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Phytochemical screening and biological potential of methanolic extract of *Oxalis corniculata* using different parts of plant

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Abstract

The aim of present study was to assess the different phytochemicals, the antioxidant activity and anti-microbial potential of different parts of oxalis corniculata viz. leaves, stem, roots and seeds. The presence of phytochemical constituents was checked by qualitative screening using different chemical tests indicated the occurrence of alkaloids, phenols, tannins, flavonoids, terpenoids and sulphates while carbohydrates, steroids and carbonates were absent. Quantitively, total content of phenol and total content of flavonoid methanolic extract was evaluated using method named Folin-Ciocalteau's and named aluminum trichloride method respectively. We concluded that leaves have highest flavonoids and phenolic content seeds whereas have least phenolic content and stem have lowest flavonoids content. Antioxidant activities of methanolic extracts of leaves, stems, roots and seeds of different plant parts were estimated using two methods from % age inhibition of DPPH and hydrogen peroxide we concluded that in both methods leaves had greater antioxidant activity and seeds had lesser antioxidant activity.Antibacterial potential was evaluated by Diffusion well method against two bacterial strains as Rhodococcus species and Pseudomonas species using ampicillin as a reference standard. By the calculation of zone of inhibition, concluded that leaves exhibited highest antibacterial effect and lowest by seeds. The results obtained from study indicate that leaves of oxalis corniculata have highest pharmacological applications for further study.

Keywords: *Oxalis corniculata*, phytochemical, antioxidant, antimicrobial.

Introduction

Medicinal plants are commonly known as "Chemical Goldmines" as they have natural phytochemical known as secondary metabolites, which are beneficially acceptable to human being and biological systems[1]. The plants are used to treat disease is almost worldwide among the peoples, and is more affordable than purchasing more expensive modern pharmaceuticals. Recently, the World Health Organisation (WHO) evaluates that approximately, 80% of the world's inhabitants currently depend on herbal medicine for primary health[2,3]. The total number of 2,50,000 higher plant species present on the earth, out of these more than 80,000 are medicinal[4]. Plants have been source of medicines throughout human history among ancient civilization. India is well suited for development of drugs from medicinal plants, because it is rich in unique medicinal plants, traditional knowledge and heritage of herbal medicines[5]. The properties of plants are due to presence of phytochemical compounds present in leaves, roots, flowers and seeds. These are extracted and used for production of human and veterinary medicines[6].

Regulars are increasingly avoiding meals equipped with preservation of chemical sources. Presently, the medical company and the patients have progressively started by means of plants to overcome an assortment of illness and suffering mainly to prevent the side effects encountered in usage of recent drugs[7]. For this reason, the significance and claim for natural antioxidants has grown over the recent years. Plants are potential source of natural antioxidants. Majority of the diseases or disorders are mainly linked due to excessive production of free radicals. Antioxidant constituents of plants are operating as scavenger of free radical and helps for conversion of free radicals to not as much of reactive species[8]. The desired response of biological activity is not due to one component but rather to a combination of bioactive secondary metabolites.

Oxalis corniculata belongs to the family oxildacea. The oxildaceae is the major group of Angiosperm having 8 generas. The genus oxalis have approximately 900 species which are mostly dispersed in temperate and sub-tropical regions of central South America including some other parts of world common in, China, Africa, Phillipines, warmer parts of Pakistan and India[9]. Among the medicinal plants reported *oxalis corniculata* have wide medicinal important used for curing of anthelmintic, styptic, astringent, diarrhea, dysentery, dysmenorrhoea, hepatitis, amenorrhoea and burning sensation, antimicrobial activity[10,11], recent studies reported to posses the antitumor activity, antiepileptic and anxiolytic activities[12].

Materials and methods

Collection of plant: The plant was obtained from the local tropical region of Patiala, Punjab, India during the month of

September and October. The plant *Oxalis* is easily growing and available in the tropical and subtropical regions. The plant species authenticated from Botany department of Punjabi University. The plant materials were dried under shade in the chemistry laboratory for usage of experiments.

Materials: DPPH and reagent named folin-ciocalteu's were purchased from Sigma Aldrich, Gallic acid from Avra synthesis and all were methanol, quercetin, copper sulphate, magnesium metal, alpha-naphthol, potassium iodide, mercuric chloride, iodine solution, picric acid, tannic acid, sodium hydroxide pallets, ferric chloride, lead acetate, acetic anhydride, Fehling solution A & B, benedict's reagent, potassium permagnate, sulphuric acid, hydrochloric acid, sodium carbonate, zinc dust, chloroform, sodium nitrite, aluminum trichloride, methanol, nutrient agar, ampicillin, trichloroacetic acid purchased from Loba chemie. These all chemicals with analytical grade are used.

The antibacterial species (*Pseudomonas and Rhodococcus*) collected from biotechnology department of Punjabi university Patiala. Leaves, roots, stems, seeds of *O.corniculata* were collected from Punjabi University Patiala.

Evaluation of moisture content: The evaluation of moisture content of leaves, stem, root and seeds was done by thermal drying method. In this method sample is placed in the oven at 105°C for 3 hours. The moisture content (MC) is calculated by expressing the weight loss upon drying as a fraction of the initial weight of the sample used. The formula is used for this is -

$$MC\ (\%) = (W_0/W_i) \times 100$$ [13]

W_0 = weight (gm) loss on drying, W_i= initial weight of sample (gm).

Method used for preparation of extracts: Extraction was carried out using methanol. The whole plant was thoroughly washed and dried under shade. The powdered sample was prepared each of leaves, stem, root and seeds differently with the help of mortar and pestle. Each powdered sample was soaked into the methanol which was used as a solvent in different conical flasks and allowed to stand for 10 days with occasional shaking.

After 10 days each solution of leaves, stem and seeds along with components were collected differently and filtered by using Whatman $N_{o.}1$ filter paper. Traces of solvent methanol from the extract were removed by keeping the extract on a water bath at low temperature. The dried extracts of different plant parts were preserved in the refrigerator in the air tight amber glass vials at low temperature for further use.

Phytochemical screening: The qualitative and quantitative analysis of plants extracts is crucial especially if there are some ethno medical claims on the plant.

Qualitative Analysis: The prepared extracts of different parts of plant were screened for the presence of Alkaloids, Flavanoids, Tannins, phenolic components, terpenoids, organic acids and Glycoside according to standard procedures of analysis and were identified using characteristic color changes.

Quantitative analysis: **Determination of Total Phenolic Content:** The phenols present in plants are act as highly effective free radical scavengers and antioxidants. The total phenolic content in methanol extracts of different plant parts was determined spectro- photometrically by using Folin-Ciocalteu method. The compounds of phenol transfer electrons in alkaline medium and other reduces the molybdenum, forming blue complex that can be detected spectrophotometrically at 760-765 nm. The FC reagent is not specific to phenolic compounds and will react with any reducing species.

$Mo(VI)(yellow) + e^-$ (from AD) $\rightarrow$ Mo(V)(blue), λmax at. = 765nm

Where, the oxidizing agent is a molybdophosphotungstic reagent, Mo (VI) is reduced to Mo (V) with an electron donated by an antioxidant represented as AD.

Determination of total flavonoids content: Quantitively total Flavonoids content is determined by Aluminum chloride's method. In this method Quercetin is used as a reference and total Flavonoids content is determined in terms quercetin equivalents at absorbance 510 nm. In this method 1.5 ml of plant extract is taken and 2.5 ml methanol and 65 µl of 3% $NaNO_2$ solution added. After span of time 6 min, volume 150 µl of 10% $AlCl_3$ .6 H_2O is added to the mixture solution, which is kept for 7 minutes at room temperature.

In the end, 1M solution of NaOH 0.5 ml added and total volume of solution was made up to 5 ml with the addition of water. Then solution is mixed well by shaking and immediately, the absorbance was measured at λmax - 380 nm on UV-Visible spectrophotometer. A standard calibration curve at different concentrations 100-500 µg/mL was prepared. The flavonoid content was expressed in terms of mg equivalents of quercetin (QE) per gram of extract.

Biological potential: Antioxidant Activity: Anti-oxidant is a substance which is prevents or delays oxidation of that substrate even in the presence of low concentration compared to that of an oxidizing substrate.

Hydrogen peroxide (H_2O_2) scavenging activity: The ability of the methanolic extracts of *O.corniculata* to scavenge hydrogen peroxide was evaluated by hydrogen peroxide. A solution of hydrogen peroxide (43mM) was prepared by using phosphate buffer (pH 7.4). Extracts of different parts of plant with various concentrations (5-25µg/mL) were added to a hydrogen peroxide solution (0.6 mL, 40mM). Absorbance was measured at 230 nm after 10 minutes against a blank solution containing the

phosphate buffer without extract using spectrophotometer. The percentage of hydrogen peroxide scavenging of extracts of different plant parts were calculated using following equation:
% age inhibition of $[H_2O_2] = [(A_0–A_1)/A_0] \times 100$ [14].

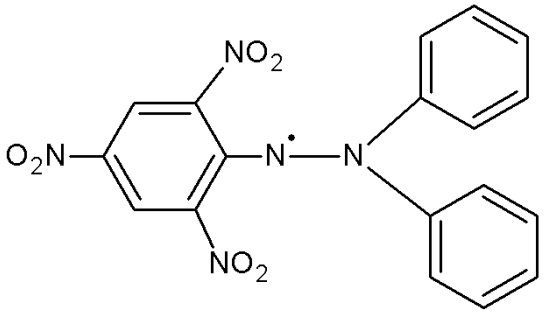
Where: A_0 represents the absorbance of the control and A_1 represents absorbance containing methanolic extracts of *O.corniculata*.

DPPH radical scavenging activity: The free radical scavenging activity of methanolic extract of different plant parts of *O.corniculata* were calculated *in vitro* using reduction of purple colored solution of DPPH. The methanolic extract of different plant parts at various concentration of serial solution (100 µl to 500 µl) to every test tube and made the volume up to 3 ml with addition of methanol and the reaction mixture well shakes and incubated for 15 min in the dark. The absorbance was recorded at 515nm using UV-Vis. spectrometer. Ascorbic acid prepared in methanol used as reference standard for the comparison of percentage inhibition.

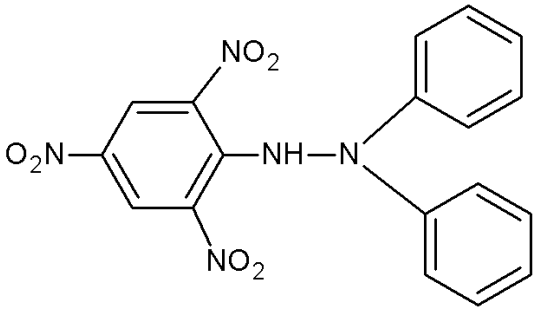
The scavenging activity was estimated based on the percentage of DPPH radical scavenged as the following equation:

%age inhibition = $[(A_0–A_1)/A_0] \times 100$[15]

Where: A_0 represents absorbance of the control and A_1 represent absorbance in the presence of the samples of methanolic extracts of *Oxalis corniculata.*



(a) Diphenylpicrylhyrdazyl (free radical)



(b) Diphenylpicrylhydrazine nonradical)

Figure-1: Structure of DPPH radical and nonradical.

Antimicrobial activity: The diffusion well method was used for estimation of antibacterial activity of methanolic extracts of different parts of plant. In this method, the petri plates filled with agar and then spread bacterial strains on surface of the filled agar in the Petri plates.

After the growth of bacterial strains antibiotic is applied to a number of wells on the plate as a standard solution. Different concentrations of plant extracts could be applied. Time is needed to allow the bacteria to grow. If there is a clearing around the plant extract, then the bacteria growth have been inhibited by the plant extract. The size of the inhibition zone can be calculated using verniar caliper and related to standards, in order to evaluate whether the bacterial strain is sensitive or not to the plant extract. The well diffusion technique allows bacteria to be screened in a practice, inexpensive and time saving style for the detection of plant extract resistance against bacterial strains.

Results and discussion

Description of plant: The plant identified from botany department estimated that, it is small, annual, tap rooted, bushy, the upper portion is ascending or weakly erect smooth or hairy somewhat appeared delicate. The plant estimated approximately 0.1-0.5 m long in length.

Moisture content: Different parts of plant were first studied for their moisture content as the moisture content is an important parameter for the extraction purposes.

Table-1: Moisture content of different parts of plant.

Sample	Leaves	Roots	Stem	Seeds
Initial weight in gram	13.12	11.30	11.89	9.60
Final weight in gram	2.0	2.8	2.64	2.5
Loss in weight	11.2	8.5	9.25	7.1
Moisture content	85.36	75.221	77.79	73.95

Phytochemical Screening: The qualitative phytochemical screening of the plants constituents were assessed by using different chemical tests. Study included the tests for alkaloids, phenolic acids, flavonoids, terpenoids, tannins, carbohydrates, inorganic acids and sterols.

Quantitative analysis of phytochemicals: Total phenolic content: Quantity estimation of total phenol content of methanolic extracts of different parts of plant articulated as Gallic acid equivalents (GAE) was calculated at different concentrations of Gallic acid from the calibration curve. The calibration curve of gallic acid measured at 765 nm was found to be linear with regression coefficient $R^2 = 0.994$ and line equation y =0.028 x as shown in Figure-2.

Table-2: Presence and absence of phytochemical constituents.

Tests Performed	Leaves	Stem	Seeds	Root
Tests for Alkaloids				
Mayer's Test	+	+	+	+
Wagner's Test	+	+	+	+
Hager's Test	+	+	+	+
Tannic acid Test	+	+	+	+
Tests for phenol compounds				
Ferric chloride ($FeCl_3$) Test	+	+	+	+
Alkaline reagent Test	+	+	+	+
Lead (Pb) Acetate Test	+	+	+	+
Tests for Flavonoids				
Shinoda's Test	+	+	+	+
Alkaline reagent Test	+	+	+	+
Zinc hydrochloric reduction Test	+	+	+	+
Tests for carbohydrates				
Molish's Test	-	-	-	-
Benedict's Test	-	-	-	-
Fehling's Test	-	-	-	-
Test for Terpenoids				
Libermann-burchard Test	+	+	+	+
Salkowski's Test	+	+	+	+
Tests for Tannins				
Ferric chloride Test	+	+	+	+
Lead acetate $(PbCH_3OO)_2$Test	+	+	+	+
Tests for sterols				
Sulphuric acid Test	+	+	+	+
Tests for Inorganic acids				
Sulphate Test	+	+	+	+
Carbonate Test	+	+	+	+

(+): presence of compounds (-): absence of compounds.

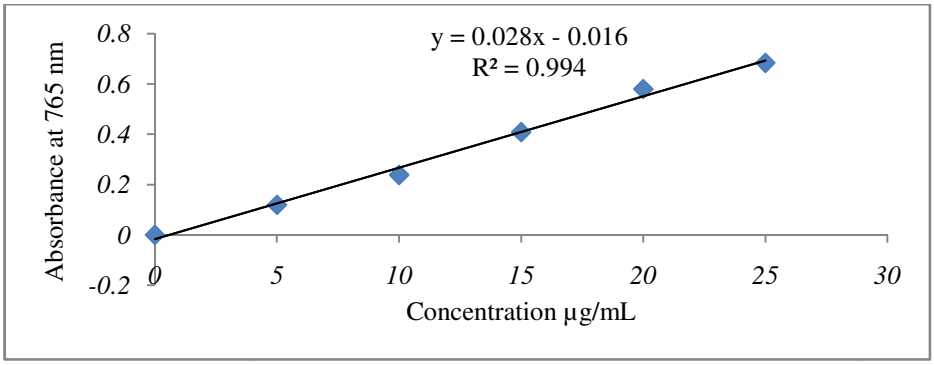


Figure-2: A Standard calibration curve of for Gallic acid at 765 nm.

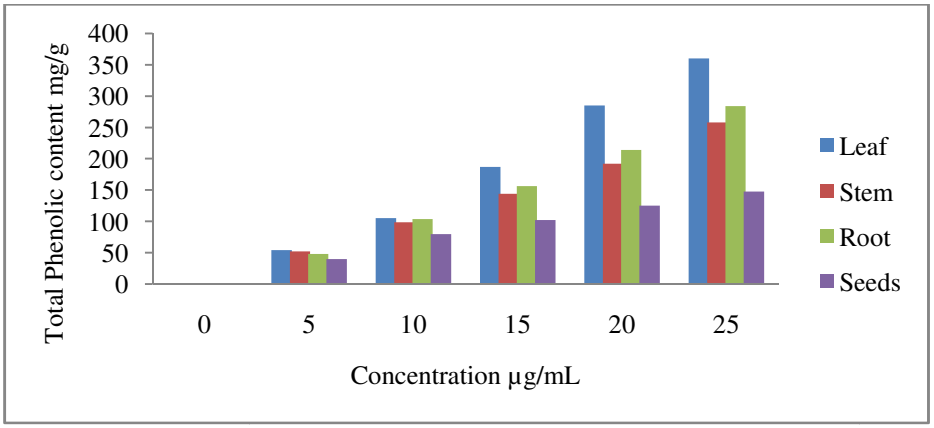


Figure-3: A comparative total phenol content of different parts of plant (root, stem, leaf, and seeds) as GAE.

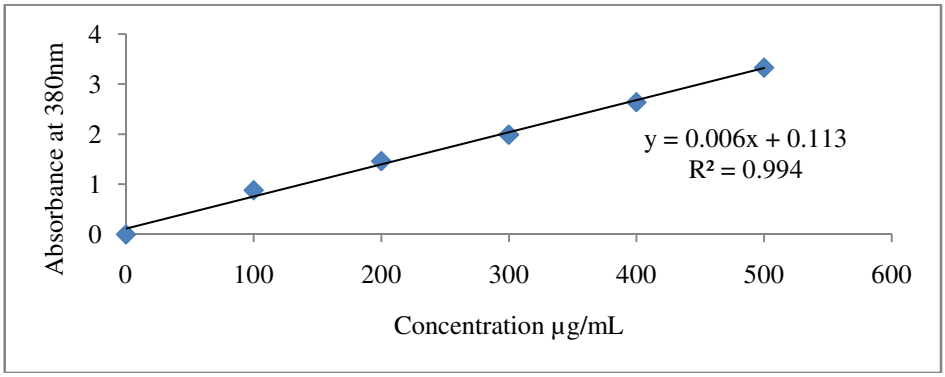


Figure-4: Standard calibration curve of for quercetin at 380 nm.

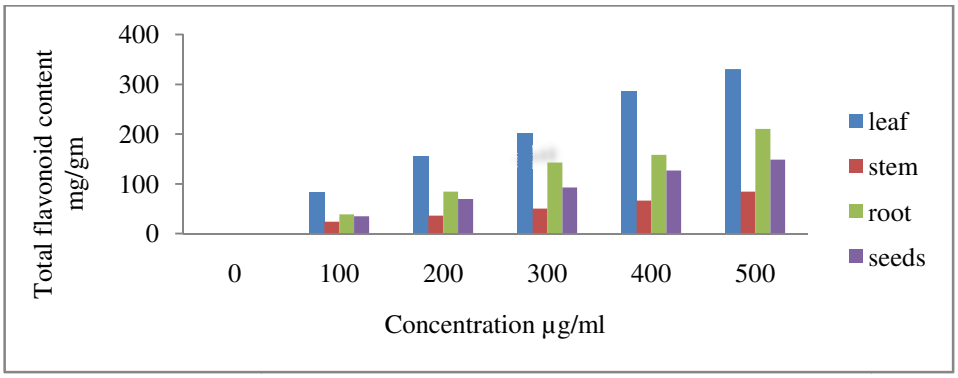


Figure-5: A comparative total Flavonoids content of different parts of plant (root, stem, leaf, and seeds) as quercetin equivalent.

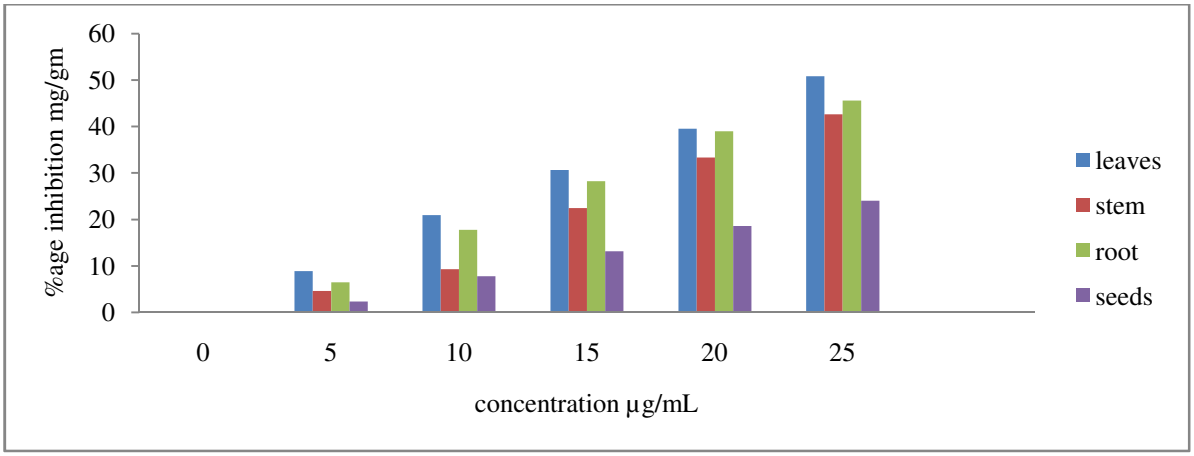


Figure-6: A graphical representation of Antioxidant Activity of different parts of plant (root, stem, leaf, and seeds) as %age inhibition of H_2O_2.

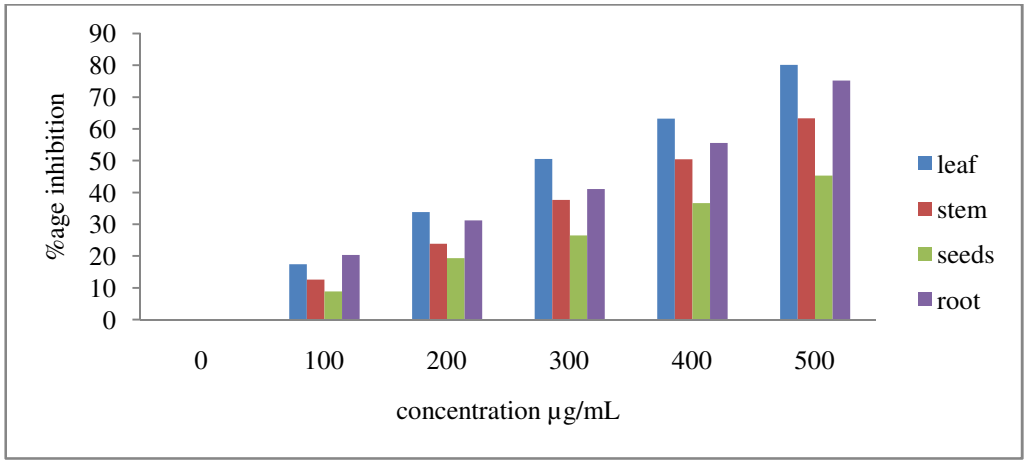


Figure-7: A comparative graphical representation total antioxidant activity of different parts of plant (root, stem, leaf, and seeds) as %age inhibition.

Antibacterial activity

Table-3: Shows the Zone of inhibition using methanolic extracts of different parts of plant *Oxalis corniculata* against different *Pseudomonas aeruginosa.* and *Rhodococcus fascians* bacterial strains. Ampicillin by taking as the control.

Sample	Pseudomonas sp.	Rhodococcus sp.
Ampicillin	37	37
Leaves	28	27
Stem	15	17
Root	25	22
Seeds	11	10

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 *in vitro* studies indicate that extracts of different parts of plant are good source of secondary metabolites which showed natural antioxidants and antibacterial activities. Therefore, further research need to be carried out to isolate and identify the important compounds present in the plant extracts.

References

1. Farhan H., Rammal H., Hijazi A., Hamad H. and Badran B. (2012). Phytochemical screening and extraction of polyphenol from stems and leaves of a Lebanese Euphorbia macrolada schyzoceras Boiss. *Ann Biol Res.,* 3(1), 149-156.

2. Eloff J.N. (1998). which extraction should be used for screening and isolation of antimicrobial componenets from plants?. *J.Ethnopharmacol.,* 60, 1-8.

3. Javed Hina, Erum Shazia, Tabassum Sobia and Ameen Farhana (2013). An overview on medicinal plant importance of *Thymus vulgaris. JASR.,* 3(10), 974-982.

4. Edeega H.O., Okwu D.E. and Mbaebie B.O. (2005). Phytochemical constituents of some Nigerian medicinal plant. *Afr.J.. biotechnol.,* 4(7), 685-688.

5. Ncube B., Finnie J.F. and Staden J.V. (2012). Quality from thefield: The impact of environmental factors as quality determinants in medicinal plants. *S.Afr. J. Bot.*, 82, 11-20.

6. Maity Pallab, Hansda Dhananjay, Bandyopadhyay Uday, Kumar Mishra Dipak (2009). Biological activities of crude extracts and chemical constituents of *Aegle marmelos. Indian J. Exp. Biol.,* 47, 849-861.

7. Gaikwad S.A., Kamble G.S., Devare S., Deshpande N.R., Salvekar J.P. (2011). In vitroevaluation of free radical scavenging potential of Cassia auriculata. *Journal of Chemical and Pharmaceutical Research*., 3(4), 766-772.

8. Devasagayam T.P.A., Tilak J.C., Boloor K.K., Ketaki Sane S., Ghaskadbi Saroj S. and Lele R.D. (2004). Free Radicals and Antioxidants in Human Health: Current Status and Future Prospects. *JAPI*, 52, 794-804.

9. Hall David W., Vandiver Vernon V. and Sellers Brent A. (1996). Creeping Wood Sorrel, Oxalis corniculata. Southern Yellow Wood Sorrel, Oxalis florida' *Salisb Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida*., 37, 01-02.

10. Kathiriya A., Das K., Kumar E.P. and Mathai K.B. (2010) Evaluation of antitumor and antioxidant activity of oxalis corniculata Linn. against Ehrich Ascites Carcinoma on Mice. *Iran J Cancer Prev.,* 3(4), 157-165.

11. Kumar Ashwani, Rani Niketa Sapna and Somiya Sagwal (2012). An Absolute Review on Oxalis corniculata. *Int J Pharma Bio Sci.,* 3(3), 1173-1188.

12. Sampath Sai T., Santosh P., Lahkar M., Ajaygodwin P., Kumar Pavan S. and Lingesh A. (2011). Anxiolytic Effect of Ethanolic Extract of Oxalis corniculata in Mice. *Inter J Pharm Bio Sci.*, 2(3), 281-90.

13. Singh Arvinder and Laishram Nomita (2010). Drying of Flowers and Other Ornamental Plant Parts in India. *Floriculture and Ornamental Biotechnology,* 4 (Special Issue 1)72-78

14. Borah A., Yadav R.N.S. and Unni B.G. (2012). Evaluation of antioxidant activity of different solvent extracts of Oxalis corniculata. *J Pharm Res.,* 5(1), 91-93.

15. Kumar S., Pooja M., Harika K., Haswitha E., Nagabhushanamma G. and Vidyavathi N. (2013). In-Vitro antioxidant activities, total phenolic and flavonoids contents of whole plant of *Hemidesmus indicus*. *Asian J Pharm Clin Res,* 6(2), 249-251.