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ANTIOXIDANT AND HEMOLYTIC QUANTIFICATION OF *THEVETIA* FLOWERS IN DIFFERENT EXTRACTS

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ABSTRACT

Aims: *The aim of this study is to quantify antioxidant and hemolytic activity of Cascabela thevetia flowers. For that, flower part of the plant was studied.* ***Methods:*** *Flowers were collected, air dried and subjected to extraction with acetone and Ethyl acetate. Extract was assessed for Phenolic and flavonoid contents. Quantification of antioxidant and hemolytic property were tested by using DPPH radical, Nitric Oxide radical and Superoxide radical assay, hemolytic activity assay with different concentrations and compared with the standard Ascorbic acid, Quercetin respectively.* ***Result and conclusion****: IC50 values for three different assays of Ethyl acetate extract for flower were found to be 90.52 µg/ml, 63.45 µg/ml and 60.41µg/ml. IC50 values from Acetone extract for flower were found to be 59.60µg/ml, 45.10µg/ml and 36.98µg/ml respectively. IC50 value for hemolytic activity of Ethyl acetate extract shown to be 78.31µg/ml and acetone extract was 50.97µg/ml. This study concluded flower extract possess the good antioxidant properties and has shown least amount of hemolysis. This observation suggests that acetone extract exhibit great potential antioxidant property and inhibit hemolytic activity, thus can be useful to treat and control many diseases.*

KEY WORDS

Ethyl acetate, Acetone, phenolics, flavonoid, Antioxidants, Hemolysis.

INTRODUCTION

Medicinal plants are of great importance to the health of individuals and communities. The medicinal plant products, which are derived from plant parts such as stem bark, leaves, fruits and seeds have been part of phytomedicine that produce a definite physiological action on the human body. The most important of these natural bioactive constituents of plants are alkaloids, tannins, flavonoid and Phenolic compounds [1]. A number of reactive molecules are generated through various biological redox reactions such as superoxide radical (O_2 -), nitric oxide (NO.) and DPPH (2, 2-diphenyl-1-picrylhydrazyl) which can directly react with biological macromolecules such as proteins, lipids and DNA of healthy human cells and cause cell membrane disintegration, DNA mutation and protein damage and can further create cancer, atherosclerosis, cardiovascular disease, liver injury, ageing and inflammatory disease. [1].

Antioxidants in biological systems have multiple functions, including depending against oxidative damage and in the major signaling pathways of cells. The major action of antioxidants in cells is to prevent damage caused by the action of reactive oxygen species (ROS). ROS such as superoxide radical (O_2), hydroxyl radical (OH), peroxide radical (ROO·) and nitric acid radical are generated in living organisms during excessive metabolism1 and involved in extensive oxidative damage to the cells that leads to age related degenerative diseases, cancer and wide range of other human diseases [2].

In vitro hemolytic activities are becoming a new area of research in drug lead discovery. Researchers are exploring ethno botanically important plants to find out

potential natural products with antiaggregant action. These studies are important because some patients have become resistant to the already existing drug e.g. aspirin and/or conventional medication (warfarin, aspirin) in association with medicinal plant formulations. This is posing a serious problem to the society. Moreover, the constant use of synthetic drugs is leading the society to face great danger. In recent years, many antiplatelet aggregating agents have been isolated from plants and have demonstrated potent activity [3].

Cascabela thevetia belongs to the family Apocynaceae, commonly known as Indian oleander. It is an evergreen tropical shrub. Its leaves are willow like in appearance. They are covered in waxy coating to reduce water loss. This plant contains a milky sap containing a compound called thevetin that is used as a heart stimulant. The roots and leaves are used in Vata, pitta, cough and bronchitis, renal and skin diseases due to spider bite. This plant is also used for snake bite treatment.

Most of the plants could not have appropriate studies of their components, which could have biotechnological applications [4]. Hence, the focus of this study was to quantify flower extract of *Cascabela thevetia* for the presence of antioxidant and hemolytic activity though the mentioned plant is an ethno medicinally important plant, no scientific work has been done yet on its bioactivities.

MATERIAL AND METHODS

The present study was completed at the department of Biochemistry, Shri Shivaji college of Arts, Commerce and Science, Akola.

Collection of Plant material

The plant material was collected from nearby local area of Shegaon tahsil of Buldhana district. Collected flowers were washed and air dried to complete removal of soil from it. Materials were also dried in incubator at 40 ^{0}C. Dried samples were ground into a uniform powder using a blender and stored in plastic bottles at 4 ^{0}C for future use in experiment.

Extraction of plant parts

Extracts prepared with different solvents according to their polarities by Soxhlet apparatus at elevated temperature. The extraction was carried out separately for 24 hrs for each solvent. Dried extracts were kept in the refrigerator at 4 ^{0}C for future uses.

Estimation of Total Phenolic content

The total phenolic content in the extracts were determined with Folin-ciocalteu reagent by using UV-Spectrophotometer technique. 0.2 ml sample extract were mixed with 1.0 ml of 10% (v/v) Folin-ciocalteu reagent and was vortex for 3 min followed by addition of 0.8 ml of 7.5% (w/v) Sodium carbonate. This reaction mixture was incubated for 30 min at room temperature. The absorbance was measured at 765 nm. Same procedure was carried out for Gallic acid standard curve and results were expressed as mg Gallic acid equivalent/ ml of extract [5].

Estimation of Total Flavonoid Content

The amount of total flavonoid in the extracts was measured spectrophotometrically as previously reported. Briefly, 500 µl of each extract was mixed with 1.50 ml of 95% ethanol, 0.10 ml of 10% aluminium chloride ($AlCl_3.6H_2O$), 0.10 ml of sodium acetate ($NaC_2H_3O_2.3H_2O$) (1M) and 2.80 ml of distilled water. After incubation for 40 min, absorbance was measured at 415 nm using a spectrophotometer. To calculate the concentration of flavonoid, a calibration curve was prepared using Quercetin as standard. The flavonoid concentration is expressed as Quercetin equivalents in mg per ml of extract. All assays were carried out in triplicate [6].

Analysis of Antioxidant activity

Analysis of Antioxidant activity was done by different methods such as DPPH assay, Nitric oxide radical assay, Superoxide radical assay using UV-VIS spectrophotometer technique.

DPPH Radical assay:

0.5 ml fractions of the solution (10, 20, 40, 60, 80, 100, 200, and 400 µg/ml in ethanol) was added to 0.5 ml of a DPPH solution (0.1mM in ethanol). After a 30 min of reaction at room temperature, the absorbance of the solution was measured at 517 nm. The free radical scavenging activity of each fraction was determined by comparing its absorbance with that of a blank solution (no sample). Ascorbic acid was used as standard. IC50 values denote the concentration of sample required to scavenge 50% of DPPH radical. The ability to scavenge the DPPH radical was calculated using the following equation:

$$\textbf{DPPH scavenging activity (\%)} = [(A_c - A_t) / Ac] \times 100$$

Where A_c is the absorbance of the control and A_t is the absorbance of test sample. [7, 8]

Nitric Oxide radical assay:

The assay is the nitric oxide radical scavenging assay. The extracts were prepared from a 10 mg/mL ethanol crude extract. These were then serially diluted with ethanol to make concentrations from 10−400 µg/ml of the plants and the standard Ascorbic acid. Griess reagent was prepared by mixing equal amounts of 1% sulphanilamide in 2.5% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride in 2.5% phosphoric acid immediately before use. A volume of 0.5 ml of 10 mM sodium nitroprusside in phosphate buffered saline was mixed with 1 ml of the different concentrations of the ethanol extracts (10−400 µg/ml) and incubated at 25°C for 180 mins. The extract was mixed with an equal volume of freshly prepared Griess reagent. Control samples without the extracts but with an equal volume of buffer were prepared in a similar manner as was done for the test samples. The colour tubes contained ethanol extracts at the same concentrations with no sodium nitroprusside. The absorbance was measured at 546 nm using UV-Vis spectrophotometer. Ascorbic acid was used as the positive control. The percentage inhibition of the extract and standard was calculated and recorded. IC50 values denote the concentration of sample required to scavenge 50% of Nitric Oxide radical. The percentage nitrite radical scavenging activity of the ethanol extracts and Ascorbic acid were calculated using the following formula:

Nitric oxide scavenging Activity % = (Abs of Control – Abs of Sample/Abs of Control) × 100

[9].

Superoxide radical assay:

The scavenging activity of the extract on superoxide radicals was determined by the pyrogallic acid method. Volumes of 4.5 ml Tris-HCl buffer (0.05 mol, pH 8.2), 1 ml of sample solution, and 0.4 ml pyrogallic acid (3.0 mM) were added together; the solution was incubated at 25°C for 15 minutes, 0.5 mL of thick hydrochloric acid was added for termination the reaction, and it was determined at 525 nm. Ascorbic acid was used as the positive control. IC50 values denote the concentration of sample required to scavenge 50% of superoxide.

Superoxide radical Scavenging activity (%) = {(Ao − Ai)/Ao} x 100

Where, Ao is the absorbance without sample and Ai the absorbance with sample [10].

Analysis of hemolytic effect

Preparation of erythrocytes suspension:

Five milliliters of blood was collected from a healthy individual in EDTA vacutainer. The blood was centrifuged at 3000 rpm for 10 minutes in a laboratory centrifuge. Plasma (supernatant) was discarded and the pellet was washed 3-4 times with sterile phosphate buffer saline solution (pH 7.2±0.2) by centrifugation at 3000 rpm for 10 min. The cells were resuspended in 0.9 % normal saline.

Hemolytic activity assay:

In vitro hemolytic activity was performed by spectrophotometer method with some modifications. A volume of 0.5 ml of the cell suspension was mixed with 0.5 ml of the plant extracts (10, 50, 100, 200 and 250 µg/ml concentrations in phosphate buffer saline). The mixtures were incubated for 30 min at 37˚C in an incubator. The mixture was centrifuged at 3000 rpm for 10 min in a laboratory centrifuge. The free hemoglobin in the supernatant was measured in UV-Vis spectrophotometer at 540 nm. Phosphate buffer saline and distilled water were used as minimal and maximal hemolytic controls. Each experiment was performed in triplicates at each concentration. The level of percentage hemolysis by the extracts was calculated according to the following formula:

% Hemolysis = 100 - (Sample/ Control) × 100

Statistical Analysis:

All tests were conducted in triplicate. Data are reported as means ± standard deviation (SD). Results were analyzed statically by using Microsoft Excel 2007 [11, 12].

RESULT AND DISCUSSION

Natural products have been shown to be a tremendous and consistent resource for the development of new drugs. Sometimes plant derived natural compounds have gained attention because of their potential to act as cytotoxic and chemopreventive activity. Various plants have already been proved to possess high antioxidant property containing high amounts of phenolics and flavonoid [3].

Obtained mean values of the Phenolic and flavonoid content of *Cascabela thevetia* Flower (EA) are 0.164mg/ml, 0.185mg/ml respectively and *Cascabela thevetia* Flower (A) are 0.176 mg/ml and 0.198mg/ml respectively. Phenolics and flavonoids have shown to be high concentration in acetone extract than ethyl acetate

extract (Table No.1). Calibration curve for standard Gallic acid and Quercetin have been shown graphically (Figure No.1 and 2).

IC_{50} value represents the concentration of test extract or compound where the inhibition of test activity reached 50%. IC50 values for DPPH radical, Nitric Oxide radical and Superoxide radical of Ethyl acetate extract showed higher potential as compare to Acetone. Comparative IC50 values of standard Ascorbic acid for DPPH radical assay, Nitric Oxide radical assay and Superoxide radical assay are 8.03µg/ml, 13.29µg/ml and 9.93µg/ml respectively. Both the flower extracts showed IC50 values higher than standard. Data are summarized in the Table no 2 and Figure no. 3.

Table No 1: Concentration of Phenolic and Flavonoid contents in mg/ml

Contents	STD	CTF (EA)	CTF (A)
Phenolic	0.2	0.164	0.176
Flavonoid	0.2	0.185	0.198

STD = Standard, CTF (EA) = *Cascabela thevetia* Flowers in Ethyl acetate
CTF (A) = *Cascabela thevetia* Flowers in Acetone

Figure no.1: Calibration plot for Total phenolics determination:

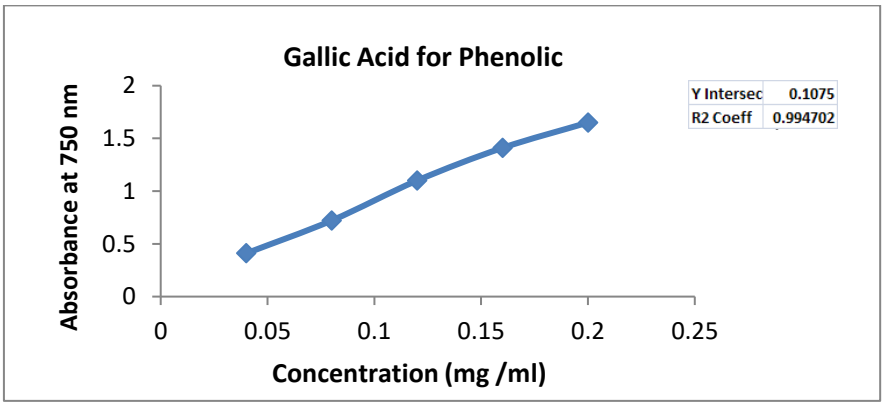


Figure No 2: Calibration plot for Total flavonoid determination:

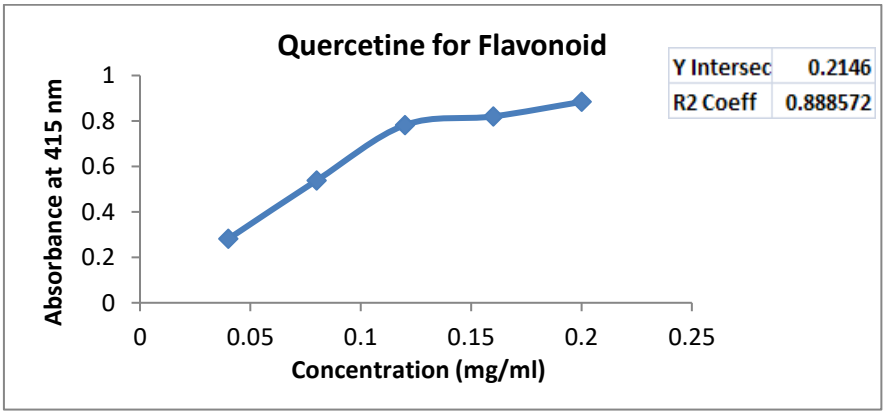


Figure No.3: Comparison of Antioxidant activity of flower extracts

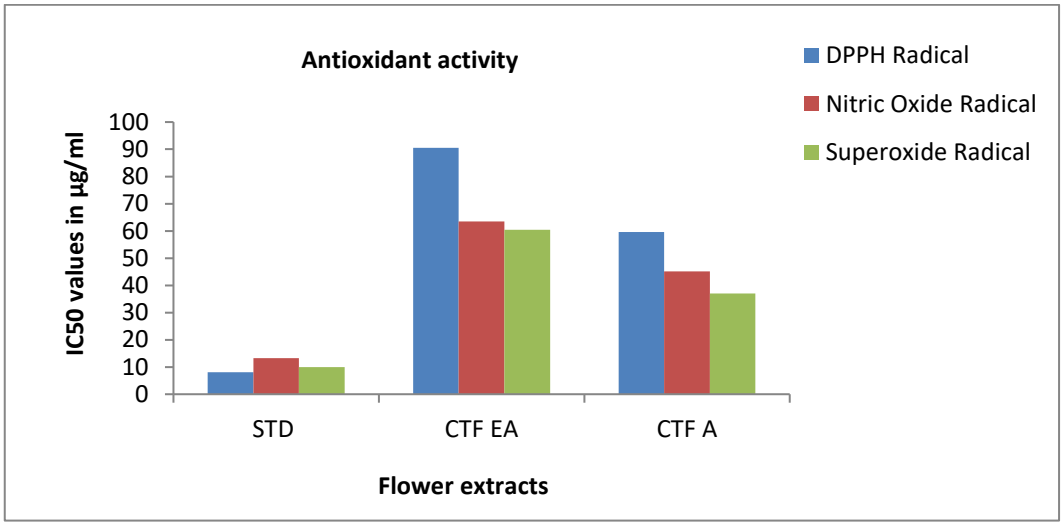


Table No.2: IC50 values of three different antioxidant assays in µg/ml

ASSAY	STD	CTF(EA)	CTF(A)
DPPH Radical	8.03	90.52	59.6
Nitric Oxide Radical	13.29	63.45	45.1
Superoxide Radical	9.93	60.41	36.98

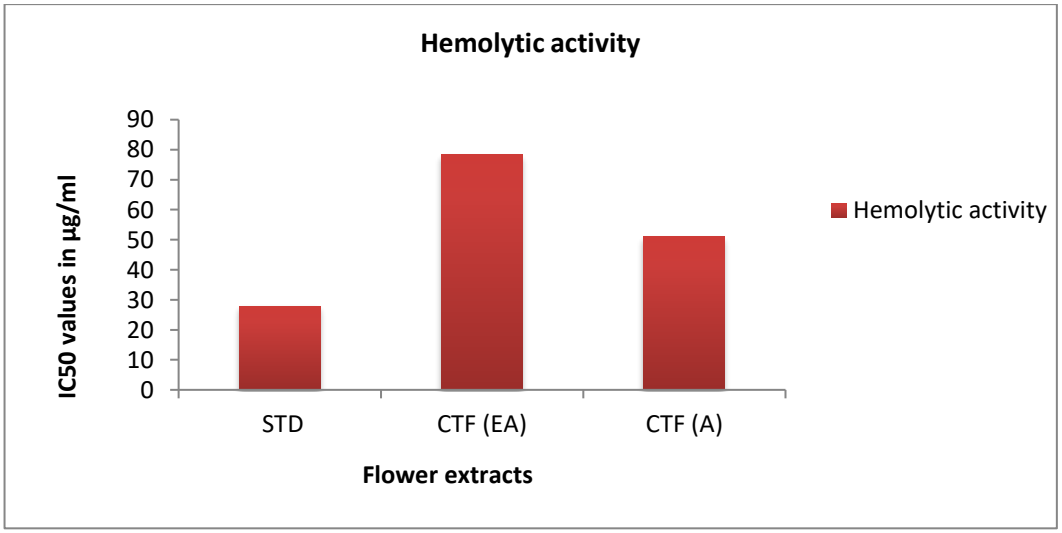
STD = Standard, CTF (EA) = *Cascabela thevetia* Flowers in Ethyl acetate
CTF (A) = *Cascabela thevetia* Flowers in Acetone

Table No. 3: IC_{50} values for Hemolytic activity in µg/ml

ASSAY	STD	CTF (EA)	CTF (A)
Hemolytic activity	27.92	78.31	50.97

STD = Standard, CTF (EA) = *Cascabela thevetia* Flowers in Ethyl acetate
CTF (A) = *Cascabela thevetia* Flowers in Acetone

Figure No. 4: Comparison of Hemolytic activity in Flower extracts



***Cascabela thevetia* flowers**

***Cascabela thevetia* leaves**

Ethyl acetate extract showed higher inhibitory concentration than acetone extracts, but both the extracts showed a dose dependent increase in activity.

The chemical nature of the active principles responsible for antioxidant activity is not known. However, preliminary phytochemical screening has confirmed the presence of flavonoids, ascorbic acid which might be responsible for such activity [13].

IC_{50} values for Hemolytic assay of Ethyl acetate and Acetone extract are expressed in the table no. 3 in comparison of standard Quercetin. Ethyl acetate extract was shown to be high inhibitory concentration as compared to acetone extract. Thus, it exhibits low hemolytic activity.

Measuring hemolytic activity is important as it is an indicator for cytotoxicities. The *in vitro* hemolysis test has also been employed by many different groups for the toxicological evaluation of different plants. Mechanical stability of erythrocytes membrane is good indicator of various in-vitro cytotoxicities. Performing hemolytic assay is important to determine whether a drug possessing antioxidant and other bioactivities can be used in pharmacological applications [3].

CONCLUSION

The result of this study concluded that flower extracts of ethyl acetate and acetone quantifies strong antioxidant activity. Obtained IC50 values for *Cascabela thevetia flower* extracts and Ascorbic acid indicates that these extracts possess efficiency to neutralize free radicals higher than Ascorbic acid. It also shows that flower extracts are less toxic to the human erythrocytes. With the help of obtained results, study revealed that *Cascabela thevetia* flowers can be used as a good source of health promoting antioxidants and pharmaceutical products. Further studies of the plant flower by extracting with different solvents in evaluating the antioxidant and low hemolytic potential, both in vitro and in vivo may be undertaken.

REFERENCES

1. Dipankar Chaudhari *et al*, 2012, Phytochemical analysis and evaluation of antioxidant and free radical scavenging activity of *Withinia somnifera* root. *Asian Journal of Pharmaceutical and clinical research*, Vol.5, suppl 4, 193-199.
2. Kannan R. Ragupathi Raja *et al*, 2010. Thin layer chromatography analysis of antioxidant constituents from seagrasses of Gulf of Mannar Biosphere Reserve, South India. *International Journal of ChemTech Research*, Vol.2, No.3, pp 1526-1530.
3. Amrita Mukherjee et al, 2010, In vitro hemolytic activity of Allium steracheyi Baker. Journal of Pharmacy Research 3 (5): 1160-1162.
4. Caramori *et al*, 2004, Biochemical characterization of selected plant species from Brazilian Savannas. *An International Journal, Brazilian archives of Biology and Technology*. Vol.47, n.2: pp. 253-259, June 2004.
5. Kesin-Sasic *et al*., 2012. Total phenolic content and antioxidant capacity of fruit juices. Bulletin of the Chemists and Technologists of Bosnai and Herzegovina, 39, 25-28.
6. Anelise Samara Nazari Formagio *et al.,* 2014. Evaluation of Antioxidant Activity, Total Flavonoids, Tannins and Phenolic Compounds in *Psychotria* Leaf Extracts. Antioxidants (Basel). 2014 Dec; 3(4): 745–757 Published online 2014 Nov 10. doi: 10.3390/antiox3040745.
7. Charles Lekhya Priya *et al.,* 2010. Antioxidant activity of *Achranthus aspera* Linn stem extracts. Pharmacologyonline 2: 228-237.
8. Shisode K. S. and B.M. Kareppa, 2011. In vitro antioxidant activity and phytochemical studies of *Boerhaavia diffusa* linn roots, ISPSR Vol.2 (12):3171-3176. Agatonovic-Kustrin *et al.,* 2015. Thin-Layer Chromatography - Bioassay as Powerful Tool for Rapid Identification of Bioactive Components in Botanical Extracts. *Mod Chem appl*, 3:1,
9. Fadzai Boora, Elaine Chirisa, and Stanley Mukanganyama, 2014. Evaluation of Nitrite Radical

Scavenging Properties of Selected Zimbabwean Plant Extracts and Their Phytoconstituents. *Journal of Food Processing,* Article ID 918018, 7 pages.

10. Chang Jian Liuab, Qiu Liub Junde Suna, Bo Jiangb Jianfang Yanb 2014. Extraction of water-soluble polysaccharide and the antioxidant activity from *Semen cassia*. *Journal of Food and Drug Analysis*, Volume 22, Issue 4, Pages 492-499.
11. Bhaskara Rao *et al.,* 2011. Haemolytic activity of Indian medicinal plants toward human erythrocytes: an in vitro study. *Elixir Appl. Botany,* 40 (2011) 5534-5537.
12. Shabbir Maria *et al.,* 2013. Assessment of phytochemicals, antioxidant, anti-lipid peroxidation and anti-hemolytic activity of extract and various fractions of *Maytenus royleanus* leaves. BMC Complementary and Alternative Medicine, 2013, 13:143.
13. D.K. Pal *et al*, 2008, A study on the antioxidant activity of *Semecarpus anacardium* L. F. nuts. Vol.8/2, 160-163.

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