INTERNATIONAL JOURNAL OF PROGRESSIVE RESEARCH IN ENGINEERING MANAGEMENT AND SCIENCE (IJPREMS)
(Int Peer Reviewed Journal)
Vol. 05, Issue 09, September 2025, pp : 1119-1124

www.ijprems.com
editor@ijprems.com

e-ISSN : 2583-1062
Impact Factor : 7.001

ANTIOXIDANT AND ANTI-INFLAMMATORY POTENTIAL OF *BUTEA MONOSPERMA* AND *CALOTROPIS GIGANTEA* AQUEOUS EXTRACTS

Apurva Dubey[1], Dr. Satish Mohabe[2]

[1,2]Department Of Botany, Faculty Of Science And IT, Madhyanchal Professional University, Ratibad, Bhopal, M.P. India.

Email ID: satish.nbri09@gmail.com

ABSTRACT

This study evaluates the antioxidant and anti-inflammatory activities of aqueous extracts from *Butea monosperma* and *Calotropis gigantea* leaves using multiple in vitro assays. Soxhlet extraction with distilled water was employed to obtain bioactive compounds, which were tested for their ability to scavenge free radicals (DPPH, ABTS, NBT assays), reduce ferric ions (FRAP assay), and inhibit protein denaturation (BSA and egg albumin assays). Both extracts exhibited dose-dependent antioxidant activity, with *B. monosperma* showing slightly higher efficacy than *C. gigantea*. At 160 mg/mL, *B. monosperma* achieved 76.5%, 77.9%, 66.1%, and 57.6 µmol $FeSO_4$ Eq./mL in DPPH, ABTS, NBT, and FRAP assays, respectively, compared to *C. gigantea*'s 70.4%, 71.4%, 61.4%, and 52.2 µmol $FeSO_4$ Eq./mL. Anti-inflammatory assays showed both extracts inhibited protein denaturation, with *B. monosperma* and *C. gigantea* achieving 71.0% and 73.6% inhibition in the BSA assay, and 65.8% and 69.0% in the egg albumin assay at 160 mg/mL, respectively, compared to diclofenac's higher inhibition. While less potent than reference standards (ascorbic acid and diclofenac), both extracts demonstrated significant bioactivity, suggesting potential as natural therapeutic agents for oxidative stress and inflammation-related conditions.

Keywords Antioxidant, Anti-Inflammatory, *Butea Monosperma*, *Calotropis Gigantea*, Protein Denaturation, Radical Scavenging.

1. INTRODUCTION

Oxidative stress and inflammation are pivotal in the pathogenesis of chronic diseases such as cardiovascular disorders, cancer, and neurodegenerative conditions [1]. The quest for natural antioxidants and anti-inflammatory agents has intensified due to their potential to mitigate these conditions with fewer side effects compared to synthetic drugs [2]. Plant-derived extracts, rich in bioactive compounds like polyphenols, flavonoids, and alkaloids, have demonstrated significant potential in neutralizing free radicals and modulating inflammatory responses [3]. *Butea monosperma* and *Calotropis gigantea*, widely utilized in traditional medicine, are two such plants with reported therapeutic properties, including antioxidant and anti-inflammatory activities [4,5]. However, comprehensive evaluations of their aqueous extracts remain limited, necessitating further research to validate their efficacy and elucidate their mechanisms.

Butea monosperma, commonly known as the flame of the forest, is a deciduous tree native to South Asia. Its leaves, bark, and flowers have been employed in Ayurvedic medicine to treat inflammation, oxidative stress, and microbial infections [4]. The leaves are particularly rich in flavonoids and phenolic compounds, known to scavenge free radicals and inhibit inflammatory pathways [6]. Similarly, *Calotropis gigantea*, a perennial shrub, is valued in traditional medicine for its anti-inflammatory, analgesic, and antioxidant properties [5]. Its leaves contain bioactive compounds such as cardiac glycosides, flavonoids, and terpenoids, contributing to its therapeutic potential [7]. Despite their traditional applications, comparative studies on the efficacy of their aqueous extracts in standardized assays are scarce.

This study aims to assess the antioxidant and anti-inflammatory potential of aqueous leaf extracts of *B. monosperma* and *C. gigantea* using in vitro assays. Antioxidant activity was evaluated through DPPH, ABTS, NBT, and FRAP assays, which measure free radical scavenging and reducing power. Anti-inflammatory activity was assessed using BSA and egg albumin denaturation assays, which evaluate the ability to prevent protein denaturation, a key mechanism in inflammation [8]. These assays provide a comprehensive understanding of the extracts' bioactivity, targeting different aspects of oxidative and inflammatory processes. The use of aqueous extracts aligns with traditional preparation methods and ensures the isolation of polar bioactive compounds, which are often safer for therapeutic applications [3]. By comparing the extracts' performance against standard references (ascorbic acid for antioxidant assays and diclofenac for anti-inflammatory assays), this study seeks to establish their potential as natural therapeutic agents. The results could guide further research into their phytochemical composition and clinical applications, contributing to the development of plant-based treatments for oxidative stress and inflammation-related disorders

www.ijprems.com
editor@ijprems.com
INTERNATIONAL JOURNAL OF PROGRESSIVE RESEARCH IN ENGINEERING MANAGEMENT AND SCIENCE (IJPREMS)
(Int Peer Reviewed Journal)
Vol. 05, Issue 09, September 2025, pp : 1119-1124
e-ISSN : 2583-1062
Impact Factor : 7.001

2. MATERIALS AND METHODS

Plant Material Preparation and Extraction

Leaves of *Butea monosperma* and *Calotropis gigantea* were washed with distilled water and shade-dried at room temperature for 7 days until fully dry. The dried leaves were ground into a fine powder using an electric grinder. Soxhlet extraction was performed with 50 g of powdered leaves in a thimble, using distilled water as the solvent at 100°C for 6 hours. The aqueous extracts were filtered through Whatman No. 1 filter paper, concentrated in a rotary evaporator at 40–50°C, and lyophilized to obtain dry extracts. These were stored in amber-colored glass vials at 4°C until use.

Concentration Preparations

Dried extracts were dissolved in dimethyl sulfoxide (DMSO) and diluted with distilled water to prepare five concentrations ranging from 10 to 200 µg/mL for biological assays. Solutions were sterilized using a 0.22 µm syringe filter.

Antioxidant Activity Assays

DPPH Radical Scavenging Assay

The DPPH assay evaluated free radical scavenging activity. A 100 µL aliquot of each extract concentration was mixed with 3.5 mL of DPPH solution and incubated in the dark at room temperature for 30 minutes. Absorbance was measured at 517 nm using a UV-Vis spectrophotometer. The percentage inhibition of DPPH radicals was calculated based on the absorbance difference between control and sample.

ABTS Radical Cation Scavenging Assay

The ABTS assay assessed the neutralization of ABTS radical cations. ABTS radicals were generated by incubating ABTS with potassium persulfate for 12–16 hours in the dark. The radical solution was diluted to a predetermined absorbance, mixed with the extract, and incubated for 30 minutes. Absorbance was measured at 734 nm, and the percentage inhibition was calculated to quantify antioxidant activity.

Nitrotetrazolium Blue (NBT) Assay

The NBT assay measured superoxide radical scavenging. A reaction mixture containing NBT, NADH, PMS, and varying extract concentrations was incubated at room temperature. Absorbance was measured at 560 nm, with reduced formazan formation indicating superoxide scavenging capacity.

Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay evaluated reducing power by measuring the conversion of ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions. Extracts were mixed with FRAP reagent and incubated at 37°C for 4 minutes. Absorbance was measured at 593 nm, and results were expressed as Trolox equivalents.

Anti-inflammatory Activity Assays

BSA Denaturation Assay

The BSA denaturation assay assessed anti-inflammatory activity by measuring inhibition of protein denaturation. A 1 mL aliquot of extract was mixed with 2 mL of 0.02% BSA solution and incubated at 37°C for 30 minutes. Denaturation was induced by adding 2 mL of 1% acetic acid and heating at 50°C for 30 minutes. Absorbance was measured at 660 nm, with lower absorbance indicating inhibition of denaturation.

Egg Albumin Assay

The egg albumin assay evaluated prevention of heat-induced denaturation. Extracts were mixed with 2 mL of egg albumin and heated at 70°C for 15 minutes. Turbidity was measured at 660 nm, and the percentage inhibition of denaturation was calculated by comparing test and control samples.

3. RESULTS

Anti-inflammatory Activity

The anti-inflammatory potential of *Butea monosperma* and *Calotropis gigantea* aqueous extracts was evaluated using BSA and egg albumin denaturation assays, with diclofenac as the reference inhibitor.

Table 1: BSA Denaturation Inhibition

Concentration (mg/mL)	*B. monosperma* Inhibition (%)	*C. gigantea* Inhibition (%)	Diclofenac Inhibition (%)
10	38.0	32.1	72.0

INTERNATIONAL JOURNAL OF PROGRESSIVE RESEARCH IN ENGINEERING MANAGEMENT AND SCIENCE (IJPREMS)
(Int Peer Reviewed Journal)
Vol. 05, Issue 09, September 2025, pp : 1119-1124
www.ijprems.com
editor@ijprems.com
e-ISSN : 2583-1062
Impact Factor : 7.001

20	45.5	48.3	78.5
40	53.8	56.2	82.1
80	62.4	64.3	84.6
160	71.0	73.6	86.5

Both extracts exhibited dose-dependent inhibition in the BSA assay. At 10 mg/mL, *B. monosperma* achieved 38.0% inhibition and *C. gigantea* 32.1%, compared to diclofenac's 72.0%. At 160 mg/mL, inhibition increased to 71.0% for *B. monosperma* and 73.6% for *C. gigantea*, approaching diclofenac's 86.5%. *C. gigantea* showed slightly higher inhibition at higher concentrations, indicating a stronger anti-inflammatory effect in this assay (Figure 1).

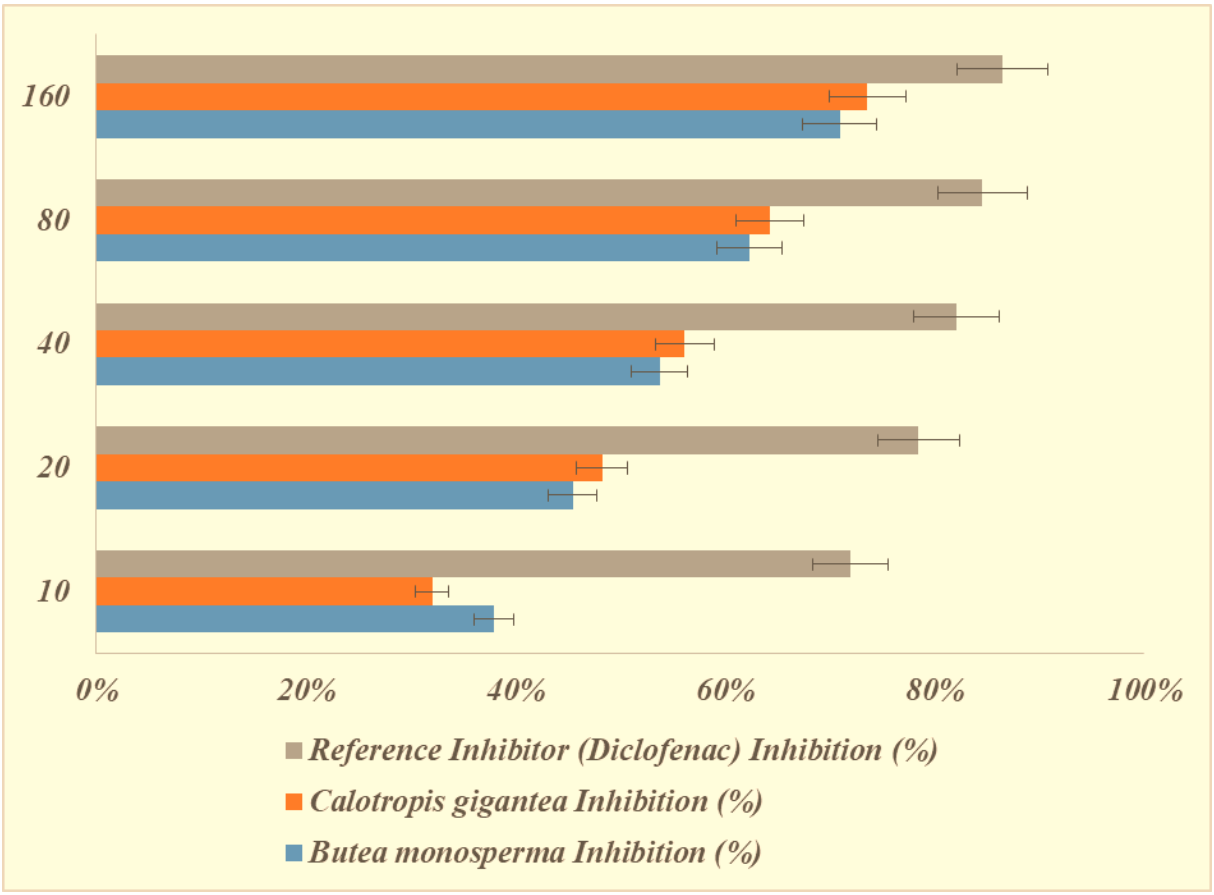

Figure 1: Comparative analysis of the protein denaturing by BSA assay.

Table 2: Egg Albumin Denaturation Inhibition

Concentration (mg/mL)	***B. monosperma* Inhibition (%)**	***C. gigantea* Inhibition (%)**	**Diclofenac Inhibition (%)**
10	28.7	31.5	68.0
20	43.6	47.2	75.3
40	51.9	54.1	79.4
80	58.3	61.4	82.8
160	65.8	69.0	85.0

In the egg albumin assay, a similar dose-dependent trend was observed. At 10 mg/mL, *B. monosperma* and *C. gigantea* showed 28.7% and 31.5% inhibition, respectively, compared to diclofenac's 68.0%. At 160 mg/mL, inhibition reached 65.8% for *B. monosperma* and 69.0% for *C. gigantea*, against diclofenac's 85.0%. *C. gigantea* again outperformed *B. monosperma* at higher concentrations (Figure 2).

INTERNATIONAL JOURNAL OF PROGRESSIVE RESEARCH IN ENGINEERING MANAGEMENT AND SCIENCE (IJPREMS)
(Int Peer Reviewed Journal)
Vol. 05, Issue 09, September 2025, pp : 1119-1124
www.ijprems.com
editor@ijprems.com
e-ISSN : 2583-1062
Impact Factor : 7.001

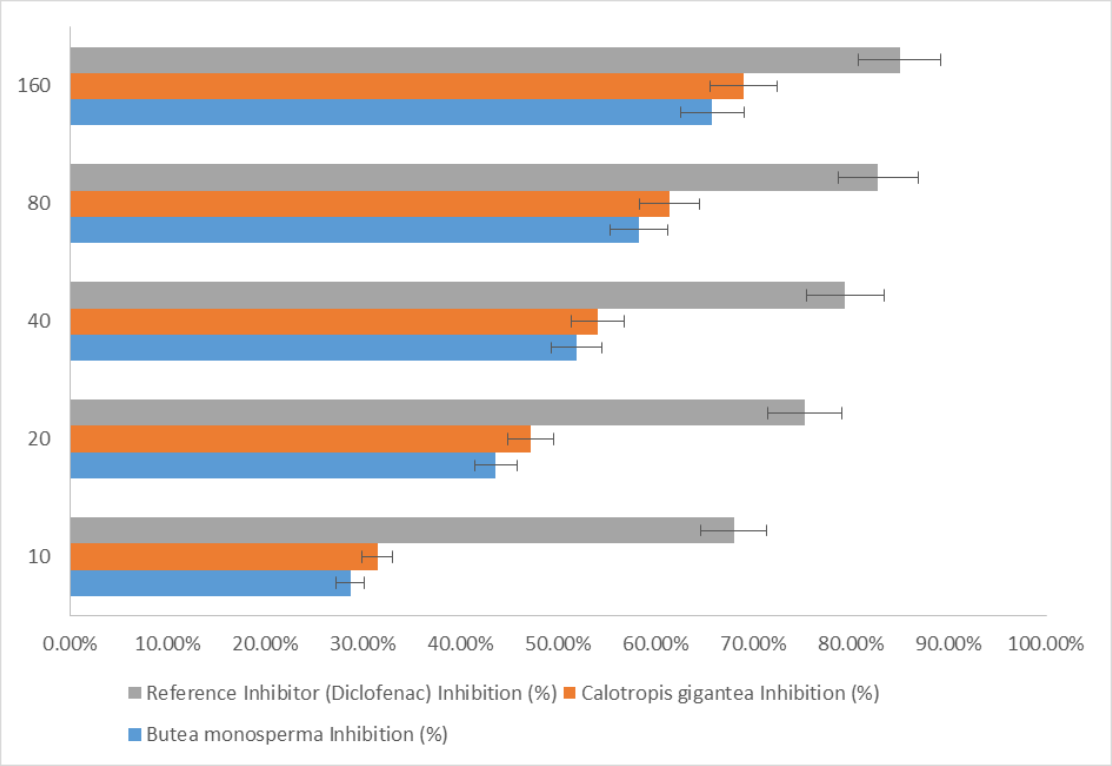

Figure 2: Comparative analysis of the protein denaturing by egg albumin assay.

Antioxidant Activity

Antioxidant activity was assessed using DPPH, ABTS, NBT, and FRAP assays, with ascorbic acid as the reference standard.

Table 3: DPPH Radical Scavenging Activity

Concentration (mg/mL)	*B. monosperma* Inhibition (%)	*C. gigantea* Inhibition (%)	Ascorbic Acid Inhibition (%)
10	25.3	22.5	70.0
20	38.6	34.1	75.3
40	51.1	46.2	80.2
80	63.7	58.5	85.5
160	76.5	70.4	90.0

Both extracts showed dose-dependent DPPH radical scavenging. At 10 mg/mL, *B. monosperma* exhibited 25.3% inhibition and *C. gigantea* 22.5%, compared to ascorbic acid's 70.0%. At 160 mg/mL, *B. monosperma* reached 76.5% and *C. gigantea* 70.4%, against ascorbic acid's 90.0%. *B. monosperma* consistently showed higher activity.

Table 4: ABTS Radical Scavenging Activity

Concentration (mg/mL)	*B. monosperma* Inhibition (%)	*C. gigantea* Inhibition (%)	Ascorbic Acid Inhibition (%)
10	28.4	26.7	72.0
20	41.9	38.2	77.5
40	53.5	49.6	82.3
80	65.3	60.5	87.9
160	77.9	71.4	92.0

In the ABTS assay, *B. monosperma* achieved 28.4% inhibition and *C. gigantea* 26.7% at 10 mg/mL, compared to ascorbic acid's 72.0%. At 160 mg/mL, inhibition increased to 77.9% for *B. monosperma* and 71.4% for *C. gigantea*, against ascorbic acid's 92.0%. *B. monosperma* showed higher efficacy.

INTERNATIONAL JOURNAL OF PROGRESSIVE RESEARCH IN ENGINEERING MANAGEMENT AND SCIENCE (IJPREMS)
(Int Peer Reviewed Journal)
Vol. 05, Issue 09, September 2025, pp : 1119-1124
www.ijprems.com
editor@ijprems.com
e-ISSN : 2583-1062
Impact Factor : 7.001

Table 5: FRAP Assay

Concentration (mg/mL)	*B. monosperma* (µmol $FeSO_4$ Eq./mL)	*C. gigantea* (µmol $FeSO_4$ Eq./mL)	Ascorbic Acid (µmol $FeSO_4$ Eq./mL)
10	13.5	11.4	44.3
20	22.7	18.9	56.8
40	33.4	30.2	69.1
80	45.1	41.5	80.5
160	57.6	52.2	92.3

In the FRAP assay, *B. monosperma* recorded 13.5 µmol $FeSO_4$ Eq./mL and *C. gigantea* 11.4 µmol $FeSO_4$ Eq./mL at 10 mg/mL, compared to ascorbic acid's 44.3 µmol $FeSO_4$ Eq./mL. At 160 mg/mL, *B. monosperma* reached 57.6 µmol $FeSO_4$ Eq./mL and *C. gigantea* 52.2 µmol $FeSO_4$ Eq./mL, against ascorbic acid's 92.3 µmol $FeSO_4$ Eq./mL.

Table 6: NBT Reduction Assay

Concentration (mg/mL)	*B. monosperma* Reduction (%)	*C. gigantea* Reduction (%)	Ascorbic Acid Reduction (%)
10	18.2	16.5	65.3
20	30.1	28.0	70.2
40	41.5	39.2	75.0
80	53.8	50.3	80.5
160	66.1	61.4	85.0

In the NBT assay, *B. monosperma* achieved 18.2% reduction and *C. gigantea* 16.5% at 10 mg/mL, compared to ascorbic acid's 65.3%. At 160 mg/mL, *B. monosperma* reached 66.1% and *C. gigantea* 61.4%, against ascorbic acid's 85.0%.

4. DISCUSSION

The aqueous extracts of *Butea monosperma* and *Calotropis gigantea* demonstrated significant antioxidant and anti-inflammatory activities, though less potent than reference standards ascorbic acid and diclofenac. The dose-dependent increase in antioxidant activity in the DPPH, ABTS, NBT, and FRAP assays suggests the presence of bioactive compounds, likely flavonoids and phenolics, capable of neutralizing free radicals and reducing oxidative stress [3]. *B. monosperma* consistently outperformed *C. gigantea* in antioxidant assays, with higher inhibition percentages (e.g., 76.5% vs. 70.4% in DPPH at 160 mg/mL) and greater reducing power (57.6 vs. 52.2 µmol $FeSO_4$ Eq./mL in FRAP). This may be due to a higher concentration of polyphenolic compounds in *B. monosperma* [6]. However, both extracts were less effective than ascorbic acid, indicating a lower potency compared to synthetic standards.

In anti-inflammatory assays, *C. gigantea* showed slightly higher inhibition of protein denaturation, particularly in the BSA assay (73.6% vs. 71.0% at 160 mg/mL). This suggests that *C. gigantea* may contain specific compounds, such as cardiac glycosides or terpenoids, that are more effective at stabilizing proteins under denaturing conditions [7]. Both extracts were less effective than diclofenac, a non-steroidal anti-inflammatory drug with a targeted mechanism [9]. The ability to inhibit protein denaturation supports their traditional use in managing inflammatory conditions.

The differences in bioactivity may be linked to phytochemical profiles. *B. monosperma*'s higher antioxidant activity could stem from its richer flavonoid content, while *C. gigantea*'s stronger anti-inflammatory effects may be due to glycosides [4,5]. The use of aqueous extracts may have limited the extraction of non-polar compounds, potentially underestimating their full therapeutic potential [3]. Future studies should explore solvent variations and identify specific bioactive compounds. These findings highlight the therapeutic potential of both plants as natural antioxidants and anti-inflammatory agents, suggesting their use in complementary therapies for oxidative stress and inflammation-related disorders, with further optimization needed to enhance potency.

5. CONCLUSION

The aqueous extracts of *Butea monosperma* and *Calotropis gigantea* exhibit significant dose-dependent antioxidant and anti-inflammatory activities, with *B. monosperma* showing superior antioxidant potential and *C. gigantea* slightly higher anti-inflammatory efficacy. While less potent than ascorbic acid and diclofenac, both extracts demonstrate

promise as natural therapeutic agents. These findings support their traditional use and warrant further research into their phytochemical composition and clinical applications for managing oxidative stress and inflammation.

Abbreviations

ABTS: 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); BSA: Bovine Serum Albumin; DPPH: 2,2-Diphenyl-1-picrylhydrazyl; DMSO: Dimethyl Sulfoxide; FRAP: Ferric Reducing Antioxidant Power; NBT: Nitroblue Tetrazolium; PMS: Phenazine Methosulfate; NADH: Nicotinamide Adenine Dinucleotide.

6. REFERENCES

[1] Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: how are they linked? Free Radic Biol Med. 2010;49(11):1603-16. doi:10.1016/j.freeradbiomed.2010.09.006

[2] Phaniendra A, Jestadi DB, Periyasamy L. Free radicals: properties, sources, targets, and their implication in various diseases. Indian J Clin Biochem. 2015;30(1):11-26. doi:10.1007/s12291-014-0446-0

[3] Krishnaiah D, Sarbatly R, Nithyanandam R. A review of the antioxidant potential of medicinal plant species. Food Bioprod Process. 2011;89(4):217-33. doi:10.1016/j.fbp.2010.10.008

[4] Sharma N, Garg V. Antidiabetic, antioxidant and antihyperlipidemic activities of Butea monosperma leaves extract in alloxan-induced diabetic rats. Indian J Pharm Sci. 2014;76(4):344-9.

[5] Kumar G, Karthik L, Rao KVB. Antibacterial activity of aqueous extract of Calotropis gigantea leaves – an in vitro study. Int J Pharm Sci Rev Res. 2010;4(2):141-4.

[6] Gupta P, Sharma V, Garg N. In vitro antioxidant activity of Butea monosperma leaves. Int J Pharm Sci Res. 2016;7(4):1521-6. doi:10.13040/IJPSR.0975-8232.7(4).1521-26

[7] Meena AK, Yadav AK, Niranjan US, Singh B, Nagariya AK, Sharma K, et al. A review on Calotropis gigantea: a multipotential medicinal plant. Int J Pharm Sci Rev Res. 2011;9(2):55-62.

[8] Mizushima Y, Kobayashi M. Interaction of anti-inflammatory drugs with serum proteins, especially with regard to their displacement and denaturation. J Pharm Pharmacol. 1968;20(3):169-73. doi:10.1111/j.2042-7158.1968.tb09718.x

[9] Gan TJ. Diclofenac: an update on its mechanism of action and safety profile. Curr Med Res Opin. 2010;26(7):1715-31. doi:10.1185/03007995.2010.486301