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Anti-oxidant and Anti-inflammatory Activity of *Cassia auriculata* (Flowers)

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: To analyze the antioxidants and anti-inflammatory potential of ethanolic crude extract of *Cassia auriculata* flowers.
Study Design: 2 kg of fresh flowers were soaked with 90% ethanol at room temperature (25ºC-30ºC). After 72 hrs the ethanolic extract was filtered. This extract was distilled and finally the crude was obtained. This ethanolic crude to get required concentrations and were used for screening antioxidants and anti-inflammatory activities.
Place and Duration of Study: PG & Research Department of Chemistry, Periyar E.V.R. College (Autonomous), Trichy, Tamilnadu, India. During the month of August and September 2014.
Methodology: Fresh flowers of *Cassia auriculata* were extracted with ethanol and evaluated for

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antioxidant activities by 2,2-Diphenyl 1-picryl hydrazyl solution (DPPH), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sul- phonic acid (ABTS) assay and anti inflammatory activites by humen blood cell (HRBC) membrane stabilization method and Inhibition of albumin denaturation method.

Results: The results obtained showed that the ethanolic crude extract of *Cassia auriculata* flowers can be considered as good sources of anti-oxidants, anti-inflammatory and can be incorporated into the drug formulations.

Conclusion: This study justifies the anti-oxidants, anti-inflammatory activity of ethanolic crude extract of *Cassia auriculata* Flowers. Further detailed analysis of this sample is required to identify the presence of bioactive compounds responsible for anti-oxidants, anti-inflammatory activities. Studies are highly needed for future drug development.

Keywords: Cassia auriculata; anti-oxidant activity; anti-inflammatory activity; albumin denaturation; DPPH(2,2-Diphenyl 1-picryl hydrazyl); ABTS(2,2'-azino-bis(3-ethylbenzthiazoline-6-sul-phonic acid)assay; total antioxidant activity etc.

1. INTRODUCTION

The search of new agents having various pharmacological activities, obtained by screening the natural sources like different plant extracts has led to the discovery of many clinically useful drugs which are immensely useful in the treatment of various human diseases [1]. Herbal drugs can therefore be considered as a better alternative to synthetic anti-inflammatory drugs [2]. *Cassia auriculata*, Linn (Caesalpiniaceae) commonly known as Tanners Cassia [Avaram] is a shrub with large bright yellow flowers, growing wild in Central Provinces and Western peninsula parts of India [3].

Cassia auriculata is an evergreen shrub that grows in many parts of India and in other parts of Asia. The flowers, leaves, stem, root and unripe fruit are used for treatment, especially in Ayurvedic medicine. People use C. auriculata for diabetes, eye infections (conjunctivitis) joint and muscle pain (rheumatism), constipation, jaundice, liver disease and urinary tract disorders. The plant has been reported to possess antipyretic, hepatoprotective, antidiabetic, antiperoxidative and antihyperglyceamic activity [4]. The flowers are used to treat urinary discharges, nocturnal emissions, diabetes and throat irritation [5]. Inflammation is the reaction of living tissues to injury, infection or irritation. Lysosomal enzymes released during inflammation produce a variety of disorders which leads to the tissue injury by damaging the macromolecules and lipid peroxidation of membranes which are assumed to be responsible for certain pathological conditions as heart attacks, septic shocks and rheumatoid arthritis etc.

The extracellular activity of these enzymes is said to be related to acute or chronic inflammation. Stabilization of lysosomal membrane is important in limiting the inflammatory response by inhibiting the release of lysosomal constituents of activated neutrophil such as bacterial enzymes and proteases which cause further tissue inflammation and damage upon extra cellular release or by stabilizing the lysosomal membrane [6]. Previous studies has proved that the chemical constituents such as flavonoids, alkaloids, tannins and terpenoids are promising agents in treatment of inflammation [7,8,9,10]. Flavonoids such as hesperidin, apigenin, luteolin and quercetin are found to be a potent anti-inflammatory constituent [11]. High content of flavonoids in methanolic extract of C. auriculata flowers is reported for its anti-inflammatory activity.

The present study involves the fresh flowers of *Cassia auriculata* were extracted with ethanol and evaluated for antioxidant activities by 2,2-Diphenyl 1-picryl hydrazyl solution (DPPH), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sul- phonic acid (ABTS) assay and anti inflammatory activites by humen blood cell (HRBC) membrane stabilization method and Inhibition of albumin denaturation method.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

Fresh flowers of *Cassia auriculata* were collected from O.Koothur Village, Ariyalur district, TamilNadu, India, during the month of November and identified by Head, PG & Research Department of Botany, Periyar E.V.R.College, Trichy, Tamil Nadu.

2.2 Flower Extraction

2 kg of fresh flowers were soaked with 90% ethanol at room temperature (25º-30ºC). After 72 hrs the ethanolic extract was filtered. This extract was concentrated in vacuum and the dry powder obtained was dissolved in DMSO (Dimethyl sulfoxide) to get required concentrations and were used for screening anti-oxidant and anti-inflammatory activities.

3. INVITRO ANTIOXIDANT ACTIVITY

3.1 DPPH (2,2-Diphenyl 1-picryl hydrazyl) Assay Method

The DPPH free radical is reduced to a corresponding hydrazine when it reacts with hydrogen donors. The DPPH radical is purple in colour and upon reaction with hydrogen donor changes to yellow colour. It is a decoloration assay, which is evaluated by the addition of the antioxidant to a DPPH solution in ethanol or methanol and the decrease in absorbance was measured at 490 nm [12].

3.2 Reagents

3.2.1 2,2-Diphenyl 1-picryl hydrazyl solution (DPPH, 100μM):

22 mg of DPPH was accurately weighed and dissolved in 100 ml of methanol. From this stock solution, 18 ml was taken and diluted to 100 ml using methanol to obtain 100 μM DPPH solution.

3.2.2 Preparation of test solutions

21 mg of the solid obtained from ethanolic extract was dissolved in distilled Dimethyl sulfoxide (DMSO) to obtain a solution of 21 mg/ml concentration. This solution was serially diluted to obtain lower concentrations.

3.2.3 Preparation of standard solutions

10 mg each of ascorbic acid and rutin were weighed separately and dissolved in 1 ml of Dimethyl sulfoxide (DMSO) to get 10 mg/ml concentrations. These solutions were serially diluted with DMSO to get lower concentrations.

3.2.4 Procedure

The assay was carried out in a 96 well microtitre plate. To 200 μl of DPPH solution, 10 μl of each of the test sample or the standard solution was added separately in wells of the microtitre plate. The final concentration of the test and standard solutions used were 1000, 500, 125 and 31.25 μg/ml. The plates were incubated at 37º C for 30 min and the absorbance of each solution was measured at 490 nm, using a micro plate reader.

3.3 Evaluation of Total Antioxidant Capacity of the Extract

The total antioxidant capacity was determined by phosphormolybdenum method and is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds and the formation of a green Mo (V) complex which has the maximal absorption at 695 nm.

3.3.1 Preparation of test and standard solutions

Weighed accurately 55 mg of the sample and the standard, ascorbic acid and dissolved in 5 ml of DMSO. The lower dilutions were made serially with Dimethyl sulfoxide(DMSO).

3.3.2 Procedure

An aliquot of 0.1 ml of the sample solution containing a reducing species in Dimethyl sulfoxide(DMSO) was combined in an Eppendorff tube with 1 ml of reagent solution (0.6 mM Sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped and incubated in water bath at 95ºC for 90min. The samples were cooled to room temperature, and the absorbance of each solution was measured at 695 nm. The total antioxidant capacity was expressed as mM equivalent of ascorbic acid (Mojca et al., 2005).

Total antioxidant activity = 372.1 μg/ml

3.3.3 ABTS (2,2'-azinobis (3-ethylbenzthiazoline -6-sul-phonic acid) radical scavenging activity

ABTS radical scavenging activity was performed as described by Re et al. (1999) with a slight modification. 7.0 mM ABTS in 14.7 mM ammonium peroxo-disulphate was prepared in 5.0 ml distilled water. The mixture was allowed to stand at room temperature for 24 hours. The resulting blue green ABTS radical solution was further diluted such that its absorbance is 0.70±0.020 at 734 nm. Various concentrations of the sample solution dissolved in ethanol (20.0 μl) were added to 980.0 μl of ABTS radical solution and the mixture was incubated in darkness for 10

min. The decrease in absorbance was read at 734 nm. A test tube containing 20.0 µl of ethanol and processed as described above was served as the control tube. Different concentrations of ascorbic acid were used as reference compound.

4. Anti- inflammatory Activity

4.1 The Human Red Blood Cell (HRBC) Membrane Stabilization Method

The method as prescribed by Gopalkrishnan et al., 2009; Sakat et al. [13] was adopted with some modifications. The blood was collected from healthy human volunteer who had not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10% suspension was made. Various concentrations of extracts were prepared in mg/ml using distilled water and to each concentration, 1 ml of phosphate buffer, 2 ml hypo saline and 0.5 ml of HRBC suspension were added. It was incubated at 37ºC for 30 minutes and centrifuged at 3,000 rpm for 20 minutes and the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. Diclofenac (100 Jg/ml) was used as reference standard and a control was prepared by omitting the extracts. The experiments were performed in triplicates and mean values of the three were considered. The percentage (%) of HRBC membrane stabilization or protection calculated using the following formula [14,15,16,13,17,18,19].

Percentage of Protection (%) = (100- OD of drug treated sample/OD of Control) X 100

4.2 Albumin Denaturation Method

The method as prescribed by Sakat et al. [13] was followed with modifications. The reaction mixture was consisting of test extracts and 1% solution of bovine albumin fraction. pH of the reaction mixture was adjusted using small amount of HCl. The sample extracts were incubated at 37ºC for 20 minutes and then heated to 51ºC for 20 minutes. After cooling the samples the turbidity was measured spectrophotometrically at 660 nm. Diclofenac sodium was taken as a standard drug. The experiment was performed in triplicates and the mean value of the three was considered. Percent inhibition of protein denaturation was calculated as follows [14,15,16,13,17,18,19].

Percentage of inhibition (%) = (OD of Control-OD of Sample/ OD of Control) X 100.

5. RESULTS

5.1 Anti-oxidant Activity

In the present study, ethanolic crude extract of *Cassia auriculata* flowers exhibited significant anti-oxidant activity when compared with DPPH assay. It is evident from the data presented in Table 1 that the sample possesses DPPH assay activity. The result showed the Percentage of cytotoxicity for 1000 µg/ml as 61.15%, 500 µg/ml as 59.03% for 125 µg/ml as 48.31%, and for 31.25 µg/ml as 29.12%, respectively when compared with ABTS assay activity. It is evident from the data presented in Table 2 that the sample possesses ABTS assay activity. The result showed the Percentage of cytotoxicity for 1000 µg/ml as 72.81 %, 500 µg/ml as 59.58%for 125 µg/ml as 46.53%, and for 31.25 µg/ml as 23.12%. Total antioxidant activity of the plant is 372.1 µg/ml.

5.2 Anti-inflammatory Activity

Ethanolic crude extract of *Cassia auriculata* flowers exhibited significant anti-inflammatory activity of the human red blood cell (HRBC) membrane stabilization exhibited data presented in Table 3. The result showed the Percentage of Inhibition in Membrane Stabilization for 100 µg/ml as 29.15±1.75%, for 200 µg/ml as 49.09±1.72%, for 400 µg/ml as 62.47±1.49%, for 600 µg/ml as 79.05±1.09% and for 800 µg/ml as 86.42±1.94%. Respectively when the Inhibition of Albumin denaturation activity exhibited data presented in Table 4. The result showed the Percentage of Inhibition in membrane Stabilization for 100 µg/ml as 29.15±1.75%, for 200 µg/ml as 49.18±1.87%, for 400 µg/ml as 58.26±1.58%, for 600 µg/ml as 85.16±0.49 and for 800 µg/ml as 89.24±1.70%.

6. DISCUSSION

The anti-inflammatory activity of various extracts of *C. auriculata* leaves was carried out using carrageenan induced rat paw edema. Carrageenan induced inflammation represents a classical model of edema formation and hyperalgesia, which has been extensively used for evaluation of anti-edemal effect of drugs [20]. The sub-planter administration of carrageenan in rat is responsible for the typical biphasic edema [21] in which the first phase observed around 0-2 hours is attributed to the release of histamine and serotonin. The second phase of swelling

which last for 2-6 hours is due to release of prostaglandin- like substances [22,23]. In the present study, ethanolic crude extract of *C. auriculata* flowers showed potent anti-inflammatory activity compared to leaves of ethyl acetate extracts. As the anti-inflammatory effect was more significant during later phase of inflammation, In accordance with previous studies steroids, flavonoids, alkaloids, terpenoids and tannins have been shown to possess of anti-inflammatory activity [24]. Thus the anti-inflammatory effect of ethanolic crude extract may be due to presence of of active constituent flavonoids. Based on the results described, it may be concluded that the ethanolic crude extract shown above the result may be due to the absence of Flavonoids.The results strongly suggest anti-inflammatory effect and anti-oxidant effects by percentage of inhibitions, which are explained in the Tables 1, 2, 3, 4 The medical use of *Cassia auriculata* as a useful remedy in various disorders could possibly be because of its excellent anti-inflammatory and antioxidant potential. However, chemical constituents and mechanism responsible for the pharmacological activities remain to be investigated.

Table 1. DPPH assay activity of ethanolic extract of flowers of *Cassia auriculata*

S. no	Concentration (µg/ml)	% CTC_{50} cytotoxicity	CTC_{50}
1	1000	61.15	201.5
2	500	59.03	µg/ml
3	125	48.31	
4	31.25	29.12	

Table 2. ABTS assay activity of ethanolic extract of flowers of *Cassia auriculata*

S. no	Concentration (µg/ml)	% CTC_{50} Cytotoxicity (µg/ml)	CTC_{50}
1	1000	72.81	483.12 (µg/ml)
2	500	59.58	
3	125	46.53	
4	31.25	23.12	

Table 3. The human red blood cell (HRBC) membrane stabilization activity of ethanolic extract of flowers of *Cassia auriculata*

S. no	Concentration (µg/ml)	% of Inhibition
		Membrane stabilization Mean±S.E.M(S-I)
1	100	28.17±1.59
2	200	49.09±1.72
3	400	62.47±1.49
4	600	79.05±1.09
5	800	86.42±1.94

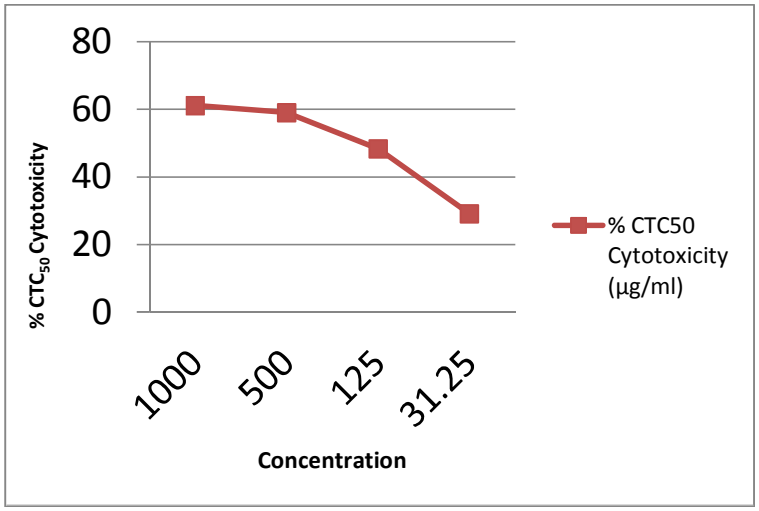

Fig. 1. Graphical representation of DPPH activity of ethanolic extract of flowers of *Cassia auriculata*

Table 4. The Inhibition of Albumin denaturation activity of ethanolic extract of flowers of *Cassia auriculata*

S. no	Concentration (µg/ml)	% of Inhibition
		Membrane Stabilization Mean±S.E.M(S-I)
1	100	29.15±1.75
2	200	49.18±1.87
3	400	58.26±1.58
4	600	85.16±0.49
5	800	89.24±1.70

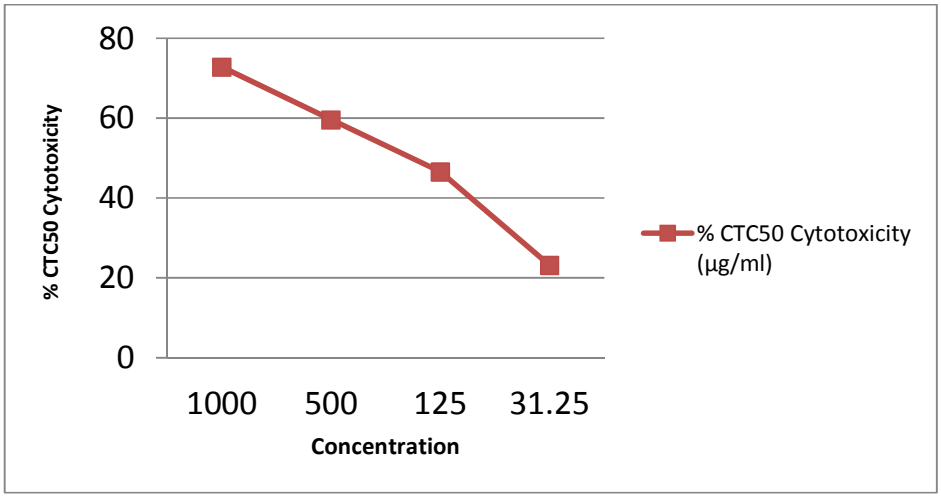

Fig. 2. Graphical representation of ABTS radical scavenging activity of ethanolic extract of *Cassia auriculata*

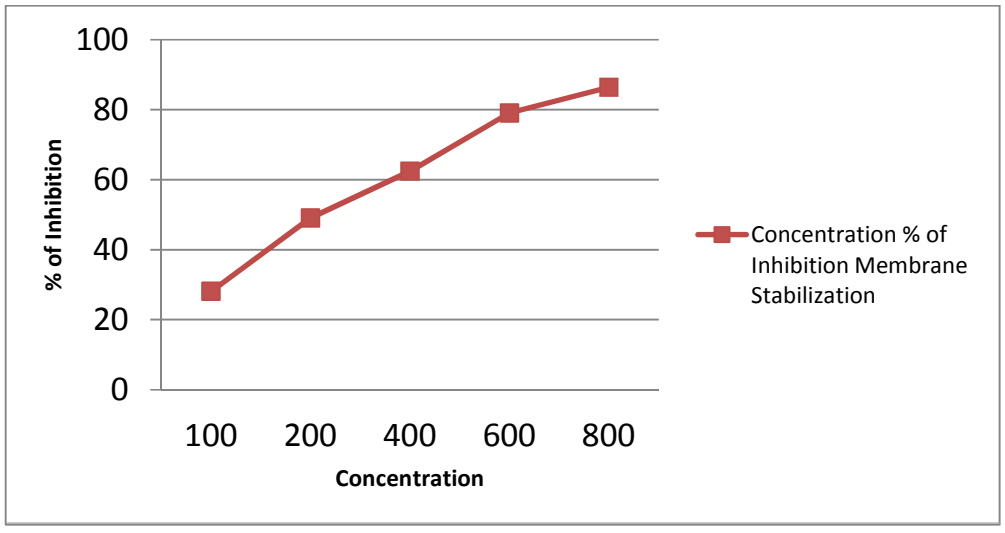

Fig. 3. Graphical representation of human red blood cell (HRBC) membrane stabilization activity of ethanolic extract of flowers of *Cassia auriculata*

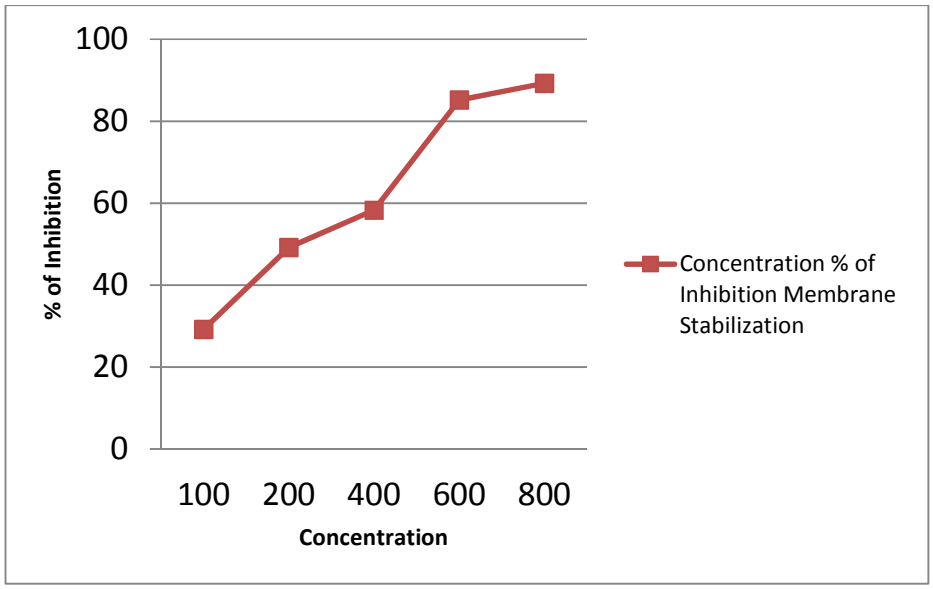

Fig. 4. Graphical representation of Inhibition of Albumin denaturation activity of ethanolic extract of flowers of *Cassia auriculata*

7. CONCLUSION

In conclusion, the present study has confirmed that DPPH assay, total anti-oxidant capacity and ABTS have showed a strong antioxidant activity and also the human red blood cell (HRBC) membrane stabilization, Inhibition of albumin denaturation indicated the anti-inflammatory activity.

In recent years, the search for phytochemicals possessing antioxidant, and anti inflammatory properties have been on the rise due to their likely use in the therapy of various chronic and communicable diseases. Epidemiology and investigational studies have implicated oxidative cellular damage arising from an imbalance between free radical generating and scavenging systems as the primary cause of cardiovascular, diseases, cancer, aging etc. [12]. Due to risk of adverse effects encountered with the use of synthetic antibiotics. In addition, a number of antibiotics have lost their effectiveness due to the development of resistant strains, mostly through the expression of resistance genes [25].

The present investigations have demonstrated a strong association between the anti-inflammatory and antioxidant activities of *Cassia auriculata* flowers. The prevention of oxidative damage to tissue could therefore be one of the mechanisms responsible for the anti-inflammatory effect shown by this plant. The medical use of *Cassia auriculata* as a useful remedy in arthritic disorders could possibly be because of its excellent anti-inflammatory and antioxidant potential. The isolation, purification and mechanism of action of phenolic and flavonoidal components and other components of *Cassia auriculata* flowers are of interest for further examination and shall be carried out in future studies.

These findings provide scientific evidence to support traditional medicinal uses and indicate a promising potential for the development of an antioxidant and anti-inflammatory agent from *Cassia auriculata* flowers. This medicinal plant by *in vitro* results appear as interesting and promising and may be effective as potential sources of novel antioxidant and anti-inflammatory drugs.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Hostettmann K. Strategy for the biological and chemical evaluation of plant extracts. Proceedings of the international Conference on Biodiversity and Bioresources: Conservation and Utilization, November 23-27. Thailand; 1997.
2. Gaikwad SA, Kale AA, Jadhav BG, Deshpande NR, Salvekar JP. Anthelmintic activity of *Cassia auriculata* L. extract: *In-vitro* study. J. Nat. Prod. Plant Resour. 2011;1:62-66.
3. Dr. Satya Prakash. Wealth of India: Raw materials. Publications and Information Directorate, New Delhi, Council of Scientific and Industrial Research. 1950;247.
4. Manickam P, Namasivayam N, Peryasamy V, Rajagopal SK. Effect of *Cassia auriculata* leaf extract on lipids in rats with alcoholic liver injury. Asia Pacific J. Cli. Nut. 2002;2(11):157-163.
5. Prakash SK. Effects of herbal extracts towards microbial activity against pathogenic *Escherichia coli* in poultry. Int. J. Poultry Sci. 2006;5:259-261.
6. Rajendran V, Lakshmi KS. *In vitro* and *In vivo* anti-inflammatory activity of leaves of *Symplocos cochinchnensis* (Lour) Moore ssp laurina. Bangladesh J Pharmacol. 2008;3:121-124.
7. Omale J, Okafor PN. Comparative antioxidant capacity, membrane stabilization, polyphenol composition and cytotoxicity of the leaf and stem of Cissus multistriata, Afr. J. Biotechnol. 2008;7:3129-3133.
8. Peraman MK, Ramalingam P, Bapatla JNN. Ant-iinflammatory and antimicrobial activities of the extracts of Edipta alba leaves. Eur. J. Exp. Bio. 2011;1:172-177.
9. Singh A, Malhotra S, Subban R. Anti-inflammatory and analgesic agents from Indian medicinal plants. Int. J. Integ. Biol., 2008;3: 57-57.
10. Doshi GM, Agarwal GV, Shanbagh PP, Shastri KV, Agarwal OK, Bhalerao B, Desai SK. Studies on *Cassia auriculata* plant parts by improved in vitro antioxidant models. J. Pharm. Biomed. Sci. 2011;10:1-2.
11. Alade PI, Irobi ON. Antimicrobial activities of crude leaf extracts of Acalypha wilkesiana. J. Ethanopharmacol. 1993;39: 171-174.
12. Halliwell B. Antioxidants in human health and disease. Annu. Rev. Nutr. 1996;6:33-50.
13. Sakat S, Juvekar AR, Gambhire MN. *In vitro* antioxidant and inflammatory activity of methanol extract of Oxalis corniculata Linn. International Journal of Pharma and Pharmacological Sciences. 2010;146-155.
14. Silva Da EJ, Hamilton A. Electronic Journal of Biotechnology. 1999;2:1-4.
15. Ilavarsan R, Mallika M, et al. Afr J Trad CAM, 2005;2(1):70-85.
16. Mizushima Y and Kobayashi M. Interaction of anti- inflammatory drugs with serum proteins especially with some biologically active proteins. J. of Pharma Pharmacol.1968;20:169-173.
17. Brown JH, Mackey HK. Inhibition of heat-induced denaturation of serum proteins by mixtures of nonsteroidal anti-inflammatory agents and aminoacids. Proc. Soc. Exp. Biol. Med. 1968;128:225-8.
18. Grant NH, Alburn HE, Kryzanaukas C. Stabilization of serum albumin by anti-inflammatory drugs. Biochem. Pharmacol. 1970;19:715-22.
19. Anitha Rani A, Mary Josephine Punitha S, Rema M. Anti-Inflammatory activity of flower extract of *Cassia auriculata* – an in-vitro study. Int. Res J Pharm. App Sci. 2014;4(1):57-60.
20. Guay J, Bateman K, Gordon R, Mancini J, Riendeau D. Carrageenan-induced paw edema in rat elicits a predominant prostaglandin E2 (PGE2) response in the central nervous system associated with the induction of microsomal PGE2 synthase-1, J Biol Chem. 2004;279:24866-24872.
21. Fabiana RN, Tais AAB, Angelica ML, Carlos ECO, Ricardo RDS, Milena BPS, Cristiane FV. Anti-inflammatory and antinociceptive activities of *Blechnum occidentale* L. extract. J. Ethnopharmacol. 2009;125:102-107.
22. Kaushik ML, Jalalpure SS. Evaluation of anti-inflammatory effect of ethanolic and aqueous extracts of *Curcuma zedoaria* rose root. Int. J. Drug Der. & Res. 2010;3:360-365.
23. Cuman RKN, Bersani-Amadio CA, Fortes ZB. Influence of type-2 diabetes on the inflammatory response in rats, Inflam. Res. 2001;50:460-465.

24. Lilian EP, Teresita G, Americo OJ, Eduardo G. Acute and chronic anti-inflammatory effects of plant flavonoids, Il Farmaco. 1998;53:421-424.

25. Berahou AA, Auhmani A, Fdil N, Benharref A, Jana M, Gadhi CA. Antibacterial activity of *Quercus ilex* bark's extracts. J. Ethnopharm. 2007;112:426-429.

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