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Scientific Validation of HPTLC Method for Quantitative Estimation of Alkaloid and Flavonoids from *Oxalis corniculata*

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Authors' contributions

This work was carried out in collaboration among all authors. Author Ranjani designed the study, performed the statistical analysis, wrote the protocol and wrote the draft of the manuscript. Authors Patharaj and Kannan managed the analyses of the study. Author Kannan managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: *Oxalis corniculata* Linn. belongs to Oxalidaceae family. It is an herbaceous plant abundant in mountain regions of India. It is one of the most adaptable and popular medicinal plants having a broad spectrum of biological activity.
Objectives: The aim of the work is to ascertain the level of an antioxidant properties of different solvent extracts of *Oxalis corniculata* plant.
Materials and Methods: A precise and accurate high-performance thin-layer chromatography method for quantitative estimation of alkaloids and flavonoids from leaf ethanol extract of *Oxalis corniculata* was developed. The method employed high-performance thin-layer chromatography aluminum plate, silica coated with fluorescent indicator F_{254} (10x10 cm) as stationary phase and the separation was achieved by using a suitable mobile phase n-Butanol - Glacial acetic acid-water (3:-1:-1), ethyl acetate-butanone-formic acid-water (5:3:1:1) and Dragendorff's reagent followed by 10% ethanolic sulphuric acid (for alkaloids) 1% ethanolic Aluminium chloride reagent (for flavonoids) were used for detection. The Peak table, Peak display and Peak densitogram were noted by using software winCATS 1.3.4 version.

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Results: Among the phytoconstitution, alkaloids and flavonoids found more abundant. Yellow, Yellowish blue fluorescent colour zone at UV 366 nm mode were there in the tracks, it was observed from the chromatogram after derivatization, which confirmed the presence of flavonoid. ABTS assay, the maximum scavenging activity was seen at a concentration of 1000 µg/ml and lowest 2 µg/ml.
Conclusion: The extracts of the aerial parts of *oxalis corniculata* are the source of natural antioxidants which can be accounted for the traditional uses in prevention of disease and health preservation.

Keywords: Oxalis corniculata; phytochemical; HPTLC; alkaloids; flavonoids; therapeutic treatment; hepatitis.

1. INTRODUCTION

Plants are the sources of many traditional medicine systems in the world for thousands of years, providing the mankind with medicine. Moreover these plants are also used in cosmetic, agricultural and food industry [1]. The most economically important bioactive constituents are alkaloids, tannins, flavonoids and phenolic compounds [2]. Phytochemical studies have gained a lot of interest among the plant scientists due to the improvement of the technology and its sophisticated techniques. These techniques play a considerable role in finding important material for pharmaceutical industry [3]. It is estimated that 14-18% of higher plant are used medicinally and related to 74% of pharmacologically active plant are discovered after following up on ethnomedicinal usage of the plants [4].

The genus Oxalis, which belongs to the wood-sorrel family Oxalidaceae, is a cosmopolitan genus comprising at least 500 species, most of them are native in South America and Southern Africa [5]. *Oxalis corniculata* has been reported highlighting its diverse ethno medicinal applications like anti inflammatory, digestive, diuretic, antibacterial, antiseptic etc [6].

Among the medicinal plants reported, corniculata have wide medicinal important used for curing of anthelmintic, styptic, astringent, diarrhea, dysentery, dysmenorrhoea, hepatitis, amenorrhoea and burning sensation, antimicrobial activity [7,8]. Recent studies reported to posses the antitumor activity, antiepileptic and anxiolytic activities [9].

2. MATERIALS AND METHODS

2.1 Preparation of Plant Extract

The plant, *Oxalis corniculata* (Ver. Puliyarai) was collected from Thirumoorthi hills of Thirupur, Tamil Nadu, India during the month of January 2019. The plant species is authendicated from Botanical Survey of India (BSI) Southern region Coimbatore, Tamil Nadu, India (Voucher No BSI/SRC/01/23/2019/3293).

The plant material were washed, shade dried and powdered using mixer grinder. The powdered material (10 g) was extracted with 100 ml of selected organic solvents (aqueous, chloroform, ethyl acetate, methanol and ethanol) using soxhlet apparatus (Six hours in each solvent) and filtered through Whatmann No. 1 filter paper. The filtrate was concentrated and dried under reduced pressure and controlled temperature. The concentrated extracts of the leaves were stored in small vials at -20º C and used for further analysis.

2.1.1 Qualitative phytochemical analysis of the plant extract

The phytochemical screening ethanol and methanol extracts of the plant sample was subjected to different chemical tests for the detection of different phytoconstituents using standard procedures [10,11]. To identify the presence of alkaloids, flavonoids, tannins, saponins, steroids, terpenoids, glycosides, phenols, carbohydrates and proteins.

2.2 HPTLC Analysis of Plant Sample for Alkaloid and Flavonoid Profile

2.2.1 Samples given

Test solution preparation: The given plant samples 20 mg each weighed accurately in an electronic balance (Afcoset), dissolved in 100µl of the respective solvent (Ethanol) and centrifuged at 3000rpm for 5min. These solutions were used as test solution for HPTLC analysis.

Sample application: 5 µl of test solutions and 2 µl of standard solution were loaded as 5mm band

length in the 3 x 10 Silica gel $60F_{254}$ TLC plate using HAMILTON SYRINGE and CAMAG LINOMAT-5 (swiz) instrument.

Spot development: The samples loaded plate was kept in TLC twin trough developing chamber (after saturated with Solvent vapor) with respective mobile phase (Alkaloid) and the plate was developed in the individual mobile phase up to 90 mm.

Photo-documentation: The developed plate was dried by hot air to evaporate solvents from the plate. The plate kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at visible light, UV 254nm and UV366nm.

Derivatization The developed plate was sprayed with respective spray reagent (Alkaloid) and dried at 100°C in Hot air oven. The plate was photo-documented in Visible light and UV 366nm mode using Photo-documentation (CAMAG REPROSTAR 3) chamber.

Scanning: After derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at 500nm. The Peak table, Peak display and Peak densitogram were noted. The software used was winCATS 1.3.4. version.

2.2.2 Analysis details for alkaloid

Mobile phase: n-Butanol - Glacial acetic acid-water (3: 1: 1)

Spray reagent: Dragendroff reagent (purchased from Siscoresearch laboratories Pvt. Ltd Noida) followed by 10% Ethanolic sulphuric acid reagent.

Detection: Orange-brown coloured zone at Day light mode 1 was present in the standard track, it was observed from the chromatogram after derivatization, which confirmed the Presence of Alkaloid.

2.2.3 Analysis details for flavonoid

Mobile phase: Ethyl acetate-Butanone-Formic acid-Water (5:3:1:1)

Spray reagent: 1% Ethanolic Aluminium chloride reagent.

Detection: Yellow, Yellowish blue coloured fluorescent zone at UV 366 nm mode were present in the tracks, it was observed from the chromatogram after derivatization, which confirmed the Presence of Flavonoid in the given standard and may be present in the sample.

2.3 Antioxidant Activity Preparation of Stock Solution

Oxalis corniculata leaf stock solutions were prepared in methanol at a concentration of 1000 µg/ml (1 mg/ml). From the stock solution various concentrations *viz* 2, 4, 8, 16, 32, 64, 128, 256, 512 and 1000 µg/ml were prepared in methanol and used for antioxidant studies.

DPPH assay: The free radical scavenging activity of the leaf extract was measured using 1,1-diphenyl-2- picryl-hydrazyl (DPPH) as described [12]. Briefly, to 1ml of different concentrations of methanolic leaf extract, 1ml of DPPH 0.1 mM (millimolar = 10^{-3}mol/L) was added. The mixture was mixed and left to stand for 30 min in the dark and the absorbance was recorded at 517 nm. An equal amount of DPPH and Methanol served as control. The experiment was done in triplicate. Ascorbic acid was used as standard control. The percentage scavenging was calculated using the following formula, DPPH Scavenging effect (%) = (Acontrol-Asample) x 100

ABTS radical scavenging: The ABTS radical scavenging assay of leaf extract was carried out using the standard protocol [13]. with slight modifications. Stock solutions of ABTS (7 mM) and potassium persulfate (140 mM) in water were prepared, and mixed together to a final concentration of 2.45 mM potassium persulfate. This mixture was left to react overnight (12-14 hrs) in the dark, at room temperature. The solution was diluted with methanol to an absorbance of 0.70 ± 0.02 at 734 nm. To 20µl of the various concentrations (2-1000 µg/ml) of test compound, 2.0 ml of diluted ABTS solution was added and the absorbance taken after 6 min. For the control, methanol was used instead of the test compound. Ascorbic acid was used as standard.

3. RESULTS

3.1 Phytochemical Constituents

Various phytochemicals were screened for their presence of alkaloid, flavonoids, saponins, tannins, phenols, terpenoids, steroids, carbohydrates glycosides amino acids and

proteins. Our investigation, phytochemical constituents of dried leaf extract of *Oxalis corniculata* revealed the presence of major phytoconstitution such as alkaloid, tannin, phenol, steroid and flavonoids. Saponin was not detected in methanol and chloroform. Among the phytoconstitution alkaloid and flavonoids found more abundant in *Oxalis corniculata* (Table 1).

Detection of alkaloid: Orange-brown coloured zone at Day light mode 1 was present in the standard track, it was observed from the chromatogram after derivatization, which confirmed the Presence of Alkaloid in the given standard and brownish violet coloured zone at Day light mode 2 was present in the standard track, it was observed from the chromatogram after 10% ethanolic sulphuric acid reagent derivatization, which may be the Presence of Alkaloid/Nitrogen containing compounds in the given sample (Table 2).

Detection of flavonoid: Yellow, Yellowish blue coloured fluorescent zone at UV 366nm mode were present in the tracks, it was observed from the chromatogram after derivatization, which confirmed the Presence of Flavonoid in the given standard and may be present in the sample (Table 3).

Table 1. Qualitative analysis of phytoconstituents present in different solvent extract of *Oxalis corniculata*

Phytochemicals	Aqueous	Ethanol	Methanol	Ethyl acetate	Chloroform
Alkaloids	++	++	+	+++	+
Phenols	+	++	+	+	+++
Flavonoids	+	+++	++	++	+
Tannins	++	+	+	++	+
Saponins	+	+	-	+	-
Terpenoids	+	+	-	_	+
Steroids	+	++	+	++	+
Carbohydrates	++	++	+	+	+
Glycosides	+	-	-	+	+
Proteins	++	+	+	+	+
Coumarone	-	-	-	-	-

+ present in small concentration; ++ present in moderately high concentration; +++ present in very high concentration; - absent

Table 2. Peak table showing presence of alkaloid in *Oxalis corniculata*

Track	Peak	Rf	Height	Area	Assigned substance
Sample I	1	0.01	10.8	110.3	Unknown
Sample I	2	0.1	13.6	290.7	**Alkaloid**
Sample I	3	0.23	50.8	684.5	Unknown
Sample I	4	0.32	61.3	1432.3	**Alkaloid**
Sample I	5	0.56	122.8	2133.9	Unknown
Sample I	6	0.96	412.3	18592.1	Unknown
Sample II	1	0.09	52.3	1967.3	Unknown
Sample II	2	0.17	79.2	2349	Unknown
Sample II	3	0.66	157.2	2292.9	Unknown
Sample II	4	0.73	172.8	3533.1	Unknown
Sample II	5	0.96	424.2	13369.9	Unknown
STD	1	0.29	128.3	2844.4	Alkaloid standard

Table 3. Peak table showing presence of flavonoids in *Oxalis corniculata*

Track	Peak	Rf	Height	Area	Assigned substance
Sample I	1	0.54	93.2	4218.7	Flavonoid
Sample II	1	0.39	18.7	639.7	Unknown
STD	1	0.62	483.9	22949.6	Flavonoid standard

Table 4. DPPH Scavenging activity of the ethanol extract of *O. corniculata*

		EC_{50} (µg mL^{-1})[a]	
S. NO	**Concentration (µg/ml)**	***O. corniculata***	**(standard) Vitamin C**
1	02	4.60 ± 0.68	26.3 ± 1.06
2	04	8.0 ± 0.25	37.95 ± 0.65
3	08	15.40 ± 0.45	41.25 ± 0.36
4	16	19.65 ± 0.15	80.3 ± 0.54
5	32	31.20 ± 1.25	85.9 ± 0.20
6	64	36.40 ± 0.45	88.0± 1.02
7	128	50.75 ± 1.05	86.6± 1.26
8	256	71.6± 0.36	86.0± 1.60
9	512	60.09± 0.24	84.46 ± 1.62
10	1000	61 ± 1.30	85.32 ± 0.82

Table 5. ABTS radical scavenging activity of the ethanol extract of *Oxalis corniculata*

		EC_{50} (µg mL^{-1})[a]	
S. NO	**Concentration (µg/ml)**	***O. corniculata***	**(standard)Vitamin C**
1	02	9.60 ± 0.67	25.3 ± 1.09
2	04	9.0 ± 0.26	37.95 ± 0.75
3	08	15.90 ± 095	40.25 ± 0.66
4	16	19.95 ± 0.25	49.3 ± 0.54
5	32	38.20 ± 1.35	60.9 ± 0.26
6	64	40.40 ± 0.40	78.90 ± 1.05
7	128	52.75 ± 1.09	79.6 ± 1.26
8	256	71.6 ± 0.28	75.0 ± 1.68
9	512	55.09 ± 0.64	70.46 ± 1.42
10	1000	68.7 ± 0.88	69.32 ± 0.09

3.2 Antioxidant Activity

The free radical scavenging activity of *Oxalis corniculata* was studied by its ability to reduce the DPPH, a stable free radical. DPPH scavenging activity of methanolic leaf extract of *Oxalis corniculata* was ranging from 4.60 ± 0.68% to 71.6 ± 0.36%. The highest scavenging activity was found at a concentration 256 µg/ml and the lowest was found at a concentration of 2 µg/ml (Table 4).

In the ABTS assay, the maximum scavenging activity was seen at a concentration of 1000 µg/ml and 2 µg/ml. The scavenging activity of *O. corniculata* was ranging from 9.1 ± 0.56% to 68.9 ± 1.76%. This wide range of antioxidant activity may be attributed to the wide variety of bioactive compounds like phenolics, flavonoids, tannins etc present in the plant. In both the antioxidant assays the extract showed efficient activity as compared to standard ascorbic acid (Table 5).

4. DISCUSSION

Phytochemical constituents of dried leaf extract of *Oxalis corniculata* revealed the presence of major phytoconstitution such as alkaloid, tannin, phenol, steroids and flavonoids. Among the phytoconstitution alkaloid and flavonoids found more abundant in *Oxalis corniculata.* It is clear that *Oxalis corniculata,* leaves in ethanol and ethyl acetate showed the significant presence of phenols, tannins, saponins, and Alkaloid. Flavonoids found more abundant in ethanol while phenol and alkaloid found more in chloroform and ethyl acetate respectively.

DPPH Scavenging activity of the ethanol extract of *O. corniculata,* The highest scavenging activity was found at a concentration of 256 µg/ml and the lowest was found at a concentration of 02 µg/ml. In the ABTS assay, the maximum scavenging activity was seen at a concentration of 256 µg/ml and lowest 02 µg/ml. This ample variety of antioxidant activity may be accredited to the wide variety of biological active compounds like phenolics, flavonoids, tannins etc present in the plant. In both the antioxidant assay the extract showed efficient activities as compared to standard ascorbic acid.

The previous study revealed that ethanolic extract of *Oxalis corniculata* at different doses

level showed significant antioxidant activity in mice [14]. Methanolic extract of *Oxalis corniculata* showed potent antioxidant activity compare to reference standard ascorbic acid. The concentration of plant extract required for 50% inhibition of DPPH radical scavenging effect (IC50) were recorded as 30 mg/ml and 37 mg/ml for Methanol extract of *Oxalis corniculata and* standard ascorbic acid. These results suggest that the *Oxalis corniculata* possess antioxidant activity compared to ascorbic acid [15].

The DPPH radical shows maximum absorbance at 517 nm. In the present study, the ethanolic leaf extract fraction exhibited the highest capability to scavenge free radicals. This is in agreement with the data reported by [16]. The high free radical scavenging activity of ethanolic leaf extract fraction may be associated to their high phenolic contents. This is in consonance with findings of other workers as well [17,18].

5. CONCLUSION

The results of different studies were indicated that the methanolic extract of leaves of *Oxalis corniculata* can be used as a medicinal supplement in pharmaceutical industry for treatment of various diseases. It is easily accessible source of natural antioxidant.

The results of the current study showed that the extracts of the aerial parts of *oxalis corniculata* which contains the highest concentration of polyphenols exhibits the greatest antioxidant activity through the scavenging of free radicals such as DPPH radical and ABTS radical scavenging.

SIGNIFICANCE STATEMENT

This study discovers the possible synergistic effect of polyphenols exhibits the greatest antioxidant activity. This wide range of antioxidant activity may be attributed to the wide variety of bioactive compounds like alkaloids, phenolics, flavonoids, tannins etc, present in the plant. These bioactive compounds can be used as a medicinal supplement in pharmaceutical industry for treatment of various human ailments. More studies should be carried out on this interesting plant, with a view to achieving more comprehensive and in- depth analysis of its pharmacological and therapeutic perspective.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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