

Phytochemical profiling and toxicological studies of *Oxalis debilis* Kunth leaves

Julfikar Ali Junejo¹, Gaurab Gogoi², Kamaruz Zaman¹, Johirul Islam³,
Gautam Hazarika⁴

¹Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh, Assam, India, ²Department of Life Sciences, Dibrugarh University, Dibrugarh, Assam, India, ³Defense Research Laboratory, Tezpur, Assam, India, ⁴Diagnostic Genetic Lab, Department of Anatomy, Assam Medical College, Dibrugarh, Assam, India

Abstract

Background: Phytochemical and toxicological studies are a vital part of modern day food analysis. *Oxalis debilis* Kunth is a cosmopolitan species and is considered as a wild edible plant having ethnomedicinal importance in India. **Objective:** The present paper synthesizes the first report related to the different phytochemical, physicochemical screening, and *in vivo* acute toxicity studies of *O. debilis* hydro-alcoholic leaves extract. **Materials and Methods:** The sample was successively extracted through different solvent systems. Preliminary physicochemical and phytochemical studies were evaluated through standard procedure and toxicity of the sample was evaluated through *in vivo* acute toxicity test as per the Organization for Economic Cooperation and Development guidelines, Section 2. **Results:** The powdered dried leaves of *O. debilis* found to exhibit fluorescence property and preliminary phytochemical screening confirmed the maximum presence of flavonoids in all the extracts. Thin layer chromatographic analysis revealed maximum distinguishable spots in the methanolic extract. *In vivo* acute toxicity studies did not show any mortality and change in behavioral pattern in the tested animals. Administration of different concentration of hydro-alcoholic extract of the plant did not alter the biochemical and hematological parameters. **Conclusion:** Acute toxicological studies are in good agreement with the traditional claim, and thus, the plant can be advocated as a safe food source.

Key words: *Oxalis debilis*, physicochemical, phytochemical, thin layer chromatography, toxicity

INTRODUCTION

Oxalis is the largest genus in the family Oxalidaceae, contributing about 89% of the total species belonging to this family. *Oxalis debilis* Kunth is a cosmopolitan, gregarious, and perennial aggressive herb having long petiole. Commonly the plant is known as pink wood sorrel and possesses great ornamental value. *O. debilis* is believed to be native to South America and at present, it has over extensive distribution in the tropical countries including Australia, Hawaii, Fiji, New Caledonia, and the Galapagos Islands.^[1] The plant got naturalized in India and now available in different parts of the country including Assam. *O. debilis* is a tristylous species and embryological studies reports the absence of seeds and plant mainly propagates through bulbils.^[2] The tiny bulbils have a prodigious ability to persist for several years in soil, which can germinate on attaining favorable conditions.^[2] *O. debilis* has uniform distribution in Assam including Dibrugarh district. Locally, it is known as *Bor-tengshi* (Assamese) and is an important wild edible plant

which is often incorporated in various traditional cuisines like sour fish and bottle gourd dishes.^[3] The plant also finds great importance in traditional medicine by its use in various major health complications. The whole plant is used to treat diarrhea, diabetes, piles, and scurvy. In various parts of the Indian subcontinent, the plant is also extensively used as antidote for toxicity.^[4]

Standardization and safety profiling is an essential analytical aspect for the study of purity and quality of edible items especially uncultivated wild edible plants.^[5] Herbal medicines are prepared according to traditional pharmacopedia; unpurified crude extracts are mostly recommended in traditional medicine system,

Address for correspondence:

Julfikar Ali Junejo, Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh - 786 004, Assam, India. Phone: +91-9085433729/7602186795. E-mail: jalijunejo@gmail.com

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which may contain several constituent. Various constituents of herbal medicine often act synergistically and affect adversely on the patient's health.^[6] Besides nutritional and proximate composition analysis, it is necessary to evaluate the toxicological and physiochemical properties of such plants to gather useful information, which is of outmost important for food safety evaluation. The documentation of standardized parameters, therefore, is an indispensable element in the development of herbal drugs from raw plant drugs by considering their desired therapeutic and safety profile. Sufficient information on pharmacological and toxicological studied of the *O. debilis* is found to be lacking in literature. Therefore, work reported here emphasis on the safety evaluation of *O. debilis* along with profiling of phytochemical and physiochemical properties. The standardized parameters reported here will be helpful for future formulation and purity studies and also it can guarantee the status of its toxicity.

MATERIALS AND METHODS

Plant Material

O. debilis was collected from different parts of Dibrugarh district, Assam, India. The plant species was identified and authenticated by Botanical Survey of India (BSI), Eastern Regional Centre, Shillong, India, and a voucher specimen (BSI/ERC/2014/Plant identification/360) was deposited in the herbal center for future reference. Leaves were separated from the collected plant materials and dried under shade at room temperature. The dried materials were coarsely powdered and stored in a desiccator until further use.

Fluorescence Analysis

Method previously described by Chase and Pratt^[7] with little modification was employed for fluorescence analysis. Leaf powder (40 mesh) was treated with different reagents such FeCl₃, CH₃CO₂H, HCl, I, HNO₃, and NaOH followed by fluorescence analysis in both daylight and ultraviolet (UV) light at 254 nm and 365 nm (UV viewer UV fluorescence analysis cabinet, MAC®, Macro Scientific Work, India).

Preparation of Plant Extracts

Powdered leaves samples of *O. debilis* were successively extracted with petroleum ether (60-80°C), chloroform, ethyl acetate, methanol, and hydro-alcohol in a soxhlet extractor. The extracted leaves were dried in room temperature for 2 h before extracting with solvent of higher polarity. Recovered extracts were then concentrated in a rotary evaporator and finally stored in airtight containers at 4°C for further analysis. The percentage yield (w/w) of each extract was calculated in terms of initial air-dried plant material.

Phytochemical Analysis

The presence of various groups of phytoconstituents was detected in different plant extracts through preliminary phytochemical analysis, following the standard procedures described by Harborne^[8] and Evans, 2002.^[9]

Thin Layer Chromatographic (TLC) Analysis

Aluminum precoated silica gel 60 F254 of 0.25 mm thickness high performance TLC plates (Merck, Germany) was used for chemical fingerprinting by TLC. The plates were developed using toluene:ethylacetate:formic acid (4:5:1) as mobile phase. One dimensional ascending method was used to develop the plates as per standard protocol (Indian Pharmacopoeia, 1996). The TLC plate was air dried and spots were visualized under UV light at 254 and 365 nm.

Experimental Animals

Wistar rats (Female, 200-250 g) were obtained from the Laboratory Animal Resources, Dibrugarh University. Rats were maintained at 22 ± 3°C and 12/12 h of light-dark cycles. They were provided adequate diet and water *ad libitum*. Animal tests were carried out according to the Principles of Laboratory Animal care (National Institutes of Health publication 85-23, revised 1985) with approval from the Institutional Animal Ethics Committee, Dibrugarh University (Approval No: IAEC/DU/50 Dt. 24.9.13, Regd. No. 1576/Go/a/11/CPCSEA dated 17.02.2012).

Acute Toxicity Test

Acute toxicity study was performed as per the Organization for Economic Cooperation and Development guidelines number 423. The animals were split in to four groups ($n = 6$) each group was given different doses of hydro-alcoholic extract of *O. debilis* (HAEOD) in corn oil by oral gavages. The groups were named as Group I (normal control), Group II (1000 mg/k HAEOD), Group III (3000 mg/k HAEOD), and Group IV (5000 mg/k HAEOD). The following observation was made which includes skin changes, hair (pilo erection), eyes, circulatory and respiratory pattern, abnormal locomotion, tremor and hypnosis, diarrhea, convulsions, coma, and mortality.^[10,11] Animals were observed individually after dosing at least once during the first 30 min, then periodically during the first 24 h with special attention given during the first 4 h and daily thereafter for 14 days.

Toxicological Studies

The animals were sacrificed and blood samples were collected by puncturing the heart. Serum was obtained by centrifuged blood at 3500 × g for 15 min at 4°C. The change

in level of different serum marker enzymes, i.e., serum glutamic oxaloacetate transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alanine aminotransferase, alkaline phosphatase (ALP), aspartate transaminase, and total bilirubin after administration of the hydro-alcoholic plant extract were estimated using commercial kits obtained from Beacon. Hematological analysis was performed as per standard procedure.

Histological Slides

The liver was obtained from all the experimental animals at 14th day and fixed in chilled 10% saline buffer and processed for routine histological examinations. Tissue samples were processed through a graded alcohol series and embedded in molten paraffin wax. Solidified blocks were kept at -20°C. The blocks were sectioned at 5 µm thicknesses using rotary microtome. The sections were stained with hematoxylin and eosin (Thermo Fisher Scientific Inc.) and washed with xylene. The observations were made in the microscope. Photomicrographs were taken with the help of camera coupled with the microscope.

Statistical Analysis

Both one-way ANOVA and Tukey's multiple comparison test were employed for statistical analysis of the data. Data were represented as a mean ± standard error of the mean, and difference below 0.05 probability level ($P < 0.05$) was considered as statistically significant. GraphPad Prism 5 was used for statistical calculations.

RESULTS

Organoleptic Characters

The organoleptic study of the plant reveals greenish leaves and stems, roots, and root bark are brownish white. Dried powdered samples of leaves are brownish green and possess aromatic property [Figure 1].

Physicochemical Properties

Different physicochemical properties like moisture content ash value, and extractive value of the powdered leaf sample were assessed. The result of the physicochemical parameters is illustrated in Table 1.

Phytochemical Screening

The preliminary phytochemical analysis of *O. debilis* leaves reveals the presence of different phytochemical groups in varying concentration in different solvent extracts. The

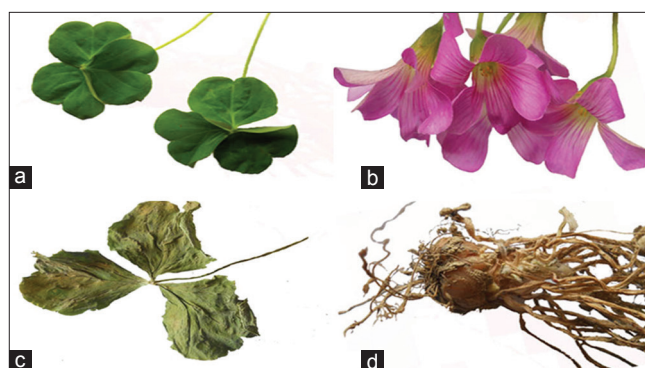


Figure 1: Organoleptic characters of *Oxalis debilis*. (a) Fresh leaves, (b) flowers, (c) dried leaves, (d) dried tuber

Table 1: Physicochemical parameters

Parameter	% w/w±SEM*
Moisture content (% LOD)	11.09±0.17
Ash values	
Total ash	34.56±0.29
Acid insoluble	7.18±0.47
Water soluble	12.56±0.33
Extractive values	
Water soluble	15.73±0.26
Alcohol soluble	8.22±0.18

*Values are expressed as mean±SEM of three replicates.
SEM: Standard error of the mean, LOD: Loss on drying

presence of flavonoids was confirmed in all the solvent extract except petroleum ether and aqueous extract, similarly phenolic compounds and tannins were present in ethyl acetate and methanolic extract; however, the presence of alkaloids and saponins was restricted to only methanolic and aqueous extract respectively [Table 2]. The extractive value of the plant material in different solvent systems is reported in Table 3.

Fluorescent Properties

The crude plant materials are often qualitatively assessed by their fluorescent characteristics which indicate the presence of certain chemical constituents under experimental conditions. The fluorescence property of powdered plant sample with different reagents is presented in Table 4.

TLC Analysis

TLC chromatograms depicted in Figure 2 show certain distinct spots with their relative intensities. The colors of the spots were recorded as yellow, yellowish green, reddish, and violet. Maximum distinguishable spots with different R_f values were obtained on the methanolic extract followed by chloroform and pet ether extracts. Table 5 reveals the TLC

Table 2: Phytochemical screening of various solvent extracts of *Oxalis debilis* leaves

Constituents	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Methanol extract	Aqueous	Hydro-alcoholic
Alkaloids	--	--	--	++	++	++
Glycosides	--	--	--	++	--	--
Phenolic compounds and tannins	--	--	++	++	--	++
Flavonoids	--	++	++	++	--	++
Carbohydrates	--	--	--	++	++	+
Proteins	--	--	--	++	++	+
Fats and oils	++	++	--	--	--	--
Saponins	--	--	--	--	++	+
Gum	--	--	--	--	--	--
Steroids	--	++	--	--	--	--
Terpenes	--	--	--	++	--	--

++: Indicates presence, --: Indicates absence

Table 3: Extractive values of different solvent extracts

Extract	Extractive values (% w/w)	Color of extract
Pet. ether extract	0.78	Light black
Chloroform extract	1.13	Green
Ethyl acetate extract	1.59	Light green
Methanolic extract	3.56	Brown
Aqueous extract	5.11	Brown

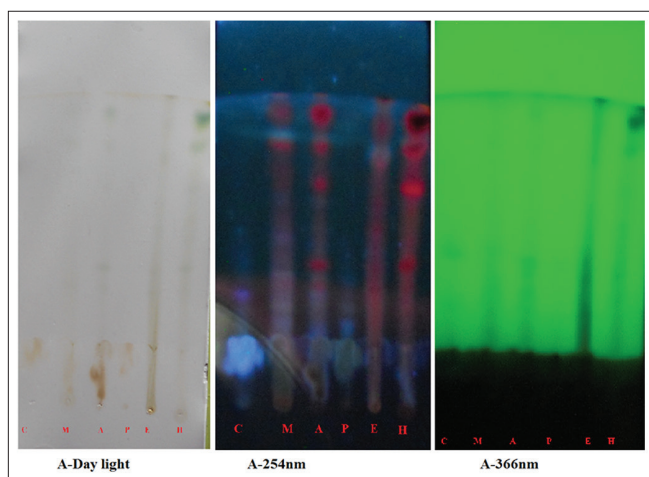


Figure 2: Thin layer chromatographic (TLC) finger print profile of *O. debilis* under 254 nm and 366 nm (P is pet. Ether extract, C is chloroform extract, E is ethyl acetate extract, M is methanol extract, A is aqueous extract, H is hydroalcoholic extract) TLC plate A was developed in toluene:ethylacetate:formic acid (5:4:1)

finger print profile of different extracts which depict the number of spots obtained with their relative R_f values.

In Vitro Toxicological Study

No toxic symptoms or mortality were observed in any animals up to 14 days of the experimental observation after the administration of hydro-alcoholic extract at a single dose up to the level of 5000 mg/kg body weight. The behavioral patterns of animals were observed after first 6 h and followed by 14 h after the administration, and the animals in both vehicle-treated and extract-treated groups were normal and did not displayed any significant changes in behavior, skin effects, breathing, impairment in food intake, water consumption, hair loss, postural abnormalities. There is also no reduction in body weight of the test animals during the experimental period in all the treated groups; however, high dose of HAEOD (Group III and IV) showed a significant increase in body weight at the end of the experiment, which needs further investigation to identify the responsible component of the plant extract in enhancement of weight. The variation in body weight of the test animals in different groups is shown in Table 6.

The level of the serum biomarker and lipid profile in different animal groups is given in Table 7. Hematological properties also remained unchanged after administration of HAEOD leaves to the test animals. Hydro-alcoholic extract as high as 5000 mg/kg did not show any significant difference in red blood cell (RBC) count, total white blood cell (WBC) count, hemoglobin (Hb) level, and mean corpuscular volume (MCV) values counts compared to the control group. However, a significant reduction in the platelets count ($P < 0.05$) was recorded in the animals treated with different concentrations of the plant extract [Table 8]. Similarly, the differential count of blood is also within the acceptable limit in the tested animals [Table 9]. The histoarchitecture of the liver did not show any pronounced degeneration or irreversible damage of the liver. Hepatocytes and centrilobular zones are normal in all the animals administered with a different dose of *O. debilis* hydro-alcoholic extract as compared to the normal untreated animal [Figure 3].

Table 4: Fluorescence analysis of powdered *Oxalis debilis* leaves

Powdered drug/treatment	Visible/day light	Short UV (254 nm)	Long UV (365 nm)
Powder drug	Green	Light green	Dark green
Powder+methanol	Blackish brown	Yellowish brown	Dark brown
Powder+1% glacial acetic acid	Light brown	Dark violet	Dark violet
Powder+10% NaOH	Light Brown	Yellowish brown	Brown
Powder+dil. NH ₃	Brown	Dark brown	Dark brown
Powder+conc. HNO ₃	Yellowish green	Yellowish brown	Green
Powder+dil. NH ₃ +conc. HNO ₃	Light brown	Dark violet	Light brown
Powder+1 M H ₂ SO ₄	Blackish green	Dark green	Black
Powder+1 M HCl	Brown	Dark violet	Black
Powder+10% FeCl ₃	Brown	Yellowish green	Light brown
Powder+acetone+methanol	Dark brown	Brown	Brown
Powder+10% iodine	Greenish brown	Dark brown	Green

Table 5: Thin layer chromatographic analysis*

Extract	Number of spots (Plate A)	R _f values (Plate A)
Pet ether	1	0.377
Chloroform	1	0.490
Methanol	3	0.943, 0.905, 0.584
Ethyl acetate	2	0.924, 0.886
Aqueous	4	0.943, 0.905, 0.792, 0.584
Hydro-alcoholic	4	0.943, 0.905, 0.792, 0.584

*Solvent system: Toluene: Ethyl acetate: formic acid (5:4:1)

Table 6: Change in body weight of animal groups after the administration of hydro-alcoholic extract of *Oxalis debilis*

Group	0 day	7 days	14 days
Control group	214.67±3.88	216.86±2.34	219.84±5.12
Group II, 1000 mg/kg	215.12±4.23*	217.97±3.76	218.53±4.12
Group II, 3000 mg/kg	216.26±2.46	219.11±2.64*	232.53±1.46**
Group III, 5000 mg/kg	215.79±3.88	220.23±1.34**	231.11±2.53**

Values are mean±SEM (n=6), statistical significance, *P<0.05, **P<0.01, compared with normal control Group I. SEM: Standard error of the mean

DISCUSSION

The widely used *O. debilis* leaf in various ethnomedicines and as a wild edible plant was investigated for various pharmacological parameters to assess the safety profile of the plant. Physicochemical, phytochemical and toxicological profiling of a plant are always the baseline investigations for systematic pharmaceutical research. Moisture content (% loss on drying) of the powdered leaf drug was found

to be 11.09 ± 0.17 (% w/w), which indicated optimum drying of the plant material. The determination of moisture content is always important due to the fact that insufficient drying may lead to possible enzymatic deterioration of active principles.^[9] The physicochemical study reveals that the amount of acid insoluble ash is less than that of water-soluble ash, whereas the amount of total ash was almost double the quantity of water soluble ash, which infers the maximum presence of organic compounds. The ash content gives an idea about the inorganic content of powdered leaves under investigation and thus the quality of the drugs can be assessed.^[12] The results of physicochemical analyses lie within the acceptable limit which in turn ascertains the leaves of the plant as pure quality in terms of physicochemical parameters. These parameters can be used in future for standardization of different experiments and formulation of drugs.

The fluorescence analysis of powdered *O. debilis* leaves exhibits fluorescent properties which may be due to the presence of fluorescence producing compound in the sample or due to the conversion into fluorescent derivatives or decomposition products by the application of different reagents.^[13,14] Further approaches in characterization and identification of the fluorescence property and the responsive compound should be evaluated to develop different fluorescence marker for rapid characterization of the plant.

Preliminary phytochemical information and separation of different plant matrices can be quickly assessed through TLC method. Analytical TLC is sometimes also useful for ascertaining the purity of crude sample by detecting the presence or absence of adulterants and substituent.^[15] Four distinct spots having respective R_f value ranging from 0.584 to 0.943 were obtained in both aqueous and hydro-alcoholic extracts developed in toluene:ethyl acetate:formic acid (5:4:1). The chromatograms (TLC) showing characteristic spots with their relative R_f values can be considered as marker component of the plant. Therefore,

Table 7: Biochemical parameters in serum of rats administered with hydro-alcoholic leaf extract of *Oxalis debilis*

Groups	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	Triglyceride (mg/dl)	Cholesterol (mg/dl)
Control group	52.87±1.32	54.87±3.23	113.81±2.93	152.76±3.87	188.43±5.32
Group II, 1000 mg/kg	51.79±2.61	53.91±5.56	114.12±1.42	151.98±1.32	187.23±2.45
Group II, 3000 mg/kg	52.68±2.47	55.21±3.57*	116.72±2.43*	153.68±2.47	189.78±2.64**
Group III, 5000 mg/kg	53.11±1.58	54.11±2.58	115.36±1.64	154.77±3.54	187.46±1.45

Values are mean±SEM (n=6), statistical significance, *P<0.05, **P<0.01, compared with normal control Group I. SEM: Standard error of the mean, SGOT: Serum glutamic oxaloacetate transaminase, SGPT: Serum glutamic pyruvic transaminase, ALP: Alkaline phosphatase

Table 8: Effect of hydro-alcoholic extract on hematological parameters

Group	WBC (10 ⁶ /mm ³)	RBC (10 ⁶ /mm ³)	Hb (g/dl)	Platelets (10 ⁶ /mm ³)	MCV (mm ³)
Control group	9.17±1.23	8.54±0.27	14.13±1.64	985.86±25.72	47.46±1.07
Group II, 1000 mg/kg	10.11±0.98	8.11±1.05	15.08±1.07	956.58±22.54**	46.81±1.33
Group II, 3000 mg/kg	9.98±0.78	8.68±1.23	14.65±1.64	959.11±33.64**	48.34±0.65
Group III, 5000 mg/kg	9.22±1.37	8.35±0.43	14.32±0.58	977.36±36.6*	46.69±1.24

Values are mean±SEM (n=6), statistical significance, *P<0.05, **P<0.01, compared with normal control Group I. SEM: Standard error of the mean, WBC: white blood cell, RBC: Red blood cell, Hb: Hemoglobin, MCV: Mean corpuscular volume

Table 9: Effect of hydro-alcoholic extract of *Oxalis debilis* on differential count of blood

Group	MCH (pg)	Eosinophils (%)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)
Control group	16.68±1.45	1.22±0.46	17.58±1.57	85.68±0.57	3.22±1.54
Group II, 1000 mg/kg	17.12±1.75 ^{ns}	1.35±1.32	18.38±1.97	86.35±0.22	2.98±1.33
Group II, 3000 mg/kg	16.23±0.46	1.64±1.41	17.91±0.36	85.89±1.46	3.65±1.51
Group III, 5000 mg/kg	16.98±1.72	1.87±0.29	19.49±1.46 ^{ns}	87.46±1.39 ^{ns}	3.43±0.41

Values are mean±SEM (n=6), statistical significance, *P<0.05, **P<0.01, compared with normal control Group I. MCH: Mean corpuscular hemoglobin, SEM: Standard error of the mean

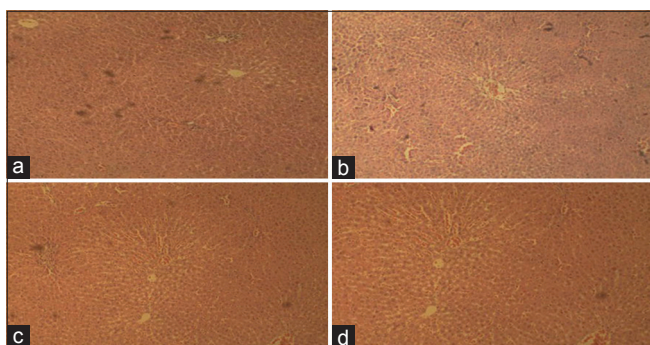


Figure 3: Photomicrographs of liver sections fixed in chilled 10% saline buffer. (a) Group I, normal untreated, (b) Group II, treated with 1000 mg/kg hydro-alcoholic *O. debilis* leaves extract, (c) Group III, treated with 3000 mg/kg hydro-alcoholic *O. debilis* leaves extract, (d) Group IV, treated with 5000 mg/kg hydro-alcoholic *O. debilis* leaves extract

the present TLC fingerprinting method can be used in chemotaxonomic identification of *O. debilis*.

Liver transaminases (SGOT and SGPT) and ALP are considered as an important biomarker for preliminary diagnosis of hepatic injury and liver dysfunction.^[16] Increased level of these enzymes and cholesterol after administration of plant extract are considered as indices of liver injury which

are a sign of toxicity. The test animal showed no significant changes in serum levels of SGOT, SGPT, ALP, triglyceride and cholesterol ($P < 0.05$) compared to the normal untreated group after oral administration of hydro-alcoholic leaves extract of *O. debilis*. The result clearly proves the probable absence of hepatotoxic agent in the hydro-alcoholic extract of the plant material. Similarly, the unchanged count of the RBC and WBC indicated that the plant extract has a little role in either production or destruction of these cells; similarly, the intact Hb level can also be inferred to the neutral role in Hb synthesis by the plant extract. The reduction of platelets due to the administration of the plant extract needs more precise research to identify its role in reduced production or destruction. The values of mean corpuscular Hb, eosinophils, neutrophils, lymphocytes, and monocytes were also found to be statistically insignificant ($P < 0.05$) in different experimental groups. The values although shows minor differences among various groups, but no statistical significance were obtained compared to the control untreated group. Hematotoxicology plays a primary role in classification of xenobiotic on their level of toxicity by predicting the exposure level that causes neutropenia.^[17] The overall findings of the hematology suggest that the plant is least toxic hematological and have a little interfering ability in biosynthesis of blood and its constituents.

CONCLUSION

The overall biochemical and hematological studies suggested that the plant showed no toxicity in any of the animals administered with the plant extract. Folklore claim on the plant as a safest source of medicine without any adverse effect is thus, validated up to some extent. However, the final validation can only be possible after complete profiling and characterization of different bioactive compounds. Furthermore, analysis on genotoxicity and cytotoxicity is necessary to confirm the safeness of the plant at regular consumption as a source of food. The preliminary phytochemical analysis of the plant extract showed the presence of various bioactive groups such as flavonoids, alkaloids, and glycosides which may exhibit activity against various diseases and disorders. The TLC analysis gave an idea of the probable number of compounds present in the extracts. Evaluation of biological activities against various disorders and diseases is under current investigation to confirm the ethnomedicinal claims and also to standardize such drug more accurately.

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