International Research Journal of Pharmaceutical and Applied Sciences (IRJPAS)
Available online at **www.irjpas.com**
Int. Res J Pharm. App Sci., 2013; 3(2):6-12

Research Article

TLC BASED PHYTOCHEMICAL AND ANTIOXIDANT ANALYSIS OF *OXALIS CORNICULATA* LINN.

Ashwani Kumar, Priya Chaudhary, Nitin Kumar Verma, Pankaj Kumar, Lalit
Department of Bioscience, Shri Ram College Muzaffarnagar, Uttar Pradesh-251002 (INDIA)
(Received: 04 April 2013; Accepted: 21 April, 2013; Published: 28 April, 2013)
Corresponding Author's email: ashwani_biotech@yahoo.co.in

Abstract: Nature has been a source of medicinal agents for thousands of years and since the beginning of the man. Thus India has a great wealth of traditional knowledge and wealth for Ayurveda. The antioxidant activity of medicinal plants is mainly related to their bioactive compounds, such as phenolics, flavonols, and flavonoids. Preliminary phytochemical analysis of aqueous and methanolic extracts of *Oxalis corniculata* revealed the presence of carbohydrates, alkaloids, flavonoids, glycosides, saponin, tannins, fixed oil and fats. The results of present investigation showed that 25-75% of major *Oxalis corniculata* resolved by means of TLC were active antioxidants supporting the previous studies of *Oxalis corniculata*. The extracts of *Oxalis corniculata* showed several resolved TLC bands with strong antioxidant activity of resolved bands (75%) showed strong antioxidant activity and few spots with weak antioxidant activity of resolved bands. However, aqueous extracts of *Oxalis corniculata* showed faint spots.

Key**words:** *Oxalis corniculata*, medicinal weed, TLC, phytochemical screening, antioxidant analysis

INTRODUCTION

Since the beginning of human civilization, man has been using many herbs and herbal like the Bible, The Iliad, The rig Vedas, History, of Herodotous, etc confirm this.). In recent years, secondary plant metabolites (phytochemical), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents [1] .Thus, it is anticipated that phytochemical with adequate antibacterial efficacy will be used for the treatment of bacterial infections [2].Since time immemorial, man has used various parts of plants in the treatment and prevention of various ailments [3]. Thus Indian medicinal plant-based industry is growing annually for pharmaceuticals, phytochemical, neutraceuticals, cosmetics and other valuable products. *Oxalis corniculata* Linn. is a small procumbent herb, with stems rooting and pubescent with apprised hairs, leaves palmately3-foliolate. This plant is well known for its medicinal value as a good appetiser and as a remover of kapha, vata and piles. It is also known to cure dysentery, diarrhoea and skin diseases [4]. *Oxalis corniculata*, the Creeping wood sorrel, also called Procumbent Yellow-sorrel or sleeping beauty resembles the common yellow wood sorrel. The bright-yellow flowers and soft-green foliage of common yellow *oxalis* adds a shade of tranquillity to any space. *Oxalis stricta* or yellow wood sorrel is found throughout North America from British Columbia to Florida. Often considered an invasive weed, this species is identifiable by its narrow, yellow-green rim of slender, white fur. Its flowers, leaves and fruit are supported by slender stalks stems and its rounded leaves. The leaves of this species of *oxalis* grow in between pale green and light pink. The fruit of common *oxalis* is typified by narrow clusters of three an elongated form that terminates in a point.[5]

Various biochemical constituents were identified in *Oxalis corniculata*, such as Potassium, Calcium Oxalic acid, Vitamin c ,Ascorbic Acid ,Carotene, Tartaric acid ,Citric acid ,Mallic acid, Isoorietin, Isovitexin, Swertsin ,2'-O(Beta-D-glucopyranosyl). It is reported to be exhibit Anti-Amoebic, Anti-Giardia, Cardio protective, Antibacterial Cream Formulation, Antitumor, Antioxidant, Anti-Epileptic, Antifungal, Abortifacient, Anti-fertility, Anti-Urolithiasis Antibacterial activity. In other studies phytochemical analysis of *Tribulus terrestris* gave positive results for carbohydrates, flavonoids, saponins, tannins and alkaloids [6]. The present study was concluded with the aim of finding out herbal remedy for exciting phytochemical analysis of secondary metabolites and antioxidant activity of *Oxalis corniculata*.

MATERIAL AND METHODS MATERIAL:

Reagent And Apparatus - Methanol, Ethanol, What man filter paper, $HgCl_2$, KI, Distilled water, Picric acid, Iodine, Ammonia, Sulphuric acid, Chloroform, Con.H_2SO_4, -Naphthol, Led acetate, Silica gel, Ethyl acetate, acetone, Acetic acid, Anisaldhyde H_2SO_4, Toluene, n-butanol, diethyl ether, Aqueous Sodium Chloride, What man filter paper, Pastel mortars, Water bath, soxhlet apparatus, Oven, Crucible, Separator funnel, Spectrophotometer, Conical flask, Magnetic Stirrer, Refrigerator.

Plant Material- The present study was carried out on aerial parts of *Oxalis corniculata*. **C**ollection of aerial parts of *Oxalis corniculata* was done between March to May 2012 from Muzaffarnagar district.

Preparation of the plant extracts- 5g. of shade dried *Oxalis corniculata* samples were ground at a high speed with blender and extracted up to clear sample in methanol with the soxhlet apparatus. First of all takes fresh healthy

stem of *Oxalis corniculata* and dried shade then until they dried properly. After dried used blend then into the blender & from a thin powder and used for the experiments. Take 5gm of powder and mix 25ml distilled water & kept it in beaker for boiling on the hot plate for 15 min also at 80-100. After boiling we kept it for cooling. Now we weight the empty crucible after heating in oven to dry at 60. Now we take filter paper for filtered the boiled sample in crucible with the help of Watt man filter paper. After filtration the sample into the crucible we weight it & than kept it into the oven for totally evaporation at 60. After evaporation the extract is remain which is used for the further experiments. In the present study, total phytochemical content was determined. The phenolic and flavonoid content in the test *Oxalis corniculata* extracts was found to be higher. In general, phenolic compounds were commonly found in plants and have reported several biological activities including potential antioxidants and free radical scavengers apart from primary defense role. Earlier reports revealed that *Oxalis corniculata* especially their flavonoids have the antioxidant activity. Weight 20 gm of powder plant of *Oxalis corniculata* and 200 ml. methanol in round bottom distillation flask. Put the sample on whattman filter paper; make the thimble of filter paper placed in the Soxhlet assembly. Placed the assembly on heating mental at 60^0C. After12 hrs the extract was filtered through whatman no.1 filter paper in a Buchner funnel. The solvent was evaporated in a rotary vaccum evaporator model then crude extracts were stored in amber glass vials in refrigerator at 4. Crude extracts were diluted with methanol for further investigation.

Preliminary Qualitative phytochemical screening –

Phytochemical analysis of all the procedure of India Pharmacopoeia 1985[7]. By this analysis the presence of several phytochemical was tested phytochemical analysis was tested as follows:

Detection of Alkaloids[8] -Dissolve 1.358 g of $Hgcl_2$ in 60 ml of water and pour into a solution of 5g of KI in 10 ml of, add H_2O distilled water to make the volume 100 ml. (White precipitate with most alkaloids in slightly acid solution). Dissolve 1g of picric acid in 100 ml of water.To one ml of the methanolic extract sample in a test tube was mixed with one ml of Hager's reagent/Wagner's reagent. The appearance of coloured precipitates indicated the presence of alkaloids.

Detection of Flavonoids[9] -Take 5ml of the dilute ammonia solution and a portion of the aqueous extract was added, followed by addition of concentrated sulphuric acid. Appearance of yellow coloration indicated the presence of flavonoids.

Detection of Terpenoids (Salkowski test)[10] -5 ml of aqueous extract was mixed with 2 ml of Chloroform and concentrated to H_2SO_4 form a layer. A raddish brown coloration on the interface showed the presence of terpenoids.

Detection of Carbohydrates [8]-Prepare reagent by dissolving o.5 g reagent grade – naphthol in 10 ml of 95% ethanol. Store the reagent at room temperature. To one ml of the sample few drops of molish reagent were added. There after con. H_2SO_4 was sided along the walls of the test tube. Appearance of purple ring at the interface indicated presence of carbohydrates.

Detection of Glycosides [11]-25% ammonia solution was made with 75 ml of distilled water in 25 ml of dilute ammonia solution. 5 ml of the extract was dried and shaken with 3 ml petroleum ether. The filtrate was added to 2 ml of a 25% ammonia solution. The mixture was shaken. Presence of red coloration was taken as indication of the presence of glycosides.

Detection of Tannins [9]-10% of lead acetate solution. Add 1 gm of lead acetate in 10 ml of distilled H_2O and mix properly. To 1 ml of sample in a test tube, 10% of lead acetate solution was added mixed well. The presence of yellow precipitates indicated tannins.

Detection of Saponins[8]-The extract was diluted with distilled H_2O and made up to 20 ml. The suspension was shaken in a graduated cylinder for 15 min. 2 cm layer of stable foam indicated the presence of saponins.

Identification of Phytochemicals & Antioxidants by TLC Thin Layer Chromatography (TLC)

The phytochemical analysis of this plant revealed the presence of flavonoids, alkaloids, carbohydrates & tannins etc. this extract was further subjected to TLC to confirm the presence of major group like alkaloids, flavonoids in the extract. Individual substances separated out based on RF value. The solvent evaporated dried extracts were redissolved in methanol. TLC performed on Merck Silica Gel 60 glass plate using different eluents analyzed the fraction obtained. The chromatograms were observed in UV/VIS before and after processing with spraying agent. The flavonoids and phytochemical were identified by comparison to co- chromatographed standards and available literature data [12].

Preparation of TLC Plate: 42 g of silica gel was dissolved in 25 ml chloroform, 25 ml methanol. Prepared the TLC plates by spreading the gel on it.

Marking the TLC Plate: The silica gel TLC plates were marked by using pencil.

Activation of TLC Plate: Placed the TLC plate in an oven at 50-60^0C for 15-20 min to "activate it". Activation involves driving of water molecules that bond to the polar sites on the plate.

Spotting the TLC Plate: The narrow end of capillary was placed into the extract. When extract rises into the capillary then touch the capillary on the silica plate very carefully. Allowed the solvent to completely evaporate from the spot.

Developing the TLC Plate: The TLC plate was placed very carefully in the developing bottle containing mobile phase solvent system. Left it for some time so that solvent front can move.

Drying The Plate: Placed the slide in an oven at Temperature 50-60 to evaporate the solvent.

TLC methods for phytochemical with different solvent systems and detecting agents

Solvent system- 1

Solvent system : Ethyl acetate: methanol: water (10:1.35:1)

Detection : By Iodine vapours gave brown red spot when dried in oven at 100^0C for 10 min.

Solvent front run : 10 cm

Solvent system- 2

Solvent system : Chloroform: Ethyl acetate: Formic acid (10:8:2)

Solvent run : 10 cm

Detection: By Iodine vapours after heating (100^0C).

Solvent system- 3

Solvent system : Benzene: Ethanol: Ammonia (18:2:0.2)

Solvent front run : 10 cm

Detection : By Iodine vapours after heating (100^0C).

Solvent system- 4

Solvent system: Toluene: Ethyl acetate(5:7)

Solvent run : 10 cm

Detection : By Iodine vapours

Solvent system- 5

Solvent system: Chloroform: Ethanol(8:2)

Solvent run : 10 cm

Detection : By Iodine vapours

Solvent system- 6

Solvent system: Toluene: Ethyl acetate(5:7)

Solvent run : 10 cm

Detection : By the spray of DPPH solution and dry about 20 Minutes at room temperature

Solvent system- 7

Solvent system : Chloroform: Ethanol(8:2)

Solvent run : 10 cm

Detection : By the spray of DPPH solution and dry about 20 Minutes at room temperature

RESULT AND DISCUSSION

The present study was carried out on the plant samples revealed the presence of medicinally important bioactive compounds. The preliminary observations on different extractions and phytochemical analysis detected the presence of glycosides, flavonoids, alkaloids, sterols, ascorbic acid and tannins and organic acid.

Aqueous extraction of *Oxalis corniculata*- Firstly two types of extracts were made, which was observed and found that 42.60% methanolic extract was sticky in nature and dark black in colour, aqueous extract characteristics was same as methanolic in nature while the yield was 7%in aqueous phase of *oxalis corniculata.*

Table-1: Nature and Percentage yield of extracts of *Oxalis Corniculata.*

Sr. no.	Name of the extract	Nature	Colour	% Yield (w/w)
1.	Methanolic	Sticky	Dark black	42.60%
2.	Aqueous	Sticky	Dark black	7%

Table-2: Results of qualitative phytochemical test:

S. NO.	PHYTOCHEMICAL	REAGENT	OBSERVATION	RESULT
TEST FOR ALKALOIDS				
1.	Alkaloids	Mayer's test	Coloured precipitates are present.	++
2.	Alkaloids	Hager's test	Coloured precipitate are present	+
TEST FOR STEROLS				
1.	Sterols	Test solution + H_2SO_4	Blue green Colour observed	++
TEST FOR GLYCOSIDES				
1.	Glycosides	Kller-Killiani Test	Red colour appear	+

TEST FOR TANNINS				
1.	Tannins	Lead Acetate test	Precipitate observation	++
TEST FOR FLAVONOIDS				
1.	Flavonoids	Ferric chloride test	Yellow Colour appear	++
2.	Flavonoids	Zn-HCL reduction test	Blue colour appear	+
TEST FOR ASCORBIC ACID				
1.	Ascorbic acid	Dichlorophenolindophenol test	Show positive result	++
TEST FOR OILS AND FATS				
1.	Oil, fats	Spot test	Fats spots are found	++
TEST FOR PROTEINS				
1.	Proteins	Million test	No reaction	-
TEST FOR CARBOHYDRATES				
1.	Carbohydrates	Molish's test	Violet ring appear	+
TEST FOR TERPENIODS				
1.	Terpenoids	Chloroform + H_2SO_4	Reddish brown colour present	+
TEST FOR SAPONINS				
1.	Saponins	Foam test	Foams are present	++

(+++) indicates Higher conc. (++) indicate moderate conc. (+) low conc. , (-) indicates negative results

For phytochemical analysis of sterols in *Oxalis corniculata* blue green colour was observed after reactions, which prove the presence of sterols. Mayer's test and Hager's test follow on the extracts and after reaction colored precipitate show the presence of alkaloids, Kller-Killiani Test used for the detection of Glycosides give the red colour after reaction show the presence of glycosides. Million's test used for the detection of the protein no reaction show the absence of proteins. Detection of Tannins used Lead acetate test extract gives yellow precipitate shown the presence of tannins in the plant. For the detection of Flavanoids two tests followed Ferric chloride test and Zn-HCL reduction test give yellow colour and blue colour with the extract positive result show the presence of flavonoids. Extract show reaction with the dichlorophenolindophenol, so ascorbic acid is present. Methanolic extract rubbed on the Watt man filter paper fats spots are found which show the presence of oil and fats in the plant. Followed the Molish' test appear violet ring show the presence of Carbohydrates.

Table 3. Thin Layer Chromatography of *Oxalis corniculata* extracts showing experimental conditions and RF values of sample constituents

Sr.no.	***Oxalis corniculata* extracts**	**Solvent system**	**Identification/Detecting reagents**	**+ Rf values**
1.	Methanolic Water	Ethyl acetate: methanol: water (10:1.35:1)	Iodine vapours	0.34 0.53
2.	Methanolic Water	Chloroform: Ethyl acetate: Formic acid(10:8:2)	Iodine vapours	0.58 0.31
3.	Methanolic Water	Benzene: Ethanol: Ammonia (18:2:0.2)	Iodine vapours	0.36 0.53
4.	Methanolic Water	Toluene: Ethyl acetate(5:7)	Iodine vapours	0.36 0.51
5.	Methanolic Water	Chloroform: Ethanol(8:2)	Iodine vapours	0.42 0.63

Table 4. TLC analysis for antioxidant compounds in *Oxalis Corniculata* used DPPH detecting agent

Sr.no.	*Oxalis corniculata* extracts	Solvent system	Identification/Detecting reagents	+ Rf values
1.	Methanolic Water	Toluene: Ethyl acetate(5:7)	DPPH	0.32 0.20
2.	Methanolic Water	Chloroform: Ethanol(8:2)	DPPH	0.42 0.38

TLC based chromatograms were produced from *Oxalis corniculata* extracts, firstly 2 spots were observed with solvent system EA: M: W having RF values 0.34, 0.53 With Iodine vapours. Solvent system Chloroform: Ethyl acetate: Formic acid (10:8:2) having Rf values 0.58, and 0.31 identification agent is iodine vapours. Solvent system Benzene: Ethanol: Ammonia (18:2:0.2) having 2 spots these RF values 0.36, 0.53 in the iodine vapours. In the Toluene: Ethyl acetate solvent system of (5:7) observed the 2 spots having 0.36, 0.51 iodine vapours is used as detecting agent. Solvent system Chloroform: Ethanol (8:2) having 2 spots RF values 0.42, 0.63, in the presence of iodine vapours.

Solvent system Toluene: Ethyl acetate (5:7) having 2 spots their Rf values are 0.32 0.20 with the DPPH identification agent. Solvent system of Chloroform: Ethanol (8:2) having five spots RF values 0.42, 0.38, 0.69, 0.72 in the detecting agent DPPH. Antioxidant activities of Methanolic extracts of *Oxalis Corniculata* was also observed which was indicated by colour change of DPPH solution due to antioxidant effect of phenolic compounds and other plant secondary metabolites. The separation of antioxidant in the extract of *Oxalis corniculata* in solvent system of Chloroform: Ethanol (8:2) maximum 5 spots having maximum RF value as 0.63. While Solvent System; Toluene: EA (5:7) have minimum spots Rf value is 0.20. These show the presence of antioxidant in *Oxalis corniculata*.

Fig 1. Detection of Carbohydrates in of *Oxalis corniculata* extract of *Oxalis corniculata*

Fig 2. Detection of Flavonoids in methanolic methanolic extract

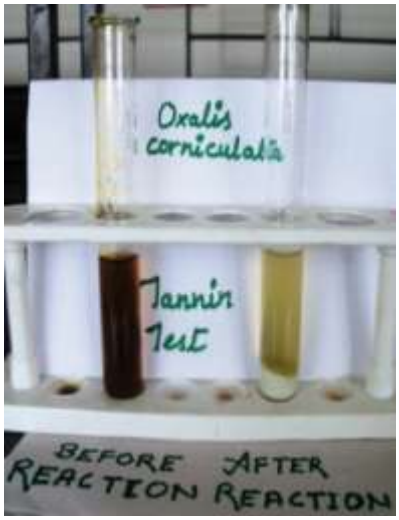
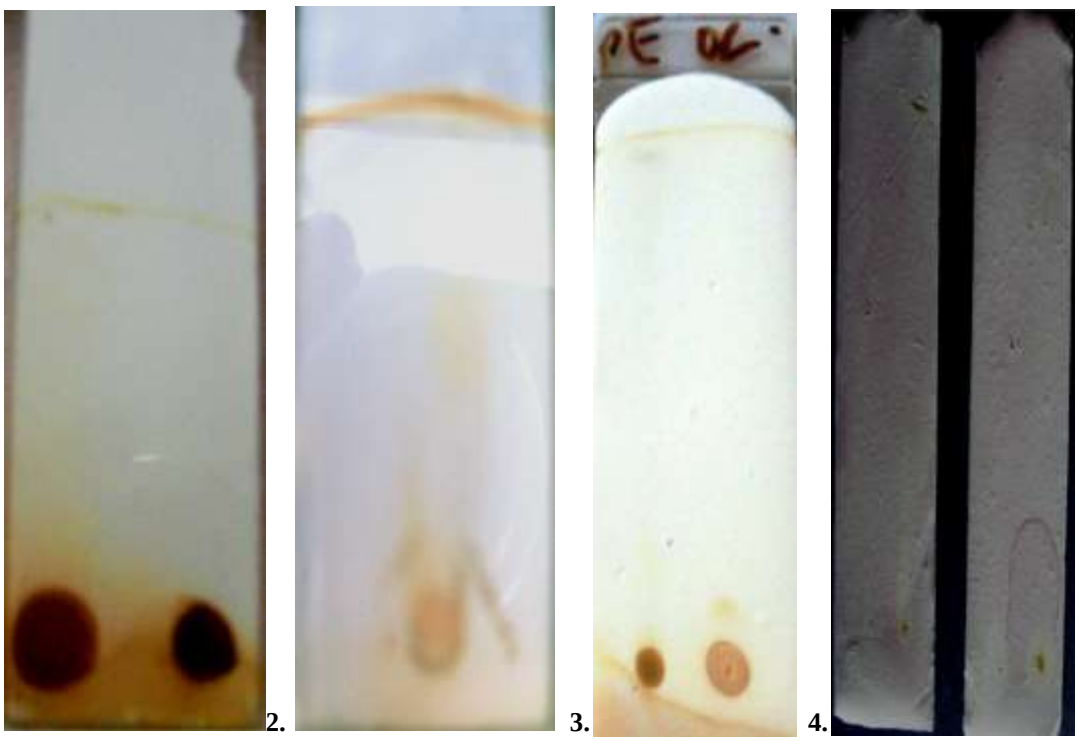


Fig 3. Detection of Tannins in methanolic *corniculata.*

Fig 4. Detection of Saponins in methanolic extract of *Oxalis* Extract of *Oxalis corniculata.*

Fig 5. Detection of Alkaloids in methanolic extract of *oxalis corniculata*



2. 3. 4.

Fig 6. TLC fingerprints of *Oxalis corniculata* with different solvent systems in 1,2,3,4, Solvent system; PE: EA (9:1), 2. Solvent system; PE: EA: M (17:2:1) 3. Solvent system; T: EA 5:7), 4.Solvent system;C:E(8:2)

DICUSSION

In the present study, total phytochemical content was determined. The phenolic and flavonoid content in the test extracts was found to be higher in *Oxalis corniculata.* In general, phenolic compounds were commonly found in plants and have reported several biological activities including potential antioxidants and free radical scavengers apart from primary defense role. Earlier reports revealed that *Oxalis corniculata* especially their flavonoids have the antioxidant activity. The nature of the active antioxidant TLC bands of the methanolic and aqueous extracts of *Oxalis corniculata* of two different solvent systems. The antioxidant activity of medicinal plants is mainly related to their bioactive compounds, such as phenolics, flavonols, and flavonoids. On the basis of antioxidant activity, a sample can be classified into one of four major groups, viz., and very high, moderate, or low antioxidant content. Preliminary phytochemical analysis of aqueous and methanolic extracts of *Oxalis corniculata* revealed the presence of carbohydrates, alkaloids, flavonoids, glycosides, saponin, tannins, fixed oil and fats. *Oxalis corniculata* extracts have been shown to have various antioxidant activities. Thus, the experimental data of these previous reports showed that *Oxalis corniculata* must have contained strong antioxidant constituents. The results of present investigation showed that 25-75% of major *Oxalis corniculata* resolved by means of TLC were active antioxidants supporting the previous studies of *Oxalis corniculata.* The extracts of *Oxalis corniculata* showed several resolved TLC with strong antioxidant activity of resolved bands (75%) showed strong antioxidant activity and few spots with weak antioxidant activity of resolved bands. However, aqueous extracts of *Oxalis corniculata* showed faint spots.

CONCLUSION

Oxalis corniculata is a wonderful plant having enormous range of different activities. In this study we have done invitro experiments for screening of phytochemicals and plant secondary metabolites related to antioxidant activity of plant. This study will help to researchers & scholars to go deep in this area as plant indicate vast range of phytochemical related to origin. *Oxalis corniculata* which is collected from different season and agro climatic zone; definitely it is assumed that research will be able to find out more suitable and specific drug plant having particular activity in specific season. Some scientist needs this data and concepts to re-research on the present scientific plant. It can really contribute to medical and pharmaceutical practices in further studies .There are still many more activities waiting for screening the drug from *oxalis corniculata.*

REFERENCES

1. Krishnaraju A V, Rao T V N, Sundararaju D. Assessment of bioactivity of Indian medicinal plants using Brine shrimp (*Artemia salina*) lethality assay. *Int J Appl Sci Eng,* **2005**; 2: 125-134.
2. Balandrin M F, Kjocke A J and Wurtele E. Natural plant chemicals sources of industrial and mechanical materials. *Science,* **1985**; 228: 1154-1160.
3. Tanaka H, Sato M and Fujiwara S. Antibacterial activity of isoflavonoids isolated from Erythrina variegata against methicillinresistant Staphylococcus aureus. *Lett Appl Microbiol,* **2002**; 35: 494- 498.
4. Kirtikar, K R and Basu B D. Indian Medicinal Plants and edn Vol 1-4. *Periodical Experts Delhi*, **1935;** 420-423.
5. Kathiriya AK, kuntal D , Manan J, Nishith M. *Oxalis corniculata* Linn- The plant of Indian subtropics. *Herbal tech industry*, **2010**; 7.
6. Kumar A, Verma N, Saggoo M I S, Kaur G, Singh M. Phytochemical analysis and antimicrobial activity of crude extract of *Tribulus terrestris* fruits; a known medicinal weed. *Ad. Plant Sci,* **2009**; 22(1): 155-8.
7. Indian Pharmacopoeia - Third edition, followed by its addenda in **1989**; **1991; and 1985**.
8. Kokate, C.K. *Plant constituents in Practical Pharmacognosy*, **2001**; 107-110.
9. Mace,Gorbach, SL. Anaerobic bacteriology for clinical laboraties *pharmacognosy*, **1963**; 23: 89-91.
10. Rajasekariah, G R, Parab, P B, Chandrashekar R, Deshpande, L and Subrahmanyam D. Pattern of *Wuchereria bancrofti* microfilaraemia in young and adolescent school children in Bassein, India, an endemic area for lymphatic filariasis. *Ann. Trop. Med. Parasitol,* **1991;** 85(6): 663-665.
11. Evans, W C. An index of medicinal plants. *A Text book of Pharmacognosy 14 ed,* **1997**; 7(5): 12-14.
12. Mabry, T J, Markham, K R and Thomas M B. The systematic identification of flavonoids. *Springer – Verlag, Berlin-Heiderbelrg New York,* **1970;** 1-102.