Research Article EMUJPharmSci ISSN 2651-3587 https://dergipark.org.tr/emujpharmsci

EMU Journal of Pharmaceutical Sciences

EMUJPharmSci

Study of *in vitro* immunomodulatory effect and quantitative evaluation of main phytoconstituents in Indian *Drosera* species

Raju Asirvatham*, Aparna Ann Mathew, Salwa Abdul Salam

St. Joseph's College of Pharmacy, Department of Pharmacology, Kerala, India.

Abstract

The present study compared the total flavonoid content in ethanol and aqueous extracts of Indian Drosera and their *in vitro* immunomodulatory activities. None of the medicinally important compounds from this species has been quantified and compared previously. HPTLC method was used to quantify plumbagin and quercetin content in the ethanol extracts of three Indian *Drosera* species.

The *in-vitro* immunomodulatory activities of both ethanol and aqueous extracts were evaluated by the inhibition of heat and hypotonic induced hemolysis, nitroblue tetrazolium (NBT) assay and by inhibition of TNF-α release in DAL cell lines.

Ethanol extract of *D. burmannii* showed significant inhibition of heat and hypotonic induced hemolysis when compared with diclofenac. In NBT reduction test, ethanol extract of *D. burmannii* showed significantly higher reduction than *D. indica* and *D. peltata*. Inhibition of TNF-α release was significantly enhanced by 400 µg/mL of *D. burmannii.* Higher concentration of flavonoid was found in the ethanol extract of *D. burmannii*. Flavonoid concentration was the least in aqueous extract of *D. indica.* The calibration curve of plumbagin and quercetin were found to be linear (200 -1000 ng/spot). Correlation coefficient of r = 0.9994 ± 8.62 and r= 0.99068 ± 13.63 was detected for plumbagin and quercetin, respectively. This is indicative of good linearity between concentration and peak area.

The identity of the plumbagin and quercetin band in the sample extracts was confirmed by comparing the UV absorption spectrum of the sample to that of the reference standard plumbagin and quercetin, using the Camag TLC scanner. Higher concentrations of plumbagin and quercetin were found in the ethanol extract of *D. burmannii*. The proposed HPTLC method provided good resolution and accuracy, and can be practiced for the rapid determination of plumbagin and quercetin in the herbal drugs. Such an approach is effective for routine quality control analysis and quantification of plumbagin. The pharmacological actions of the species investiagted are due to its chief chemical constituents.

Keywords

High-performance thin liquid chromatography, immunomodulatory, Indian *Drosera*, plumbagin, quercetin, quantification.

Article History

Submitted: 18 February 2020 Accepted: 01 March 2020 Published Online: March 2020

Article Info

***Corresponding author:** Raju Asirvatham **e-mail:** rajuasirvatham@gmail.com

Research Article:

Volume: 3 Issue: 1 March 2020 Pages: 50-63

INTRODUCTION

Drosera plants belonging to family Droseraceae are commonly known as sundew plants. *Drosera* consists of approximately 170 species (Jayaram and Prasad 2006). Three species of *Drosera* are found in India, viz., *D. burmannii* Vahl, *D. indica* L., and *D. peltata* J.E.Sm (Santapau and Henry 1976). The genus *Drosera* contains naphthoquinones such as plumbagin,7-methyljuglone and flavonoids with various important pharmacological activities. Plumbagin is used for curing bronchial infection, whooping cough, hyperglycaemia, hypolipidaemia, tuberculosis, spasms, cancer, fertility problems, arteriosclerosis, phthisis, asthma; leprosy, leishmaniasis and malaria. Moreover, it acts as immunomodulator, cosmetics, aphrodisiac, chitin synthetase inhibitor, insecticide, antifeedant and abortifacient. It enhances phagocytic activity of human granulocytes *in-vitro* and inhibits the development of insect and parasitic nematodes. 7- Methyljuglone is shown to be inhibitory to several insects and is highly toxic to fungal pathogens (Raju *et al.*, 2012, Yu He *et al.*, 2012). Literature review fails to claim the study of immunomodulatory effects (*in-vitro*) of these Indian *Drosera* species. Therefore, in this study it was aimed to evaluate the immunomodulatory potency of the three Indian *Drosera* species and quantify the variation in the amount of plumbagin and quercetin among different species. High Performance Thin Layer Chromatography (HPTLC) is an efficient tool for phytochemical evaluation of herbal drugs. However, no HPTLC method has been developed for the quantification of plumbagin and quercetin in the ethanol extracts of *D. burmannii*, *D. indica* and *D. peltata*. The HPTLC method proposed in the study is simple, precise and sensitive.

MATERIALS AND METHODS

Plant material

The whole plants of *D. burmannii* and *D. indica* L. were collected during December 2010, from the forests of Savanadurga, Karnataka, India. The plant material was identified and authenticated by Dr. S.N. Yoganarasimhan, a taxonomist and research coordinator at M. S. Ramaiah College of Pharmacy, Bangalore, Karnataka, India. The whole plant of *D. peltata* was collected from the hills of Munnar, Kerala, India. It was identified and authenticated by Dr. Madava Chetty, Asst. Professor, Department of Botany, Sri Venkateshwara University, Tirupathi. All the voucher specimens have been

deposited at the Department of Pharmacognosy, Shri Rawatpura Sarkar Institute of Pharmacy, Datia, (M.P) (Herbarium No :SRIP/COGNOSY/2011-04,05&06). Plant materials were washed, shade dried, powdered, passed through sieve no. 60 and stored in air tight containers for further experiments.

Preparation of the extracts

Shade dried, powdered samples were subjected to successive solvent extraction with petroleum ether (60-80 °C), followed by chloroform and ethanol (90 % *v/v*) in a Soxhlet extractor. The extract was concentrated in a rotary flash evaporator at a temperature not exceeding 50 ºC. The ethanol extracts were suspended in distilled water for experimental purpose.

Determination of flavonoid content

The content of flavonoids, expressed as flavones and flavonols, was quantified from the ethanol and aqueous extracts of *D. burmannii* Vahl, *D. indica* L., and *D. peltata* J.E.Sm by colorimetric method using aluminum chloride ($AlCl_3$) (Bag GC *et al.*, 2015).

Preparation of calibration curve

100 µg/mL quercetin solution was prepared in methanol as stock solution and then diluted to get final concentrations of 2, 5, 10, 15 and 20 µg/mL. To 0.5 mL of these diluted solution, 1.5 mL of methanol, 0.1 mL 1 M of potassium acetate (CH_3COOK), 2.8 mL of H_2O and 0.1 mL of $AlCl_3$ (10% in distilled water) were added. The final reaction mixtures were thoroughly mixed and kept for 30 minutes at 32ºC. After incubation, the absorbance was read at 415 nm using a UV spectrophotometer. 0.1 mL of distilled water instead of $AlCl_3$ was used as blank. Standard calibration curve for quercetin was graphed by various concentrations at X axis and absorbance at Y axis.

Sample preparation

10 mg of each ethanol and aqueous extracts of *D. burmannii*, *D. indica* and *D. peltata* were dissolved in 2.5 mL of methanol using vortex agitator. The extracts were filtered to remove the solid residue. The final volume was adjusted to 5 mL with methanol.

Procedure

An aliquot of 0.5 mL of each extract (concentration from 1 to 10 mg/mL) was added to1.5 mL of methanol, 0.1 mL 1 M of CH_3COOK, 2.8 mL of H_2O and 0.1 mL of 10% $AlCl_3$. Later, the absorbance was read at 415 nm. Total flavonoid content in the extracts was computed from the quercetin standard graph and the quantity was expressed in mg/g quercetin equivalent (QE) or % w/w of the extractives.

Study of preliminary immunomodulatory properties

The preliminary immunomodulatory properties of ethanol extracts of all Indian species were evaluated by the procedure described by Suresh Kumar *et al*. (2017).

Heat induced hemolysis

The assay mixture contains 1 mL phosphate buffer (pH 7.4, 0.15 M), 2 mL hyposaline (0.36 %), 0.5 mL human red blood cell (HRBC) suspension (10 % v/v) with 0.5 mL of all extracts and standard drug diclofenac sodium (200 µg/mL) at various concentrations (50, 100, 200, 400, 800, 1000 µg/mL). Distilled water instead of hyposaline that produces 100 % hemolysis was used as control. The assay was carried out in triplicate manner. The mixtures were incubated at 40 ^{o}C for 30 minutes and centrifuged. The hemoglobin content in the suspension was estimated using spectrophotometer at 560 nm.

The percentage of hemolysis was calculated as follows:

% Hemolysis = (Optical density of test sample / Optical density of control) X 100

The percentage of HRBC membrane stabilization can be calculated as follows:

% Protection = 100 – [(Optical density of test sample / Optical density of control) X 100].

Hypotonic induced hemolysis

Samples of the extracts were dissolved in hypotonic solution (0.2% sodium chloride). The hypotonic solutions (5 mL) of graded doses of the extracts (50, 100, 200, 400, 600, 800 and 1000 µg/mL) were taken in triplicate manner (per dose) in centrifuge tubes. Control tubes contained 5 mL of the vehicle (distilled water) and standard tubes included 5 mL of 200 µg/mL diclofenac sodium. Erythrocyte suspension (0.1 mL) was added to each of the tubes and mixed gently. The mixtures were incubated for one hour at room temperature (30°C) and afterwards, centrifuged for 3 minutes at 3000 rpm. Absorbance of the hemoglobin content in the supernatant was estimated at 540 nm using spectrophotometer. The hemolysis percentage was calculated by assuming the hemolysis produced in the presence of distilled water as 100%. The percentage of the inhibition of hemolysis by the extracts was calculated using the formula:

% Hemolysis = (Optical density of test sample / Optical density of control) X 100

The percentage of HRBC membrane stabilization was calculated as follows:

% Protection = 100 – [(Optical density of test sample / Optical density of control) X 100]

Nitroblue Tetrazolium (NBT) assay on haemocytes

The reduction of NBT to insoluble blue formazan was used as an indicative for superoxide generation, although it is not entirely specific for O_2^-. A measured

volume of haemocytes were loaded, in triplicate, in 96 well microtiter plate (Sigma M-0156) and incubated with different concentration of extracts under humid conditions for 30 minutes at room temperature for the adherence of the hemocytes. The supernatants were removed and 0.3% NBT and absolute methanol were added. The formazan deposits were solubilized in 120 mL 2 M KOH and 140 mL dimethyl sülfoksit (DMSO). After homogenization of the contents in the wells, the absorbance was read at 620 nm in spectrophotometer.

TNF-α inhibition assay

Inhibition of TNF-α release in lipopolysaccharide (LPS) stimulated DAL cells was assayed using a slight modification of procedure suggested by Weiss *et al.* (1982). Different concentrations of extracts and LPS were added to induce inflammation in measured volume of cell line and kept aside for three hours. Then, the cells ($1x10^6$ cells/mL) were transferred to a 96 well microplate and incubated at 37 °C for 18 hours. After an overnight incubation, the plate was centrifuged (1800 g, 5 minutes, 16 °C), the supernatant was collected and TNF-α was quantified by the cytokine-specific sandwich quantitative enzyme-linked immune-sorbent assay (ELISA), according to the manufacturer's instructions. The inhibition of TNF-α release by LPS-stimulated DAL cells was calculated by the ratio between the TNF-α amount (pg/mL) secreted by treated cells and nontreated cells that were stimulated with LPS. TNF-α inhibition was reported as percentage values: [1 − (cytokine secretion of treated cells/cytokine secretion of cells cultivated with solvent control) x 100].

HPTLC study

Instruments and chemicals

CamagLinomat V automatic sample spotter, Camag (Muttenz, Switzerland), Camag TLC Scanner III linked to winCATS software (Camag), Camag glass twin trough chamber (10 x 10 cm) were used. Silica gel $60F_{254}$ (E. Merck, India) coated aluminum sheet plates were employed. Toluene and glacial acetic acid were used as mobile phase. Standard plumbagin and quercetin was obtained from Sigma Aldrich.

Preparation of standard solution

Standards of plumbagin and quercetin were prepared by dissolving 1 mg of standard in 5 mL of methanol. The solutions were sonicated for 10 minutes and the volume was adjusted to 10 mL with methanol, to obtain a working standard solution of 100 µg/mL concentration.

Preparation of sample solution

Stock solutions of the samples were prepared by transferring 1 mg of accurately weighed ethanol extracts of *D. peltata, D. burmanii* and *D. indica* in 10 mL

volumetric flask and 5 mL of methanol was added. The volumetric flask was sonicated for 15 minutes at room temperature. The flask content was filtered through Whatman filter paper No. 1 (Merck, Mumbai, India). The filtrate was collected and the volume was adjusted to 10 mL with methanol.

Procedure

HPTLC experiments were carried out by following standard procedures (Madhavan *et al.,* 2008; Wagner *et al.,* 1984). The working standard solutions of plumbagin and quercetin (100 ng in 1 µl) were spotted (2 µl) to get different concentrations ranging from 200 – 1000 ng/spot, using a micro-syringe. The working standard solutions were spotted as sharp band of 6 mm width using Camag 100 µl sample syringe on precoated silica gel aluminum plate $60F_{254}$ (10cm x 10cm) using a CamagLinomat V automatic sample applicator. The bands were applied at a distance of 10 mm from the bottom edge of the plate. The distance between the two bands was 11.4 mm. Total 8 spots were applied. First 5 spots were standard plumbagin and quercetin (concentrations of 200, 400, 600, 800 and 1000 ng/spot) and the next 3 spots were 5 µl of sample solution of ethanol extracts of *D. peltata*, *D. burmanii* and *D. indica*. The plates were developed in the solvent system comprising of toluene:glacial acetic acid (55:1) in a Camag glass twin trough chamber at 25 ± 2 °C, up to a distance of 70 mm. After the development, the plates were dried by a dryer for 5 minutes. Densitometric scanning was performed on a Camag TLC scanner 3 in the reflectance absorbance mode at 254 nm for all measurements, operated by CATS 3 software.

Statistical analysis

Data were statistically analyzed by Student's t-test and $p<0.01$ was considered to be significant. For efficacy assessments, one way ANOVA and Dunnet's t test were used. All the tests were performed in triplicate.

RESULTS

The effects of ethanol extracts of *D. burmannii*, *D. indica* and *D. peltata* on the inhibition of hemolysis under two different conditions (hypotonic solution and heat) were expressed in Table 1. Ethanol extract of *D. burmannii* showed significantly ($p< 0.001$) higher inhibition of hemolysis than *D. peltata*. *D. indica* revealed the least inhibition ($p< 0.01$).

Table 1: Inhibition of hypotonic solution and heat induced haemolysis of erythrocyte membrane by Indian *Drosera* species ethanol extracts.

	Sample concentration (Ethanol extracts µg/mL)	% Inhibition of haemolysis*	
		Hypotonic solution	Heat induced
D. burmannii	50	11.6±1.4	15.6±4.2
	100	23.5±3.4	25.6±1.2
	200	42.6±3.8	46.5±3.4
	400	68.4±2.9	69.2±1.6
	800	88.4±1.3	87.8±1.9
	1000	91.5±2.2	93.6±2.4
D. indica	50	1.1±2.2	6.4±3.6
	100	8.5±3.1	12.7±1.0
	200	16.4±1.3	21.6±1.9
	400	23.5±1.1	26.5±4.2
	800	46.2±4.3	52.6±2.4
	1000	52.6±2.3	59.7±1.5
D. peltata	50	3.1±1.6	5.3±2.2
	100	10.5±2.2	11.6±1.3
	200	22.7±1.1	37.4±2.4
	400	49.4±3.1	51.6±3.1
	800	70.8±1.3	76.2±1.3
	1000	82.5±2.1	86.7±2.1
Diclofenac	200	90.4±1.0	88.8±2.4

*mean±SD (n=3)

The results of ethanol extracts of *D. burmannii*, *D. indica* and *D. peltata* with respect to NBT reduction were shown in Table 2. All of the ethanol extracts increased the NBT reduction concentration dependently. The highest increase in the reduction was detected in extracts of *D. burmanii* followed by *D. peltate* and *D. indica*, respectively.

Table 2: Effect of Indian *Drosera* species extracts on NBT reduction test.

Plant name	Sample concentration (µg/mL)	Mean (%) ±SD
D.burmanni	50	11.6±1.4
	100	23.5±3.4
	200	42.6±3.8
	400	68.4±2.9
	800	88.4±1.3
	1000	91.5±2.2
D.indica	50	1.1±2.2
	100	8.5±3.1
	200	16.4±1.3
	400	23.5±1.1
	800	46.2±4.3
	1000	52.6±2.3
D.peltata	50	3.1±1.6
	100	10.5±2.2
	200	22.7±1.1
	400	49.4±3.1
	800	70.8±1.3
	1000	82.5±2.1

Effect of ethanol extracts of *D. burmannii*, *D. indica* and *D. peltata* on the inhibition of TNF-α release by LPS-stimulated DAL cells were shown in Table 3. All the extracts inhibited the secretion of TNF-α. The higher the concentration was, the more the inhibition was observed. Ethanol extracts of *D. burmannii* was detected to have the highest percentage of inhibition; followed respectively *by D. peltata and D. indica.*

Total flavonoid content present in all the extracts was estimated by using $AlCl_3$. The amount of flavonoids present in each extracts was expressed as mg/g (QE) and shown in Table 4.

Table 3: Inhibition of TNF-α release on LPS-stimulated DAL cells.

	Concentration of extracts (µg/mL)	Inhibition of TNF-α (% ± SD)
D.burmannii	100	12.4±2.1
	200	59.6±1.4
	400	92.6±2.1
D. indica	100	9.5±2.5
	200	42.3±1.3
	400	72.3±1.8
D. peltata	100	10.3±1.4
	200	48.8±1.8
	400	90.7±2.7
Control	-	12.6±2.1
Dexamethasone	(0.1 µM)	87.5±1.3

Table 4: The total flavonoid quantity in *Drosera* species.

Concentration of extracts (µg/mL)	Total flavonoid content in mg/g (QE)*, **					
	EEDB***	AEDB***	EEDI***	AEDI***	EEDP***	AEDP***
100	0.235± 0.004	0.111± 0.003	-0.04± 0.009	-0.039± 0.02	0.242± 0.002	0.096± 0.009
250	0.448± 0.021	0.309± 0.109	0.016± 0.004	-0.03± 0.02	0.465± 0.002	0.224± 0.013
500	0.633± 0.021	0.459± 0.002	0.111± 0.004	0.024± 0.02	0.598± 0.02	0.354± 0.022
750	0.986± 0.078	0.606± 0.003	0.182± 0.064	0.143± 0.011	0.896± 0.006	0.484± 0.009
1000	1.204± 0.021	0.672± 0.028	0.402± 0.014	0.221± 0.007	1.051± 0.024	0.607± 0.012

*QE=quercetin equivalent;
**mean±SD (n=3)
***EEDB- Ethanol extract of *D. burmannii*, AEDB- Aqueous extract of *D. burmannii* ,EEDP - Ethanol extract of *D. peltata*, AEDP- Aqueous extract of *D. peltata*, EEDI- Ethanol extract of *D. indica*, AEDI- Aqueous extract of *D. indica.*

The total flavonoid content in three plants was presented as mg/g of Quercetin equivalent (QE) ± SD (n = 3). Both ethanol and aqueous extracts of *D. burmannii*, *D. indica* and *D. peltata* were tested at 1000, 750, 500, 250 and 100 µg/mL and the positive control (Quercetin) at 10, 7.5, 5.0, 2.5 and 1 µg/mL. Higher concentrations of flavonoid were found in ethanol extract of *D. burmanii* whereas the concentration was the least in aqueous extract of *D. indica*. The order of total flavonoid content with respect to plant was *D. burmannii>D. peltata>D.indica* for the concentrations

500 µg/mL and over. For 100 and 250 µg/mL, the amount of flavonoid was only slightly higher in *D. peltata* than *D. burmanii*. Ethanol extracts of all species contained significantly higher concentration of flavonoids than aqueous extracts.

The flavonoid contents were estimated from the standard calibration curve of quercetin. 10, 7.5, 5.0, 2.5 and 1 µg/mL concentration of quercetin were plotted at X axis and the corresponding absorbances were plotted at Y axis, which was represented in Figure 1.

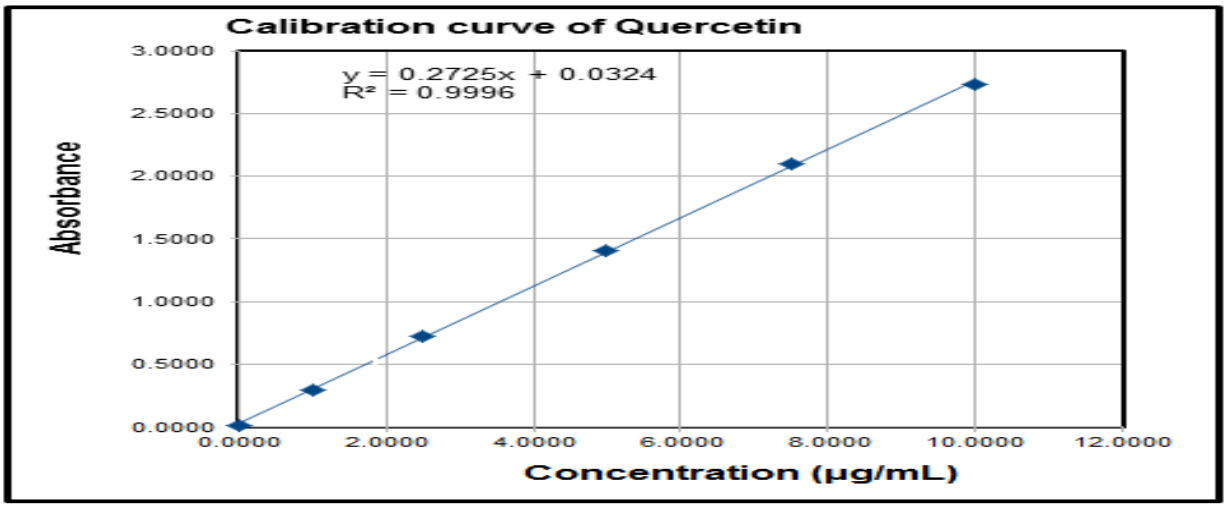

Figure 1: Calibration curve of quercetin for determination of total flavonoid content.

Plumbagin and quercetin from the sample solution and standard spots were well separated from other compounds in HPTLC condition. Their peaks displayed excellent peak resolutions as shown in Figure 2 and 3. The TLC plate was visualized under UV light at 254 nm. The HPTLC photographed chromplate is shown in Figure 4 and 5. In total flavonoid content determination, aluminum chloride reacts with carbonyl group at C4 and hydroxyl at C3 (flavonols) and C5 in flavonols and flavones to form acid stable as well as unstable complexes. Chromatographic analysis that was performed using ethanol extracts of three plants, on pre-coated silica gel HPTLC plates $60F_{254}$, using solvent system comprising of Toluene:Glacial Acetic Acid (55:1) as mobile phase gives good separation of plumbagin at R_f =0.83 to 0.88. Similarly, using a solvent system, comprising of toluene:ethyl acetate: formic acid:methanol (3:6:1.6:0.4 *v/v/v/v*) as mobile phase gives good separation of quercetin at R_f =0.76 and 0.77. Detection and quantification of plumbagin and quercetin were done by densitometric scanning at λ = 254 nm.

The calibration curves (Figure 6 and 7) of plumbagin and quercetin were found to be linear (200 - 1000 ng/spot) and dependent

on the concentration against area. The equation of best fitting line was Y = 2684 + 13.8 * X, r = 0.9994 sdv = 8.62 for plumbagin and Y = 1.309e + 004+12.1*X r= 0.99068 sdv= 13.63 for quercetin.

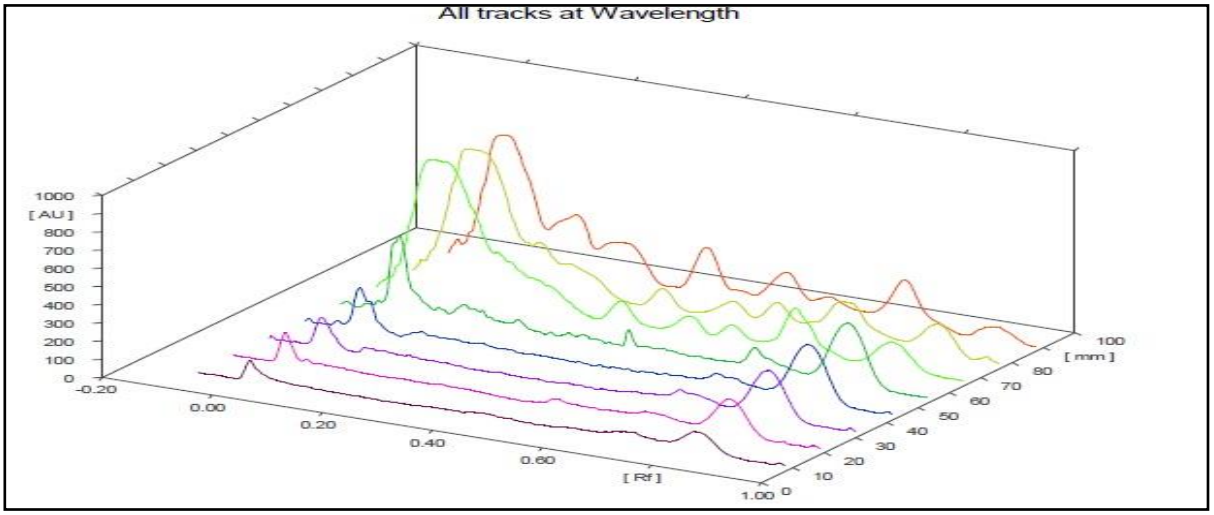

Figure 2: HPTLC chromatogram of plumbagin in all applied spots.

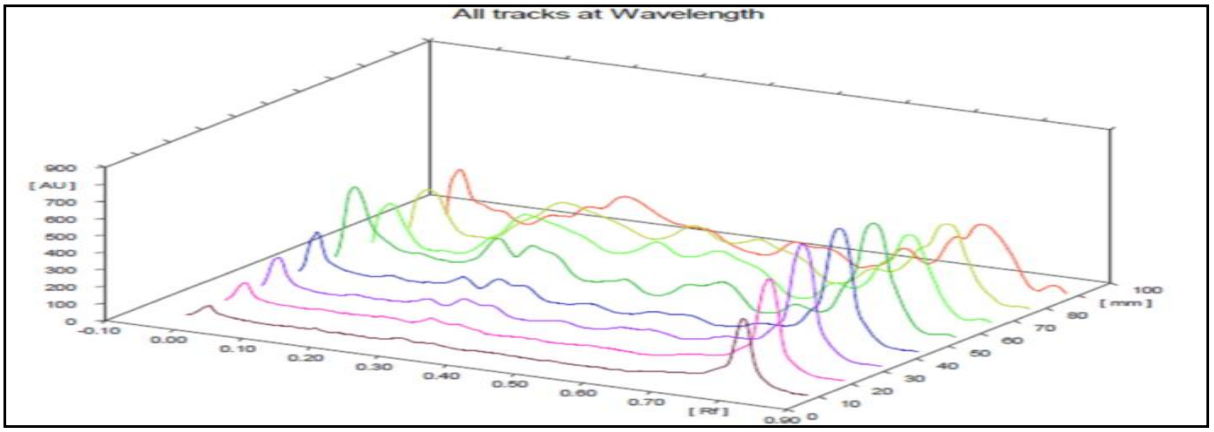

Figure 3: HPTLC chromatogram of quercetin in all applied spots.

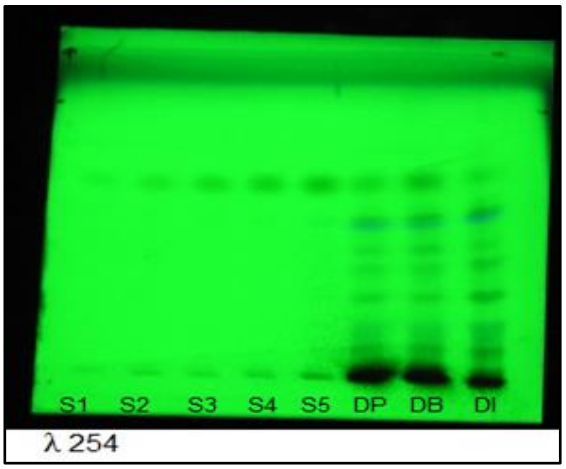

Figure 4: Photograph of plumpagin TLC plate at λ max 254.

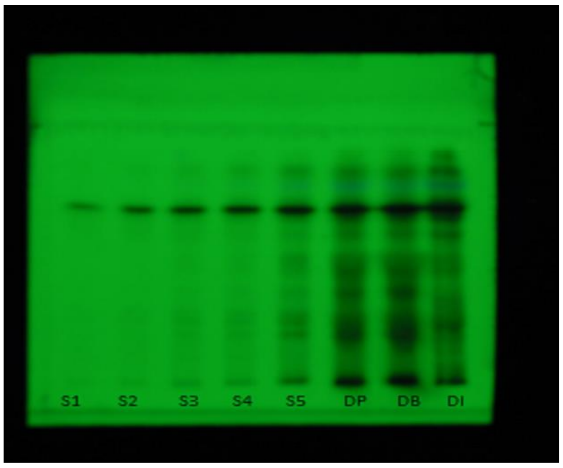

Figure 5: Photograph of quercetin TLC plate at λ max 254.

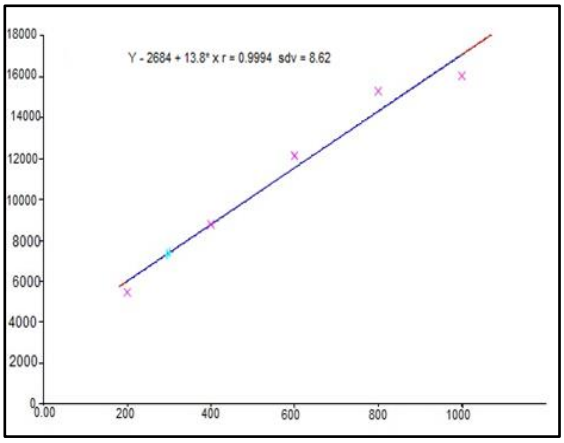

Figure 6: Quantification of plumbagin (Regression via area).

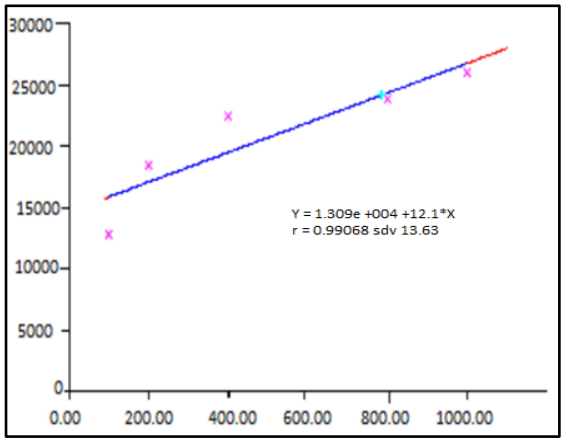

Figure 7: Quantification of quercetin (Regression via area).

From the HPTLC study, mg/g quantities of plumbagin and quercetin were calculated by regression via area, and the results are presented in Table 5. Ethanol extract of *D. burmannii* possesses higher concentration of plumbagin and quercetin when compared to *D. peltata* and *D. indica.* Plumbagin and quercetin concentration in *D. burmannii* was 1.572 and 0.6022 mg/g of extract, respectively. Similarly, plumbagin and quercetin concentration was 1.568 and 0.5884 mg/g in *D. peltata* and 0.9812 and 0.2471 mg/g for *D. indica*.

Table 5: Amount (mg) of quercetin and plumbagin found in per gram of ethanol extracts of Indian *Drosera* species.

Name of extracts	Amount of Quercetin/g of extract (mg/g)	Amount of Plumbagin /g of extract (mg/g)
EEDB*	1.572	0.6022
EEDP*	1.568	0.5884
EEDI*	0.9812	0.2471

*EEDB- Ethanol extract of *D. burmannii*, EEDP - Ethanol extract of *D. peltata*, EEDI- Ethanol extract of *D. indica*.

DISCUSSION

There is a link between cancer and inflammation because many researchers reported that naturally occurring anti-inflammatory or immunomodulatory plant metabolites have anticancer effect. The anticancer effect is due to the stimulation or inhibition of particular cellular inflammatory actions and the related molecular signaling pathways (Chien-Fu H *et al*., 2008). All the three plants investigated in the present study were reported to have anti-cancer effect on DAL and EAC cell line (Raju A *et al*., 2012). Immunomodulatory reaction was exhibited by stabilization of lysosomal membrane and thereby limiting the release from activated neutrophils of lysosomal constituents such as bactericidal enzymes and proteases. The release of lysosomal enzymes into the cytoplasm stimulates the inflammatory mediators such as oxygen radicals, prostaglandins and trigger the

inflammation (Balkwill *et al*., 2001). Many of NSAIDS stabilize lysosomal membrane and inhibit the inflammatory process by restricting the release of lysosomal enzymes. The results of the present study confirmed that all the ethanol extract of Indian *Drosera* species were able to stabilize RBC membrane against stress, like hypotonicity and heat. Inhibition of haemolysis indicates that all the extracts had the ability to prevent rupture or haemolysis of RBCs. Since there is a close similarity between RBC and lysosomal membrane, protection against hypotonic solution or heat induced lysis of RBC is often extrapolated to stabilization of lysosomal membranes. Therefore, inhibition of the haemolysis is used as a biochemical index of anti-inflammatory activity (Gandhidasan R *et al*., 1991). Researchers have already reported that, flavonoids, triterpenoids, and many other secondary plant metabolites can exhibit an algesic and anti-inflammatory effects as a result of their membrane stabilizing actions (Prasanna B *et al*., 2012). Similarly, ethanol extracts of Indian *Drosera* species exhibited statistically significant and concentration dependent membrane stabilization effect by inhibiting both hypotonicity and heat induced lysis of erythrocytes compared to the standard drug diclofenac sodium.

Furthermore, the ethanol extract of Indian *Drosera* species significantly increased the intracellular reduction of NBT dye to formazan (deep blue compound) by the neutrophils, confirming the increase in the intracellular killing property. The reduction was also determined to be concentration dependent.

As a part of host defense, upon inflammatory stimuli, such as LPS, macrophages trigger signals for the production of diverse inflammatory mediators, such as TNF-α and nitric-oxide. In tumor progression process, inhibition of TNF expression occur and is used to find out the possible mechanism of any anticancer drug. Researchers reported that a drug with anti-inflammatory or immunomodulatory property also inhibits TNF expression in host cell for defense purpose (Yamada Y *et al*., 1998). The present study results revealed that ethanol extracts of all Indian *Drosera* species possess concentration dependent inhibition on TNF-α release.

Our HPTLC results indicated that the whole plant extracts of Indian *Drosera* species contain various biologically active constituents such as plumbagin and quercetin. According to the quantity of constituents, each extract exhibited potency of inhibition and pharmacological action.

CONCLUSION

In conclusion, besides reports on various pharmacological actions of plumbagin and quercetin, no other literature is available for evaluation of their immunomodulatory activity although it has been used for the treatment of various diseases in Ayurveda, Sidha and traditional medicine. *Droserae* herbal drugs are remarkable for their anti-carcinogenic and anti-oxidant constituents such as plumbagin and quercetin. In the present research, plumbagin and quercetin content of the three Indian *Drosera* species were determined by a fast and sensitive HPTLC method. The method can be used for routine quality control analysis and for the quantitative determination of plumbagin and quercetin in other herbal and medicinal preparations.

ACKNOWLEDGMENTS

The authors gratefully acknowledge K M C H College of Pharmacy, Coimbatore, Tamil Nadu, for providing support and facilities for this research work. The authors also thank Dr. SN Yoganarasimhan for his guidance in the selection and collection of these plant materials.

REFERENCES

Bag GC, Grihanjali Devi P, Bhaigyabati TH (2015). Assessment of total flavonoid content and antioxidant activity of methanolic rhizome extract of three *Hedychium species* of manipur valley. *Int J Pharm Sci Rev Res* **30**(1): 154-159.

Balkwill, Mantovani A (2001). Inflammation and cancer: back to Virchow? *The Lancet* **357**(9255): 539–545.

Chien-Fu H, Shih-Shen L, Pao-Hsin L, Young SC, Yang CC (2008). The immunopharmaceutical effects and mechanisms of herb medicine. *Cellular and Molecular Immunology* **5**(1):23–31.

Gandhidasan R, Thamaraichelvan A (1991). Anti-inflammatory action of Lannea coromandelica by HRBC membrane stabilization. *Fitoterapia* **62**(1): 81–83.

Jayaram K, Prasad MNV (2006). *Droser aindica* L. and *D. burmanii* Vahl., medicinally important insectivorous plants in Andhra Pradesh –regional threats and conservation. *CurrSci* **91**:943-946.

Madhavan V, Basnett H, Kumar AC, Yoganarasimhan SN (2008). Fingerprinting of plumbagin in *Drosera burmannii* Vahl using high performance thin layer chromatography. *Indian J Pharm Sci* **70**:798-800.

Prasanna BG, Sameera RS, Ira TG, Weerasinghe AK, Mayuri GT, Ratnasooriya WD, Kamani HT (2012). Anti-inflammatory activity is a possible mechanism by which the polyherbal formulation comprised of *nigella sativa* (seeds), *Hemidesmusindicus* (Root), and *Smilax glabra* (Rhizome) mediates its antihepatocarcinogenic effects. *Evidence Based Complementary and Alternative Medicine*: 1-12.

Raju A , Christina AJM , Mayakrishnan A (2012). Antitumor potential of ethanol and aqueous extracts of *Drosera burmannii* Vahl against Dalton's ascitic lymphoma bearing mice. *J Pharm Res* **5**(3): 1418-1423.

Santapau H, Henry AN (1976). A Dictionary of the flowering plants in India. Publication and information Direcorate, pp.58, New Delhi, India.

Suresh Kumar Karri, Angappan Sheela (2017). Comparative in vitro Antidiabetic and Immunomodulatory evaluation of standardized five select medicinal herbs and spectral analysis of i L. (Nyctaginaceae). *PharmacognJ* **9**(3): 336-344.

Wagner H, Bladt S, Zgainski EM (1984). A Thin Layer Chromatography Atlas. In: Plant Drug Analysis – 2nd edn, pp. 276,Springer: Berlin, Germany.

Yamada Y, Webber EM, Kirillova I, Peschon JJ, Fausto N (1998). Analysis of liver regeneration in mice lacking type 1 or type 2 tumor necrosis factor receptor: requirement for type 1 but not type 2 receptor. *Hepatology* **28**(4I): 959–970.

Yu He, Zhimin He, Feng He, Haitong Wan (2012). Determination of quercetin, plumbagin and total flavonoids in *Droserapeltata* Smith var. glabrataY.Z.Ruan. *Pharmacogn Mag* **8**(32): 263–267.