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In vitro Anti-inflammatory and Antioxidant Potential of *Calotropis procera* (Aiton) Dryand Flowers

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Abstract

The plant *Calotropis procera* (Aiton) Dryand belongs to plant family Asclepiadaceae is a deciduous wild shrub. The present study aimed to evaluate the *in vitro* antioxidant and anti-inflammatory activities of metabolic extract of flowers *Calotropis procera* (Aiton) Dryand. The previous result indicates the presence of a varied group of phytochemicals such as alkaloids, Anthraquinones, Carbohydrates, Flavonoids, Glycosides, Phenols, Terpenes, Saponins. *Calotropis procera* in water extract showed the percentage of DPPH radical scavenging activity (73.29±2.020) as compared to standard Ascorbic acid (93.94 ±0.63). The Photometric in vitro antioxidant activity of *Calotropis procera* flower extracts in non-polar solvent (Hexane) was shown highest OH radical scavenging activity (62.22 ±2.020) as compared with standard ascorbic acid (57.77±0.73). The photometric in vitro anti-inflammation activity by protein denaturation of *Calotropis procera* flower extracts in Water solvent was shown to have the highest activity (87.17±1.020) as compared to standard Diclofenac sodium (92.30 ± 0.47).

The plant *Calotropis procera* is a perennial shrub, grown up to 5-6 ft in height, woody xerophytes plant. Stem become aerial, cylindrical, hairy (tomentose) exudates milky latex. Leaves are with opposite decussate phyllotaxy, auriculate, simple, thick and glaucous. Flowers are arranged on the stem axis as polychasial cymes of inflorescence. Flower is bracteates, actinomorphic, bisexual, dichlamydeous, and white with purple blotch

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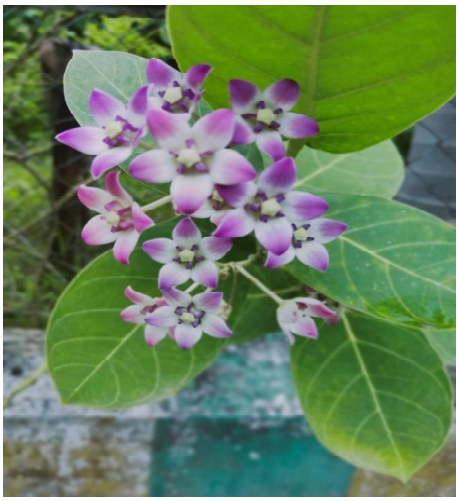

Fig. *Calotropis procera* (Aiton) Dryand

Classification
Kingdom : Plantae
Division : Magnoliophyta
Class : Magnoliopsida
Order : Gentianales
Family : Asclepiadaceae
Genus : *Calotropis*
Species : *C. procera* (Aiton) Dryand

on erect lobes[14]. The impact of herbal medicine has become most popular in ancient as well as recent years as compared to allopathic medicine. In Ayurveda, plant *Calotropis procera* (Aiton) Dryand a tropical plant reported for its medicinal importance against several infectious diseases[4]. The plant *Calotropis procera* contains phytochemicals viz., Alkaloids, Flavonoids, Carbohydrates, Phenols, Saponins, Terpenoids, and Tannins as secondary metabolites effectively used as pesticides[2]. Ethanolic extract of *Calotropis procera* flower showed antimicrobial activity against gram positive and gram negative human pathogenic bacteria[10]. Root extract in chloroform solvent of *Calotropis procera* possesses anti-inflammatory activity[3].

Plant material:

Healthy flowers of plant *Calotropis procera* were collected from different locations of Kinwat forest Nanded (MS) during their flowering season, Plant was authenticated and identified by Dr. Pund M. M (Asst. prof. & Head Dept. of Botany, Indira Gandhi Sr. College Nanded MS) with the help of Flora of Marathwada[8]. The voucher specimen of *Calotropis procera* (Aiton) Dryand (IGM 11) was deposited in the Herbarium Centre of Department of Botany, Indira Gandhi Sr. College, Nanded (MS).

Extraction of Material:

Flowers were shaded dried and coarsely grind to fine powder using blender (Bosch Pro 1000W Mixer). 15 gm of powder used for extraction in polar and non-polar solvent, crude extract was concentrated in oven and evaporated and stored in deep freezer at 4^{o}C for further experimental use[15].

Percentage Yield :

Powdered form of flower was extracted in Soxhlet apparatus in polar solvent

such as water and methanol and non-polar solvent such as Chloroform and Hexane, the extraction yield of flower was measured. The obtained crude extract of the flower was transferred in the Eppendorf tube and stored at 4°C in the refrigerator for future use. The percentage yield of extract was determined with the formula Percentage Yield= Final weight of extract/Initial dry weight of sample X 100 (Table-1).

Antioxidant activity :
DPPH radical scavenging activity :

The molecule 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity was carried out as per standard reported method and moderate modification Roesler[11] and slightly modified Shaikh[13] and Adole[1]. Briefly, 1ml of test solution (flower extract) isolated compounds was added to equal quantity of 0.1 mM solution of DPPH in ethanol. After 30 min of incubation at room temperature, measurement of absorbance was carried out at 517 nm by using UV-Vis Spectrophotometer. Ascorbic acid (1mM) was used as a reference compound.
The percentage of scavenging activity was derived using the following formula,

Percentage of inhibition (%) = [(A control – A sample) / A control] x 100
Where, A control - absorbance of DPPH

A sample - absorbance reaction mixture (DPPH with Sample)

Hydroxyl Radical Activity :

The OH radicals scavenging activity carried out as per standard reported method demonstrated by Rollet[12]. The reaction mixture contained, 60 µl of $FeCl_2$ (1mM), 90 µl of 1-10 phenanthroline (1mM) 2.4ml of phosphate buffer (0.2M, & 7.8 pH) 150 µl of hydrogen peroxide (0.17M) and 1.5 ml of flower extract (1mg/ml). The reaction was begun by adding hydrogen peroxide after 5 min. incubation at room temperature, measurement of absorbance was carried out at 560 nm by using UV-Vis Spectrophotometer. Ascorbic acid (1mM) was used as a reference compound. The percentage scavenging effect will be calculated as :
Scavenging activity = A_0 control – A_1 test / A_0 control x 100 Where A_0 is absorbance of the control (without extract), A_1 is the absorbance in the presence of the extract.

Anti-Inflammatory Activity :

The *in vitro* anti-inflammatory activity by protein denaturation was carried out by earlier method described by Elias and Rao and slightly modified by Padmanabhan and Jangle standard protocol[6,9]. The reaction mixture (10 mL) consisted of 0.4 mL of egg albumin (from fresh hen's egg), 5.6 mL of phosphate buffered saline (PBS, pH 6.4) and 4 mL of herbal extract formulation (1000µg/ml). Similar volume of double-distilled water served as control. Then the mixtures were incubated at (37°C ±2) in an incubator for 15 min and then heated at 70°C for 5 min. After cooling, their absorbance was measured at 660 nm by using the vehicle as blank. Diclofenac sodium at concentration 1000 µg/ml) was used as a reference drug and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula,

% inhibition=absorbance of control-absorbance of test / absorbance of control x 100

Statistical Analysis :

All results have been evaluated as means ± Standard deviation. ANOVA was used for evaluating differences between means[7].

The percentage yield of flower *Calotropis procera* in different solvent arranged with their order of percentage yield of flower in different solvent viz., methanol > water > chloroform > hexane with value 17.86 %>17.26 %> 08 % > and 07.66% respectively.

Table-1. Percentage yield of flower *Calotropis procera* in different solvents

Sr. No.	Name of Plants	Solvents	The yield of Extract (mg/gm)	Percentage of yield
1	*Calotropis procera* (Aiton) Dryand	Water	2.59	17.26
		Methanol	2.68	17.86
		Chloroform	1.20	08.00
		Hexane	1.15	07.66

* indicates experiment performed in triplicates.

Antioxidant Activity :
DPPH Radical Scavenging Activity :

The Photometric in vitro antioxidant activity of *Calotropis procera* flower extracts in polar solvent (water) was shown highest DPPH antioxidant activity (73.29±2.020) followed by hexane (66.77±1.980) whereas, chloroform and methanol extract of flower shown same antioxidant activity (55.59±1.980). Percentage of DPPH radical scavenging activity of flower in water (73.29±2.020) extracts a good antioxidant activity as compared to standard Ascorbic acid (93.94 ±0.63) (Table-2).

Table-2. DPPH radical scavenging activity (%) of *Calotropis procera* in different solvent

Sr. No.	Name of plant	DPPH radical scavenging activity (%) of plant sample in different solvents				
		Water	Methanol	Chloroform	Hexane	Ascorbic acid (Std.)
1	*Calotropis procera* (Aiton) Dryand.	73.29±2.020	55.59±1.980	55.59±1.980	66.77±1.980	93.94 ±0.63

The results presented here are the mean values from three independent experiments ± S.D.

Table-3. OH radical scavenging activity in different solvents

Sr. No.	Name of plant	OH radical scavenging activity (%) of plant samples in different solvents				
		Water	Methanol	Chloroform	Hexane	Ascorbic acid (Std.)
01	*Calotropis procera* (Aiton) Dryand.	37.11±2.020	NR	51.11 ±1.980	62.22±2.020	57.77±0.73

The results presented here are the mean values from three independent experiments ± S.D.

Table-4. Protein denaturation activity of flowers in different solvent

Sr. No.	Name of plant	Inhibition of Protein Denaturation by Using Colorimeter			
		Water	Methanol	Chloroform	Hexane
1	*Calotropis procera* (Aiton) Dryand	87.17±1.020	79.48±2.020	82.05±2.020	76.92±2.020
2	Diclofenac Sodium	92.30 ± 0.47			

The results presented here are the mean values from three independent experiments ± S.D.

Hydroxyl radical activity :

The Photometric in vitro antioxidant activity of *Calotropis procera* flower extracts in non-polar solvent (Hexane) was shown highest OH radical scavenging activity (62.22 ±2.020) as compared with standard ascorbic acid (57.77±0.73) followed by chloroform (51.11 ±1.980) whereas, water extract of flower shown (37.11 ±2.020). Percentage of OH radical scavenging activity. Flower in methanol extract do not show any OH radical scavenging activity. (Table-3).

Anti-Inflammatory Activity :

The photometric *in vitro* anti-inflammation activity by protein denaturation of *Calotropis procera* flower extracts in Water solvent was shown highest activity (87.17±1.020) followed by Chloroform (82.05±2.020) whereas, methanol and chloroform extract of flower shown (79.48±2.020) and (76.92±2.020)Percentage of protein denaturation activity as compared to standard Diclofenac sodium (92.30 ± 0.47).

The current research article on *Calotropis procera* with their qualitative and quantitative analysis for antioxidant and anti - inflammatory activity indicates that presence of active biomolecules exhibiting active molecules, in the form of enzymes, carbohydrates, proteins, secondary metabolites and phytocompound can be explore as therapeutics

medicinal product for treating various traditional diseases as well as very promising opportunity in recombinant r-DNA technology[5].

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References :

1. Adole, V. A., R. A. More, R. A. Shinde, S. L. Dhonnar, B. S. Jagdale, S. G. Shinde, ... and T. B. Pawar, (2021). *Vietnam Journal of Chemistry, 59*(5): 689-700.
2. Azmathullah, N. M., M. A. Sheriff, and A. S. Mohideen, (2011). *International Journal of Pharmaceutical and Biological Archives*, *26:* 1718-1721.
3. Basu, A., and A.K. Nagchaudhuri, (1991). *Journal of Ethnopharmacology 31*: 319–324.
4. Charka Samhita, (1965) Translated by Shastri Gyrijashankar Mayashankar. Published by Sastu Sahitya, Near Badthra, Ahmedabad, 1st Edn, 1952, Vide vaidya BG, Nighantu Aadarsha, vol. 2, and pp. 683-690.
5. Coêlho, M. R., R. Rivas, J. R. C. Ferreira-Neto, V. Pandolfi, J.P. Bezerra-Neto, A.M. Benko-Iseppon, and M. G. Santos, (2019). *PloS one, 14*(4): e0215729.
6. Elias, G., and M. N. Rao, (1988). *Indian Journal of Experimental Biology, 26*(7): 540-542.
7. Mead, R. and R.N. Curnow, (1982). *A simple statistical method in Agricultural and Experimental Biology*. Chapman Hall, London, 33 - 46.
8. Naik, V.N. (1998). *Flora of Marathwada.* Amrut Prakashan, Aurangabad, *1:* 237-319.
9. Padmanabhan, P., and S. N. Jangle (2012). *International journal of basic and applied medical sciences, 2*(1): 109-116.
10. Ranjit, P. M., T. Santhipriya, S. Nagasri, Y. A. Chowdary, N. Pasumarthy, and V. Gopal, (2012). *Asian J Pharm Clin Res, 5*(3): 127-31.
11. Roesler, R., L. G. Malta, L. C. Carrasco, and G. Pastore, (2006). *Journal of Food Science, 71*(2): C102-C107.
12. Rollet-Labelle, E., M. J. Grange, C. Elbim, C. Marquetty, M. A. Gougerot-Pocidalo, and C. Pasquier, (1998). *Free Radical Biology and Medicine, 24*(4): 563-572.
13. Shaikh, R., M. Pund, A. Dawane, and S. Iliyas, (2014). *Journal of traditional and complementary medicine, 4*(4): 253-257.
14. Sinha, R. K. (2010). *Practical taxonomy of angiosperms*. IK International Publishing House.
15. Sontakke KS and SL Shinde (2019). *Int. J. of. Life Sciences, 7*(4): 785-790.