with biomolecules like lipids, carbohydrates, and proteins which subsequently cause many degenerative diseases like cancer, cardiovascular dysfunctions, neurodegenerative, inflammation, and premature ageing.[1]. Thus, antioxidants are required to neutralize oxidation in the food system and the human body, thereby shielding the body from free radical toxicity.[2]. The Human body has been naturally complemented with a defense mechanism against free radicals but, under conditions of stress and ageing, this natural system becomes inefficient. Therefore, antioxidants must be supplemented through diet and drugs. Although synthetic antioxidants are frequently used, however, they are associated with potential side effects. Lately, interest has increased considerably towards plant-based natural antioxidants and replacing synthetic ones due to their toxicological effects associated with prolonged usage.[3].

A persistent metabolic condition arises either due to fault in the receptor of insulin or deficiency in insulin secretion is known as diabetes.[4]. In the 21st century, this disease is emerging as one of the greatest threats to human well-being.[5]. Besides the incidence of diabetes has significantly increased all across the world. The dramatic shift towards disease is due to major lifestyle changes, ageing, uptake of food with nutrient deficiencies, and consuming carbohydrate-rich foods.[6]. According to an estimation, over 220 million people are suffering from diabetes of which 10% are dealing with type one diabetes (insulin-dependent) and the rest is suffering from type 2 diabetes (insulin-independent).[7]. "The most prevalent one is type II diabetes which is creating a global health concern linked to co-morbidity and death".[8]. Diabetic patients come across countless

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miseries along with debilitating complications which affect their quality of life. Besides, the demand for self-care becomes overwhelming and burdensome.[9]. Treatment and management of diabetes include diet and exercise-related self-care along with insulin therapy. In addition, various conventional therapies such as metformin-biguanides, sulphonylureas, insulin, and acarbose are used to alleviate hyperglycemia.[10] Prolonged usage of these medications shows significant side effects.[11] In this context, consideration of novel plant-based therapies holds immense potential as an alternative drug as these are non-toxic with fewer side effects which will lead to improved quality of life in patients.

Genus *Oxyria* belongs to (the family Polygonaceae) and consists of just two species. *Oxyria digyna* and O. sinensis. The Former shows a diverse distribution and the latter is endemic to China.[12]. *Oxyria digyna* (mountain sorrel) is a perennial herbaceous, edible wild plant consumed as a salad.[13]. It is distributed in Artic-alpine zones, Artic Tundra, Asia, North America, and Eurasia. In India, it is present throughout the Himalayas. Herbal formulation of this species shows protective or healing potential and is used to treat various maladies by traditional healers. Earlier ethnobotanical reports reveal that *O. digyna* roots are used to cure cough, cold, and boils by locals of Himachal Pradesh.[14]

Natives of the Poonch region of Azad Jammu and Kashmir, utilise the entire plant to heal liver disorders, stomach issues, constipation, and juices are used to treat jaundice.[15] People of district Bagh Azad Kashmir use the leaves of *O. digyna* to treat scurvy and leaves, stems, and roots are used for treating dysentery.[16] In the Ladakh region, entire plant decoction is consumed two or three times a day to alleviate dyspepsia.[17] However, the research on the facet of phytochemicals and other pharmacological potentials was scanty and lacking. As an ethnomedicinal plant, *O. digyna* serves to treat various diseases. Therefore, it is crucial to investigate the phytochemical composition and constituents responsible for its essential biological actions. Therefore, we aim to evaluate pharmacological properties with particular reference to antioxidant ability and antidiabetic potential. Further, using the technique GC/MS, phytochemical identification was done. Moreover, our study is new to validate in vitro antidiabetic potential of *Oxyria digyna* collected from the high altitude Ladakh region.

2. METHODOLOGY

2.1 Collection and Verification of Herb

Plant material was gathered from the Matho village of Ladakh. Plant identification was done from the Botanical Survey of India, Dehradun, and a specimen with accession no. 811 was submitted to the herbarium.

2.2 Preparation of Crude Extract

Healthy leaves of *O. digyna*, were thoroughly cleaned and shade dried under normal temperature, and later crushed into fine powder. Plant powder was mixed with methanolic solvent 90 % and kept on a shaker for nearly 24 hours. Under similar conditions, the extraction was reproduced thrice. Filtration was done using (Whatman no1) filter paper and left open at normal temperature. The concentrated extract was further used for various analyses.

2.3 Chemicals

DPPH, DMSO, K_2HPO_4 (Potassium phosphate dibasic), NaOH "Sodium hydroxide", $AlCl_3$ (Aluminum chloride), $NaCl_2$ (Sodium chloride), $C_7H_4N_{207}$ (Dinitro salicylic acid), Na_2CO_3 (Sodium carbonate), HCL, Starch, Sodium potassium tartrate, Hydrogen peroxide, Ascorbic acid, Gallic acid, Quercetin, Enzyme alpha-amylase was purchased from sigma Aldrich, FC reagent, Aluminum chloride, Ferric chloride. The chemicals used above were all analytical grade. Methanol, Ethanol, and the majority of chemicals above were purchased from (GK Enterprises, India).

2.4 Qualitative Screening

Preliminary screening of phytochemicals was performed to identify the secondary metabolites of plant samples in different extracts; ethanolic, methanolic, and aqueous. Used protocols by.[18]

2.5 Assessment of Total Phenolic Content

O. digyna was evaluated for (TPC) employing Folin-Ciocalteu reagent according to[19]. with slight modifications. Concentrations (50 to 250 µg/ml) of plant extract were prepared in which 2.5 ml of 10% (FC) reagent was put in. The extract was added to 2.5 ml of a 7.5% (Na_2CO_3) solution. The final volume was made (6.5 ml) and further, incubated for 2 hours. Absorbance measured at 765 nm.

2.6 Estimation of Total Flavonoid Content

O. digyna was evaluated for (TFC) by $AlCl_3$ method[20]. with some minor alterations. The plant extract was prepared in methanol and different volume of extract was prepared (50 to 250 µg/ml). In addition, incorporation of, 100 µl of 10 % $AlCl_3$ and 100 µl of potassium acetate. To make the final volume of 3 ml, 80% methanol was added, incubated further for 30 minutes, and measured readings at 420 nm.

2.7 In Vitro Antioxidant Determination

2.7.1 DPPH assay

The scavenging capability of *O. digyna* was assessed with DPPH (0.1 mM) in methanol, the method is given by.[21]. DMSO was used to prepare the plant samples in concentrations of (50 to 250 µg/ml), further addition of 2.7 ml of DPPH solution. Thereafter, incorporation of DPPH solution of (2.7 ml). The solution was then placed for 30 minutes of incubation under dark conditions. Ascorbic acid served as a reference, and the lowering in samples absorbance was measured at 517 nm. Calculations are done as:

$$\% \text{ inhibition} = [(A_m - A_n) / A_m] \times 100$$

A_m Absorbance of (DPPH) solution excluding test sample

A_n Absorbance of (DPPH) solution including test sample.

2.7.2 Hydrogen Peroxide Assay

O. digyna was evaluated for H_2O_2 scavenging capability by the method given by[22]. H_2O_2 was prepared using (phosphate buffer). The different volumes of test samples (50 µg/ml to 250 µg/ml) were prepared in methanol and added to H_2O_2 solution (0.6 ml, 40 mM). A spectrophotometer was used to read the absorbance at 230nm. The formula shown below was used for calculation.

$$\% \text{ inhibition} = [(A_m - A_n) / A_m] \times 100$$

A_m Absorbance of (hydroxyl radical) excluding test sample

A^n Absorbance of (hydroxyl radical) solution including test sample.

2.8 *In Vitro* Antidiabetic Determination

The antidiabetic efficacy of *O. digyna* was determined using an enzyme inhibition assay with a slight alteration of the method[23]. Test extracts of various concentrations were prepared in DMSO. 1g of starch was mixed with phosphate buffer (pH 6.9), and mixing was done by continuously stirring and heating in a hot plate for around 15 minutes. After allowing the mixture to cool, it was brought to initial volume by adding deionized water and made 25 ml. The enzyme solution, which contained 0.001g of alpha-amylase and 100 ml of buffer solution, was prepared using buffer; 20 mM) bearing 6.7 mM sodium chloride. Different concentrations of methanolic leaf extract (50 to 250 µg/ml) were prepared. Before incubation for 30 minutes, each test sample was added 1 ml of the enzyme. Afterward addition of 1ml starch solution to all test tubes and further incubated for half an hour. The reaction samples were halted with 1 ml of DNSA. Thereafter, the solution was put in a water bath, for the next 15 minutes. Absorbance was checked immediately at 540 nm. Herein, a blank was formulated by substituting buffer in place of the enzyme. The formula given below was employed for calculations.

$$\% \text{ inhibition} = [(A_m - A_n) / A_m] \times 100$$

A_m absorbance of control

A_n absorbance of a test sample

2.9 Fourier Transform Infrared

FTIR spectra were obtained with the use of KBr (potassium bromide) pellets. The spectrophotometer used was Perkin Elmer Spectrum 400 and the range of spectral adsorption was 400 to 4000 cm^{-1}.

3. GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS

A plethora of phytochemicals present in *O. digyna* was identified through the GC-MS technique. The instrument used was Shimadzu QP 2010 ultra-mass spectrometer, with spectrum Rtx-5MS, length 30M, and dia-0.25 mm column. The instrumental temperature of the column injector and interface is 280 °C, and 230 °C for the ion source. The flow pressure was 61.3 kPa, 14.0 ml/min was the overall flow rate, and 1.00 ml/min was the column flow rate, employed for the analysis.

3.1 Identification of Compounds

The constituents of the extract were identified through the (RI) Retention Index, and (RT) Retention Time, along with molecular weight and formula. The NIST database was used for spectrum interpretation. Comparison of mass spectra of unknown compounds done with those of recognized ones kept in the (NIST) research library.

4. STATISTICAL EXAMINATION

Outcomes demonstrated as mean ± SEM of 3 duplicates. All results were prepared in MS-Excel.

5. RESULTS

5.1 Phytochemical Screening

Preliminary qualitative analysis was performed in three solvents; aqueous, ethanol, and, methanol. Table 1 depicts the existence of Tannins, Glycoside, Phenolic, flavonoids, Terpenoids, carbohydrates, amino acids, and proteins in the leaf extract of *Oxyria digyna*. However, alkaloid was absent in all three solvents and only the aqueous extract contained tannin.

Table 1. Preliminary phytochemical screening of *Oxyria digyna* in various solvents

S. No.	Phytochemicals	Methanol extract	Ethanol extract	Aqueous extract
1	Phenols	#+	#+	#+
2	Flavanoids	#+	#+	#+
3	Alkaloids	#-	#-	#-
4	Tannin	#-	#-	#+
5	Terpenoids	#+	#+	#+
6	Glycoside	#+	#+	#+
7	Carbohydrate	#+	#+	#+
8	Proteins and amino acid	#+	#+	#+

+ specifies the presence and #– specifies the absence of primary and secondary metabolites

5.2 Total Phenolic and Flavonoid Content

Phenolic and flavonoid concentration in *Oxyria digyna* is displayed in Table 2. A standard calibration curve equation was employed to find out the total phenolic and represented as gram gallic acid equivalent. Phenolic content varied from 52.35 to 108.11 µg/ml and displayed a concentration-response relationship and demonstrated that an increase in plant extract content corresponds to an increase in phenolic concentration. Results of flavonoid varied from 48.56 to 69.65 mg quercetin equivalent per gram extract range between (50 to 250 µg/ml).

Table 2. Total phenol content and Total flavonoid content in *O. digyna*.

Concentration (µg/ml)	TPC (mg GAE/100 of extract)	TFC (mg of quercetin/100 of extract)
50	52.35±0.005*	48.56±0.002*
100	63.66±0.003*	56.48±0.012*
150	72.35±0.009*	60.9±0.012*
200	91.81±0.007*	64.81±0.019*
250	108.11±0.001*	69.65±0.014*

Every value denoted as mean ± SEM of 3 duplicates

5.3 DPPH Free Radical Scavenging Activity

(Fig. 1) represents the inhibition potential of *O. digyna* along with its standard ascorbic acid. The extract of plant exerted stronger inhibition activity; at a concentration of 250 µg/ml (95.40±0.533); while at the same concentration inhibition of standard was reported as (78.94±0.36). Likewise, the IC 50 value of the plant sample was 42.55±0.7311 µg/ml; while the standard revealed IC_{50} = 128.94±0.275 µg/ml as shown in (Table 3).

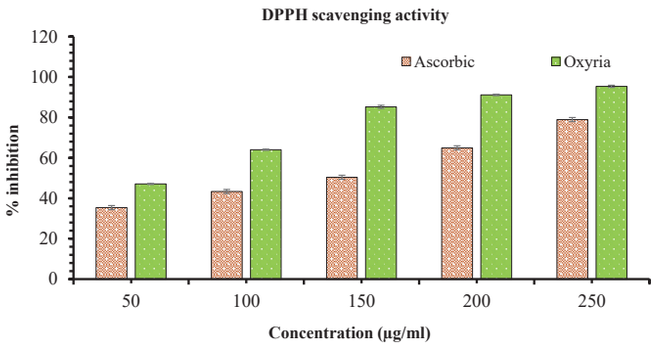

Figure 1. DPPH percentage inhibition by methanolic leaf extract of *O. digyna*. Values represent mean ± SEM of three replicates

Table 3. IC_{50} values of DPPH and H_2O_2 assays.

Plant name Standards	IC_{50} µg/ml DPPH	H_2O_2
Oxyria digyn	42.55±0.7311	51.77±1.855
Standard (ascorbic acid)	128.94±0.275	121±1.135

5.4 Hydrogen Peroxide Scavenging Ability

The H_2O_2 scavenging potential of MeOH plant extract was assessed by the percentage inhibition method and compared data with (Ascorbic acid) which is presented in Fig. 2. The % inhibition of plant sample at 250 µg/ml was found to be (95.40±0.533) and under similar concentration % inhibition of standard ascorbic acid was (80±89±0.262). IC_{50} value indicates that the plant extract showed a stronger H_2O_2 scavenging potential that is 51.77±1.855 µg/ml than the standard ascorbic acid 121±1.13, as revealed in Table 3.

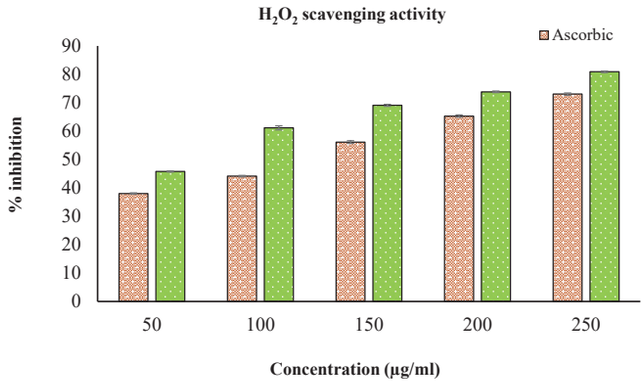

Figure 2. H_2O_2 percentage inhibition by methanolic leaf extract of *O. digyna*. Values represent mean ± SEM of three replicates

5.5 *In vitro* Antidiabetic Assay

Enzyme inhibition by *O. digyna* was found to be 50.70% at 250 µg/ml, under similar concentrations, the percentage inhibition of standard was found to be 65.27%, as shown in (Fig. 3). Extract of Oxyria showed enzyme inhibiting potential but comparatively lesser to the standard acarbose. Therefore, the result indicates that acarbose (IC_{50} value is 22.05±3.9) tends to be stronger compared to methanolic extract (IC_{50} value of 131.02±904).

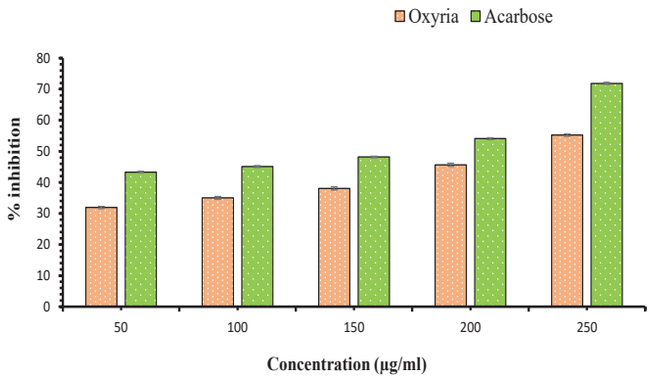

Figure 3. Percentage inhibition of enzyme alpha-amylase by methanolic leaf extract of *O. digyna* compared with standard acarbose inhibitory activity. Values represent mean ±SEM of three replicates.

5.6 FTIR Analysis

The FTIR study of *Oxyria digyna* leaf powder has shown the occurrence of numerous peaks allied with various functional groups. A functional group was represented by the presence of FTIR bands, peak values ranged from 415.92 to 393.10 and the range of spectral adsorption was 400 to 4000 cm^{-1}. The chromatogram is shown in (Fig. 4) and the functional group along with intensity is presented in (Table 4).

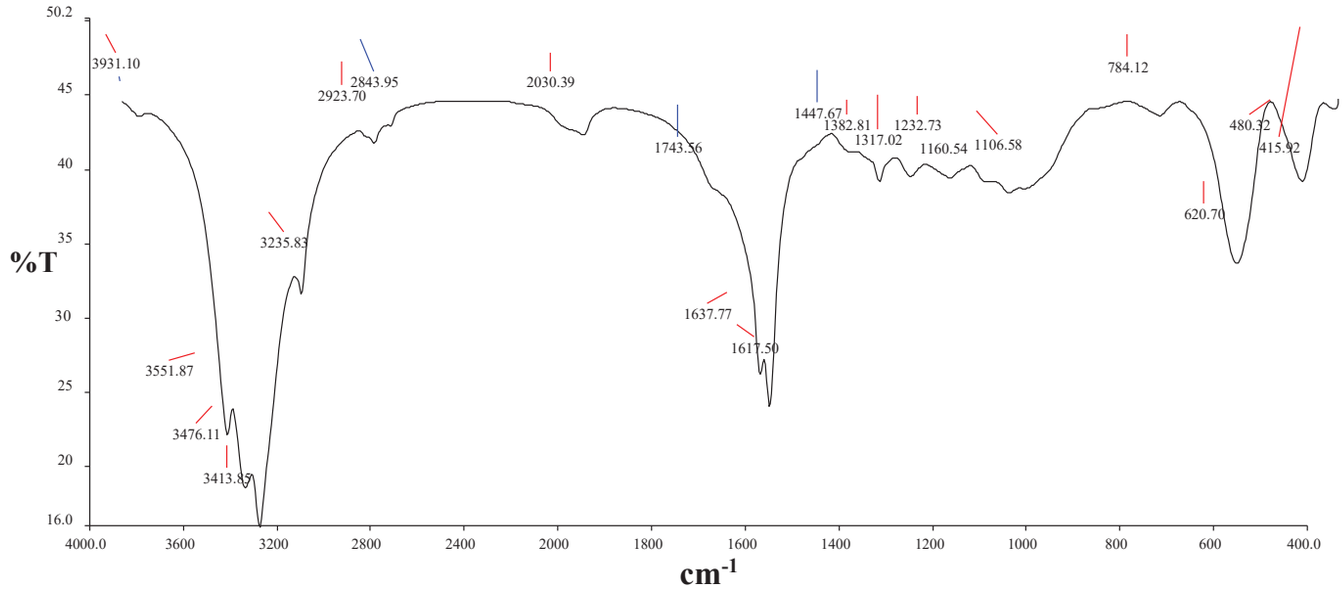

Figure 4. Fourier transformed infrared spectra of leaves powder of *O. digyna.*

Table 4. Fourier transform infrared spectroscopy of *O. digyna*

Peak value	Functional group	Functional group	Identified groups
3931.10	O-H stretching	Medium, sharp	Alcohol
3551.87	O-H stretching	Medium, sharp	Alcohol
3476.11	O-H stretching	Strong, broad	Alcohol
3413.85	O-H stretching	Strong, broad	Alcohol
3235.83	O-H stretching	Strong, broad	Carboxylic acid
2923.70	N-H stretching	Strong, broad	Amine salt
2843.95	C-H stretching	Medium	Alkane
2030.39	(C≡C) stretching	Weak	Alkyne
1743.56	C=O stretching	Strong	Esters
1637.77	C=C stretching	Strong	Alkene
1617.50	C=C stretching	Strong	α,β-unsaturated ketone
1447.67	C-H bending	Medium	Methyl group
1382.81	C-H bending	Medium	Aldehyde
1317.02	C-N stretching	Strong	Aromatic amine
1232.73	C-O stretching	Strong	Alkyl aryl ether
1160.54	C-O stretching	Strong	Tertiary alcohol
1106.58	C-O stretching	Strong	Aliphatic ether
784.12	C-H bending	Strong	1,2,3-trisubstituted
620.70	C-Br stretching	Strong	Halo compound

5.7 GC/MS Analysis

O. digyna was studied for chemical composition by GC/MS technique. Sixty constituents were detected, from the plant extract. Compound identification was carried out based on (RT) retention time, peak area, molecular formula, and molecular weight. Active compounds are represented in (Table 5). and the chromatogram in (Fig. 5). The major phytochemical detected in methanolic leaf extract of Oxyria includes; 9,12,15-Octadecatrienoic acid (Z,Z,Z) 38.33 %, followed by Diethyl phthalate 18.69 %, n- Hexadecanoic acid 9.21 %, 9,12,15-Octadecatrienoic acid, ethyl 6.28 %, Sorbitol 2.63 %. Compounds such as d-mannose, 8-Methyloctahydrocoumarin, 8-Pentadecanone were present in the least amount.

5.8 Discussion

Phytochemical screening was done in three different solvents, which showed the existence of a broad variety of compounds. Overall, aqueous extract estimated better results as compared to ethanol and methanol. Alkaloid was found to be absent in all three solvents, whereas tannin was reported only in aqueous extract. The occurrence of phytochemicals is essential to the biological effects of herbal plants including their antioxidant, antibacterial, anti-inflammatory, antidiabetic, antimalarial, and anticarcinogenic properties.[24] Flavonoids are a widely distributed class of polyphenolic chemicals which is linked to antioxidant potential in a variety of biological systems.[25] Glycosides are reported to have blood pressure-lowering properties[26]. and tannins have astringent properties and are recognized for accelerating the recovery of inflamed mucus membranes and wound healing.[27] The study done so far has shown that *O. digyna* is rich in phenolic and flavonoid compounds and owing to the occurrence of the hydroxyl group, phenolic compound exhibit scavenging capability. Plants

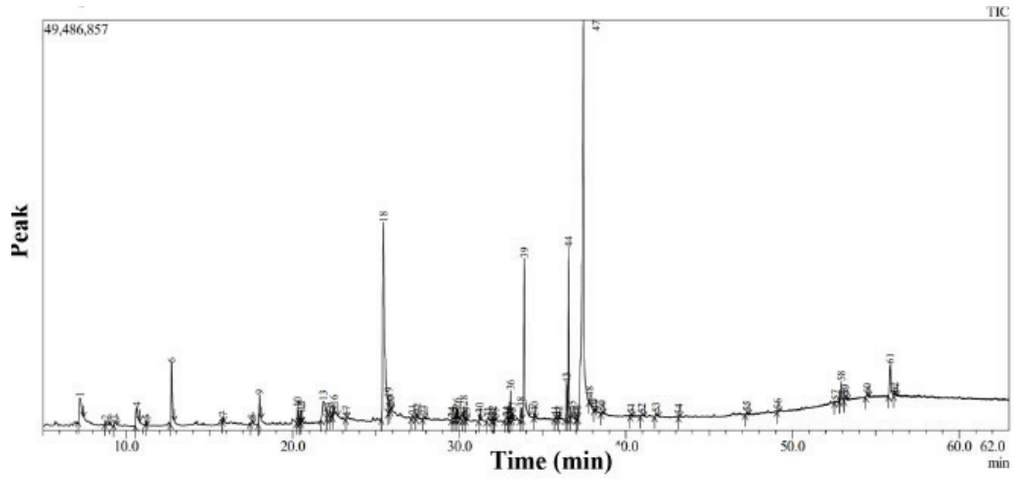

Figure 5. GC-MS Chromatogram of methanolic leaf extract of *O. digyna*.

Table 5. Identification of phytocompounds via GC-MS

S. No.	Retention time	Compound	Formula	Molecular weight	Peak %
1.	7.215	Sorbitol	$C_6H_{14}O_6$	182.1718	2.63
2.	8.754	1-Hexanol, 2-ethyl-	$C_8H_{18}O$	130.2279	0.29
3.	9.325	Benzene acetaldehyde	C_8H_8O	120.1485	0.32
4.	10.627	Thymine	$C_5H_6N_2O_2$	126.1133	1.34
5.	11.189	Undecane	$C_{11}H_{24}$	156.3083	0.15
6.	12.720	Carbaril	$C_{12}H_{11}NO_2$	201.2212	2.95
7.	15.802	Cyclohexane, hexyl-	$C_{12}H_{24}$	168.3190	0.14
8.	17.538	Phenol, 2-methyl-5-(1-methyl ethyl)-	$C_{10}H_{14}O$	150.2176	0.17
9.	17.996	2-Methoxy-4-vinyl phenol	$C_9H_{10}O_2$	150.1745	1.20
10.	20.289	1-Hexadecanol	$C_{16}H_{34}O$	242.4406	0.48
11.	20.460	Benzene, 1-chloro-4-methoxy-	C_7H_7ClO	142.583	0.54
12.	20.513	Pentadecane	$C_{15}H_{32}$	212.4146	0.20
13.	21.829	Dimethyl phthalate	$C_{10}H_{10}O_4$	194.1840	2.56
14.	22.097	d-Mannose	$C_6H_{12}O_6$	180.1559	0.43
15.	22.368	Cycloheptasiloxane, tetradecamethyl-	$C_{14}H_{42}O_7Si_7$	519.0776	0.09
16.	22.478	1-Methyl-2-methylene cyclohexane	C_8H_{14}	110.1968	0.91
17.	23.218	Pentadecane	$C_{15}H_{32}$	212.4146	0.07
18.	25.437	Diethyl Phthalate	$C_{12}H_{14}O_4$	222.2372	18.69
19.	25.750	1,2-Benzenedicarboxylic acid	$C_{26}H_{42}O_4$	418.6093	0.78
20.	27.190	Dodecylcyclohexane	$C_{18}H_{36}$	252.4784	0.11
21.	27.461	8-Pentadecanone	$C_{15}H_{30}O$	226.3981	0.14
22.	27.851	3-Buten-2-one, 4-(4-hydroxy-2,2,6-trimethyl-7-oxabicyclo.[4.1.0]hept-1-yl)	$C_{13}H_{20}O_3$	224.2961	0.14
23.	29.550	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.3709	0.16
24.	29.690	Benzoic acid, 3,4,5-trimethoxy-	$C_{10}H_{12}O_5$	212.1993	0.18
25.	29.860	1-Cyclohexene-1-propanol	$C_{13}H_{24}O$	196.3291	0.62
26.	29.997	Cyclononasiloxane	$C_{18}H_{54}O_9Si_9$	667.3855	0.13
27.	30.262	Behenic alcohol	$C_{22}H_{46}O$	326.6000	0.50
28.	30.414	Heneicosane	$C_{21}H_{44}$	296.5741	0.10

29.	31.211	Phytyl stearate	$C_{38}H_{74}O_2$	562.9930	0.24
30.	31.733	cis-9-Hexadecenal	$C_{16}H_{30}O$	238.4088	0.25
31.	31.998	10-Nonadecanone	$C_{19}H_{38}O$	282.5044	0.13
32.	32.135	Citronellyl isobutyrate	$C_{14}H_{26}O_2$	226.3550	0.11
33.	32.824	Benzoic acid, 4-(dodecyloxy)-	$C_{19}H_{30}O_3$	306.4397	0.22
34.	32.957	9-Hexadecenoic acid, methyl ester, (Z)-	$C_{17}H_{32}O_2$	268.4348	0.12
35.	33.080	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.4507	1.06
36.	33.183	Benzenepropanoic acid., 3,5-bis(1,1-dimethylethyl) -- 4-hydoroxy-, methyl ester	$C_{18}H_{28}O_3$	292.4131	0.25
37.	33.702	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.3435	0.46
38.	33.898	n- Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4241	9.21
39.	34.505	Behenic alcohol	$C_{22}H_{46}O$	326.6000	0.18
40.	35.764	Octanoic acid, 2-propenyl ester	$C_{11}H_{20}O_2$	184.2753	0.09
41.	35.967	Cyclooctasiloxane, hexadecamethyl-	$C_{16}H_{48}O_8Si_8$	593.2315	0.05
42.	36.444	9,12-Octadecadienoic. acid (Z,Z)- methyl ester	$C_{19}H_{34}O_2$	294.4721	1.26
43.	36.559	9,12,15-Octadecatrienoic. acid, ethyl ester, (Z , Z, Z)-	$C_{20}H_{34}O_2$	306.4828	6.28
44.	36.788	Phytol	$C_{20}H_{40}O$	296.5310	0.43
45.	37.084	Heptadecanoic acid., 16 methyl-, methyl ester	$C_{19}H_{38}O_2$	298.5038	0.09
46.	37.444	9,12,15-Octadecaterienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278.4296	38.33
47.	37.813	Octadecanoic acid	$C_{18}H_{36}O_2$	284.4772	0.22
48.	38.143	10-Undecenal	$C_{11}H_{20}O$	168.2759	0.05
49.	38.555	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	667.3855	0.09
50.	40.306	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292.4562	0.11
51.	40.938	Tetracosamethyl-cyclododecasiloxane	$C_{24}H_{72}O_{12}Si_{12}$	889.8473	0.17
52.	41.817	Hexadecanamide	$C_{16}H_{33}NO$	255.4393	0.24
53.	43.181	Cyclodecasiloxane, eicosamethyl-	$C_{20}H_{60}O_{10}Si_{10}$	741.5394	0.08
54.	49.113	Tetracosamethyl-cyclododecasiloxane	$C_{24}H_{72}O_{12}Si_{12}$	889.8473	0.07
55.	52.583	1-Naphthalenepropanol., α-ethenyldecahydro-2-. hydroxy-α,2,5,5,8a-pentamethyl-, [1R-[1α(R*),2β,4aβ,8aα]]-	$C_{20}H_{36}O_2$	308.4986	0.12
56.	52.937	dl-α-Tocopherol	$C_{29}H_{50}O_2$	430.7061	0.93
57.	53.183	Phytyl stearate	$C_{38}H_{74}O_2$	562.9930	0.26
58.	54.487	Corticosterone	$C_{21}H_{30}O_4$	346.4605	0.17
59.	55.874	Lathosterol	$C_{27}H_{46}O$	386.6535	2.23
60.	56.077	Phytyl stearate	$C_{38}H_{74}O_2$	562.9930	0.19

The phytocompounds in the extract were identified using the Retention index, Retention time, along with molecular weight and formula. Compounds were tentatively identified based on computer matching of mass spectra peaks with libraries such as NIST.

contain a variety of phenolic compounds, chief among them are flavonoids. Studies on flavonoid derivatives have revealed numerous activities including antibacterial, anti-allergic, antiviral, anti-inflammatory, and anti-cancer.[28] The Literature review revealed that the phytochemical analysis of this plant has not yet been investigated. However, many biological activities associated with this species have been documented. The powered aerial portions of Oxyria is used to alleviate indigestion and as a liver tonic.[29] DPPH is one of the extensively employed assay to investigate the antioxidant ability of natural products or compounds to scavenge DPPH radicals in solution.[30] During the procedure, the deep violet color DPPH radical gets reduced to a yellowish product due to an antioxidant compound, exhibiting a decline in the absorbance at 517 nm. The lower IC_{50} value of plant extract represents better antioxidant potential. The outcomes of our study indicate that the plant under study is an efficient scavenger of DPPH radical. Literature is scanty about reducing power in *O. digyna*. One of the authors reported that the ethanolic extract of *O. digyna* showed a lower antioxidant effect despite its rich phenolic content[31].

The difference in results might be due to the difference in the solvent. H_2O_2 itself is less reactive, yet it may lead to toxicity of cells by producing hydroxyl radicals in cells.[32] Further, removing such radicals is essential to protect the system from cytotoxic effects. There has been no study on *O. digyna* for reducing property by this method. Therefore, our data represent the first result to determine the methanolic plant extract for antiradical activity by this method and our study shows a promising result. The antidiabetic potential of methanolic plant extract of *O. digyna* was assessed for their potential alpha-amylase inhibitory assay, and it was compared with acarbose which served as standard. The enzyme alpha-amylase is secreted by the salivary gland and pancreas and is regarded as the key enzyme responsible for the catalysis of starch into simple sugars. Hence, inhibition of alpha-amylase will reduce the starch breakdown thus resulting in the reduction of glucose adsorption.

The capability of the methanolic leaf extract of *Oxyria digyna* to inhibit alpha-amylase is presented in Table 4. Observed results suggest a dose-dependent response differentiated according to concentration. Drugs that lower post-prandial hyperglycemia by subduing the breakdown of carbohydrates are found valuable in the control of DM.[33] FTIR is an effective, versatile technique utilized for the chemical characterization of various compounds. Our study revealed various functional groups, predominating ones are O-H stretching and C-H stretching, followed by C-O Stretching. The frequencies of O-H stretching were observed at peak values ranging between (3931.10 to 3235.83) and C-H stretching was observed at various peak such as 2843.95,1447.67,1382.81 and 784.12.

The presence of a large number of functional groups may account for its therapeutic benefits. GC/MS, a highly efficient, and effective chromatographic technique utilized for the identification and quantification of unidentified chemicals contained in a sample, was used to analyse the chemical profile of the methanolic leaf extract of *Oxyria digyna*. The results demonstrated the existence of many chemicals with diverse biological characteristics. "Compounds such as 9,12,15- Octadecatrienoic acid, (Z, Z, Z) shows anticancerous, anti-inflammatory, hepatoprotective, antihistaminic, antiarthritic, and nematicide properties"[34]. In previous studies, this compound is found in Tiliacora acuminata hook F & Thomas (85.6%).[35] Diethyl" phthalate (18%) shows antimicrobial activity.[36] N-hexadecenoic acid (9.21%) shows antioxidant, antiandrogenic and 5-alpha-reductase inhibitor[37] "followed by 9,12,15- Octadecatrienoic acid, ethyl ester (6.28) shows hepatoprotective, antiandrogenic, antihistaminic, anticancer and antiarthritic"[38] and the rest are present in less concentration and exhibit various biological properties.

Limited studies have been conducted on Oxyria with respect to chemical characterization. So, there are very few prior publications, five known compounds were isolated, i.e., Orientin, Stigmasterol, Quercetin-3-O-beta-D-glucopyranoside, and Vitexin.[39] GC/MS characterization of our study shows a distinct compound that hasn't been reported earlier, this might be due to differences in plant extraction, climatic condition, altitude variations, and, many other factors.

6. CONCLUSIONS

O. digyna has traditionally been used for edible and medicinal purposes. The current analysis demonstrates that *O. digyna* consists of a promising source of phenols and flavonoids and also reported an outstanding free radical scavenging potential, this was supported by the existence of a high amount of phenolic compound. Rich sources of antioxidants will encourage the consumption of underutilized plants with potent health benefits. Moreover, the first-time extract was evaluated for antidiabetic potential and showed moderate alpha-amylase inhibiting activity. Further examination by GCMS revealed the presence of a wide number of medicinally important phytocompounds which need to be further isolated, to assess their therapeutic potential.

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REFERENCES

1. Astley, S.B. Dietary antioxidants-past, present and future. *Trends. Food Sci. & Technol*., 2003, **3**(14), 93-98. doi:10.1016/S0924-2244(02)00281-9
2. Dhaiwal, K.; Chahal, K.K.; Kataria, D. & Kumar, A. Gas chromatography-mass spectrometry analysis and *in vitro* antioxidant potential of ajwain seed (*Trachyspermum ammi* L.) essential oil and its extracts. *J. of Food Biochem*., 2017, **41**(3), e12364. doi:10.1111/jfbc.12364
3. Babich, H.J. Butylated hydroxytoluene (BHT): a review. *Environmental Research*., 1982, **29**(1), 1-29. doi:10.1016/0013-9351(82)90002-0
4. Patel, D.K.; Kumar, R.; Laloo, D. & Hemalatha, S. Diabetes mellitus: an overview on its pharmacological aspects and reported medicinal plants having antidiabetic activity. *Asian Pac. J. of Trop. Biomed*., 2012, **2**(5), 411-420. doi:10.1016/S2221-1691(12)60067-7
5. Zimmet, P. Globalization, coca-colonization, and the chronic disease epidemic: can the Doomsday scenario be averted. *J. of Inter. Med*., 2001, **249**(S741), 17-26. doi:10.1046/j.1365-2796.2001.00625.x
6. De Souza Schmidt Goncalves, A.E.; Lajolo, F.M. & Genovese, M.I. Chemical composition and antioxidant/antidiabetic potential of Brazilian native fruits and commercial frozen pulps. *J. of Agric. & Food Chem.,* 2010, **58**(8), 4666-4674.

doi:10.1021/jf903875u
7. Ali, A.M.D. Anti-diabetic potential of phenolic compounds: A review. *Int. J. of Food Prop*., 2013, **16**(1), 91-103. doi:10.1080/10942912.2011.595864
8. Bhupathiraju, S.N. & Hu, F.B. Epidemiology of obesity and diabetes and their cardiovascular complications. *Circulation research*., 2016, **118**(11), 1723-1735. doi:10.1161/CIRCRESAHA.115.306825
9. Mosaku, K.; Kolawole, B.; Mume, C. & Ikem, R. Depression, anxiety and quality of life among diabetic patients: a comparative study. *J. of the National Med. Association*., 2008 **100**(1), 73-78. doi:10.1016/S0027-9684(15)31178-0
10. Moller, D.E. New drug targets for type 2 diabetes and the metabolic syndrome. *Nature*., 2001, **414**(6865), 821-827.
11. Stein, S.A.; Lamos, EM. & Davis, S.N. A review of the efficacy and safety of oral antidiabetic drugs. *Expert Opin. Drug Saf*., 2013, **12**(2), 153-175. doi:10.1517/14740338.2013.752813
12. Xing-yu, L.I.; Chun-lin, L.O.; Yue-hu, W.A. & Rong, G.U. Chemical Constituents of Oxyria digyna. *Nat. Product Res. & Dev*., 2008, **20**(5).
13. Angmo, D.; Puri, R.; Mehta, M. & Devi, G. Ethnobotanical Survey of Wild Edible Plants of Leh District, Ladakh. *Def. Life Sci. J*., 2022, **7**(4), 257-266.
14. Rana, D.; Bhatt, A.; Lal, B.; Parkash, O.; Kumar, A. & Uniyal, S.K. Use of medicinal plants for treating different ailments by the indigenous people of Churah subdivision of district Chamba, Himachal Pradesh, India. *Environ. Dev. and Sustain*., 2021, **23**(2), 1162-241.
15. Mehmood, S.I.; Majeed, S.; Jannat, Z. & Habib, T. Imaging-based ethnobotanical studies of district Poonch, Azad Jammu, and Kashmir. *Int. J. of Herb. Med*., 2018, **6**(6), 81-91.
16. Shaheen, H.; Shinwari, Z.K.; Qureshi, R.A. & Ullah, Z. Indigenous plant resources and their utilization practices in village populations of Kashmir Himalayas. *Pak. J. of Bot*., 2012, **44**(2), 739-745. http://173.208.131.244:9060/xmlui/handle/123456789/14371
17. Rinchen, T. & Pant, S. Ethnopharmacological uses of plants among inhabitants surrounding Suru and Zanskar valleys of cold desert, Ladakh. *Int. J. of Pharma Bio Sci*., 2014, **5**, 486-494.
18. Krishnaiah, D.; Devi, T.; Bono, A. & Sarbatly, R. Studies on phytochemical constituents of six Malaysian medicinal plants. *J. med. Plant. Res*., 2009, **3**(2), 067-72.
19. Saeed, N.; Khan, M.R. & Shabbir, M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L. *BMC Complementary Altern. Med*., 2012, **12**(1), 1-2.
20. Chang, C.C.; Yang, M.H.; Wen, H.M. & Chern, J.C. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. of Food Drug & Anlys*., 2002, **10**(3).
21. Brand-Williams, W.; Cuvelier, M.E. & Berset, C.L. Use of a free radical method to evaluate antioxidant activity. *LWT-Food Sci. & Technol*., 1995, **28**(1), 25-30. doi:10.1016/S0023-6438(95)80008-5
22. Bozin, B.; Mimica-Dukic. N.; Samojlik, I.; Goran, A. & Igic, R. Phenolics as antioxidants in garlic (Allium sativum L., Alliaceae). *Food Chemistry*., 2008, **111**(4), 925-932.
23. Ali, H.; Houghton, P.J. & Soumyanath, A. α-Amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to Phyllanthus amarus. *J. Ethnopharmacol*., 2006, **107**(3), 449-455. doi:10.1016/j.jep.2006.04.004
24. Negi, J.S.; Singh, P. & Rawat, B. Chemical constituents and biological importance of Swertia: a review. *Current Res. in Chemisty*.; 2011, **3**(1), 1-5.
25. Morel, I.; Lescoat, G.; Cogrel, P.; Sergent, O.; Pasdeloup, N.; Brissot, P.; Cillard, P. & Cillard, J. Antioxidant, and iron-chelating activities of the flavonoids catechin, quercetin and diosmetin on iron-loaded rat hepatocyte cultures. *Biochemical pharmacology*., 1993, **45**(1), 13-19. doi:10.1016/0006-2952(93)90371-3
26. Vindhya, K.; Sampath, Kumara, K.K.; Neelambika, H.S. & Leelavathi, S. Preliminary phytochemical screening of Gardenia latifolia Ait. and Gardenia gummifera Linn. *Research J. of Pharml. Biol. Chem. Sci*., 2014, **5**(2), 527-532. http://www.rjpbcs.com/pdf/2014_5(2)/[57]
27. Yadav, M.; Chatterji, S.; Gupta, S.K. & Watal, G. "Preliminary phytochemical screening of six medicinal plants used in traditional medicine." *Int. J. of Pharm. Pharm. Sci.* 2014, **6**(5): 539-542.
28. Di Carlo, G.; Mascolo, N. & Izzo, A.A. & Capasso, F. Flavonoids: old and new aspects of a class of natural therapeutic drugs. *Life sciences*., 1999, **65**(4), 337-53. doi:10.1016/S0024-3205(99)00120-4
29. Usha, D. & Pankaj, S. Study on ethnomedicinal plants of Kibber Wildlife Sanctuary: A cold desert in Trans Himalaya. *India J. Med. Plants Res*., 2013, **7**(47), 3400-19. doi:10.5897/JMPR2013.5298
30. Kedare, S.B. & Singh, R.P. Genesis and development of DPPH method of antioxidant assay. *J. Food Sci. Technol*., 2011, **48**(4), 412-22.
31. Orhan, I.; Kartal, M.; Abu-Asaker, M.; Şenol. F.S.; Yilmaz, G. & Şener, B. Free radical scavenging properties and phenolic characterization of some edible plants. *Food Chemistry*., 2009, **114**(1), 276-281. doi:10.1016/j.foodchem.2008.09.005
32. Halliwell, B. Reactive oxygen species in living systems: source, biochemistry, and role in human disease. *The American J. of Medicine*., 1991, **91**(3), S14-22. doi:10.1016/0002-9343(91)90279-7
33. Tiwari, A.K. & Rao, J.M. Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. *Current science*., 2002, 10, 30-38. https://www.jstor.org/stable/24106071
34. Sermakkani, M. & Thangapandian, V. GC-MS analysis of Cassia italica leaf methanol extract. *Asian J. of Pharm. Clin. Res*., 2012, 90-94.
35. Nishanthini, A.; Mohan, VR. & Jeeva, S. Phytochemical, ft-ir, and gc-ms analysis of stem and leaf of tiliacora acuminata (lan.) Hook f & Thomas (menispermaceae). *Int.*

J. Pharm. Sci. and Res., 2014, **5**(9), 3977. 10.13040/IJPSR.0975-8232.5(9).3977-3986
36. Premjanu, N. & Jaynthy, C. Antimicrobial activity of diethyl phthalate: An insilico approach. *Asian J. of Pharm. Clin. Res*., 2014, **7**(4), 141-2.
37. Prabhadevi, V.; Sahaya, S.S.; Johnson, M.; Venkatramani, B. & Janakiraman, N. Phytochemical studies on Allamanda cathartica L. using GC–MS. *Asian Pac. J. of Trop. Biomed*., 2012, **2**(2), S550-4. doi:10.1016/S2221-1691(12)60272-X
38. Mamani, R. & Alhaji, N.M. GC-MS analysis of phytocomponents in methanolic extract of Coleus aromaticus. *J. of Pharmacogn. Phytochem*., 2019, **8**(4), 106-109.
39. Zhou, Y.; Zhang, G.L.; Li, B.G. & Chen, Y.Z. Three new compounds from Oxyria digyna (L.) Hill. *Indian J. of Chemisty including Medicinal Chemistry*., 2002, **33**(16).

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