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A PHARMACOGNOSTIC, PHYTOCHEMICAL, AND ANTIOXIDANT POTENTIAL OF OXALIS TRIANGULARIS

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ABSTRACT
Background*:* To evaluate *Oxalis triangularis* with pharmacognostical parameters (macroscopy, microscopy & physico-chemical analysis), phytochemical analysis, and to investigate the in-vitro antioxidant capacity of different extracts obtained. **Methods:** Using specific standard protocols, the following tests were performed: loss on drying, extractive value, ash value, t.s. & powder microscopy, fluorescence analysis, and phytochemical screening. The Folin-Ciocalteu technique was adopted to ascertain the amount of phenolic compounds. In-vitro antioxidant activity was evaluated using 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) & 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assays. **Results:** The extractive values varied between solvents, ranging from 1.28% (ethyl acetate) to 18.8% (water), while the ash values were 16.25% (total), 2.9% (acid insoluble), and 10.625% (water-soluble). Numerous vascular systems, lignified trichomes, and calcium oxalate crystals were visible under a microscope. Saponins, steroids, flavonoids, phenols, anthocyanins, and alkaloids were detected by phytochemical screening; glycosides were not detected. At 200 µg/ml, the phenolic concentration of the ethanolic extract was the highest, at 1151.7 ± 59.22 µg/ml. The ethanolic extract exhibited greater DPPH (IC_{50} = 2.403 µg/ml) and ABTS (IC_{50} = 22.94 µg/ml) radical scavenging activity in comparison to the aqueous extract, i.e., (DPPH, IC_{50}= 76.67 µg/ml & ABTS, IC_{50}= 43.52 µg/ml). In contrast, both extracts showed notable antioxidant activity. **Conclusion:** A comprehensive examination of *Oxalis triangularis* revealed a rich reservoir of bioactive chemicals, such as phenols, which make the plant rich in antioxidant properties. This may serve as a foundation for the discovery of novel medicines.

INTRODUCTION

The availability and consumption of proper drugs have proven to be one of the aspects that influence the effectiveness of the healthcare system [1]. Since the earliest days of human civilization, the only practical and accessible approach for regulating health care has come from plant-based medicine [2]. Utilization of plant-based medicine/natural cures has not provided immediate danger to public health or the environment [3]. Reliable sources of medicinal plants and their metabolites have been utilized since ancient times for treating

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various human ailments [4]. In many communities, traditional medicine is still considered the main healthcare option. Approximately 80% of people in poor nations and over 60% worldwide rely only on plants for their medical requirements [5]. Natural medicine has become so prevalent because of its safety and affordability [6]. Nowadays, plants with possible medicinal properties have been the subject of global investigation by researchers. Plant parts from various genera and species, including leaves, fruits, seeds, stems, and bark, are used for *in vivo* and *in vitro* examinations. Therefore, the potential of herbal remedies for discovering novel therapeutic molecules or their derivatives for treating medical conditions should be considered [7]. *Oxalis* is the most prominent genus of the wood sorrels family, consisting of 5 genera and 570 species. Among all species, *Oxalis corniculata*, native to India, has flourished with varied traditional and pharmacological utilities. Traditionally, *O corniculata* have been utilized for diarrhea, dysentery, treating worms, etc [8]. Many reproducible pharmacological activities have also been reported, including anti-oxidants, anti-bacterial, anti-diabetes, anti-venom, and anti-cancer [9-11]. On the other hand, falling under the same genus Oxalis triangularis with varied traditional utilities is not under the limelight of researchers worldwide. There have been reports that the plant possesses phytochemicals like glycoside and anthocyanin. It has already been established for its antibacterial potential, and isolation of acylated anthocyanins and flavon C-glycoside have been reported [12-14]. This fact entails the need for in-depth study in the genus Oxalis because many species studied earlier showed good potential against diseases like diabetes, neuro-protective, cancer, etc.

The gap for the study of *Oxalis triangularis* is the main backbone of the research. *Oxalis triangularis*, traditionally used as a remedy for various illnesses, has not been accessed for its other pharmacological potential except for antibacterial activity. Given all of this, the current study aims to provide helpful information about the standardization and identification of oxalis triangularis and its antioxidant potential. The data that might provide relevant work to the authenticity, quality, and purity of the plant.

MATERIALS AND METHODS

Chemicals and solvents

The investigation utilized analytical-grade chemicals and solvents from S D Fine Chemical Limited and Thermo Fisher Scientific Ind Private Limited in Mumbai.

Plant material collection and authentication

On August 1, 2023, the plant was collected from Majhitar, Rangpo, East Sikkim, and the herbarium specimen was prepared. The competent authority Botanical Survey of India, Sikkim Himalayan Regional Centre, Gangtok, authenticated the plant specimen, and the identification number SHRC-5/02/2023-24/Tech was provided for the specimen.

Morphological evaluation

The plant's size, color, shape, smell, texture, and taste were evaluated visually, and observations were made per WHO [15].

Microscopical evaluation

The fresh leaves stem and powdered drug of *O. triangularis* were subjected to histochemical and microscopic evaluation following methods outlined by Kokate and Khandelwal [16-18]. The WESWOX (HL-5/6) binocular compound microscope was used for the study.

Physico-chemical analysis

British Pharmacopeia prescribed the protocol to determine the drug's ash and extractive values [19]. The approach outlined by Sohail I *et al.* and Das K. P *et al.* was employed to assess the fluorescence of powdered drugs. A pinch of powder was mounted over grease-free glass slides, and a few drops of prepared reagents, i.e., acids, alcohols, or alkalis, were added, mixed with the powder with minimal agitation, and kept aside for a few minutes. The glass slides were then observed under visible light, ultraviolet light (254 nm), and ultraviolet light (365 nm). The hue observed under the same conditions was noted [20-21]. Powder microscopy was done as per the protocol outlined by Hole AD with minor modifications. Microscopic slides were prepared by soaking a pinch of powder in distilled water for 30 minutes and staining with safranin, phloroglucinol, or iodine for 2-3 minutes. A drop of glycerin was used to eliminate air bubbles [22]. The WESWOX (HL-5/6) binocular compound microscope was used to draw the results, and pictures were clicked.

Preparation of plant extract

Soxhlet apparatus extracted the dried powder of the aerial part of Oxalis triangularis by solvents in an increasing polarity (petroleum ether, chloroform, ethyl acetate, ethanol, and water). Approximately 250g of powder was used for extraction. Initially, the powder was subjected to defatting with pet. ether and the same defatted residues were subjected to extraction with

other solvents. A rotary evaporator (HAHNVAPOR, HS 3000) concentrated each extracted material at a low temperature (40–50ºC) and low pressure. The dried extract was collected and calculated in terms of % w/w, which was then stored at 4ºC for future use.

Preliminary phytochemical evaluation

The freshly generated extracts were qualitatively examined for chemical components. The procedure highlighted by Shaikh JR and Patil M was adopted [23].

Determination of total phenolic content:

The protocol based on the Folin-Ciocalteau (FC) reagent was used for the study. 1.0gm of ethanolic and aqueous extract of *oxalis triangularis* was weighed and dissolved in 1000ml of distilled water, and the label was made as a stock solution. From the stock solution, two dilutions were made for concentration with approximate concentrations of 40 & 200 µg/ml in distilled water. A test tube containing 0.5 ml of the prepared dilution was filled with 2.5 ml of Folin-Ciocalteau (FC) reagent (diluted with distilled water in the ratio of 1:10) was added and agitated. The reaction mixture was kept aside for a few minutes, and 2ml 7.5% sodium carbonate solution was added, mixed thoroughly, and incubated at room temperature for 30 min. The developed fusion's absorbance at 760 nm was measured following 30 minutes of development. Three records of the absorbance were made. Using the calibration curve's Y= mx + c equation of gallic acid, the quantity of phenolic component was calculated and represented in mg of gallic acid equivalents, or GAE, per gram of sample material [24-26].

***In-vitro* evaluation of antioxidant activity**

In-vitro antioxidant study of aqueous and ethanolic plant extracts was carried out using the DPPH and ABTS radical methods. The former advocates a decrease in the absorbance of DPPH and ABTS in the presence of extracts or test samples, which infers antioxidant activity.

DPPH radical method

A solution of 0.1mM DPPH (2, 2-diphenyl-1-picrylhydrazyl) was produced in methanol. The stock solution of the sample (aqueous and ethanolic extract) and standard (gallic acid) was produced at concentrations of 0, 1.56, 3.125, 6.25, 12.5, 25, 50, and 100 µg/ml. 5µl of stock solution was mixed with 0.1ml DPPH solution in 96-well plates. The reaction was set in triplicate, and a duplicate of the blank was prepared with 0.2ml methanol and 10 µl of standard/sample with mentioned concentration. Wells without treatment, i.e., reaction mixture containing deionized water, were deemed control, whereas wells without reagent (DPPH) were termed blank. The plate was incubated in darkness for 30 minutes. After incubation, the decolorization was quantified at 517 nm with a microplate reader (iMark, BioRad). The result was calculated concerning negative control. % Inhibition was calculated using the following formula:

$$\%RSA = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100 \ldots\ldots \text{Equation 1}$$

Where RSA = Radical Scavenging Activity,

$Abs_{Control}$ = Absorbance of control

Abs_{Sample} = Absorbance of sample

The IC50 value was calculated using graph pad prism 10.3.0. The data obtained was tabulated, and a graph was plotted, with sample concentration on the X-axis and % inhibition compared to control on the Y-axis [27-28].

ABTS radical method:

ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) free radical reagent was generated by combining 2.45 mM APS (ammonium per sulphate) with 7 mM ABTS solution, which was then diluted 100 times to yield the final radical solution. 10µl of different concentrations (0, 0.78, 1.56, 3.125, 6.25, 12.5, and 50 µg/ml) of gallic acid (standard) and (0, 1.56, 3.125, 6.25, 12.5, 50, 100 µg/ml) of samples (ethanolic and aqueous extract) was added to the 200µl of ABTS free radical reagent in 96 well plates, and incubated in the dark for 10 min. The wells without treatment were considered as controls. After incubation, a microplate reader (iMark, BioRad) was used to assess absorbance at 750nm to determine decolorization. The result was calculated concerning negative control. % Inhibition was calculated using equation 1.

The IC50 value was calculated using graph pad prism 10.3.0. The data obtained was tabulated, and a graph was plotted, with sample concentration on the X-axis and % inhibition compared to control on the Y-axis [29- 30].

RESULTS

Morphological description

As a perennial plant, *Oxalis triangularis* is a member of the Oxalidaceae family. Its leaves are carried by a long petiole, which is cylindrical and ranges to 20 cm in length, that emerges at the base of a tuberous rhizome, which ranges to 5 cm long,

over 10 to 15 mm in diameter, and is completely coated with scales. The leaf is trifoliate and consists of three smooth, sessile, obtriangular to obovate-triangular leaflets placed in the same plane perpendicular to the petiole. Plant has no stipules. The leaves show a "sleep motion" symbol, i.e., open when there is light and close when it gets dark. The flowers are small, with 5 petals and sepals attached to a long flower stalk, which ranges to 20 cm in length and is cylindrical in shape. The sepals are densely haired, pointed and ranges to 4-5.5 mm in length. It is green to yellowish in color and slightly reddish in pointed top. The petals are purplish pink, oblong-lanceolate, and range to 2-3 inches in length. The flower is bisexual and possesses 10 stamens arranged in 2 rings; outer ring includes 5 stamens which are opposite to the petals, and the other 5 lie in inner whorls alternate to petals, along with 5 styles in a single ring. The flower generally blooms during the spring season. The captured photographs are provided below in Figures 1 & 2.

Figure 1: Characteristics of the reproductive organs of *Oxalis triangularis*: (a) Inflorescence, (b) Intact flowers, (c) Seals, (d) Petals, (e) Stamens and pistils.

Organoleptic evaluation:

Organoleptic analysis of the aerial part of the plant was carried out for the following sensory-based characteristics: smell, taste, color, and touch. The results are reported in Table 1.

Table 1: Table representing physical attributes of *Oxalis triangular* aerial portion.

Sl.no.	Parameters	Observation
1	Color of leaves	Dark purple
2	Color of stalk	Light green to Purple
3	Color of flower	White to Pink
4	Taste	Tangy sour
5	Odour	Odorless
6	Texture	Smooth

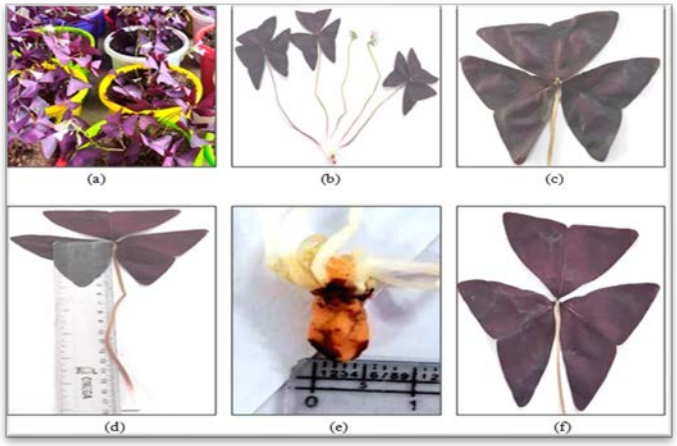


Figure 2: Characteristics of the vegetative organs of Oxalis triangularis; (a) Plant grown in basket, (b) Whole plant, (c) Leaf front, (d) Leaves and petioles, (e) Rhizome, (f) Back of leaf.

Microscopical evaluation:

The lower and upper epidermis, phloem, xylem, and bundle sheath were visible in the transverse slice of the leaf. Similarly, T.S. of stem showed the presence of epidermis, parenchyma, xylem, phloem, and pith. Moreover, lignified unicellular and multicellular trichomes, calcium oxalate crystals, sclerenchymatous fibers, phloem fibers, spiral vessels, and scleriform vessels were all detected by powder microscopy. The pictures captured for all contents are depicted below in Figures 3, 4, & 5, respectively.

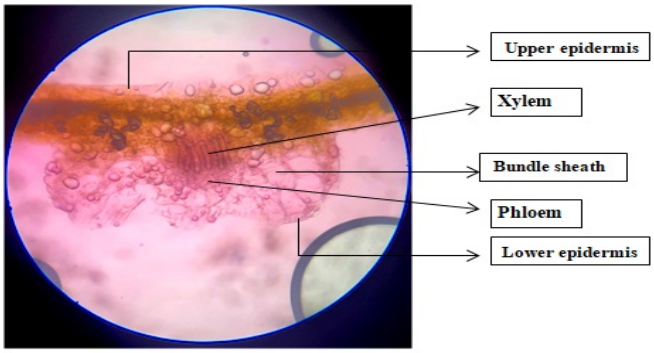


Figure 3: Transverse section of leaf of *Oxalis triangularis*.

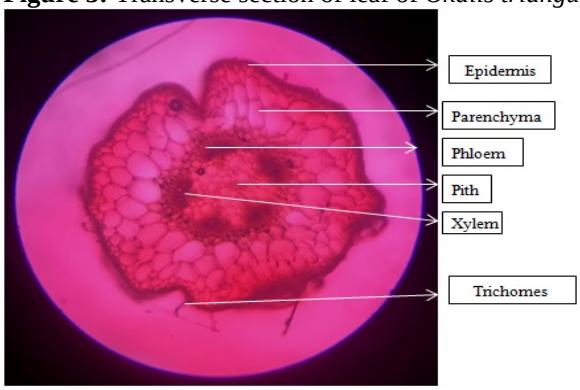


Figure 4: Transverse section of the stem of *Oxalis triangularis*.

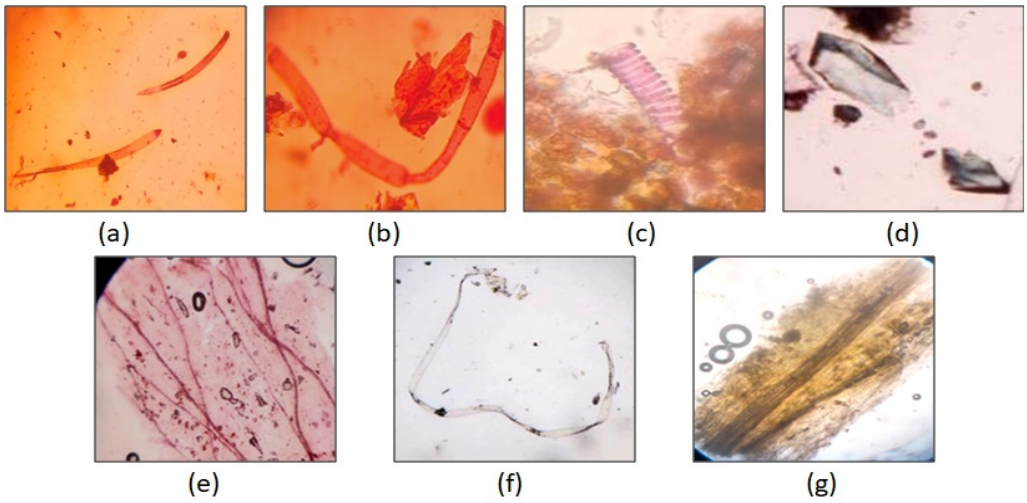


Figure 5: Powder microscopy revealing (a) lignified unicellular trichomes; (b) lignified multicellular trichomes; (c) spiral vessels; (d) prismatic calcium oxalate crystals; (e) sclerenchymatous fibers; (f) phloem fiber; (g) scleriform vessel

Physico-chemical analysis:

The values of all determinations are summarized in Tables 2, 3, & 4.

Table 2: Table showing the ash value of *O. triangularis* powder.

Ash Value	Values in % (w/w) (mean ± SEM)
Total ash	16.33 ± 0.08
Water Soluble ash	10.65 ± 0.02
Acid insoluble ash	2.9 ± 0.01

Table 3: Extractive values of powdered aerial part of *O. triangularis.*

Solvent Used	Physical nature	% yield (w/w) (mean ± SEM)
Petroleum ether	Sticky	5.5 ± 0.05
Chloroform	Sticky	3.04 ± 0.08
Ethyl acetate	Semisolid	1.33 ± 0.01
Ethanol	Semi solid	4.43 ± 0.03
Distilled water	Sticky	18.69 ± 0.11

Table 4: Table representing fluorescence analysis of powdered aerial part of *O. triangularis.*

Sl. No.	Treatment	Visible light	UV light (254)	UV light (365)
1	Powder as such	Olive green	Dark green	Light green
2	Powder + hot water	Light brown	Dark brown	Light brown
3	Powder + cold water	Light brown	Dark brown	Light brown
4	Powder + 1N NaOH (aq)	Yellowish brown	Dark brown	Light brown
5	Powder + 50% HCl	Dark brown	Black	Dark brown
6	Powder + 50% H_2SO_4	Dark brown	Black	Black
7	Powder + 50% HNO_3	Purple brown	Blackish brown	Blackish brown
8	Powder + Ammonia	Dark green	Deep brown	Greenish brown
9	Powder + Petroleum ether	Faded brown	Black	Light brown
10	Powder + Ethyl acetate	Olive green	Black	Dark brown
11	Powder + Ethanol	Moss green	Light brown	Light brown
12	Powder + Methanol	Moss green	Black	Dark brown
13	Powder + 50% KOH	Yellowish brown	Dark brown	Light brown
14	Powder + Acetic acid	Reddish brown	Black	Black
15	Powder + Iodine in water (1%)	Reddish brown	Black	Black
16	Powder + $FeCl_3$	Light brown	Black	Black
17	Powder + Picric acid	Light brown	Black	Black

Table 5: Phyto-chemical screenings of extracts of aerial part of *O. triangularis.*

Sl. No.	Phytoconstituents	Tests	Observation				
			PEE	CHE	EAE	EE	AE
1	Alkaloids	Dragendorff's test	-	-	-	-	-
		Wagner's test	-	-	-	+	+
		Mayer's test	-	-	-	+	+
		Hager's test	-	-	+	+	+
2	Flavonoids	Alkaline reagent test	-	+	+	+	+
		Ferric Chloride test	-	-	-	+	+
		Lead acetate test	-	+	-	+	+
		Conc. H_2SO_4 test	-	-	-	+	-
3	Carbohydrates	Fehling's test	-	-	+	+	+
		Barfoed's test	-	-	-	-	-
		Molisch's test	-	-	+	+	+
		Benedict's test	-	-	+	+	+
4	Glycoside	Keller-killiani test	-	-	-	-	-
		10% NaOH test	-	-	-	-	-
		Legal's test	-	-	-	-	-
		Conc. H_2SO_4 test	-	-	-	-	-
5	Steroids	Salkowski test	-	+	-	-	-
		H_2SO_4 test	-	+	+	-	-
		Sulphur test	-	+	+	+	-
6	Protein	Biuret test	-	-	-	+	-
		Milon's test	-	-	-	+	+
		Ninhydrin test	-	-	-	-	-
		Xantoprotic test	-	-	-	+	-
7	Tannins	5% Ferric Chloride test	-	-	-	+	+
		10% NaOH test	-	-	-	-	-
		Bromine water test	-	-	+	+	+
		Lead acetate test	-	-	-	+	+
8	Saponins	Foam test	-	-	-	+	+
		Froth test	-	-	-	+	+
		$NaHCO_3$ test	-	-	-	+	+
9	Phenols	Lead acetate test	-	+	-	+	+
		Ferric chloride test	-	-	+	+	+
		Iodine test	-	+	+	+	+
		Ellagic acid test	-	+	-	+	-
10	Fixed oils and fats	Spot test	+	+	-	-	-
11	Gums & Mucilage	Alcohol test	+	+	-	-	-
12	Anthocyanins	HCl test	-	-	-	+	+

(Note: PEE: petroleum ether; CHE: chloroform; EAE: ethyl acetate; EE: ethanol; AE: aqueous).

Preliminary phytochemical evaluation:

Following the phytochemical test, the existence of alkaloid compounds, saponins, flavonoids, phenols, and other phytoconstituents in the aerial part of the plant was highlighted in Table 5.

Total phenolic content

The experiment yielded results (Table 6) showing that the 200µg/ml ethanolic extract had the most substantial phenolic substance of the two extracts and concentrations. Although the aqueous extract at the same concentration has also shown reproducible results, a larger standard error makes it inconsistent. Conversely, both extracts at 40µg/ml exhibited consistent levels of phenolic content.

Table 6: Data representing Total phenolic content in mg GAE/g extract (mean ± SEM).

Samples	Concentration (µg/ml)	Total phenolic content mg GAE/g extract (mean ± SEM)
Ethanolic extract	40	366.45 ± 14.83
	200	1151.7 ± 59.22
Aqueous extract	40	365.38 ± 7.75
	200	1235.25 ± 179

Tests for antioxidant activity:

DPPH radical method:

Tables 7 and 8 list the standard and explored samples' DPPH radical neutralization activities.

Table 7: Data representing mean absorbance, % inhibition wrt control (mean ± SEM) & IC50 (mean ± SEM) value of gallic acid for DPPH assay.

Sample concentration Gallic acid (µg/ml)	Mean Absorbance 517nm	% Inhibition wrt control (mean ± SEM)	IC_{50} (mean ± SEM)
0	0.542	0.00 ± 0.58	3.499 ± 0.73
0.78	0.475	12.32 ± 1.00	
1.56	0.418	22.89 ± 0.20	
3.125	0.314	41.95 ± 0.50	
6.25	0.192	64.47 ± 0.38	
12.5	0.106	80.39 ± 0.46	
25	0.067	87.59 ± 0.50	
50	0.058	89.20 ± 0.58	

Table 8: Data representing mean absorbance, % inhibition wrt control (mean ± SEM) & IC50 (mean ± SEM) value of ethanolic and aqueous extract of *Oxalis triangularis* for DPPH assay.

Sample conc. (µg/ml)	Mean Absorbance 517nm		% Inhibition wrt control (mean ± SEM)		IC_{50} (mean ± SEM)	
	EE	AE	EE	AE	EE	AE
0	0.537	0.53	0.00 ± 0.24	0.00 ± 0.54	2.403 ± 16.125	76.67 ± 1.59
1.56	0.515	0.53	3.96 ± 0.76	0.37 ± 0.16		
3.125	0.273	0.48	49.15 ± 24.24	9.98 ± 0.48		
6.25	0.263	0.47	51.04 ± 24.43	12.46 ± 0.41		
12.5	0.236	0.46	56.05 ± 21.36	14.10 ± 0.15		
25	0.221	0.45	58.84 ± 19.53	15.93 ± 0.33		
50	0.187	0.44	65.15 ± 13.61	18.17 ± 0.16		
100	0.174	0.39	67.62 ± 12.59	26.98 ± 0.26		

Note: EE: ethanolic extract, AE: aqueous extract, SEM: standard error mean, wrt: with respect to.

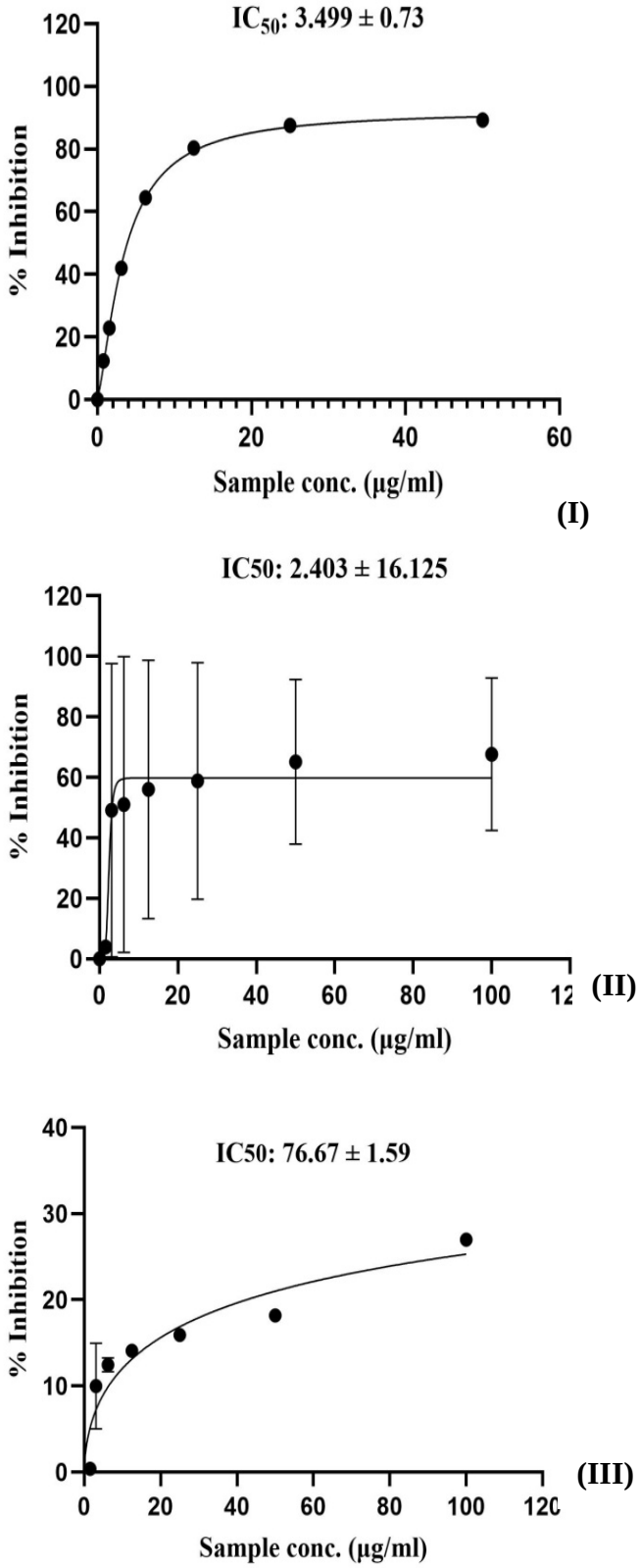


Figure 6: Graph representing IC50 (mean ± SEM) value of (I) Gallic acid, (II) Ethanolic extract & (III) Aqueous extract of *oxalis triangularis* for DPPH assay.

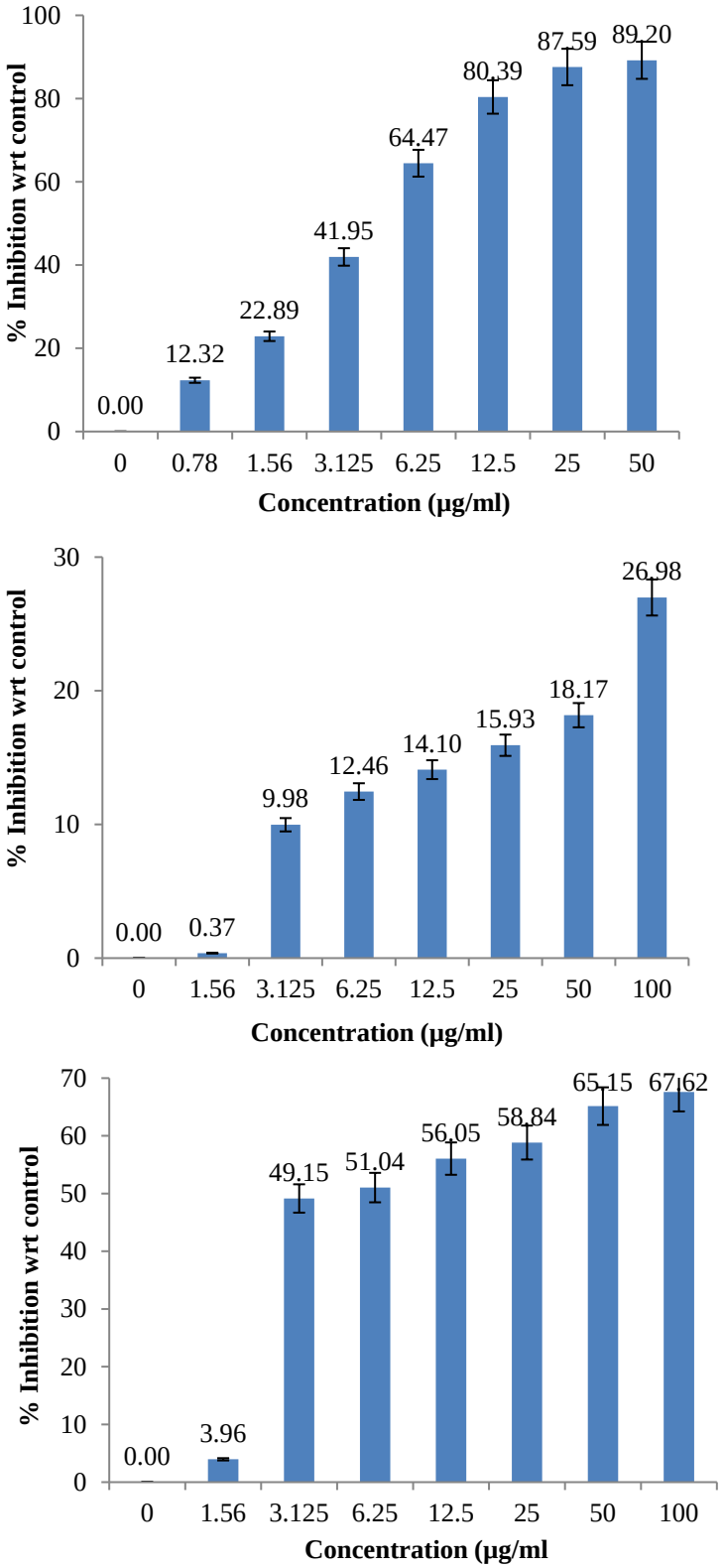


Figure 7: Mean % of DPPH scavenging activity of different extracts of aerial part of *O. triangularis*.

ABTS radical method:

The results obtained are tabulated in Tables 9 and 10.

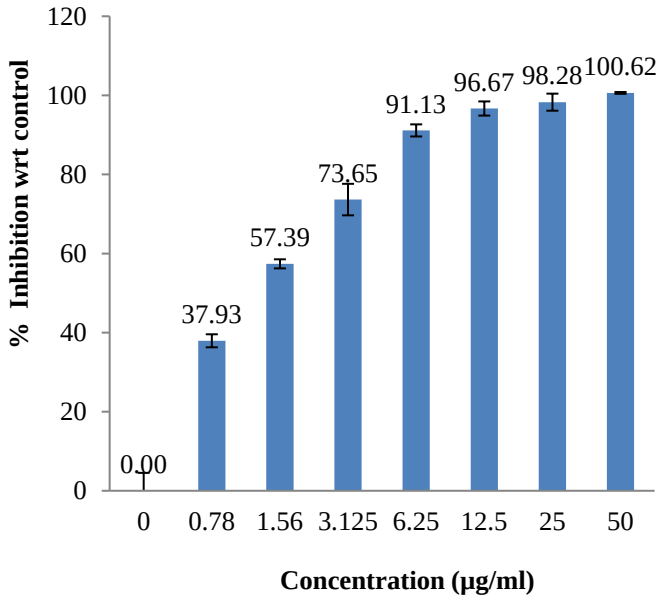
Table 9: Data representing mean absorbance, % inhibition wrt control (mean ± SEM) & IC50 (mean ± SEM) value of gallic acid for ABTS assay.

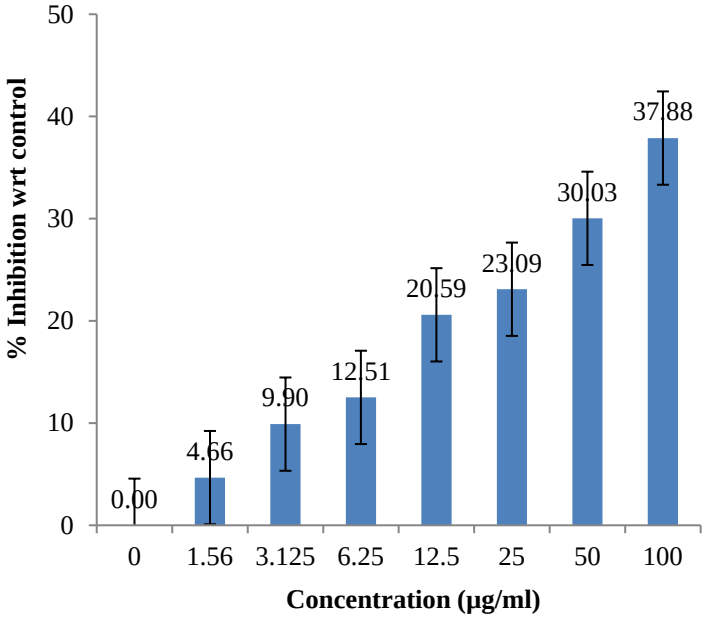
S. No.	Sample concentration Gallic acid (µg/ml)	Mean Absorbance 750nm	% Inhibition wrt control (mean ± SEM)	IC_{50} (mean ± SEM)
1	0	0.203	0.00 ± 4.53	1.266 ± 2.44
2	0.78	0.126	37.93 ± 1.64	
3	1.56	0.086	57.39 ± 1.14	
4	3.125	0.053	73.65 ± 3.98	
5	6.25	0.018	91.13 ± 1.53	
6	12.5	0.007	96.67 ± 1.79	
7	25	0.0035	98.28 ± 2.15	
8	50	-0.00125	100.62 ± 0.23	

Table 10: Data representing mean absorbance, % inhibition wrt control (mean ± SEM) & IC50 (mean ± SEM) value of ethanolic and aqueous extract of *Oxalis triangularis* for ABTS assay.

S. No.	Sample conc. (µg/ml)	Mean Absorbance 750nm		% Inhibition wrt control (mean ± SEM)		IC_{50} (mean ± SEM)	
		EE	AE	EE	AE	EE	AE
1	0	0.198	0.220	0.00 ± 0.32	0.00 ± 1.61	22.94 ± 1.06	43.52 ± 1.09
2	1.56	0.189	0.209	4.67 ± 1.38	4.66 ± 0.29		
3	3.125	0.187	0.198	5.67 ± 0.63	9.90 ± 0.32		
4	6.25	0.181	0.191	8.45 ± 0.78	12.51 ± 0.29		
5	12.5	0.166	0.174	16.02 ± 0.92	20.59 ± 1.97		
6	25	0.148	0.169	25.09 ± 1.58	23.09 ± 0.60		
7	50	0.138	0.154	30.39 ± 0.20	30.03 ± 0.39		
8	100	0.124	0.136	37.33 ± 1.31	37.88 ± 0.29		

Note: EE: ethanolic extract, AE: aqueous extract, SEM: standard error mean, wrt: with respect to.





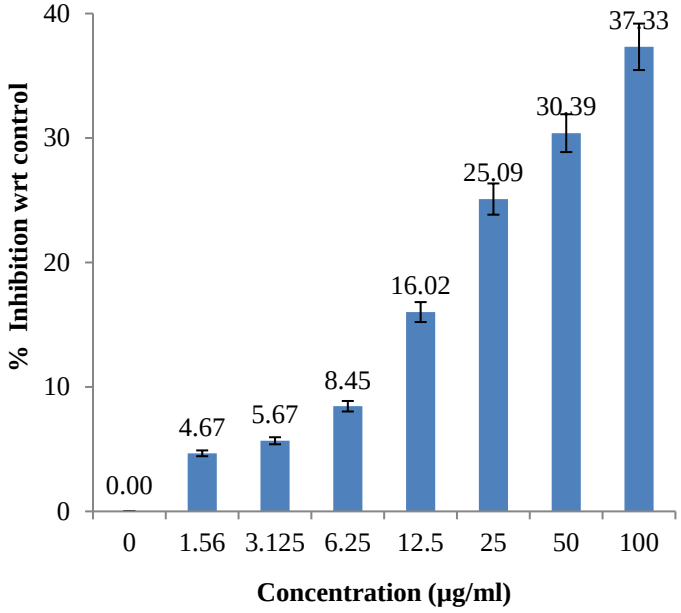
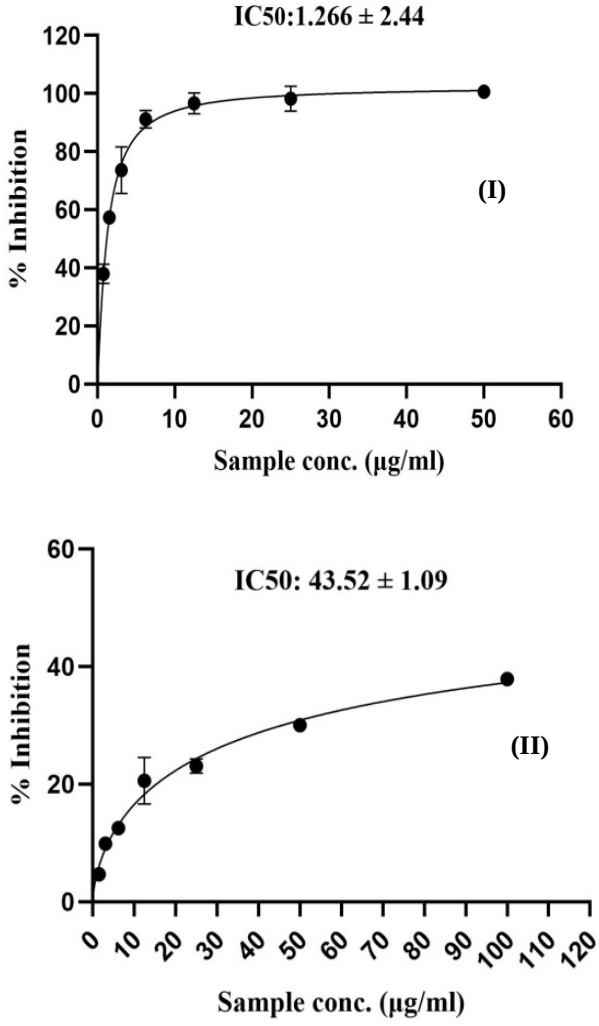


Figure 8: Mean % of ABTS scavenging activity of different extracts of aerial part of *O. triangularis*.



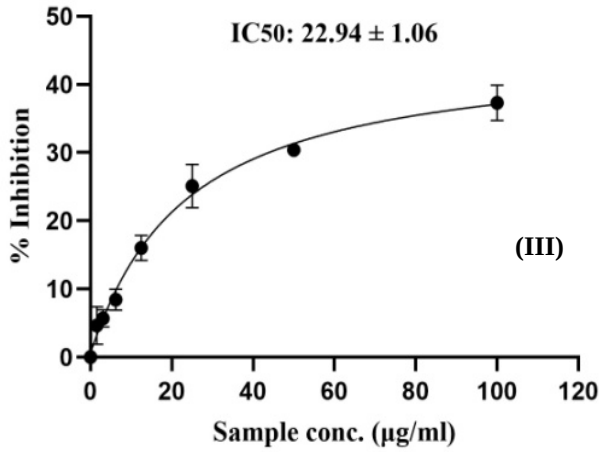


Figure 9: Graph representing IC50 (mean ± SEM) value of; (I) Gallic acid, (II) Aqueous extract & (III) Ethanolic extract of *oxalis triangularis* for ABTS assay.

DISCUSSION

Just like quality checks in allopathic treatment, standardizing herbal medications is essential to ensuring the drug's quality because alternative or fake herbal compounds are commonly utilized in formulas for commercial purposes. These evaluations aid in confirming the drug's identification, caliber, purity, and safety for usage in humans [31]. For *Oxalis triangularis*, several parameters have been investigated, including microscopic, macroscopic, physicochemical, and fluorescence investigations. A morphological study was carried out to identify the plant. The physical traits of the plant verified its identity. The morphological characteristics of the aerial sections of *Oxalis triangularis*, including flavor, hue, size, shape, smell, and appearance, were investigated in the current study highlighted in Table 1.

The study also included microscopic, histochemical, and physiochemical examinations of the plant. Its genuineness was further supported by a fluorescence examination done on the powdered aerial portion of *O triangularis*. Even though there are many sophisticated methods for assessing crude drugs, microscopic techniques are still among the easiest and reasonably priced ways to identify the source material [32] reliably. Numerous unique anatomical characteristics, including lignified unicellular and multicellular trichomes, calcium oxalate crystals, sclerenchymatous fibers, parenchyma, phloem, and xylem, were spotted amid microscopy of T.S & powder form *O triangularis*. Furthermore, physicochemical parameter

assessment helps determine how pure and high-quality crude medications are. By giving an estimate of the chemical constituents present in different solvents, the extractive values assist in selecting suitable extraction solvents [33]. The result highlighted in current investigations entails that aqueous solvent has a higher affinity towards compounds within plant materials with a % yield of 18.69, followed by the petroleum ether, ethanol, chloroform, and ethyl acetate with a % yield of 5.5, 4.43, 3.04 & 1.33, respectively. Ash values constitute a significant quantifiable marker used to assess raw drugs' purity, authenticity, and quality. An elevated ash value could represent evidence of drug cross-contamination or counterfeiting. The current analysis yielded the following ash values: water-soluble ash (10.65%), acid-insoluble ash (2.9%), and total ash (16.33%). Since many phyto-constituents in plant materials exhibit fluorescence, fluorescence analysis helps identify and standardize herbal remedies. When a sample glows, certain phytoconstituents glow in daylight or when exposed to UV light [34].

In this work, a range of organic solvents were used to analyze the fluorescence of dried powder. Table 4 displays the results of the fluorescence analysis of the *O. triangularis* aerial component powder, which displayed distinct colors after being treated with several chemical reagents. Finding bioactive chemicals in plants may be done effectively and economically using preliminary phytochemical screening. The plant may be used as a source of precursors for synthetic medications since it offers a rapid and easy method to identify different phytochemicals [23]. Glycosides were not detected in the phytochemical examination of *O. triangularis* aerial component extracts, but saponins, steroids, flavonoids, phenols, anthocyanin, and alkaloids were reported in almost every extract. The absence of glycoside in preliminary screening may be because of practical error or species differences among the genus of *Oxalis*. Polyphenols, which exist in plants, fruits, vegetables, cereals, and beverages, help protect against the damaging effects of ultraviolet light. The antioxidant qualities of polyphenols in the diet have been linked over time to a lower chance of osteoporosis, diabetes, cardiovascular disease, cancer, and neurological disorders. Plant-based phenolic compound-rich dietary supplements are on the market, and studies investigating their potential health advantages and biochemical impacts are being conducted [35]. On this foundation, quantification of total phenols has been done on two extracts, i.e., ethanolic and aqueous, of aerial parts of *O triangularis* and found that ethanolic extract at 200µg/ml had the most substantial quantity of phenolic substance of 1151.7±59.22. On the contrary, aqueous extract at the same concentration has also shown a reproducible result of 1235.25±179, but a more significant standard error makes it inconsistent. The more standard significant mistake for aqueous extract and higher phenolic content in the ethanolic extract can be attributed to several reasons, i.e., solubility difference of phenolic compound, nature of compound extracted, extraction efficacy of solvents, etc. Conversely, both extracts at 40µg/ml exhibited consistent levels of phenolic content of 366.45 ± 14.83 for ethanolic extract and 365.38±7.75 for aqueous extract, respectively.

Oxidative stress brought on by reactive oxygen species (ROS) can harm membrane proteins and threaten the structural strength of cell membranes. This can then lead to the promotion of oncogenic transformation and the emergence of several illnesses, such as cancer and disorders of the heart and liver [36]. Recently, there has been an increasing curiosity about the functioning of ROS (reactive oxygen species) in multiple disorders. Antioxidants that can scavenge radicals have thus drawn interest due to their potential for use in disease prevention and therapy. Measuring an organic compound's ABTS or DPPH radical-scavenging function is one way to assess its electron-donating capacity [37]. The DPPH assay result in IC_{50} entails ethanolic extract, indicating the most muscular DPPH radical scavenging activity with 2.403 ± 16.125. However, it has shown higher error than aqueous extract with 76.67 ± 1.59. Both extracts have shown reproducible results compared to standard Gallic acid, i.e., 3.499 ± 0.73. On the other hand, the ABTS assay result in IC50 entails ethanolic extract, indicating the strongest ABTS radical scavenging activity with 22.94 ± 1.06, compared to aqueous extract with 43.52 ± 1.09. Compared to standard Gallic acid, i.e., 1.266±2.44, both extracts have also shown reproducible results. The aqueous extract showed weaker antioxidant potential in both assays, which may have occurred because of non-phenolic antioxidants in ethanolic extract or because of the influence of other bioactive components like anthocyanins or flavonoids.

CONCLUSION

Setting physico-chemical criteria ensures herbal remedies' efficacy, legitimacy, and purity. Standardizing herbal remedies is, therefore, essential to guaranteeing the supply of high-grade

drugs. The investigation's approach is necessary for locating and gathering authentic medications while removing fakes. To ensure the manufacturing of authentic and standardized medicines for therapeutic use, the characteristics examined in the present investigation can be used for quality assessment and setting standards for crude drugs. The results of this study indicate that *Oxalis triangularis* may be identified by their unique and essential characteristics. The outcomes of various investigations support the creation of quality control guidelines and guarantee the purity of drugs; phytochemicals, for instance, are essential components of medication quality. When employing the material in traditional medicine, even untrained persons can benefit from these straightforward, affordable, and trustworthy criteria. Strong antioxidant activity was found in these substances, and the various phytochemical makeups like phenols and flavonoids of plant extracts account for their distinct actions.

Despite the genus *Oxalis* having more than 500 species, several species have caught the attention of researchers, leading to numerous studies. This effort is the first for *Oxalis triangularis* regarding antioxidant activity and medicinal material standardization. Many more studies to identify the bioactive constituent and other pharmacological actions are under process for the plant.

FINANCIAL ASSISTANCE
NIL

CONFLICT OF INTEREST
The authors declare no conflict of interest.

AUTHOR CONTRIBUTION
Every author contributed equally to the work. Safal Sharma developed the concept, performed the experiments, prepared the manuscript, and analyzed the results. Nihar Ranjan Bhuyan provided technical assistance checked the manuscript in terms of grammar and plagiarism, and Jyochhana Priya Mohanty conceived and designed the analysis. All the authors create the final manuscript.

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