

Genetic Profiling of *Cynodon dactylon* Species Using ISSR Markers and Its Pharmacological Activities

Chandrika S. Tanthry¹, Saswati Mandal² and S. D. Shruthi^{2*}

¹Department of Biotechnology, NMAM Institute of Technology, Nitte, Karkala, 574110, India.

²Microbiology and Molecular Biology Lab, Bio Edge Solutions, Bangalore, 058, India.

Authors' contributions

This work was carried out in collaboration among all authors. Author CST has designed the study, performed the statistical analysis and wrote the protocol. Author SM has written the first draft of the manuscript. Author SDS has managed to supervise the overall process to bring till final publication of this work. All authors read and approved the final manuscript.

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ABSTRACT

Aim: *Cynodon dactylon* is a grass variety of the tropical and sub-tropical region belonging to the family Poaceae. In this study genetic profiling of 3 different varieties of *Cynodon dactylon* were done using ISSR primers.

Methodology: Polymorphism patterns were observed by amplifying DNA with 8 sets of primers such as OPA16, OPB11, OPC06 etc. PyElph tool was used to understand the genetic similarity of the samples and accordingly the relatedness was shown by dendrogram construction by DendroUPGMA. The grass samples were also used to check their antibacterial activity against potential pathogenic bacteria like *Pseudomonas aeruginosa*, *Salmonella typhi*, *Chrysobacterium gleum* etc. The plant species had showed a very satisfactory inhibition against the bacteria. *In vitro* anti-diabetic activity of *Cynodon dactylon* was checked by calculating its ability to inhibit the enzyme alpha-amylase and the anti-inflammatory activity was carried out by calculating the inhibition percentage of albumin degradation.

*Corresponding author: E-mail: sdshruthi@gmail.com;

Outcome: All these experiments were done to study the pharmacological property as well as to gather knowledge on the genetic make-up of *Cynodon dactylon*. Overall, the final outcome supports the ethanobotanical claim of this plant.

Keywords: Anti-diabetic; anti-inflammatory; Bermuda grass; ISSR primers; dendrogram; polymorphism.

1. INTRODUCTION

Cynodon dactylon popularly known as the Bermuda grass [1] is a short-lived, prostrate and perennial grass. It grows on the soil very rapidly and forms a dense mat covering the soil. It is the main source of food for grazing cattle and many other herbivores. The culms of the plants are 5-45 cm long [2]. It is very much resistant to drought condition as the rhizome of the plant can survive by drought induced dormancy over a period of more than 7 months. After the drought is over and when normal conditions prevail it can again re-sprout with the help of stolons and rooted runners. Stolons and rhizomes play an important role in placement of leaves and roots in new microhabitats. In case of stolons and rhizomes their branching ability gets greatly reduced under lower light intensity and lower nutrient availability and branching ability is greatly accelerated under higher light intensity [3,4]. It has the ability to withstand floods and also can revive after fire. It can control functions of the ecosystem by altering fire regimes, hydrological cycles, biophysical dynamics, nutrients cycles, and community composition [5]. Studies on normal rats and streptozotocin induced diabetic rats showed that aqueous extract of *Cynodon dactylon* exhibit antidiabetic effect. Among several doses 500mg/kg reduced 31% blood glucose level after 4 hour administration in normal rats and 23% in mild diabetic rats during Glucose Tolerance Test (GTT) [6]. It has high therapeutic and pharmacological effects as the phytochemical constituents of this species are flavanoids, alkaloids, glycosides, terpenoides, triterpenoides steroids, saponins, tannins, resins, phytosterols, reducing sugars, carbohydrates, proteins, volatile oils and fixed oils [7]. Genetic profiling or DNA fingerprinting of this grass species is done using ISSR primers. Inter simple sequence repeat or ISSR are widely used for genetic variation studies. It is used as no prior information on DNA sequence is not required for the primer and moreover their cost of synthesizing is low and can be used for any plant species. Genetic profiling using ISSR primers are done to study ploidy complex and often to know the geographic origin of some plant

species. DNA profiling studies have been successfully used in analyzing relatedness of *Cynodon* species includes DNA amplification fingerprinting (DAF), RAPD, AFLP, ISSR, SSR and chloroplast specific simple sequence repeat length polymorphism (CpSSRLP) [8].

The plant is highly useful in its medicinal properties. Aqueous extract of the whole plant as well as leaves and rhizome exhibit properties like Antipyretic, Analgesic, Antihelminthic, Anticataleptic, Antimicrobial, Antidiuretic and Anti-inflammatory. Thorough studies resulted that aqueous and ethanol extract of the whole plant showed antiepileptic and antidiabetic activity; hydro alcoholic extract of the aerial parts of the plant exhibited antioxidant activity and 50% aqueous ethanolic extract could reduce kidney stones. This plant could be used in cough, cramps, diarrhea, headache, warts, wounds, measles etc. as a traditional medicinal plant [9,10]. Our present study involves analyses of the genetic profiling of plant samples of 3 different species using ISSR primers and their dendrogram construction to understand the relatedness between the samples. Antidiabetic, antibacterial and anti-inflammatory activities were also investigated using leaf extracts.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Fresh green grass samples were collected surrounding GKVK, Bangalore and stored. Three types of samples- Lawn grass (CD1), randomly available samples (CD2) and bigger leaflets (CD3) were taken for the genetic profiling studies.

2.2 Isolation of DNA

DNA was isolated from the fresh samples by CTAB method. 500 mg of the samples were ground with 1mL of pre-heated CTAB buffer [3% Cetyltrimethyl ammonium bromide, 5M NaCl,

0.5M EDTA (pH 8.0), 1M Tris-HCl (pH 8.0)]. The homogenate was obtained and incubated in dry bath at 60°C for 30 minutes, and was cool down to room temperature after completion of incubation. Chloroform and Isoamylalcohol in a ratio 24:1 was added in equal volume and gently mixed. It was then centrifuged in refrigerated centrifuge at 10,000 rpm for 10 minutes. After centrifugation the top layer was carefully transferred to another fresh tube and added equal volume of ice-cold isopropanol. The contents were centrifuged at 10,000 rpm for 10 minutes and the pellet was retained. 500 µL of 70% Ethanol was added to the pellet and gently tapped to mix the pellet. It was then spun in refrigerate centrifuge at 10,000 rpm for 10 minutes. The pellet was collected and dried for about 45 minutes thereafter the pellet was mixed with TE buffer. Gel-electrophoresis was done to observe the desired isolated DNA.

2.3 Polymerase Chain Reaction and Electrophoresis

After gel-electrophoresis samples were subjected for PCR using random primers to understand the polymorphism between species. The PCR reaction mixtures and the primers used are as shown in Table 1 and Table 2. The initial denaturation was done at 94°C for 1 minute followed by 40 cycles of denaturation at 92°C for 2 minutes; primers were annealed at 35°C for 1 minute and elongation at 72°C for 2 minutes followed by extended elongation at 72°C for 5 minutes. After amplification the products were electrophoresed for analysis on 1.5% agarose gel in 1X TBE buffer at 100V for 35 minutes. The gel was then visualized under UV transilluminator and photographed.

Table 1. Characteristics of the used primers in this study

| SL. No. | Primers | Annealing temperature (°C) |
|---------|---------|----------------------------|
| 1. | OPA 16 | 25 |
| 2. | OPB 11 | 25 |
| 3. | OPC 06 | 25 |
| 4. | OPC 16 | 25 |
| 5. | OPC 18 | 25 |
| 6. | OPD 13 | 27 |
| 7. | OPL 05 | 25 |
| 8. | OPW 04 | 25 |

Table 2. Composition of PCR reaction mixture

| Components | Quantity (µL) |
|--------------------|---------------|
| Primer | 0.4 |
| Template DNA | 0.5 |
| DNTP's | 0.4 |
| 5X Buffer | 1 |
| Taq buffer | 0.2 |
| Nuclear free water | 7.5 |

2.4 Dendrogram Construction

A dendrogram is a hierarchical tree which shows clustering of genes or samples. Hierarchical clustering is a popularly used method for detecting clusters in genomic data. Clusters can be described as cutting branches off the Dendrogram [11]. It consists of data points which are connected by U-shaped lines. Dendrograms are of two types- column and row, both the types comprises of Clades and Leaves. Using the web server DendroUPGMA dendrograms were constructed based on UPGMA (Unweighted Pair Group Method using Arithmetic Mean) or WPGMA (Weighted Pair Group Method using Arithmetic Mean). UPGMA is a simple agglomerative hierarchical clustering method applied for the construction of dendrogram from distance matrix. The UPGMA method utilizes a sequential clustering algorithm, where local topological relationships are inferred in descending order of similarity and a dendrogram is built in a gradual way. WPGMA is another algorithm that measures the distance between clusters is as a simple average [12]. Gel image was taken and bands were matched using PyElph software, binary matrix was generated. Using binary matrix Dendrogram was constructed through UPGMA method.

2.5 In-vitro Antibacterial Activity

Fresh leaves of *Cynodon dactylon* were collected from local shops in Bangalore, India to check its effect against urinary tract infection causing bacteria *Chryseobacterium gleum*. From the leaf powder plant samples was extracted using the solvent ethanol. The plant extract was then used for testing antibacterial activity. It was done by agar well method where DMSO was used as negative control. Plant extracts were mixed with 2 concentrations of DMSO 100 µg/mL and 200 µg/mL and added to the wells made on the plate containing bacterial culture. Development of the zone of inhibitions was checked after 24 hours incubation at 37°C.

2.6 *In-vitro* Antidiabetic Activity

From 10 grams of the powdered sample extract was prepared from ethanol for 24 hours. The extraction was done twice to make sure that we will not lose any phytochemicals. The extract was dried in a desiccator and used for testing *in-vitro* antidiabetic activity. In this method mixture of starch solution and plant extract was incubated at 25°C for 10 minutes. After that 1 mL of 1% starch solution prepared in 2 mL phosphate buffer was added and incubated at 37°C for 5 minutes to allow the reaction to happen. The reaction was terminated by the addition of 2 mL of 3,5 dinitro- salicylic acid reagent. Now the activity was measured by taking the absorbance at 540 nm with Glucobay 25 mg (1mg/mL). Inhibition of the enzyme α -amylase was noted in percentage. The formula for calculation of percentage of inhibition $I\% = (Ac-As)/Ac \times 100$

Ac= absorbance of control; As= absorbance of sample [13]

2.7 *In-vitro* Anti-inflammatory Activity

The above prepared methanol extract was used to estimate its *in-vitro* anti-inflammatory activity. The activity is determined by the inhibition of albumin denaturation. 5 ml of reaction mixture was prepared containing 0.2ml of bovine albumin, 2.8ml of phosphate buffered saline (PBS, pH 6.9) and 2ml of varying concentration of extracts (100-500 ppm). Equal volume of double distilled water was used as negative control. The reaction mixture was incubated at 37°C for 15 minutes followed by heating at 70°C for 5 minutes. Absorbance was observed at 660 nm using blank. Diclofenac sodium was used as the reference drug and treated for absorbance observation. The percentage of inhibition of protein denaturation was calculated. The formula for calculation of percentage of inhibition is $I\% = (Ac-As)/Ac \times 100$

Ac= absorbance of control; As= absorbance of sample [14]

3. RESULTS AND DISCUSSION

DNA which was isolated by CTAB method was further column purified to get rid of impurities and purity of the DNA was checked in spectrophotometer. The wavelength used was 260/280 nm and the concentration of DNA obtained was satisfactory to carry out other

procedures. Table 3 shows the concentration and purity of isolated DNA when compared with standard 1kb ladder. The bands obtained for ISSR primers range from 60 bp to more than 100 bp and for CD1 7 bands, CD2 6 bands and CD3 4 bands were observed. Close observation of the gel revealed that each lane have bands which were similar with the other 2 bands though the morphology of the grass samples were different. This polymorphism of bands showed the variation among genotypes and this study would help in the investigation of other relatives of the specimen. Using the PyElph software similarity between the samples was checked by band match (Fig. 2). UPGMA by PyElph showed that CD1 and CD2 shared more similarity than that of CD3 (Fig. 3). Dendrogram was constructed using the online tool Dendro UPGMA and tree obtained was as shown in Fig. 4. Matrix was loaded in the online tool setting the parameters as similarity index used to compare between the variables - Jaccard index (Tanimoto) and clustering method - UPGMA (Unweighted Pair Group Method with Arithmetic mean) and then submitted. The results obtained depicted that the primers OPC06, OPC18, OPL05 and OPW04 have worked and the rest didn't work. The corresponding bands for p1, p2, p4 and p6 shared a common clade but didn't polymerize the product fragment in all plant specimens. While the bands for p7 and p3 sharing a common clade polymerized with CD1 and CD2 only and the bands for p5 and p8 shared a common clade as well as polymerized with CD1 only.

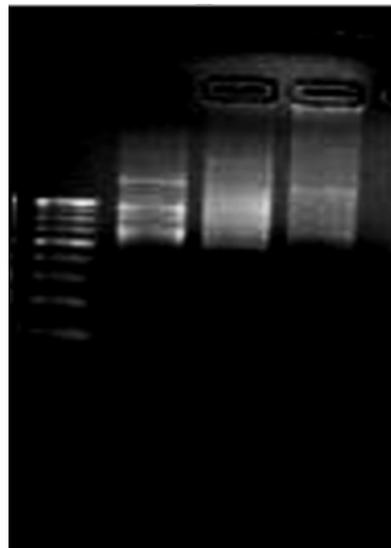


Fig. 1. Polymorphic bands on agarose gel for different primers

Table 3. Showing concentration and purity of isolated DNA

| Sample-ID | Scientific name | 260/280 | Conc(ng/μl) |
|-----------|----------------------------|---------|-------------|
| CD1 | <i>Zoysia pacifica</i> | 1.79 | 71.8 |
| CD2 | <i>Cynodon dactylon</i> | 1.72 | 93.4 |
| CD3 | <i>Cynodon nlemfuensis</i> | 1.77 | 184.1 |

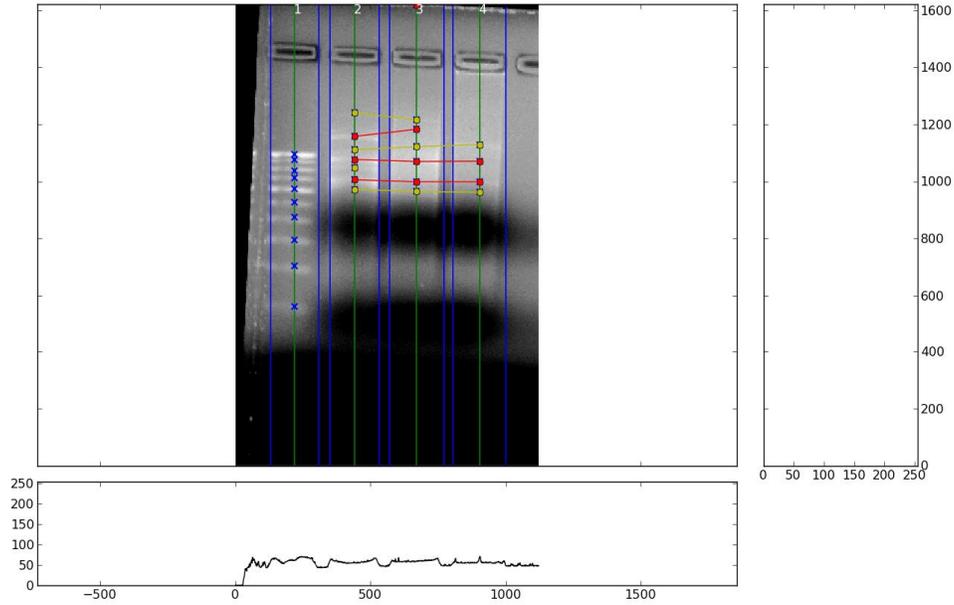


Fig. 2. Band match using PyElph software [lane 1- ladder, 2- CD1, 3-CD2, 4- CD3 specimens]

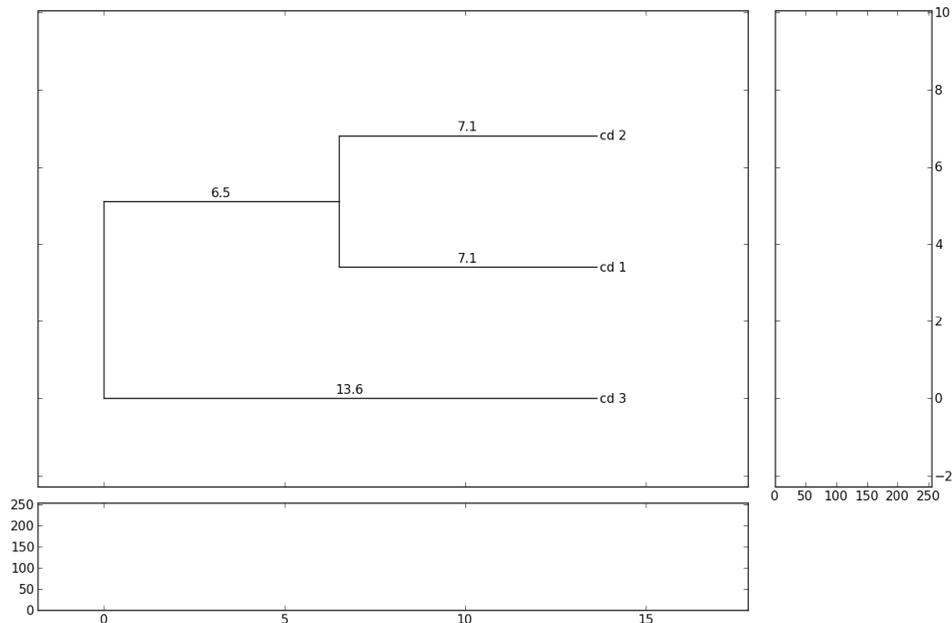


Fig. 3. UPGMA results got by PyElph software

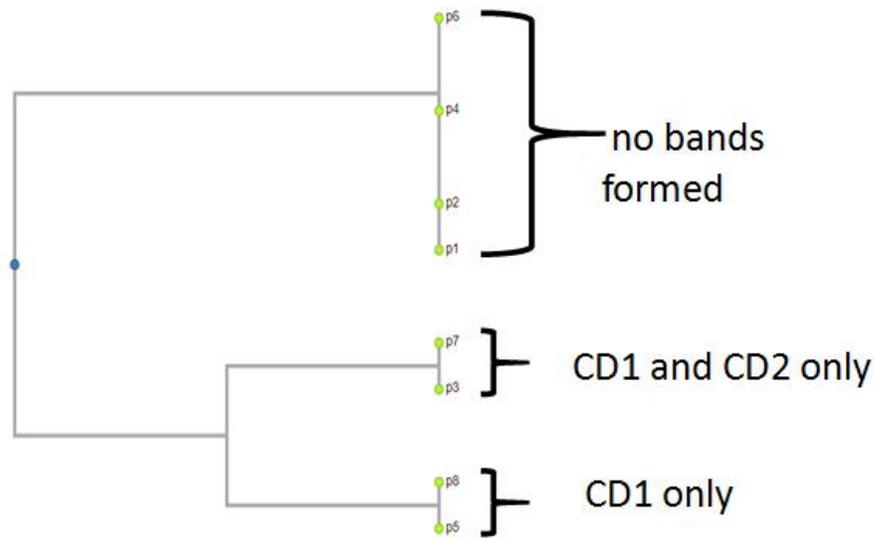


Fig. 4. UPGMA and cophenetic correlation coefficient results from the online tool DendroUPGMA

3.1 Antibacterial Studies

The antibacterial property of the samples were tested on plates cultured with *Chrysobacterium gleum*, *Klebsella pneumoniae*, *Pseudomonas aeruginosa*, *Pseudomonas syringae*, *Salmonella typhi* and *Xanthomonas campestris*. All these bacteria are gram negative, pathogenic and cause disease in either in plants or animals the most lethal among these is *Pseudomonas aeruginosa* which is a multi-drug resistant bacteria as well as an antibiotic resistant [15] one while *Pseudomonas syringae* is a very interesting bacteria as it is an atmospheric “biological ice nucleator” which can freeze the water of leaves and cause an injury. It is a potential plant pathogen and acts as model to study host-microbe interactions, bacterial virulence mechanisms, host adaptation of pathogens, as well as microbial evolution,

ecology and epidemiology [16]. *Chrysobacterium gleum* is a gram-negative, non-fermenting bacteria found widely in soil and water. It is an emerging pathogen and can be acquired through nosocomial infection. It is a metallo β-lactamase producing bacterium [17,18]. This bacteria is yellow pigmented on blood agar, catalase positive, oxidase positive, non-motile, non-glucose fermenting Gram negative bacilli on MacConkey agar. *Chryseobacterium* spp are resistant to several antibiotics such as aminoglycosides, chloramphenicol, tetracyclines, clindamycin, teicoplanin, and erythromycin [19].

Antibacterial studies revealed that *Cynodon dactylon* extract has significant effect on *Chrysobacterium gleum* forming a zone of inhibition with 3 mm diameter in 200 µg/ml concentration. It has also affected the other bacteria which are as tabulated in Table 4.

Table 4. Respective microorganism and inhibition zone formed by extract dissolved in DMSO

| Micro organism | Gram staining | Inhibition zone (mm) | |
|--------------------------------|---------------|----------------------|--------------|
| | | 1 (200µg/mL) | 2 (100µg/mL) |
| <i>Chrysobacterium gleum</i> | negative | 3 | 1 |
| <i>Klebsella pneumoniae</i> | negative | 4 | 1 |
| <i>Pseudomonas aeruginosa</i> | negative | 1 | 3 |
| <i>Pseudomonas syringae</i> | negative | 1 | 3 |
| <i>Salmonella typhi</i> | negative | 2 | 1 |
| <i>Xanthomonas comperttris</i> | negative | 0 | 0 |

3.2 Antidiabetic Studies

A graph was obtained by calculating the inhibition percentage of α -amylase. The control used for this study Glucobay has an active amylase inhibition ingredient called Acarbose which is a synthetic chemical. 1 mg/ml concentrated Acarbose showed 79% inhibition rate and 400 ppm of *Cynodon dactylon* extract showed 72%. This result opened a new modus operandi for antidiabetic drug synthesis as *Cynodon dactylon* is a plant which can act as a superb alternative to synthetic drugs as shown in Fig. 5.

3.3 Anti-inflammatory Studies

Anti-inflammatory activity is based on the capability of inhibiting albumin degradation. The graph obtained by calculating the percentage showed that with increasing concentration of the plant extract capacity of inhibiting albumin degradation also increased. The control Sodium diclofenac, a standard drug showed 79 % inhibition rate at 1mg/ml concentration and plant extract with 500 ppm of concentration showed 62% of inhibition rate as shown in Fig. 6.

Cynodon dactylon is a medicinal plant and is used to treat inflammations in traditional

medicine. In a study doses ranging from 200, 400 and 600 mg/kg of the aqueous extract of the plant was administered on carrageenan, serotonin, histamine and dextran induced rat paw edema. The study concluded that all the doses are safe when taken orally and considerably reduced ($P < 0.001$) the edema formation induced by carrageenan, serotonin, histamine and dextran after 3 and 5 hours. [20] The plant exhibits antidiabetic activity and can be used as an alternative. Studies had shown that aqueous, chloroform, ethanol and methanol extracts when treated on alloxan induced diabetic rats considerably reduce blood glucose level. Among all these solvents methanol extract had reduced the blood glucose level of the diabetic rats to 108 ± 10.0 mg/100ml which is similar to that of the normal rats. [21] In another study it is observed that *Cynodon dactylon* has antibacterial activity. It was observed that aqueous and chloroform extract of this plant inhibited *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae* [22]. In this study also it was observed that *Cynodon dactylon* possess antibacterial activity showing immense sensitivity to *Chrysobacterium gleum*. It also had anti-diabetic and anti-inflammatory property when checked with respect to acarbose and diclofenac sodium respectively.

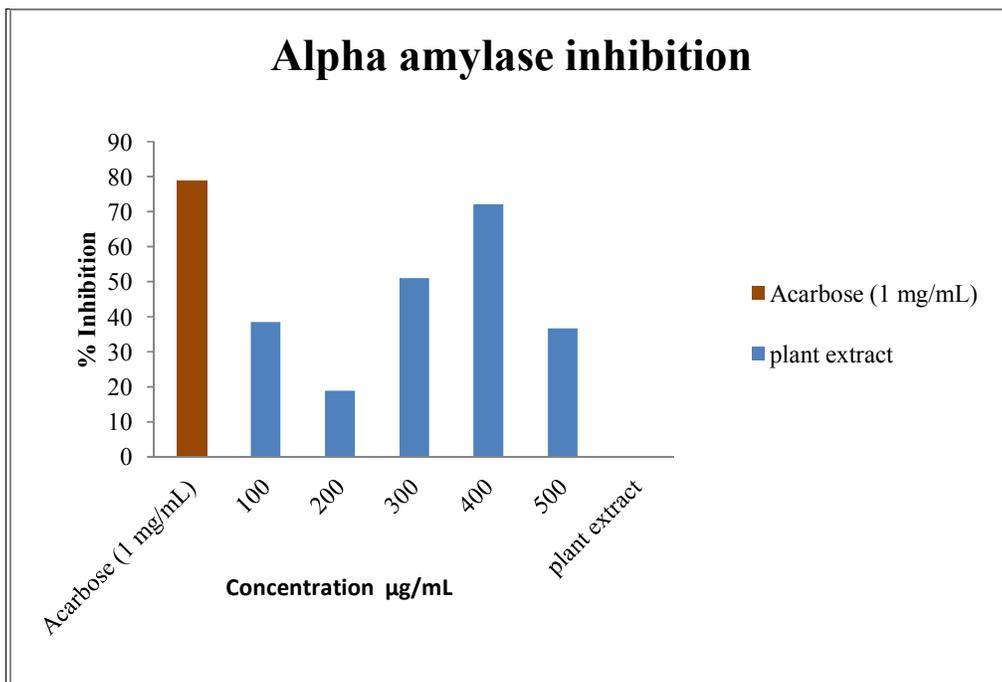


Fig. 5. Alpha amylase inhibition showed by *Cynodon* extract

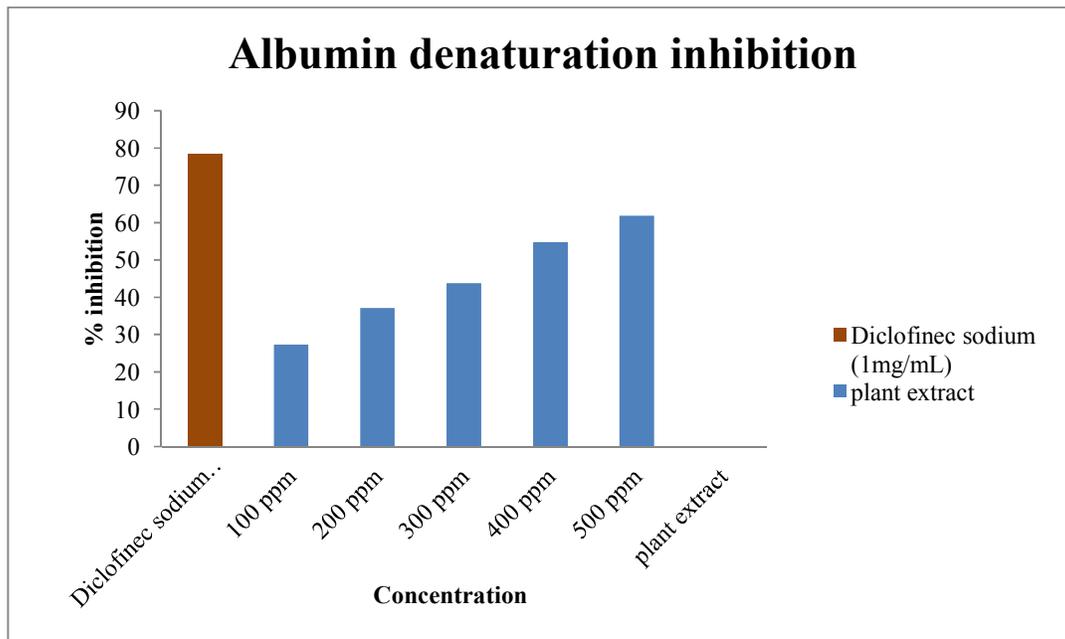


Fig. 6. Albumin denaturation inhibition showed by *Cynodon* extract

A species without enough genetic diversity is thought to be unable to cope with changing environments or evolving competitors and parasites [23]. In addition, the ability of a population to respond adaptively to environmental changes depends on its level of genetic variability or diversity [24]. Thus, genetic diversity in indigenous breeds is a major concern considering the necessity of preserving what may be a precious and irreplaceable richness, regarding new productive demands. Conservation should be based on a deep knowledge of the genetic resources of the specific breed. Therefore, it is important to try to genetically characterize indigenous breeds [25] and the applications of molecular genetics have many important advantages [26]. The ISSR is a molecular marker method which does not need the genome sequence information and leads to multi-loci and highly polymorphic patterns [27]. Each ISSR band corresponds to a DNA sequence delimited by two inverted microsatellites. The ISSR loci are dominant markers with the assumption of only two alleles per locus. It has been shown that the ISSR markers are universal, quick, easy to apply, highly reproducible and polymorphous [28]. The ISSR method has been used in genetic diversity studies in several species such as cattle [29], cattle, goat and sheep [27], sheep [28], fish [30], silkworm *Bombyx mori* [31] et al., 1999, mouse [32] and has proved to be universal.

4. CONCLUSION

Cynodon dactylon or Bermuda grass rapidly grows in warm climate and forms a dense mat by underground rhizomes and surface stolons. Since it is a lawn grass it needs regular mowing and if left unmowed can reach up to 2 feet high. It is a C4 plant and has various useful properties. This study is very useful in understanding the pharmacological property of the plant. Since the study showed that this plant has active anti-diabetic activity it can be used as a potential alternative against synthetic compounds moreover it has antibacterial activity and can be used to synthesize antibiotic to treat potential pathogens. This study is very promising and will surely unveil new methods of treatment in the medical world.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Feedipedia.org. Bermuda Grass (*Cynodon dactylon*); 2020.
2. Tropical.the ferns.info. *Cynodon dactylon* - Useful Tropical Plants; 2020.
3. Dong M and de Kroon H. Plasticity in Morphology and Biomass Allocation in *Cynodon dactylon*, a Grass Species Forming Stolons and Rhizomes. *Oikos*. 1994;70(1):99.
4. Dong M. Morphology and growth of stolons and rhizomes in three clonal grasses, as affected by different light supply. Springer Link; 1995.
5. Cabi.org. *Cynodon dactylon* (Bermuda Grass); 2020.
6. Singh S, Kesari A, Gupta R, Jaiswal D, Watal G. Assessment of antidiabetic potential of *Cynodon dactylon* extract in streptozotocin diabetic rats. *Journal of Ethnopharmacology*. 2007;114(2):174-179.
7. Al-Snafi P. Chemical constituents and pharmacological effects of *Cynodon dactylon*- A Review. *IOSR Journal of Pharmacy (IOSRPHR)*. 2016;06(07):17-31.
8. Farsani T, Etemadi N, Sayed-Tabatabaei B, Talebi M. Assessment of Genetic Diversity of Bermuda grass (*Cynodon dactylon*) using ISSR Markers. *International Journal of Molecular Sciences*. 2011;13(1):383-392.
9. Ashokkumar K. *Cynodon dactylon* (L.)Pers. An updated review of its phytochemistry and pharmacology. *Journal of Medicinal Plants Research*. 2013;7:7.
10. Nagori B, Solanki R. *Cynodon dactylon* (L.) Pers.: A Valuable Medicinal Plant. *Research Journal of Medicinal Plant*. 2011;5(5):508-514.
11. Langfelder P, Zhang B, Horvath S. Defining clusters from a hierarchical cluster tree: the Dynamic Tree Cut package for R. *Bioinformatics*. 2007;24(5):719-720.
12. Vallvé S, Puigbò P. DendroUPGMA: A dendrogram construction utility [Ebook]. 2009;14. Available: https://usuaris.tinet.cat/debb/UPGMA/DendroUPGMA_Tut.pdf
13. Sabitha V, Panneerselvam K, Ramachandran S. In vitro alpha-glucosidase and alpha-amylase enzyme inhibitory effects in aqueous extracts of *Abelmoscus esculentus*(L.) Moench. *Asian Pac J Trop Biomed*. 2012;2(2):1624.
14. Sakat S, Juvekar AR, Gambhire MN. In vitro antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. *International Journal of Pharma and Pharmacological Sciences*. 2010;2(1):146-155.
15. Al-Marzoqi A, Al Taei Z. *Pseudomonas aeruginosa*: Antibiotic resistance pattern to different isolates in Al-Hillah city, Iraq [Ebook] (3rd ed., p. 7). *Journal of Natural Sciences Research*; 2013.
16. Xin X, Kvitko B, He S. *Pseudomonas syringae*: what it takes to be a pathogen. *Nature Reviews Microbiology*. 2018;16(5):316-328.
17. Baby TGR, Das SP, RKG. *Chryseobacterium Gleum* Urinary Tract Infection. *Genes Review*. 2015;1(1):1-5.
18. Easow J, Rajendran P, Muthusamy S, Balaji V, Rakesh G. Urinary tract infection due to *Chryseobacterium gleum*, an uncommon pathogen. *Indian Journal of Pathology and Microbiology*. 2016;59(4):551.
19. Abdalhamid B, Elhadi N, Alsamman K, Aljindan R. *Chryseobacterium gleum* pneumonia in an infant with nephrotic syndrome. *Idcases*. 2016;5:34-36.
20. Garg V, Paliwal S. Anti-Inflammatory Activity of Aqueous Extract of *Cynodon dactylon*. *International Journal of Pharmacology*. 2011;7(3):370-375.
21. Ramya S, Vijayanand N, Rathinavel S. Antidiabetic activity of *Cynodon dactylon* (L.) Pers. Extracts in alloxan-induced RATS [Ebook]. 2014;6:5.
22. Suresh Kumarasamy P, Deepa R, Harisaranraj V, Vaira Achudhan. Antimicrobial and Phytochemical Investigation of the Leaves of *Carica papaya* L., *Cynodon dactylon*(L.) Pers., *Euphorbia hirta* L., *Melia azedarach* L. and *Psidium guajava* L. [Ebook]. 2008;12:8.
23. Khodabakhshzadeh R, Mohammadabadi MR, Esmailizadeh AK, Moradi Shahrehabak H, Bordbar F, et al. Identification of point mutations in exon 2 of GDF9 gene in Kermani sheep. *Polish Journal of Veterinary Sciences*. 2016;19:281-289.
24. Askari N, Mohammadabadi MR, Baghizadeh A. ISSR markers for assessing DNA polymorphism and genetic characterization of cattle, goat and sheep

- populations. Iranian Journal of Biotechnology. 2011;9:222-229.
25. Shojaei M, Mohammad Abadi MR, Asadi Fozi M, Dayani O, Khezri A, et al. Association of growth trait and Leptin gene polymorphism in Kermani sheep. Journal of Cell and Molecular Research. 2010;2:67-73.
 26. Mousavizadeh A, Mohammad Abadi MR, Torabi A, Nassiry MR, Ghiasi H, et al. Genetic polymorphism at the growth hormone locus in Iranian Talli goats by polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP). Iranian Journal of Biotechnology. 2009;7:51-53.
 27. Zamani P, Akhondi M, Mohammadabadi MR, Saki AA, Ershadi A, et al. Genetic variation of Mehraban sheep using two inter simple sequence repeat (ISSR) markers. African Journal of Biotechnology. 2011;10:1812-1817.
 28. Mohammadabadi MR, Esfandyarpoor E, Mousapour A. Using Inter Simple Sequence Repeat Multi-Loci Markers for Studying Genetic Diversity in Kermani Sheep. Journal of Research and Development. 2017;5(2):154
 29. Ghasemi M, Baghizadeh A, Mohammadabadi MR. Determination of genetic polymorphism in Kerman Holstein and Jersey cattle population using ISSR markers. Australian Journal of Basic Applied Science. 2010;4:5758-5760.
 30. Zhigileva ON, Baranova OG, Pozhidaev VV, Brol IS, Moiseenko TI. Comparative analysis of using isozyme and ISSR-PCR markers for population differentiation of Cyprinid fish. Turkish Journal of Fishery and Aquatic Science. 2013;13:159-168.
 31. Damodar Reddy K, Nagaraju J, Abraham EG. Genetic characterization of the silkworm Bombyx mori by simple sequence repeat (SSR)-anchored PCR. Heredity. 1999;83:681-687.
 32. Renny M, Julio NB, Bernardi SF, Gardenal CN, Oyarzabal MI. Estimation of genetic variability level in inbred CF1 mouse lines selected for body weight. Journal of Genetics. 2014;93:483-487.

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