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PHARMACOGNOSTICAL AND PHYTOCHEMICAL STUDIES OF OXALIS CORNICULATA

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ABSTRACT

The present study aimed to perform pharmacognostic, physicochemical and phytochemical investigations on crude powders and different types of extracts of various parts of *Oxalis corniculata* (Family: *Oxalidaceae*). The sections of different parts of the plant were stained and examined using a light microscope. Powders of different parts were also examined under light microscope. The behavior of the powders under ultraviolet light was also investigated, before and after treating with certain reagents. The powders were analyzed for the determination of proximate parameters, primary metabolites and Fourier Transform Infrared (FTIR) spectra. The extracts of different parts were investigated for primary and secondary metabolites. FTIR spectra of leaves stem and fruit showed bands at 3435–3400 cm^{-1}, 2918 cm^{-1}, 1654–1651 cm^{-1} and 1052–1049 cm^{-1}, however, the fingerprint region of the spectrum indicated the difference in chemical constituents. The stained cross-sections of different parts and their powder's microscopy, TLC profiles and primary and secondary metabolites showed distinct pharmacogostic features that could be used for identification. The observed moisture content values of leaves and stems were 5.5% and 6.0%, respectively which showed less quantity of moisture in the crude powders. The results of the present study may be used for the identification of the plant.

Keywords: *Oxalis corniculata*, *Oxalidaceae*, Pharmacognostic, Physicochemical, Phytochemical investigations

INTRODUCTION

Oxalis corniculata (Family: *Oxalidaceae*), a sub-tropical annual herb, is well-known traditional medicinal uses. As a folklore remedy, juice and extracts of aerial parts are used for treating stomachache, datura poisoning, scorpion sting and wound bleeding (Muhammad and Mir, 2000) whereas roots are used for treating diarrhea, dysentery and giddiness, and expulsion of worms. The leaves in various forms are used for curing fever, coughs, cold, mouth ulcers, eczema, headache, stomachache, expulsion of gastrointestinal worms, jaundice and hepatitis (Chopra *et al*., 1986; Hebbar *et al*., 2004; Abinash *et al*., 2006; Unni *et al*., 2009 and Abbasi *et al*., 2009). Nevertheless, Pakistani and Indian villagers quite frequently consume aerial parts of the plant as such or in the form of a paste - *chatney* - to improve digestion. The plant has been investigated extensively for a vast array of pharmacological activities such as antibacterial activity (Unni *et al*., 2009; Valsaraj *et al*., 1997; Raghvendra *et al*., 2006 and Satish *et al*., 2008), anti-protozoa activity (Manna *et al*., 2010), anti-fungal activity (Iqbal *et al*., 2001), wound healing activity (Taranalli *et al*., 2004), anti-implantation and abortifacient activity (Sharangouda and Patil, 2007), cardio-protective activity (Achola *et al*., 1995), nematocidal activity (Taba *et al*., 2008), hypoglycemic activity (Sharma, 2004), anti-inflammatory activity (Sakat *et al*., 2012), anxiolytic

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activity (Kumar *et al*., 2012), anti-tumor activity (Kathiriya *et al*., 2010), anti-epileptic activity (Kumar *et al*., 2010) and hepatoprotective activity (Das *et al*., 2011 and Khan *et al*., 2012). Phytochemical investigations indicated that the plant had a number of fatty acids, amino acids, proteins, carbohydrates, glycosides, phytosterols, flavonoids and volatile oils (Unni *et al*., 2009; Raghvendra *et al*., 2006; Han, 1998; Daniel, 2006 and Hioki *et al*., 2008). Fresh leaves contain 86% water, 7-12% oxalate, 8.2% carbohydrates, 0.8% fats, 150 mg calcium, 78 mg phosphorous, 78 mg vitamin C, 8 mg iron, 0.6 mg niacin and 6.05 mg β-carotene (Pal *et al*., 2003). Based on its flavonoids, vitamins and minerals contents, the plant is considered as a good candidate for preparing antioxidant and hepatoprotective products. However, it could not get popularity due to high oxalate contents, and there were no reports in the literature about lowering oxalates in extracts according to best of our knowledge. Therefore, there is a dire need to prepare extracts of the plant having either lower or no oxalates.

There were two reports of hepatoprotective studies on different types of extracts of dried aerial parts of the plant, wherein Das *et al.* (2011) used aqueous and ethanol extracts using thioacetamide to induced oxidative stress and Khan *et al.* (2012) had investigated sequential extracts and fractions of methanol extracts using CCl_4 intoxication. Both of the agents used in these studies did not have clinical relevance, therefore, there was a need to perform hepatoprotective studies using agents which had clinical usage such as isoniazid and rifampicin. Moreover, the extraction procedure for reducing oxalates and hepatoprotective investigation of extracts of different parts of the plant are being described for the first time.

A well-known hepatoprotective drug, silymarin, has been used in the present study as a positive control, which has been selected due to its activity and safety profile (Saller *et al*., 2001) and well-established mechanisms of antioxidant, inhibition of lipid peroxidation, stimulation of protein synthesis, anti-inflammatory and anti-fibrotic (Pradhan and Girish, 2006). The findings of the present study may be helpful in preparing oxalate free extracts of *Oxalis corniculata* that can be used as antioxidant and hepatoprotective products.

MATERIALS AND METHODS

Plant materials

The plant was collected from Warburton, district Nankana Sahib, Punjab, Pakistan, and got authenticated by Prof. Dr. Zahee-ur-Din Khan, Department of Botany, Government College University (GCU), Lahore, Pakistan. A voucher specimen (GC/Herb/Bot/863) was deposited in herbarium of GCU. The leaves, stems and fruits were separated and dried under shade. The dried parts were pulverized to coarse powder and stored in well closed glass containers.

Chemicals

Chemicals and solvents were of analytical grade which include safranin and gallic acid (China National Chemical, China), fast green, petroleum ether, potassium sodium tartrate, and anthrone reagent (BDH, England), Canada balsam (Syn, UK), chloral hydrate, NaOH, HCl, H_2SO_4, HNO_3, KBr, folin-ciocalteau, Na_2CO_3, bovine serum albumin, acetyl chloride, sulfur powder, zinc dust, lead acetate, copper sulphate, potassium hydroxide, ethyl acetate, formic acid, glucose, quercetin, aluminium nitrate and potassium acetate (Merck, Germany), triton X-100 (Uni-Chem, China), ethanol, n-hexane, chloroform, methanol, acetic acid, ferric chloride, calcium chloride and bromine (RDH, Germany), pyridine (Mallinckrott, USA), betulinic acid, flavonone and piperine (Sigma-Aldrich, Germany), Molisch's reagent, Barfoed's reagent, Benedict's reagent, Mayer's reagent, Wagner's reagent, Hager's reagent, Dragendorff's reagent, Millon's reagent and Ninhydrin reagent (Lab. Prepared reagents).

Instruments

Instruments included Rotary microtome (Nippon, Japan), Compound microscope (Labomed, USA), Handhold UV lamp (UVGL-58, UVP, UK), Analytical balance (Mettler Toledo, Switzerland), Microwave oven (Memmert, Germany), Electric carbolite furnace (Sheffield, UK), Electric stirrer (Sybron, USA), Carver laboratory press (Carver, USA), FTIR Spectrophotometer (Thermo Nicolet-6700, USA), Rotary evaporator (Heidolph Laborata 4002-control, Germany), Centrifuge machine (Sigma 2-16k, Germany), Soxhlet apparatus (Quickfit, England), Ultrasonic mixer (Elma, Germany), Freeze dryer (Alpha 1-4 LD plus, Christ, Germany), Freezer (Sanyo, Japan), and UV/Visible Spectrophotometer (UV – 1700, Shimadzu, Japan).

Macroscopic, microscopic and fluorescence analysis

Macroscopic studies of plant leaves and stems were performed according the method described by Kashyap and Joshi (1936). Microscopic studies of fresh plant leaves and stems were done by cutting transverse sections. Transverse section of leaf was cut with rotary microtome whereas petiole and stem transverse sections were cut with ordinary razor; fixed with ethanol and double stained with safranin and fast green solutions. The transverse sections were mounted with Canada balsams and observed under compound microscope. Microscopic studies of dried powders of leaves and stems were done by taking a pinch on glass slide and

mounted with chloral hydrate solution. The slides were observed under low (10x) and high (40x) objectives and photographed with the help of Sony cyber shot, DSC-W220, 12.1 mega pixels by mounting on the compound microscope.

Fluorescence analysis was performed as per procedure illustrated by Kumar *et al.,* (2010). The dried powders of leaves, stems and fruits were examined under ordinary and UV light with and without mixing 1N NaOH (aqueous), 1N NaOH (methanolic), 1N HCl (aqueous), 50% H_2SO_4, and 50% HNO_3 solvents.

Physicochemical analysis

Physicochemical analysis of crude powders of leaves and stems were performed for moisture contents, total ash, acid insoluble ash, acid soluble ash, sulphate ash, water soluble extractives and ethyl alcohol soluble extractives according to the specifications of USP (2009).

FTIR Spectroscopy of crude powders

The IR spectroscopy of crude powders were performed by taking one milligram of each powdered leaves, stems and fruits materials by mixing with 100 mg KBr and made into discs with carver laboratory press under hydraulic pressure. The pellets were used to get IR spectra by FTIR spectrophotometer at wave length range of 4000-550 cm^{-1} using OMNIC 6.0a software.

Phytochemical analysis of crude powders

Crude powders of leaves and stems were analyzed for the estimation of their total contents of lipids, proteins, carbohydrates and oxalates. Total lipids estimation was done by a method of Besbes *et al.* (2004); Total proteins estimation was performed by a method of Rasool *et al.* (2010), Hussain *et al.* (2008) and Lowry *et al.* (1951). Total carbohydrates were determined by a method of Al-Hooti *et al.* (1998). Total Oxalates were determined by gravimetric method.

Extraction

Hundred gram crude powders of leaves and stems were successively extracted by n-hexane, chloroform and methanol using soxhlet apparatus. Each of the extracts dried under reduced pressure at 40°C. All extracts were dried and weighed to calculate the percentage yield.

Phytochemical analysis of extracts

Aqueous, ethanol, n-hexane, chloroform and methanol extracts of leaves and stems were used for phytochemical analysis. The test solutions of each of the extracts having concentration of (0.1 mg/mL) were prepared by dissolving the extracts in distilled water. Aliquots were then used for the tests of sterols, triterpenoids, glycosides, flavonoids, saponins, carbohydrates, alkaloids, tannins and proteins according to the specifications of USP (2009).

Detection of photochemical groups using TLC

The thin layer chromatography (TLC) of ethanolic extract of leaves and stems was performed. The best solvent system chloroform : ethyl acetate : formic acid (5 : 4 : 1, v/v/v) was used for the detection of triterpenoids, flavonoids and alkaloids along with their reference standards i.e. betulinic acid, flavonone and piperine after spraying anisaldehyde, potassium hydroxide and dragendorff's reagents respectively.

UV Spectroscopy of extracts

n-hexane, chloroform and methanolic extracts of leaves and stems were dissolved in methanol to prepare stock solution of concentration 1 mg/mL. Working solutions were prepared by diluting 1 mL stock solution to 10 mL with methanol (0.1 mg/mL) and scanned by UV/Visible spectrophotometer at 200-800 nm using methanol as a blank.

Estimation of primary and secondary metabolites

Methanolic extracts of leaves and stems were analyzed for the estimation of primary metabolites (proteins and carbohydrates) and secondary metabolites (polyphenols, flavonoids and glycosaponins). Total proteins estimation was performed by a method of Rasool *et al.* (2010), Hussain *et al.* (2008) and Lowry *et al.* (1951). Total carbohydrates estimation was performed by a method of Hussain *et al.* (2008). Total polyphenols estimation was performed by a method of Singleton and Slinkard (1997). Total flavonoids were determined by a method of Chang *et al.* (2002). Total glycosaponins were determined by a method of Hussain *et al.* (2008).

RESULTS AND DISCUSSION

Macroscopic characters

Macroscopic characters are same as mentioned in introduction.

Microscopic characters

Transverse section of leaf

Epidermis is on both upper and lower side. Epidermal cells are larger in size. There is a single row of palisade mesophyll which is columnar in shape, vertically oriented below the upper epidermis. The sponge mesophyll is 2-3 layered. Spongy mesophyll cells are irregular in shape and loosely arranged having intercellular spaces between the cells. The vascular bundles are co-lateral. Xylem is present on the upper side of vascular bundle and phloem present below. Bundle

sheath is present. Stomata are present on the lower side. The transverse section of leaf is shown in Figure 1.

Transverse section of petiole

The overall shape of the stalk of leaf is rounded on ab axial while it is more or less triangle on ad axial. A number of non-glandular trichomes are visible on both sides. The trichomes are more or less uniseriate with unicellular or bi-cellular in appearance. There is a larger area of cortical tissues having lot of resin ducts. The resin ducts are composed of unicellular large canal like structure arrange in a circular manner surrounded by cortical tissues.

Cortical tissues are surrounded by epidermal cells containing thick cortical layer all around. Middle portion of the section is thin walled parenchyma comprising of pith cells. The vascular bundles are present in between the cortical area and pith region. Phloem and xylem are visible in this area. Both these tissues are exarch. Xylem which is stained red toward the center or pith whiles the phloem which stained green toward peripheral or cortical region. The transverse section of petiole is shown in Figure 2.

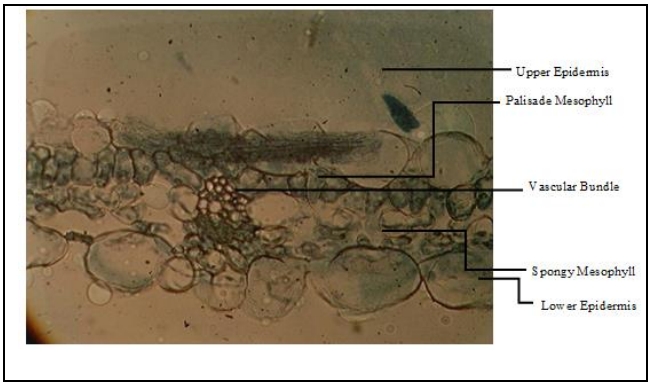

Figure 1: Transverse section of leaf of *Oxalis corniculata*

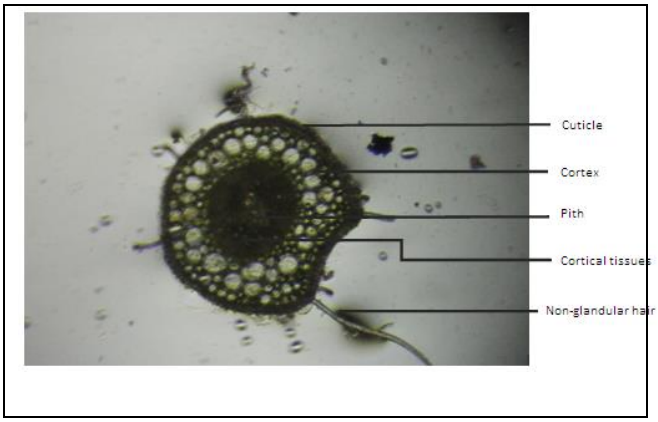

Figure 2: Transverse section of petiole of *Oxalis corniculata*

Transverse section of stem

The xylem stained with safranin, phloem stained with fast green. Cortical tissues and pith region are also light green. There is a layer of thick walled sclerenchymatous tissues all around the vascular area. The sclerenchyma tissues are also stained with safranin and probably 2–3 layered in thickness. The cells of this layer are moderately thickened. The pith region has largest size cells than other areas. Some of these cells have packed starch grains and crystals. Xylem is towards the center of the section while phloem is outside. Cambium is not fully visible. Few stomata are visible on the epidermis tissue. Guard cells and subsidiary cells are not fully visible. Non glandular trichomes are seen in the form of single layer. They are uniseriate. The transverse section of stem is shown in Figure 3.

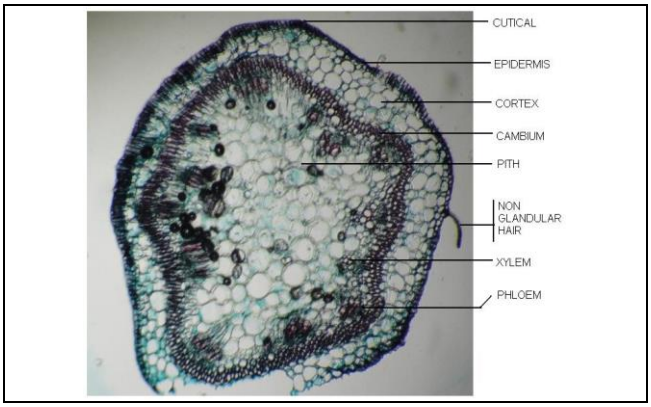

Figure 3: Transverse section of stem of *Oxalis corniculata*

Powder characteristics of leaves

Microscopic examination indicated the presence of spiral and annular vessels. These vessels were covered by parenchymatous tissues. Parenchyma tissues were composed of simple thin walled cells with greenish chlorophyll in it. Some crystals and other cellular organelles were present in the powder. Non glandular uniseriate with two distinct cells were also visible. Epidermal cells were more or less rectangular with transparent cell contents. These cells had limited and small inter cellular spaces thus closely packed. No distinct stomata were seen. Few of the cells had emergences in the form of non-glandular trichomes which were uniseriate. Pollen grains had double cell wall with outgrowth. The microscopic picture of leaves powder is shown in Figure 4.

Powder characteristics of stems

Simple uniseriate non-glandular trichomes were visible which made up of 4 cells. The upper one is tapering while lower one is broadened. Glandular trichomes were also visible with a simple unicellular head and

unicellular stalk. Thin walled parenchyma tissues were also observed. The cells were of irregular shapes having distinct nuclei. Fibro vascular tissues were seen embedded in the parenchyma. Spiral vessels and simple annular vessels were all observed in the xylem tissues.

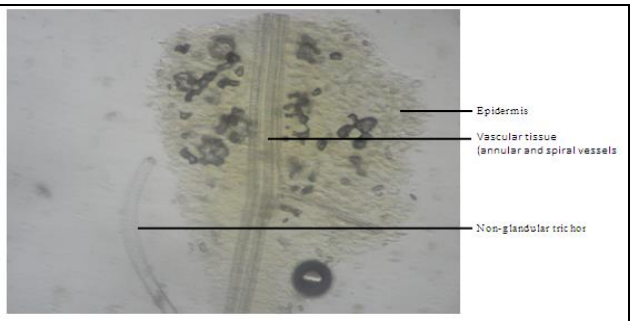

Figure 4: Powder characteristics of leaves of *Oxalis corniculata*

Xylem was associated with sclerenchyma tissues on either side. These sclerenchyma tissues were fibrous with tapping end walled. Epidermis tissues were seen in the form of irregular compact masses of cells joint together, no intercellular space. In the powder, stomata were not visible. Pollen grains, starch grains and micro crystals were also visible. The microscopic picture of stems powder is shown in Figure 5.

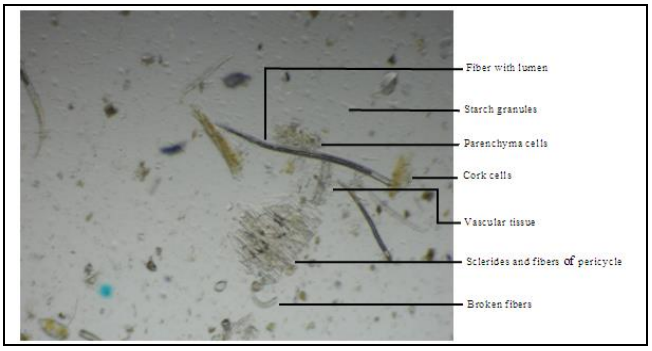

Figure 5: Powder characteristics of stems of *Oxalis corniculata*

Fluorescence Analysis

The fluorescence analysis of leaves, stems and fruits powders were performed with and without mixing some reagents. The analysis was done under ordinary light and UV light. The results of fluorescence analysis powdered leaves, stems and fruits are shown in Table I. The results of the table showed variation in colors that might be used to identify original herbal products and discriminate adulterated / substandard herbal products.

Table I: Fluorescence analysis of leaves, stems and fruits powders of *Oxalis corniculata*

Treatment	Leaves		Stem		Fruit	
	Day light	**UV light**	**Day light**	**UV light**	**Day light**	**UV light**
Powder as such	Dark green	Green	Brown	Green	Green	Light brown
Powder + 1N NaOH (Aq)	Green	Dark green	Brownish green	Light green	Brownish green	Dark green
Powder + 1N NaOH (MeOH)	Dark green	Pink	Light brown	Light pink	Light green	Green
Powder + 1N HCl (Aq)	Green	Dark brown	Brown	Light brown	Light brown	Brown
Powder + 50% H_2SO_4	Green	Green	Light brown	Brown	Light green	Brown
Powder + 50% HNO_3	Green	Light green	Reddish brown	Dark green	Light brown	Dark green

Aq (Aqueous); MeOH (Methanol)

Physicochemical studies

The results of physicochemical properties like moisture content, ash values and total extractives of crude powder of leaves and stems are mentioned in Table II. The moisture content plays an important role in stability of crude drug. The moisture content should be minimized to prevent drug's degradation and microbial growth. The observed moisture content values of leaves and stems are 5.5% and 6.0% respectively which shows less quantity of moisture in the crude powder.

The ash values are helpful to determine quality and purity of crude drugs. The objective of ash test is to remove all traces of organic matter which may interference analysis. On incineration, crude drugs leave an ash comprising carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium. The total ash of crude drug reflects the care taken in its

preparation. If crude drug has high concentration of silica or calcium oxalate then acid insoluble ash test is performed. Some analysts prefer mixing of sulphuric acid with powdered crude drug before ashing because sulphated ash is normally less fusible than ordinary ash (Brain and Turner, 1975). The observed total ash, acid insoluble, acid soluble and sulphated values were in normal limits.

The extractive values are helpful to determine quantity of active constituents that can be extracted by using suitable solvent in plant materials. The criteria to choose suitable solvent depend upon the extractive potential of a solvent. The observed extractive values showed that ethanol had more extractive potential than that of water.

Table II: Physicochemical analysis of powdered leaves and stems of *Oxalis corniculata*

Sr. No.	Physicochemical properties	Leaves % w/w	Stem % w/w
1	Moisture contents	5.5	6.0
2	Total ash	4.5	14.5
3	Acid insoluble ash	4.0	13.5
4	Acid soluble ash	0.5	1.0
5	Sulphated ash	14.1	24.35
6	Water soluble extractives	7.14	3.68
7	Ethanol soluble extractives	8.26	4.92

FTIR Spectroscopy of crude powders

IR spectra of crude powders of different parts of the plant belonging to different batches were used to evaluate the qualitative difference, if any. IR spectra of leaf powder indicated high density bands at 3435-3400 cm^{-1} (amide, alcohol), 2918 cm^{-1} (alkane, aldehyde), 1654–1651 cm^{-1} (aromatic amine, alkene, amide) and 1052-1049 cm^{-1} (andydride, ether, halogen compounds). IR spectra of stem powder indicated high density bands at 3399-3389 cm^{-1} (alcohol), 2918 cm^{-1} (alkane, aldehyde), 1650–1637 cm^{-1} (aromatic amine, alkene, amide), 1324-1322 cm^{-1} (alkene, alcohol) and 1035-1034 cm^{-1} (ether, halogen compounds).

IR spectra of fruit powder indicated high density bands at 3401-3393 cm^{-1} (amide, alcohol), 2918 cm^{-1} (alkane, aldehyde), 1654–1648 cm^{-1} (aromatic amine, alkene, amide) and 1054-1052 cm^{-1} (anhydride, ether, halogen compounds). Similarly, the IR spectra of leaf, stem and fruit were compared to know the difference in chemical constituents. The overlay spectrum of three parts of the plant is shown in Figure 6. All the three parts showed bands at 3435–3400 cm^{-1}, 2918 cm^{-1}, 1654–1651 cm^{-1} and 1052–1049 cm^{-1} are similar. However, the fingerprint region of the spectrum indicated difference in chemical constituents.

Phytochemical contents of crude powders

The results of total contents of lipids, proteins, carbohydrates and oxalates are mentioned in Table III the contents of total lipids were determined by gravimetric method which verified the results of Unni *et al.* (2009). The total proteins contents were determined by Spectrophotometry method. The contents were calculated by standard curve; attained from bovine serum albumin (0.1 mg/mL) solution in concentrations of 0.0075, 0.015, 0.03, 0.06 and 0.09 mg/mL. The linear regression equation was found to be Y = 0.3035x + 0.0005 with R^2 0.9826. The observed contents verified the results of Unni *et al.* (2009). The total carbohydrates contents were calculated by subtracting mean values of moisture contents, total ash, lipids and proteins from hundred.

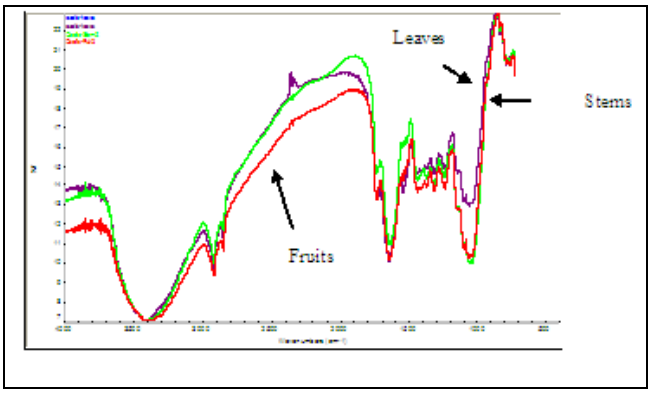

Figure 6: Overlay FTIR spectra of leaves, stems and fruits powders of *Oxalis corniculata*

The contents of total oxalates were also determined by gravimetric method. The powder treated with 10% $FeCl_3$ and 10% $CaCl_2$ solutions showed no significant reduction in oxalate contents. The aqueous extract also treated with 10, 20 and 30% $FeCl_3$ and $CaCl_2$ solutions and no precipitation of oxalates occurred. Results showed that contents of oxalates are in minor quantity and $FeCl_3$ and $CaCl_2$ did not make precipitation with oxalates.

Extraction

Different extracts of leaves obtained by sequential extraction using solvents in the order of increasing polarities. The observed extractive values showed that methanol has more extractive potential than n-hexane and chloroform. The percentage yield of leaves and stem extracts are mentioned in Table IV. The extracts color and consistency as mentioned in Table V might be used to identify and discriminate adulterated / substandard extracts.

Table III: Phytochemical contents of crude powder of leaves and stems of *Oxalis corniculata*

Sr. No.	Phytochemical contents	Leaves % w/w	Stem % w/w
1	Total lipids	6.45	5.01
2	Total proteins	18.00	15.00
3	Total carbohydrates	65.55	59.49
4	Total oxalates (aqueous extract)	0.00012	0.00001
5	Total oxalates (ethanolic extract)	0.00030	0.00028
6	Total oxalates (10% $FeCl_3$)	0.00010	-
7	Total oxalates (10% $CaCl_2$)	0.00075	-

Phytochemical analysis of extracts

The qualitative chemical analysis of aqueous, ethanol, n-hexane, chloroform and methanol extracts of leaves and stems were performed to determine various chemical constituents including sterols, triterpenoids, glycosides, flavonoids, saponins, carbohydrate, alkaloids, tannins and proteins. The results mentioned in Table VI and VII showed the presence of sterols, flavonoids, saponins, carbohydrates, alkaloids and proteins. The presence of these compounds verified earlier phytochemical studies (Raghvendra *et al.,* 2006; Unni *et al.* 2009; Pal, 2000).

Table IV: Percentage yield of extracts of leaves and stems of *Oxalis corniculata*

Sr. No.	Extracts	Leaves % w/w	Stem % w/w
1.	n- hexane	9.18	2.45
2.	Chloroform	2.43	0.80
3.	Methanol	18.03	10.42

Table V: Color and consistency *Oxalis corniculata* leaves and stem extracts

Extracts	Leaves		Stem	
	Color	Consistency	Color	Consistency
n-hexane	Blackish green	Waxy	Brownish black	Semi solid
Chloroform	Black	Solid	Black	Solid
Methanol	Dark black	Oily	Dark black	Oily

The pharmacological activities of this plant are only due to presence of these phytochemical constituents. Different phytochemical constituents have different therapeutic indications. The presence of sterols, flavonoids, saponins, carbohydrates, alkaloids and proteins indicates the importance and usefulness of this plant.

UV/VIS Spectroscopy of extracts

UV/Visible spectroscopy was performed to analyze and compare phytochemical constituents of n-hexane, chloroform and methanol extracts of leaves and stems. The extracts exhibited maximum absorption at 236 – 238 nm. Such scans were used as fingerprints to compare the extracts qualitatively.

Table VI: Phytochemical analysis of leaves extracts of *Oxalis corniculata*

Phytochemical tests	Leaves extracts				
	aqueous	ethanol	n-hexane	chloroform	methanol
Tests for sterols	-	+	+	+	+
Tests for triterpenoids	-	-	-	-	-
Tests for glycosides	-	-	-	-	-
Tests for flavonoids	+	+	-	-	+
Tests for saponins	+	-	-	-	-
Test for carbohydrates	+	+	+	+	+
Tests for alkaloids	+	+	+	+	+
Tests for tannins	-	-	-	-	-
Tests for proteins	-	-	-	-	+

+ (present) ; - (absent)

The scan of overlay of n-hexane, chloroform and methanol extracts of leaves and stem is given in Figure 7 and 8. As it is obvious from the scan, n-hexane and chloroform extracts were having similar profiles, whereas the chemical profile of methanol extract was entirely different than of the two other extracts. Thus study indicated that the activity of methanol extract will be different than that of the n-hexane and chloroform extracts. methanol leaves and stems will be having similar pharmacological activity.

Table VII: Phytochemical analysis of stem extracts of *Oxalis corniculata*

Phytochemical tests	Stem extracts				
	aqueous	ethanol	n-hexane	chloroform	methanol
Tests for sterols	+	+	+	+	+
Tests for triterpenoids	-	-	-	-	-
Tests for glycosides	-	-	-	-	-
Tests for flavonoids	+	+	-	-	+
Tests for saponins	+	-	-	-	+
Test for carbohydrates	+	+	+	+	+
Tests for alkaloids	+	+	-	-	-
Tests for tannins	-	-	-	-	-
Tests for proteins	-	-	-	-	+

+ (present) ; - (absent)

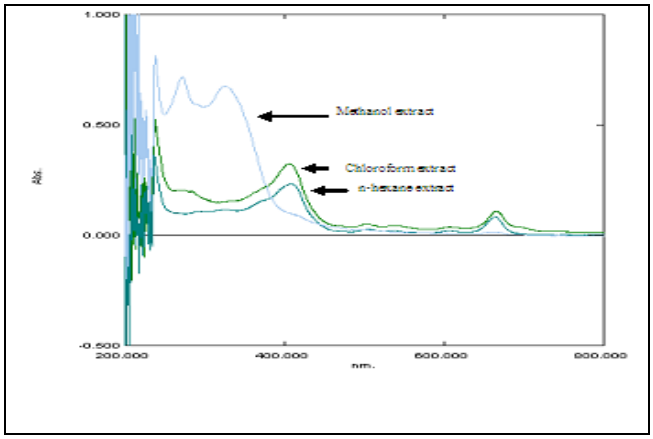

Figure 7: Overlay UV/VIS spectra of extracts of leaves of *Oxalis corniculata*

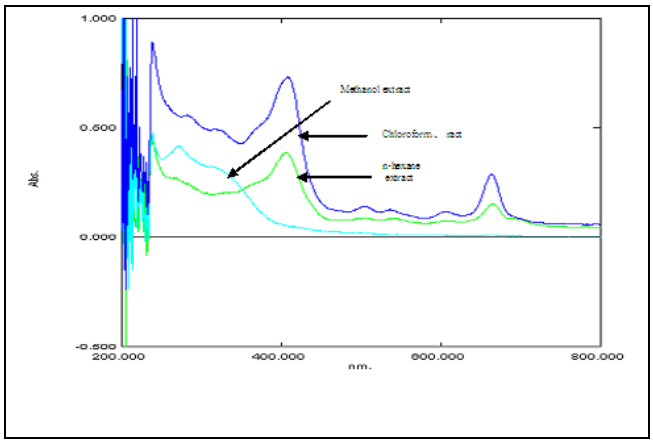

Figure 8: Overlay UV/VIS spectra of extracts of stems of Oxalis corniculata

The overlay scan of n-hexane leaves and stems, chloroform leaves and stems and methanol leaves and stems extracts having almost similar profile. Therefore, we can predict that the n-hexane, chloroform and methanol extracts of leaves and stem will be having similar pharmacological activity.

Estimation of primary and secondary metabolites

The primary metabolites (proteins and carbohydrates) and secondary metabolites (polyphenols, flavonoids and glycosaponins) were estimated quantitatively by spectrophotometry. The methanolic extract of leaves and stems showing higher *in vitro* antioxidant activity was analyzed to determine total contents of proteins, carbohydrates, polyphenols, flavonoids and glycosaponins.

The results mentioned in Table VIII show the contents (mg/g) of such metabolites. The results showed that contents (mg/g) of all the metabolites were higher in leaves than stems. The contents in the extract were in the order as proteins › flavonoids › carbohydrates › polyphenols › glycosaponins.

Table VIII: Estimation of primary and secondary metabolites of methanolic extract of leaves and stems of *Oxalis corniculata*

Sr. No.	Metabolites	Percentage contents	
		Leave extract (mg/g)	Stem extract (mg/g)
1.	Proteins	168.00	76.00
2.	Carbohydrates	74.70	67.05
3.	Polyphenols	27.50	23.00
4.	Flavonoids	153.50	85.50
5.	Glycosaponins	27.40	65.20

The plant produces secondary metabolites from primary metabolites to regulate biological function and protect itself from diseases. The secondary metabolites can be used as therapeutic agents. These compounds in the same plant vary depending upon the age, climate, growing conditions, harvesting methods and storage conditions. Due to this reason, it is very hard to maintain the chemical composition of natural products that we produce from raw material of different sources. Therefore, chemical estimation of total primary and secondary metabolites can provide an easy tool to the comparison of extracts.

CONCLUSION

The local plant, *Oxalis corniculata* has been investigated for a number of studies. The pharmacognostic evaluation will be helpful for the identification of plant. Such studies will also be helpful to maintain batch to batch reproducibility of herbal drugs prepared from the plant. The results of physicochemical analysis are within the specified limits. FTIR fingerprints of crude powders of leaves, stems and fruits are found to be similar indicating similarity of chemical constituents.

The qualitative analysis of extracts indicated the presence of sterols, flavonoids, saponins, carbohydrates, alkaloids and proteins. The thin layer chromatography of extract also confirmed the presence of flavonoids and alkaloids. The UV spectra of methanol extract were found to be different than that of the n-hexane and chloroform extracts.

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