PROSPECTS
IN PHARMACEUTICAL SCIENCES

Prospects in Pharmaceutical Sciences, 23(3), 29-35
https://prospects.wum.edu.pl/

Original Article

EXPERIMENTAL INVESTIGATION OF *CYNODON DACTYLON* ETHANOLIC EXTRACT AGAINST ALLERGIC CONJUNCTIVITIS IN RATS

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Received: 04.03.2025 / Received in revised form: 22.03.2025 /Accepted: 28.03.2025 / Published: 22.07.2025

ABSTRACT

Allergic conjunctivitis refers to inflammation of the conjunctiva, often treated with allopathic drugs that may cause side effects. Plant-based preparations are considered safer alternatives; therefore, this study aims to evaluate the anti-allergic conjunctivitis effect of ethanolic extract of *Cynodon dactylon* (EECD) in rats. The allergic conjunctivitis was induced in the rats, for that we administered the 0.6 ml of saline solution which contains 10^{10} killed *Bordetella pertussis* cells, aluminium hydroxide (2 mg) and egg albumin (1 mg) by intra-peritoneal injection on the first day. On 5th day we administered egg albumin (0.5 mg) in 1 ml saline solution subcutaneously at 10 places on the back side of rats. The rats were then given extracts (EECD) at 100 mg/kg, 200 mg/kg, and 400 mg/kg given orally, corresponding to their group from days 14 to 42. In contrast, the standard group received treatment with cetirizine hydrochloride at a dose of 10 mg/kg p.o., whereas 1% w/v carboxymethyl cellulose (CMC) solution was given to the control group. Five microliters of egg albumin in a physiological solution (10 mg/ml) were administered an hour after the dose via micropipette into each of the eyes to achieve local sensitization. Subsequently, the number of eye scratching behaviour was counted for five minutes following local sensitization, while allergic symptoms, such as conjunctival hyperemia and edema, were noted at five and twenty minutes, respectively. In the results we found that, treatment with the ethanolic extract of *Cynodon dactylon* at 200 and 400 mg/kg significantly decreased ($p<0.001$) allergy symptoms and eye scratching behaviour and its effects were found in dose dependent manner. Based on the current investigation, it was concluded that the ethanolic extract of *Cynodon dactylon* significantly reduced the signs of conjunctivitis caused by allergies.

KEYWORDS: *Cynodon dactylon*; Antioxidants; Anti-allergic conjunctivitis; Allergic symptoms; Eye scratching behaviours; Cetirizine hydrochloride.

1. Introduction

Inflammation of the conjunctiva caused by allergies is known as allergic conjunctivitis, and it most commonly affects infants and children in the late summer and spring seasons. Seasonal and perennial allergic conjunctivitis are the two most prevalent and milder varieties of allergic conjunctivitis, but vernal and atopic kerato-conjunctivitis are less common but can cause blindness [1,2]. Although laboratory testing can be used to confirm the diagnosis, clinical diagnosis is the primary method used to diagnose ocular allergies. Allergists can use scratch tests as a skin test for specific allergens. Testing for IgE antibodies to allergens in vitro is a routine procedure. By enabling the distinction between intrinsic and extrinsic forms, allergy testing facilitates more straightforward therapy [3]. Treatments for allergic conjunctivitis include a variety of drugs, including corticosteroids, non-steroidal anti-

inflammatory drugs (NSAIDs), antihistamines, dual-acting antiallergics, anti-leukotrienes, anti-IgE, mast cell stabilisers, and several other plant derivatives [4].

Medicinal plants are the sources of some commonly used medications and serve as the substitute for manufactured medications. Various parts of the plant-such as the bark, leaves, roots, stems, and seeds-contain a variety of phytochemical constituents that exhibit distinct pharmacological activities. *Cynodon dactylon* is a common perennial herbaceous creeping grass-distributed throughout the India and belongs to the Poaceae family. It is also known as Bermuda-grass and most significant plant from the genus *Cynodon* due to its inescapable dissemination in hotter parts of the world [5,6]. Entire herb and its root stalk are therapeutically active and it is local to north and east Africa, Asia and Australia [7,8]. It contains carbohydrates, proteins, minerals (calcium, magnesium, potassium, iron, zinc), terpenoids (limonene, geraniol, phytol, ß-sitosterol), vitamin C, palmitic acid, alkaloids (paspalitrem B, ergonovine, ergine) [9] and flavonoids like apigenin, vitexin [10,11]. It also contain carotenoids [12], volatile oils [13], saponins, and glycosides [14]. Its leaves show the presence of glycerine and linoleic acid [15,16]. It is traditionally used to treat diarrhoea, urogenital problems, calculus, dropsy, hemorrhage, sores, coughing, migraines, tumors, cystitis, diarrhea, seizures, hemorrhoids, high blood pressure, hysteria, snakebite, stones, and dandruff [17,18].

There are some pharmacological actions of *Cynodon dactylon* that have been reported like antioxidant activity [19], antidiabetic activity [20], diuretic activity [21], anticancer activity [22], analgesic and anti-pyretic activity [23], wound healing activity [24], antiarrhythmic activity [25], hepatoprotective activity [26], antiulcer activity [27], antimicrobial activity [28], anticonvulsive activity [29], immunomodulatory activity [30], antifertility activity [31], cardio-protective [32], haemostatic effect [33], anthelmintic activity [34], angiogenic effect [35] and anti-diarrhoeal activity. Based on the literature survey, it was found that there is no study related to the effects of *Cynodon dactylon* on conjunctivitis, so this study aimed to evaluate its anti-allergic conjunctivitis effects [36].

2. Materials and Methods

2.1. Drugs and Chemicals

The following reagents were indicated: egg albumin, carboxy methyl cellulose (SD fine chemicals, Mumbai), aluminium hydroxide, chloroform, formaldehyde (CDH Ltd, New Delhi), *Bordetella pertussis* inactive microorganism suspension (Sigma-Aldrich), cetirizine hydrochloride (GSK Ltd, Baddi), sterile water for injection (Nirlife Health Care, Mumbai).

2.2. Identification, collection and authentication of plant material

With reference number 2016/SOS/BOT/24, the plant was verified and taxonomically identified by the Botany department of IFTM University Moradabad, Uttar Pradesh, India. After gathering the plant material (aerial portion), it was dried for two weeks at room temperature before being pulverized into a coarse powder.

2.3. Extraction of plant material

The plant's dried coarse powder was first put through a 20-mesh sieve before going through several solvent extraction steps through hot extraction process using Soxhlet apparatus. Firstly, the powder was extracted with petroleum ether at 50°C for 10 extraction cycle to remove the colour and fat of the plant material and then final extraction was done in ethanol at 60°C for 15 extraction cycle to extract-out the compound to be used. The semisolid mass that was obtained from the solvent's evaporation at lower pressure and then solid residues were obtained by vacuum drying of ethanolic extract of *Cynodon dactylon* (EECD). The dried extract was then stored in air-tight container throughout the study.

2.4. Preliminary phytochemical screening

Several chemical tests were performed on the extract (EECD) to identify the phytoconstituents present in the extract such as alkaloids, carbohydrates, glycosides, protein, amino acids, tannins, flavonoids and triterpenoids [37,38].

2.5. *In-vitro* antioxidant activity (DPPH scavenging method)

Antioxidant either transfers a hydrogen atom or electron to DPPH (2,2-diphenyl-1-picrylhyorazyl), hence neutralizing its free radical character [39]. The solutions of extract at 50, 100, 150, 200, and 250 µg/ml concentrations in 95% methanol were prepared. The DPPH solution (0.5 mM) was prepared in 95% methanol. To start the reaction, 0.2 ml of the extract solution was added in 2 ml of DPPH solution and the reaction was allowed to complete for 30 minutes, then at 517 nm the absorbance was measured and correlated with ascorbic acid reference. The activity percentage was calculated using the following formula.

$$\% \text{ Inhibition} = [(A_0-A_1)/A_0] \times 100$$

where A_0 represents the control's absorbance (blank, extract-free) and A1 represents the extract's or standard's absorbance [40,41].

2.6. Experimental animals

The Wister albino rats, weighing between 140 to 150 grams and six weeks old, were used. The animals were obtained from the I.F.T.M. University's animal house in Moradabad. Water and ordinary lab chow were administered to the rats. The IFTM University in Moradabad's Institutional Animal Ethics Committee accepted the experimental protocol with resolution no. 2016/837ac/MPh/09. The Animal Care and Use Committee's rules were followed in all procedures involving the animals.

2.7. Acute oral toxicity study

Using albino Wistar rats, acute oral toxicity experiments for ethanolic extracts of *Cynodon dactylon* were carried out in accordance with organisation for economic co-operation and development (OECD) guidance No. 420. The rat was given 2000 mg/kg of extract orally after it had been fasted and its physical parameters assessed. The rat was then closely watched for any clinical symptoms for four hours. The animals' weight was once again measured six hours after the test was administered,

and during the following fourteen days, an extensive clinical exam would be conducted every day [42].

2.8. Evaluation of anti-allergic conjunctivitis activity of extract

Five groups of five rats each were used to assess the anti-allergic conjunctivitis impact of EECD. The first group, designated as the control group, received a daily dose of 1 ml of 1% CMC solution. The second group, designated as the standard group, received 10 mg/kg of cetirizine hydrochloride treatment. EECD treatments of 100 mg/kg, 200 mg/kg, and 400 mg/kg were administered to Groups III, IV, and V.

All groups of the rats were sensitized by injection (i.p.) with 0.6 ml of saline solution which contains 10^{10} killed *B. pertussis* cells, aluminium hydroxide (2 mg) and egg albumin (1 mg) by intraperitoneal injection on the first day. On 5th days we administered egg albumin (0.5 mg) in 1 ml saline solution subcutaneously at 10 places on the back side of rats. Then from days 14 to 42, the local sensitization was carried out with administration of egg albumin at 10 mg/ml in physiological saline using a micropipette, 5 µl were administered to each eye.

For the estimation of anti-conjunctivitis activity, the actively sensitized rats were treated with their respective drug from day 14 to 42. Subsequently, using a scoring system, the number of episodes of eye scratching behavior, such as an unbroken cluster of fast forelimb movements directed towards the ocular surface, was counted for five minutes following topical antigen challenge, while allergic symptoms, such as conjunctival hyperemia and edema, were noted at five and twenty minutes, respectively (Table 1) [43,44]

Table 1. The grading method used for determining the severity of conjunctivitis

Score	Symptoms	
	Hyperaemia	**Edema**
0	No symptom	No symptom
1	Mild hyperaemia in one eye	Mild edema in one eye
2	Mild hyperaemia in both eyes	Mild edema in both eyes
3	Serious hyperaemia in one eye while mild hyperaemia in another eye	Serious edema in one eye while mild edema in another eye
4	Serious hyperaemia in both eye	Serious edema in both eyes

3. Results

3.1. Preliminary phytochemical screening

After performing the different chemical test for identification of types of chemical compounds present in extract we observed the presence of following compound as alkaloids, carbohydrates, glycoside, protein, amino acid, tannins, flavonoids while chemical test for triterpenoids was found to be negative (Table 2).

Table 2. Phytochemical investigation of extract (EECD)

Phytochemicals	Test	Results
Alkaloid	Dragendroffs test	+
	Mayers test	+
	Wagner test	+
Carbohydrate	Fehling test	+
	Benedict test	+
	Molisch test	+
Glycoside	Keller-killiani test	+
	Dinitro benzoic acid test	+
Protein	Biuret test	+
	Millions test	+
Amino acid	Ninhydrine test	+
	Millons test	+
Tannin	Ferric chloride test	+
	Iodine test	+
Flavonoid	Alkaline reagents test	+
	Zinc hydrochloride test	+
Triterpenoid	Salkowski test	–
	Libermann-burchard test	–

Where, (+) = Present, (–) = Absent

3.2. *In-vitro* antioxidant activity (DPPH scavenging method)

During DPPH scavenging experiment of the extract, the extract was found to be less effective when compared to the standard drug and it was observed that the EECD has dose dependent DPPH scavenging activity (Table 3). From the graphical representation of data, we observed that the IC_{50} value of EECD was found at 165.5 µg/ml (Figure 1).

Table 3. Evaluation of DPPH scavenging activity of extract (EECD)

S. No.	Concentration (µg/ml)	Extract (EECD) % of inhibition	Ascorbic acid % of inhibition
1	50	30.81 ± 0.74	61.44 ± 0.65
2	100	39.26 ± 0.57	84.35 ± 0.75
3	150	47.87 ± 0.23	90.37 ± 0.35
4	200	54.72 ± 0.86	97.87 ± 0.32
5	250	61.36 ± 0.38	99.45 ± 0.45

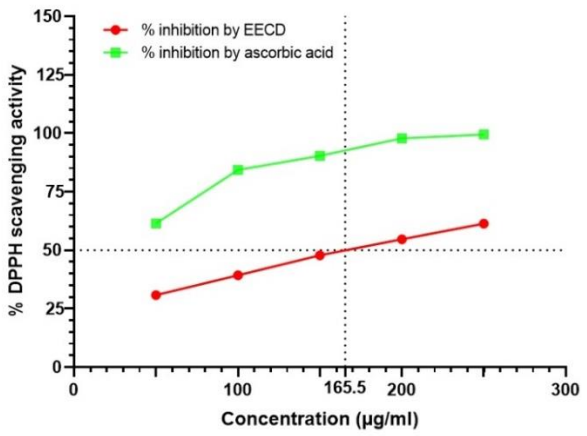


Figure 1. Evaluation of DPPH scratching activity of EECD

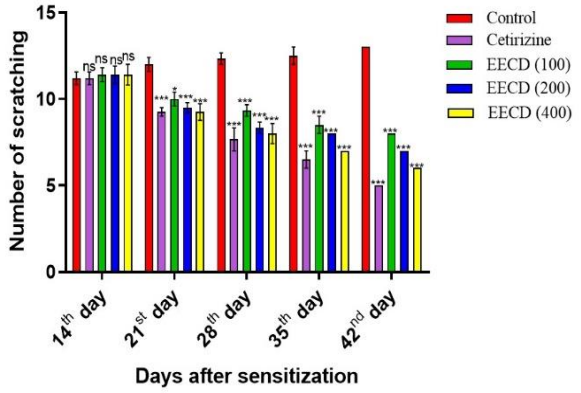
3.3. Acute oral toxicity study

All the animals lived for 14 days after the EECD was administered, and no noticeable signs of acute oral toxicity were seen over the whole observation period. The observation brought about the conclusion that the LD50 was higher than 2000 mg/kg and that EECD was safe up to 2000 mg/kg.

3.4. Anti-conjunctivitis activity

3.4.1. Evaluation of eye scratching behaviour

Following the antigen challenge, a notable alteration in the scratching behavior of the eyes was detected. Once the antigen has been applied topically, eye scratching behavior was seen right away and persisted for five minutes. Repeated topical application of antigen up to 21 days significantly elevated the frequency and severity of eye-scratching behavior and after that a significant decrease in eye scratching behavior was observed from days 21 to 42 in the treatment group in contrast to the group under control. The impact of EECD was noted to be dose dependent (Figure 2).

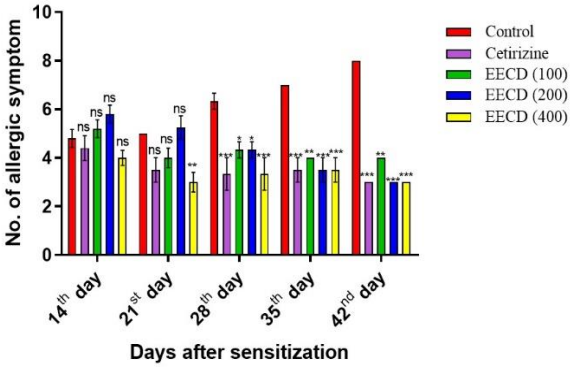


Vertical bar represent the mean ± SEM of 5 animal for eye scratching behaviour. ANOVA followed by dunnet's test *p<0.05 and ***p<0.001 against control group of rats

Figure 2. Evaluation of eye scratching behavior

3.4.2. Evaluation of allergic symptoms

Allergic symptoms like hyperaemia and edema were observed after 5 min and 20 min after local sensitization respectively. We found that the extract diminished both hyperaemia and edema and their action is started from the first week of the study and comparative outcomes were seen when compared to the standard group. We observed a dose dependent effect of extract in maintaining the allergic reaction and these signs maintained throughout local sensitization as well, and a significant impact was observed (Figure 3).



Vertical bar represent the mean ± SEM of 5 animal for allergic symptom of eye. ANOVA followed by dunnet's test *p<0.05, **p<0.01 and ***p<0.001 against control group of rats

Figure 3. Evaluation of allergic symptoms

4. Discussion

Considering the important role that oxidative stress plays in the pathophysiology of a few neurological illnesses and creating evidence of the antioxidant qualities of plants, exploring plant-based therapies could offer promising neuroprotective strategies. Natural medicines are great source of water-soluble antioxidants [45,46]. 2,2-diphenyl-1-picrylhyorazyl (DPPH) is a free radical and mostly utilized in the study of plant extract scavenging activity [47,48]. Since DPPH either adds an electron or a hydrogen molecule, neutralizing the free radical character of the compound, it is typically employed as a reagent to assess antioxidant effectiveness [41,49]. In this research we observed that ethanolic extract of *Cynodon dactylon* (EECD) produced DPPH scavenging activity with the IC_{50} value at 165.5 µg/ml.

Plant remedies are frequently used for self-medication since they are safe and effective in healthcare. Still, information regarding the toxicological profile and negative effects of these substances is lacking [50,51]. Rats treated with EECD did not exhibit any morbidity or death in the acute toxicity investigation up to 2000 mg/kg.

Allergic conjunctivitis is the biphasic allergic reaction, early by degranulation of mast cells and later by cell infiltration, mainly eosinophils [52,53]. Rats' eye scratching behavior is a more accurate way to measure itching in allergic conjunctivitis than it is in mice or guinea pigs. The observation that hyperemia and edema were noted concurrently with eye-scratching behavior supported this theory [54,55]. The model of allergic conjunctivitis induced by ovalbumin in rats has been utilize for screening of anti-allergic agents [56,57]. It is commonly known that the conjunctiva contains mast cells and that when conjunctivitis occurs, eosinophils enter in the conjunctiva. Since histamine is a component of both mast cells and eosinophils, high affinity IgE receptors are expressed on their surface [58,59]. The conventional medication, cetirizine (10 mg/kg p.o.), reduced all allergy indications in the current research, and it was additionally noted that cetirizine possesses H1 inhibitory action [60,61]. As demonstrated in this work, H1 antagonists nearly completely prevented antigen-induced eye scratching behavior. Thus, we hypothesised that eye itches brought on by an antigen-antibody response might be caused by histamine produced from mast cells and eosinophils. We found that the EECD significantly reduced allergic symptoms and scratching of the eyes, leading us to conclude that the treatment had a strong, dose-dependent inhibitory impact on allergic conjunctivitis in rats caused by antigen.

5. Conclusion

Up to the dosage level of 2000 mg/kg, no animal death was noted during the acute oral toxicity assessment of the extract, suggesting that it was mostly harmless. The EECD produced in-vitro antioxidant activity observed by DPPH scavenging method. EECD, 100 mg/kg, 200 mg/kg, and 400 mg/kg in oral administration, significantly decreased allergic symptoms and eye scratching behaviour. Therefore, we concluded that the current research demonstrated that the EECD produced a significant anti-allergic conjunctivitis activity.

Author Contributions: Conceptualization, A.K.P., P.C. and N.S.; methodology, N.S. and A.K.P.; validation, A.K.P., P.C. and V.C.B.; investigation, A.K.P. and S.K.; resources, N. Singh and V.C.B.; data curation, A.K.P. and N. Singh; writing-original draft preparation, A.K.P. and P.C.; writing-review and editing, P.C. and N.S.; visualization, S.K. and P.C.; supervision, N.S. and P.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Informed Consent Statement: The Wistar albino rats used in this study were provided by the Institutional Animal Ethics Committee of IFTM University, Moradabad, which approved the experimental protocol under resolution no. 2016/837ac/MPh/09.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Leonardi, A.; De Dominicis, C.; Motterle, L.J.C. Immunopathogenesis of ocular allergy: a schematic approach to different clinical entities. *Curr. Opin. Allergy Clin. Immunol.* **2007,** *7(5)*, 429-435.
2. Patel, A.K.; Chandra, P.; Sachan, N.; Singh, N. A review on pathophysiology and managements of allergic conjunctivitis. *Indian J. Clin. Exp. Ophthalmol.* **2024,** *10*, 13-20. DOI: 10.18231/j.ijceo.2024.003
3. Friedlaender, M.H. Ocular allergy. *Curr. Opin. Allergy Clin. Immunol.* **2011,** *11(5)*, 477-482. DOI: 10.1097/ACI.0b013e32834a9652
4. La Rosa, M.; Lionetti, E.; Reibaldi, M.; Russo, A.; Longo, A.; Leonardi, S.; Tomarchio, S.; Avitabile, T.; Reibaldi, A. Allergic conjunctivitis: a comprehensive review of the literature. *Ital. J. Pediatr.* **2013,** *39*, 18. DOI: 10.1186/1824-7288-39-18
5. Rai, P.K.; Rai, N.K.; Rai, A.; Watal, G.J.I.S.; Technology. Role of LIBS in elemental analysis of Psidium guajava responsible for glycemic potential. *Instrum. Sci. Technol.* **2007,** *35(5)*, 507-522.
6. Harlan, J. Cynodon species and their value for grazing and hay. *Herbage Abstracts* **1970,** *40*, 233-238.
7. Asthana, A.; Kumar, A.; Gangwar, S.; Dora, J.J.R. Pharmacological perspectives of *Cynodon dactylon*. *Res. J. Pharmaceut. Biol. Chem. Sci.* **2012,** *3(2)*, 1135-1147.
8. Raghunāthan, K.; Mitra, R. *Pharmacognosy of indigenous drugs*. Central council for research in Ayurveda and Siddha, 1999.
9. Solanki, R.; Nagori, B.J.I.J. Physicochemical and phytochemical investigation of whole plant of *Cynodon dactylon*. *Int. J. Comp. Pharm.* **2012,** *3(10),* 1-4.
10. Annapurna, H.V.; Apoorva, B.; Ravichandran, N.; Arun, K.P.; Brindha, P.; Swaminathan, S.; Vijayalakshmi, M.; Nagarajan, A. Isolation and in silico evaluation of antidiabetic molecules of *Cynodon dactylon* (L.). *J. Mol. Graph. Model.* **2013,** *39,* 87-97. DOI: 10.1016/j.jmgm.2012.10.009
11. Johnson, A.W.; Snook, M.E.; Wiseman, B.R.J. Green leaf chemistry of various turfgrasses: differentiation and resistance to fall armyworm. *Crop Sci.* **2002,** *42(6)*, 2004-2010.
12. Chapman Jr, G.W.; Burdick, D.; Higman, H.C.; Robertson, J.A.J.J. Steam volatiles from coastal bermudagrass. *J. Sci. Food Agric.* **1978,** *29(4)*, 312-316.
13. Kumar, A.; Gnananath, K.; Kiran, D.; Reddy, A.; Ch, R.J.I.J. Antidiabetic activity of ethanolic extract of *Cynodon dactylon* root stalks in streptozotocin induced diabetic rats. *Int. J. Adv. Pharm. Res.* **2011,** *2(8)*, 418-422.
14. Jananie, R.; Priya, V.; Vijayalakshmi, K.J.N. Determination of bioactive components of *Cynodon dactylon* by GC-MS analysis. *New York Sci. J.* **2011,** *4(4)*, 16-20.
15. Kaleeswaran, B.; Ilavenil, S.; Ravikumar, S.J.I.J. Screening of phytochemical properties and antibacterial activity of *Cynodon dactylon* L. *Int. J. Curr. Res.* **2010,** *3,* 83-88.
16. Chopra, R.N.; Nayar, S.L.; Chopra, I.C. *Glossary of Indian medicinal plants*. Council of Scientific & Industrial Research, 1992.
17. Agharkar, S. *Medicinal plants of Bombay Presidency*. Scientific Publisher, Jpghpur, India, 1991.
18. Karthikeyan, R.J.T.B. Isolation of Anticancer Bioactive and In Vitro Evaluation of Antioxidant and Anticancer Activity of *Cynodon dactylon* (L). Pers. *Transl. Biomed.* **2015,** *6(3),* 23-31.
19. Rahman, M.S.; Akter, R.; Mazumdar, S.; Islam, F.; Mouri, N.J.; Nandi, N.C.; Mahmud, A. Antidiabetic and antidiarrhoeal potentials of ethanolic extracts of aerial parts of *Cynodon dactylon* Pers. *Asian Pacific J. Trop. Biomed.* **2015,** *5(8)*, 658-662.
20. KP, S.G.; CM, M.; Kumar, V. Study on the diuretic activity of *Cynodon dactylon* root stalk extract in albino rats. *Res. J. Pharm. Technol.* **2009,** *2(2)*, 338-340.
21. Kanimozhi, D.; Bai, V.R. In vitro anticancer activity of ethanolic extract of *Cynodon dactylon* against HT-29 cell line. *Int. J. Curr. Sci.* **2013,** *5*, 74-81.
22. Garg, V.K.; Khosa, R.J.P. Analgesic and anti-pyretic activity of aqueous extract of Cynodon dactylon. *Pharmacologyonline* **2008,** *3*, 12-18.
23. Kamal, Z.; Bairage, J.J.; Moniruzzaman, P.R.D.; Tabibul, M.; Islam, M.O.F.; Islam, M.R.; Paul, P.K.; Mou, S.M.; Rahmatullah, M.J.W. Folk medicinal uses of some plants in Tangail district, Bangladesh. *World J. Pharm. Pharm. Sci.* **2014,** *3(11)*, 52-63.
24. Najafi, M.; Nazemieh, H.; Ghavimi, H.; Gharahkhani, A.; Garjani, A. Effects of hydroalcoholic extract of *Cynodon dactylon* (L.) pers. on ischemia/reperfusion-induced arrhythmias. *J. Pharm. Sci.* **2008,**
25. Surendra, V.; Prakash, T.; Uday, R.; Goli Divakar, G.D.; Snehal, D.; Kotresha, D. Hepatoprotective activity of aerial parts of *Cynodon dactylon* against CCl4-induced in rats. *Pharmacogn. Mag.* **2008,** *4(16)*, S195-S201.
26. Patil, M.; Jalalpure, S.; Prakash, N.; Kokate, C. Antiulcer properties of alcoholic extract of *Cynodon*

dactylon in rats. in *III WOCMAP Congress on Medicinal and Aromatic Plants-Volume 6: Traditional Medicine and Nutraceuticals, Acta Hort.* **2003**, *680*, 115-118 (2003).

27. Kanimozhi, D.; Bai, V.J.I. Evaluation of Phytochemical antioxidant antimicrobial activity determination of bioactive components of ethanolic extract of aerial and underground parts of *Cynodon dactylon* L. *Int. J. Sci. Res. Rev.* **2012,** *1*, 33-48.
28. Pal, D.K.J. Determination of brain biogenic amines in *Cynodon dactylon* Pers. and *Cyperus rotundus* L. treated mice. *Int. J. Pharm. Pharm. Sci.* **2009,** *1(1),* 190-197.
29. Devi, K.S.; Annapoorani, S.; Ashokkumar, K.J.J.M. Hepatic antioxidative potential of ethyl acetate fraction of *Cynodon dactylon* in Balb/c mice. *J. Med. Plants Res.* **2011,** *5(6)*, 992-996.
30. Nayanatara, A.; Alva, A.; Kottari, S.; Soofi, A.A.; Rejeesh, E.; Bhagyalakshmi, K.; Shetty, S.B.; Kini, R.D.; Pai, S.R.J. Effect of *Cynodon dactylon* extract on estrous cycle and reproductive organs in female wistar rats. *Int. J. Anal. Biomed. Pharm. Sci.* **2012,** *1(3)*, 10-15.
31. Garjani, A.; Afrooziyan, A.; Nazemiyeh, H.; Najafi, M.; Kharazmkia, A.; Maleki-Dizaji, N.J.B.c.; medicine, a. Protective effects of hydroalcoholic extract from rhizomes of *Cynodon dactylon* (L.) Pers. on compensated right heart failure in rats. *BMC Complement. Altern. Med.* **2009,** *9*, Art. No: 28. DOI: 10.1186/1472-6882-9-28
32. Hugar, L.; Ramesh, H.J.J. Evaluation of haemostatic effect of *Cynodon dactylon* pers in albino rats. *J. Evol. Med. Dental Sci.* **2014,** *3(11)*, 2711-2714.
33. Abhishek, B.; Thakur, A. Anthelmintic activity of *Cynodon dactylon*. *J. Pharmacogn. Phytochem.* **2012,** *1(3)*, 1-3.
34. Soraya, H.; Moloudizargari, M.; Aghajanshakeri, S.; Javaherypour, S.; Mokarizadeh, A.; Hamedeyazdan, S.; Esmaeli Gouvarchin Ghaleh, H.; Mikaili, P.; Garjani, A.J.D.J.o.P.S. Angiogenic effect of the aqueous extract of *Cynodon dactylon* on human umbilical vein endothelial cells and granulation tissue in rat. *DARU J. Pharm. Sci.* **2015,** *23(1)*, Art. No: 10. DOI: 10.1186/s40199-015-0093-x
35. Babu, D.R.; Neeharika, V.; Pallavi, V.; Reddy, M.B.J. Antidiarrheal activity of *Cynodon Dactylon*. pers. *Pharmacogn. Mag. [Internet]* **2009,** *5(19S), 23-27*.
36. Rad, A.K.; Rajaei, Z.; Mohammadian, N.; Valiollahi, S.; Sonei, M.J.U. The beneficial effect of *Cynodon dactylon* fractions on ethylene glycol-induced kidney calculi in rats. *Urol. J.* **2011,** *8(3)*, 179-184.
37. Evans, W.C.; T., E. Pharmacognosy, Nanomedicine, and Contemporary Issues. Volume 2. *Harcourt Publisher Limited, Mumbai* **2002**, 262-263.
38. Kokate, C.J.V. Practical Pharmacognosy. 3rd ed. New Delhi. *VPBN.* **1991,** *3*, 107-111.
39. Pan, Y.; Wang, K.; Huang, S.; Wang, H.; Mu, X.; He, C.; Ji, X.; Zhang, J.; Huang, F.J.F. Antioxidant activity of microwave-assisted extract of longan (*Dimocarpus Longan* Lour.) peel. *Food Chem.* **2008,** *106(3)*, 1264-1270. DOI:10.1016/j.foodchem.2007.07.033
40. Blois, M.S.J.N. Antioxidant determinations by the use of a stable free radical. *Nature* **1958,** *181(4617)*, 1199-1200.
41. Rekka, E.; Kourounakis, P.N. Effect of hydroxyethyl rutosides and related compounds on lipid peroxidation and free radical scavenging activity. Some structural aspects. *J. Pharm. Pharmacol.* **1991,** *43(7)*, 486-491. DOI: 10.1111/j.2042-7158.1991.tb03519.x
42. OECD. 425: acute oral toxicity-fixed dose procedure. *OECD Guidelines for the Testing of Chemicals* **2001,** *2*, 12-16.
43. Chandra, P.; Patel, A.K.; Sachan, N.; Kabra, A.; Singh, N.; Alshammari, A.; Albekairi, N.A.; Murthy, H.C.A. Evaluation of Antiallergic Conjunctivitis Effects of Extract of *Nelumbo nucifera* Rhizome and Its Isolated Constituents Against Chemical-Induced Allergic Conjunctivitis in Experimental Rats. *J. Chem.* **2025,** *2025(1)*, Art. No: 6747119. DOI: 10.1155/joch/6747119
44. Minami, K.; Kamei, C. A chronic model for evaluating the itching associated with allergic conjunctivitis in rats. *Int. Immunopharmacol.* **2004,** *4(1)*, 101-108. DOI: 10.1016/j.intimp.2003.10.013
45. Deba, F.; Xuan, T.D.; Yasuda, M.; Tawata, S.J.F. Chemical composition and antioxidant, antibacterial and antifungal activities of the essential oils from *Bidens pilosa* Linn. var. Radiata. *Food Control* **2008,** *19(4)*, 346-352.
46. Muscolo, A.; Mariateresa, O.; Giulio, T.; Mariateresa, R. Oxidative Stress: The Role of Antioxidant Phytochemicals in the Prevention and Treatment of Diseases. *Int. J. Mol. Sci.* **2024,** *25(6)*, Art. No: 3264. DOI: 10.3390/ijms25063264
47. Bhuiyan, M.; Hoque, M.; Hossain, S.J.W. Free radical scavenging activities of *Zizyphus mauritiana*. *World J. Agricult. Sci.* **2009,** 5(3), 318-322.
48. Baliyan, S.; Mukherjee, R.; Priyadarshini, A.; Vibhuti, A.; Gupta, A.; Pandey, R.P.; Chang, C.M. Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of *Ficus religiosa*. *Molecules* **2022,** *27(4)*, Art. No: 1326. DOI: 10.3390/molecules27041326
49. Anitha, S.; Krishnan, S.; Senthilkumar, K.; Sasirekha, V. A comparative investigation on the scavenging of 2,2-diphenyl-1-picrylhydrazyl radical by the natural antioxidants (+) catechin and (-) epicatechin. *J. Mol. Struct.* **2021,** *1242*, Art. No: 130805. DOI: 10.1016/j.molstruc.2021.130805
50. Lal, R.; Sharma, M.; Behera, S.; Regar, R.K.; Tripathi, D.; Kumar, G.N.; Singh, S.; Verma, D.; Gupta, P.; Kaushik, S.J.T. Safety Evaluation of Arsenicum Album in Acute and Sub-Acute Toxicity Studies in Rats. *Toxicol. Int.* **2023**, *30(2)*, 233-247.
51. Patel, A.K.; Chandra, P.; Sachan, N. Oral toxicity study of *Nelumbo nucifera* Gaertn. rhizome extract in rats. *Ann. Phytomed.* **2023**, *12(2)*, 713-719.
52. Bielory, B.P.; O'Brien, T.P.; Bielory, L. Management of seasonal allergic conjunctivitis: guide to therapy. *Acta Ophthalmol.* **2012,** *90(5)*, 399-407. DOI: 10.1111/j.1755-3768.2011.02272.x

53. Zdilar, A.M. Thesis. University of Zagreb. School of Medicine. Department of Internal Medicine (2024).

54. Davies, M.G.; Greaves, M.W. The current status of histamine receptors in human skin: therapeutic implications. *Br. J. Dermatol.* **1981**, *104(5),* 601-606. DOI: 10.1111/j.1365-2133.1981.tb08181.x

55. Nakazawa, Y.; Oka, M.; Takehana, M. Model for Studying Anti-Allergic Drugs for Allergic Conjunctivitis in Animals. *Open Med. (Wars)* **2017**, *12*, 231-238. DOI: 10.1515/med-2017-0034

56. Shoji, J.; Kiyo, N.G. Histological study of allergic conjunctivitis report 2. Time course of allergic conjunctival inflammation. *Nihon Ganka Kiyo (Folia Ophthalmol. Jpn)* **1995**, *46,* 1015-1020.

57. Choi, S.; Jung, M.-A.; Hwang, Y.-H.; Pyun, B.-J.; Lee, J.Y.; Jung, D.H.; Ji, K.-Y.; Kim, T. Anti-allergic effects of *Asarum heterotropoides* on an ovalbumin-induced allergic rhinitis murine model. *Biomed. Pharmacother.* **2021**, *141,* 111944. DOI: 10.1016/j.biopha.2021.111944

58. Hamid, Q.; Tulic, M.K.; Liu, M.C.; Moqbel, R. Inflammatory cells in asthma: mechanisms and implications for therapy. *J. Allergy Clin. Immunol.* **2003**, *111(1),* S5-S12; discussion S12-17. DOI: 10.1067/mai.2003.22

59. Labib, B.A.; Chigbu, D.I. Therapeutic Targets in Allergic Conjunctivitis. *Pharmaceuticals (Basel)* **2022,** *15(5)*, Art. No: 547. DOI: 10.3390/ph15050547

60. Nonaka, H.; Otaki, S.; Ohshima, E.; Kono, M.; Kase, H.; Ohta, K.; Fukui, H.; Ichimura, M. Unique binding pocket for KW-4679 in the histamine H1 receptor. *Eur. J. Pharmacol.* **1998,** *345(1)*, 111-117. DOI: 10.1016/s0014-2999(97)01620-8

61. Bulsara, K.G.; Patel, P.; Cassagnol, M.: Amlodipine. in *StatPearls [Internet]* (StatPearls Publishing), 2024.