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FORMULATION AND EVALUATION OF TRANSDERMAL PATCH OF *CYNODON DACTYLON* ROOT EXTRACT FOR IT'S ANTICOAGULANT ACTIVITY

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ABSTRACT

Cynodon dactylon, a tropical grass has been traditionally used in folk medicine to treat various ailments. The present study aimed to extract the root part of *Cynodon dactylon* and evaluate the anticoagulant activity. The extract was prepared by using maceration method with aqueous & non aqueous solvent and evaluation of it's anticoagulant activity by using blood clotting time measurement method. The extract was subjected to preliminary photochemical screening which gives the confirmation of presence of active metabolite like phenolic compound, alkaloid, flavonoid etc. The result showed that the root extract of *Cynodon dactylon* exhibited significant anticoagulant activity. The study suggests that the root extract of *Cynodon dactylon* may be a potential natural anticoagulant agent. This study explores the development and evaluation of a transdermal patch containing *Cynodon dactylon* extract for anticoagulant activity. This may offer a novel approach for preventing and treating thrombotic disorders. This may provide a convenient and effective delivery system for *Cynodon dactylon*'s anticoagulant compounds.

KEYWORDS: Anticoagulant, *Cynodon dactylon*, PBS, Patch, Folding endurance, etc.

INTRODUCTION

In recent decades, medicinal plants and their derivatives have been extensively taken as valuable substitutes for chemically synthesized drugs. Comparing of synthetic drugs, which are frequently associated with undesirable side effects, including occasional psychological impacts, herbal medicine has been shown to offer greater safety having minimal adverse effects. Nevertheless, many natural products have remedial efficiency is still burdened by their low selectivity for targeted cells. There have been increasing efforts to produce natural compounds with improved and efficacy potency and specificity.[1,2]

Cynodon dactylon Pers. (Poaceae), widely recognized as Bermuda grass, is an essential in warmth season perennial grass predominantly utilized for turf and forage.

Traditionally, it has been taken as folk medicine as well as potential source of bioactive medicinal compounds with massive health benefits for humans. *Cynodon dactylon* is considers as one of the holiest plants in India, second only to Tulsi, and is utilized by Hindus in worships of the lord Ganesha with the leaves of Durva.[3]

Plant Profile

(A) *Cynodon dactylon* has been classified based on its taxonomic position

Kingdom	Plantae
Subkingdom	Tracheobionta
Spermatophyta	Seed
Division	Magnoliophyta– Flower
Class	Liliopsida
Subclass	Commelinidae
Order	Cyperales
Family	Poaceae/Gramineae

(B) Common Names of *Cynodon dactylon*

Tamil	Arugampullu
Kannada	Garikoihallu
Sanskrit	Haritali
Marath	Durua
Telugu	Garikagoddi
Bengali	Durba
Punjabi	Dhubkhabbal

Fig. 1: Root part of *Cynodon dactylon*.

(C) MORPHOLOGY

Stem: It is slight, creeping, about 1.0 mm thick, jointed, and covered with leaves. Their surface is appears yellowish-green color and exceptionally smooth. Oval shaped have a slight indentation on one side. There fibers are short, thick-walled, having a narrow lumen and pointed ends.[4,5]

Leaves: *Cynodon dactylon* leaves are light green color, soft, and smooth, with a narrow, linear shape. They range from 2 to 10 cm in length and 1.25 to 3 mm in width.

Stolon: The inter node length of the stolon is associated by the soil pH. The most significant action is observed in plants that are six months or older.[6]

Roots: Small, hair-like roots emerge from the main root system, which are fibrous, cylindrical, and up to 4 mm in thickness.[7]

Chemical Constituents

- Chemical constituents present in roots- Hydroquinone (69.49 %), levoglucosenone, (2.72 %) and furfural (6.0%), Cinnamic acid 4-hydroxy-3-methoxy.[8]
- Chemical constituents present in the leaves are Alkaloids, Flavonoids, Steroids.[9]
- Chemical constituents present in rhizomes- Cellulose, lignin, Saturated fatty acids including Palmitic acid (C16:0; 40.36%), Stearic acid (C18:0; 4.10%) Unsaturated fatty acids including oleic acid (28.26) and linoleic acid (17.01).[10]

Therapeutic activity

Cynodon dactylon is reported to possess various therapeutic properties, including anti-arrhythmic,[11] anti-arthritic,[12] anti-cancer,[13] anti-diarrheal,[14] anti-diabetic,[15,16] anti-

diuretic,[17] anti-malarial,[13] cardio-protective,[18] immune modulatory,[19] and gastro-protective,[14] activities.[20]

Coagulation and Anti-coagulation

Hemostasis is a changing phenomenon in which coagulation of blood begins and concludes swiftly while being tightly regulated. This process relies on three key components: the vascular walls, platelets aggregation and the coagulation cascade. These elements are crucial for normal hemostasis, serving two primary objectives: maintaining blood in a body fluid, clot-free state and rapidly forming a localized clot at the site of vascular injury.

The hemostatic reaction includes spontaneous vasoconstriction, blood clotting, platelet aggregation and fibrinolysis (clot breakdown). Blood clotting is a complex cascade of reactions involving multiple factors (Proteins) that must function in a precise sequence to produce clot formation. This process is both rapid and efficient and required regulation on it. if an imbalance could lead to excessive clotting and thrombosis. Any disruption in the equilibrium between coagulation and its inhibition, favoring either excessive clot formation or prevention, can result in life-threatening conditions like thromboembolism or hemorrhage. In various clinical situations, drug interventions are required to regulate coagulation, that aim to prevent tissue damage caused by reduced blood flow when clot formation obstructs the blood supply to an organ or tissue.

Anticoagulants are chemical compound that interact with the body's natural coagulation system to prevent and treat abnormal blood clot formation, such as Deep Vein Thrombosis (DVT) and Pulmonary Embolism (PE), both of which fall under Venous Thromboembolism (VTE), as well as Atrial Fibrillation. These drugs are widely used to manage blood coagulation in both healthy individuals and patients with conditions such as cardiovascular disease, diabetic patient and cancer. As a result, there is growing research interest in the discovery of natural anticoagulant drugs that exhibit lower toxicity and fewer side effects.[21]

Transdermal drug delivery system

Transdermal drug delivery systems are also known as rate controlled drug delivery systems which is delivers a specific medication dose through the skin into our blood circulation that promotes demulcent on injured areas of the body. The primary objective of designing transdermal dosage is to improve the rate through the skin into bloodstream and simultaneously reduce the retention of drug and their metabolism in the skin. This dosage

form does not affect to the gastrointestinal tract so there is no loss due to first pass metabolism. The medicament can be delivered without interfering with pH, enzymes and intestinal bacteria. This dosage form contains penetration enhancers that improve the permeation of medicament across the skin. TDDS gives more benefits over injectables and oral routes due to non-invasiveness, improved patient compliance.

Polymers are used during skin preparations, marking on the basis of transdermal drug delivery systems. The surface and bulk characteristics of that polymer can give the desired interfacial, mechanical, chemical and biological function. Hydrophilicity, lubricity, smoothness and surface energy are surface property which is directs with biocompatibility with tissues and blood. There physical properties like durability, permeability and degradability. *The* polymer's choice and it's physicochemical properties depend on the need for extensive biochemical characterization and specific preclinical tests for improving their safety.[22]

MATERIAL AND METHOD

Chemical required

All the requirements used in this study were of standard pharmaceutical ranking. Ethanol, Polyvinyl pyrrolidone, Poly vinyl alcohol, Distilled water, Glycerol, Polyethylene glycol 400 etc were obtained from Pharmacy College, Azamgarh and of analytical ranking.

Apparatus required

The apparatus were required for making a patch Beaker, Measuring cylinder, Butter Paper, Weighing balance, Spatula, Petri dish, Hot air oven etc.

Collection of plant material

Root of *Cynodon dactylon* were obtained from the garden of Pharmacy College Azamgarh. About 250 gm of freshly roots was collected and washed in running water and shade dried for about two weeks at ambient condition.

Pre-authentication

Pre-authentication of *Cynodon dactylon* (Durva) was carried out from the Department of Botany, Banaras Hindu University (BHU), to confirm their botanical identity. This process ensured accurate plant material verification essential for reliable research outcomes involving

phytochemical analysis, antimicrobial studies and other biological evaluations of these medicinally valuable species and voucher specimen no. is **Poa. 2024/01.**

Micrometric studies

• Angle of Repose

A measurement of the flow properties of powder is its angle of repose. We used a formula for calculating the angle of repose. The finely pulverized powder was transferred via a funnel onto graph paper with the height of funnel kept constant. The angle formed between the powder heap's surface and horizontal plane. The height and base of the powder heap that had developed were measured and equation was used for calculating the angle of repose accordance with USP.

$$\text{Tan}\theta = h/r$$

$$\theta = \tan^{-1}(h/r) \quad \text{Eq. 1}$$

Where, θ represents the angle of repose,

H is height in cm and R is radius/base in cm

• Bulk density

This is the ratio of the bulk volume of powder to its total volume. Precisely weight 20 gm of powdered and then pour it into a 250 ml container graduated barrel. The powder's initial apparent volume (Vo) is meticulously leveled. For calculating the bulk density we used following formula and result is represented in g/ml.

$$\boldsymbol{\rho}b = M/Vb \quad \text{Eq. 2}$$

Where ρb=bulk density,

M=bulk weight of blend,

Vb= bulk volume of the blend.

• Tap density (TD)

This is the proportion of the powder's total mass to its tapped volume. Weigh precisely 40 gm of the powder combination, which was placed in a 100 ml container cylinder for measuring. After three manual taps (1250, 750, and 500) on the sample-containing cylinder, the final tapped volume (Vf) was determined. The formula for calculating tapered bulk density can be used, and the result is stated in g/ml.

$$\boldsymbol{\rho}t = M/Vt \quad \text{Eq. 3}$$

Where, ρt=Tapped density,

M=weight of blend,

Vb= tapped volume of the blend.

• Compressibility Index (Carr's Index)

The ratio of the bulk density to the tapped density and the difference between the two is known as the compressibility index. It is expressed in and quantifies the flowability of powder proportion.

$$\text{Carr's Index (\%)} = (Dt-Db/Dt) *100 \quad \text{Eq. 4}$$

Where Dt = Tapped density of the powder, Db=bulk density of the powde

• Hausner ratio

The Hausner ratio is a number that is correlated to the flowability of a powder or granular material. It is an indirect index to measure the ease of powder flow. A measure of a powder or granular material's flowability is called the Hausner ratio. It is a metric that measures the powder flow's easiness indirectly.

$$\text{Hausner ratio} = \boldsymbol{\rho}b/\boldsymbol{\rho}t \quad \text{Eq. 5}$$

Where, ρb=Tapped density of the powder,

ρt =Bulk density of the powder

• Foaming index

Most of medicinal plants have saponin which is responsible for foam when an aqueous decoction is shaken and their foaming ability is known as foaming index.

By using a sieve (Size no. 1250) sieved 1 gm of the plant material to a coarse powder and transfer to a 500 ml conical flask have 100 ml of boiling water and boil for 30 minute. Cool and filter into a 100 ml volumetric flak and add sufficient water through the filter to dilute to volume. Poured the decoction into 10 stoppered test tubes in successive portion of 1 ml, 2 ml, 3 ml etc up to 10 ml and adjust the volume of liquid in each tube with water to 10 ml. Stopper the tubes and shake them in a lengthwise motion for 15 seconds, 2 shakes per seconds. Allow to stand for 15 minutes and measure the height of foam.

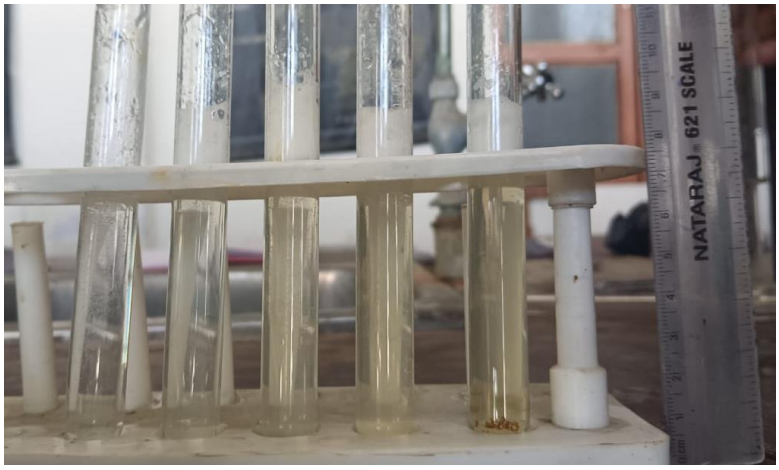


Fig. 2: Foaming index.

Powder characteristic

• Fluorescence Test

The fluorescence analysis in this test, plant material (like leaves) is dried, powdered, and then treated with various solvents or reagents such as ethanol, chloroform, hydrochloric acid (HCl), sulfuric acid (H2SO4), and water. The treated samples are observed under ultraviolet (UV) light to detect fluorescence, which can help in the identification and characterization of chemical constituents present in the plant material.

• T.S. Study of root part of *Cynodon dactylon*

A transverse section (TS) of a plant often referred to as a cross section, reveals the internal structure of a plant part when viewed from a perpendicular angle. Observation of various tissue and their arrangement. TS of roots exhibit unique structures aiding in understanding plant anatomy and function.

Fig. 3: T.S. of root of *Cynodon dactylon*.

• Ash Value Determination

To determine the total ash value of plant leaf dried powder, start by accurately weighing 2-3 grams of the sample. Place the sample in a pre-weighed crucible and gradually incinerate it by increasing the heat to 500-600°C in a muffle furnace until the sample turns white, indicating the complete removal of carbon. After incineration, allow the crucible to cool in a desiccator to prevent moisture absorption. Once cooled, weigh the crucible containing the ash. The total ash value is then calculated using the formula:

%Ash = [(ashed wt.) – (crucible wt.) \ (crucible and sample wt.) – (crucible wt.)] × 100 Eq. 6

• Powder Microscopy

The powder microscopy, involves examining powdered sample under a microscope to analyse their physical and chemical properties. This technique is commonly used in field such as pharmacognosy, forensic science and materials science. It helps to analyse particle size and shape, Crystallinity and crystal structure, Purity and contaminants, homogeneity, chemical composition, surface morphology and texture, and thermal properties.[23,24]

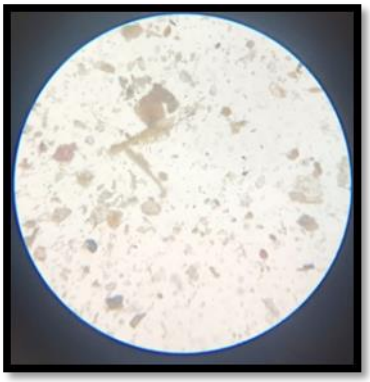


Fig. 4: Powder microscopy.

Extract of root part of *Cynodon dactylon*

- The well dried roots were then pulverized into coarse powder using grinder or mortar pestle.
- A 500 ml of beaker was taken and 40 gm of coarse powder were transferred in it and mixed with methanol at a ratio of 1:10 (wt./vol.) for 48 hours with an intermittent mixing for every 1 hours.
- Seal the container with the lid and gently shake it to ensure thorough mixing of the solvent and plant material.

- Store the sealed container in a cool, dark place for an extended period, usually several days to allow for efficient extraction.
- Periodically shake the container to facilitate the extraction process
- The mixture was then filtered through a fine muslin cloth followed by whatman No. 1 filter paper.
- Collect the liquid extract in a glass beaker
- To remove extra methanol, the mixture was concentrated by using water bath at 35℃ and the extract was eventually dried under vacuum in a dryer at 40℃ and kept in closed impermeable container for further use.(25)

Phytochemical analysis of root

Ethyl alcohol is commonly employed as a standard solvent in phytochemical screening to detect various bioactive compounds present in the plant extract.

- **Organoleptic evaluation:** The grinded coarse powder was evaluated on organoleptic parameters like odor, texture, color.
- **Test for alkaloid:** 2 ml of the extract was mixed with 2 ml of Wagner's reagent. The formation of a brown precipitate was confirmed the presence of alkaloid.
- **Flavonoid test:** 2 ml of extract was mixed with 2 ml of 10% lead acetate. Appearance of yellowish-green coloration was confirmed the presence of flavonoid.
- **Saponin detection:** 1 ml of extract was combined with 5 ml distil water and shake for 15 minutes. Development of stable foam was confirmed the presence of saponin.
- **Reducing sugar detection:** The extract was first mixed with distilled water and filtered. The resulting filtrate was boiled with Fehling's solution A and B for a few minutes. Appearance of orange-red precipitation was confirmed the presence of reducing sugar.
- **Test for Glycoside:** The extract was hydrolyzed by hydrochloric acid and then neutralized with sodium hydroxide solution. A few drops of Fehling's solution A and B were added. Appearance of red precipitate was confirmed the presence of glycoside.

- **Test for Phenolic compound**

I. The extract was mixed with lead acetate and appearance of white precipitation was confirmed the presence of phenolic compound.

II. The extract was mixed with dilute nitric acid and appearance of reddish to yellow color was confirmed the presence of phenolic compound (24).

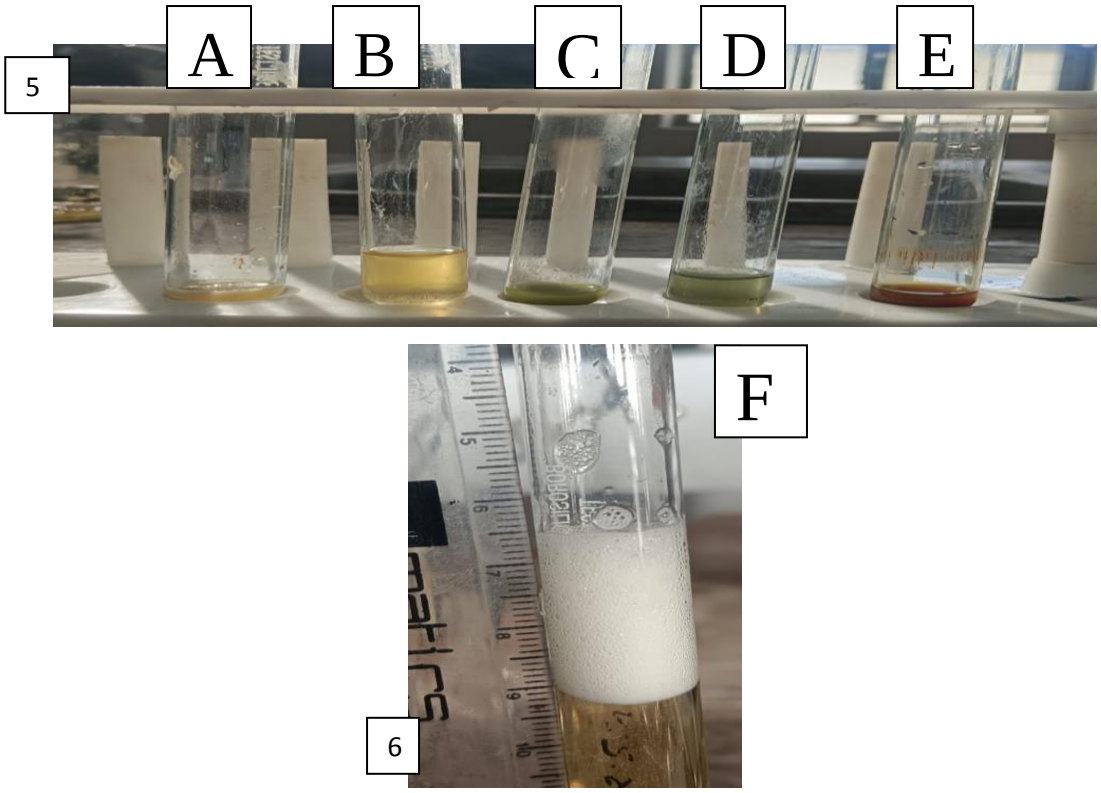


Fig. 5 & 6: Phytochemical test; A&B for Phenolic compound, c for Flavonoid, D for Glycoside & Reducing sugar, E for Alkaloid and F for Saponin.

Collection of blood

- Firstly, we selected a person who was good in health like disease free, then that person was seated on a chair comfortably.
- A suitable vein was selected in their arm (e.g. median cubital vein).
- Their skin was cleaned by using disinfectant with an antiseptic solution.
- A tourniquet was applied above the selected vein to help it swell with blood.
- A sterile needle was inserted into vein and blood was collected into syringe.
- The required amount of blood was collected and the tourniquet was removed.
- The needle was removed and pressure was applied to the puncture site to stop bleeding and a bandage was applied on the puncture site.[26]

In-vitro Activity testing using blood clotting time measurement

The blood clotting time measurement was carried out by modified method of Lee and White that reported by Osoniyi and Onajobi.[27]

- Take 5 clotting tubes and add Phosphate buffered saline (PBS) around 2 ml in every tube.
- Separate 3 tubes for test, one for control and last for standard.

- For test added 0.5 ml each of crude extract and fractions of root of *Cynodon dactylon* (100mg, 125mg 150mg).
- For control tube only PBS was taken and for standard Aspirin tablet was kept.
- Every tube were incubated on water bath at 37℃. Freshly drawn blood (0.5 ml) was carefully added by running it down the side of the incubated tubes, simultaneously starting a stopwatch.
- At every 30s interval, the tubes were gently slanted to an angle of 45º to check for clot formation of blood.
- The time of clot formation for every tube were recorded.
- The stopwatch was stopped instantly and clotting time was recorded as the final clotting time.[28]

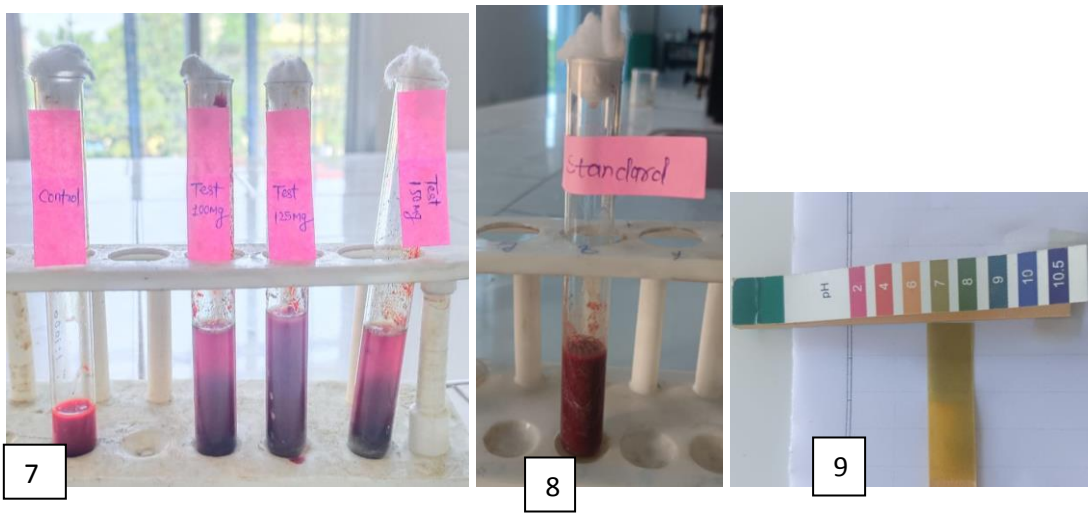


Fig. 7 & 8 Blood clotting time measurement Fig. 9 pH of PBS.

Formulation of Patch

The patches were prepared by using solvent evaporation method. Take 2 gm of PVA and 2 gm of PVP were mixed in 20 ml of distilled water over a hot water bath until dissolved. Then cooled down that mixture and added root extract about 0.5 ml, propylene glycol (0.5 ml), glycerol (0.5 ml) and mixed together with using mechanical stirrer at 800 rpm for 15 minutes under occluded condition. Then poured that mixture into a glass petri dish and dried in by placing funnel over it for 24 hrs. The films were removed by using sharp blade by inserting along the edge of the film.[29]

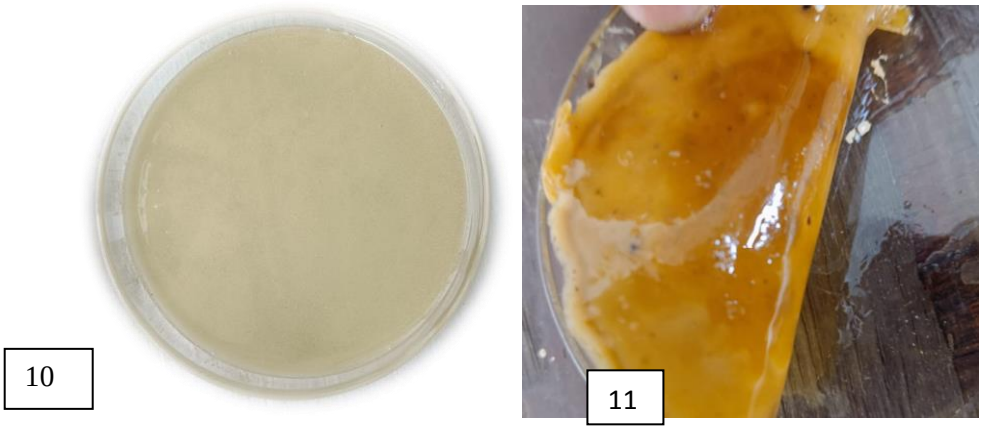


Fig. 10 & 11: Formulation of Patch.

Evaluation of transdermal patch

I. Organoleptic Characteristics

The physical appearance of developed patch was evaluated by using a naked-eye examination for its appearance, colour, clarity, flexibility, and smoothness.

II. Physico-Chemical Evaluation

A. Thickness of Patch – A Vernier caliper was used to measure patch thickness uniformity at 3 different places.

B. Determination of Surface pH –The pH of the patch is evaluated by swelling it with 1 ml of distilled water for two hours at room temperature before use. Then place the pH electrode on the surface of patch to record the pH value and make them to adjust itself for few minute.

C. Folding endurance - Folding endurance is the number of times the patches can be folded in the same area without breaking.

D. Uniformity of weight - Each of the three patches were weighted for each batch, and the mean weight was determined (30).

E. Percent elongation test – It was determined from the below mentioned formula.

Elongation percentage = $\{(L_1 - L_2) / L_2\} \times 100$ (31)

RESULT AND DISCUSSION

On the behalf of our research we perform in vitro testing of anticoagulant testing method through manually. We formulated the anticoagulant patch of *Cynodon dactylon* and evaluated the patch on their evaluation parameters and their result are shown below -

Table 1: Preformulation study.

Sr. No.	Parameters	Result
1.	Angle of repose	38.65^0
2.	Bulk density	0.2 gm/ml
3.	Tap Density	0.28 gm/ml
4.	Compressibility Index	28.57 %
5.	Hausner ratio	1.4
6.	% Ash	9 %
7.	Foaming index	Less than 100

Table 2: Fluorescence Test.

Sr. No.	Chemical name	Long UV	Short UV	Visible
1.	Sample + Chloroform	LG	DG	DG
2.	Sample + Hydrogen peroxide	DG	DG	LG
3.	Sample + Lactic acid	DG	DG	LG
4.	Sample + Benzene	BL	DG	LG
5.	Sample + Ethyl acetate	BL	DG	DG
6.	Sample + Sulphuric acid	BL	BL	BL
7.	Sample + Acetone	BL	DG	GR
8.	Sample + Methanol	BL	DG	GR
9.	Sample + Hydrochloric acid	BL	DG	GR
10.	Sample + Water	BL	GR	LG

LG – Light green, DG – Dark Green, BL – Black, GR - Green

Table 3: Organoleptic test.

Color	Odor	Taste	Texture
Typical yellow	Characteristic	Mild bitter	Soft and plush to rough and prickly

Table 4: Phytochemical test of plant.

Sr. No.	Test for		Result
1.	Alkaloid		+
2.	Flavonoid		+
3.	Saponin		+
4.	Reducing sugar		-
5.	Glycoside		-
6.	Phenolic compound	I.	+
		II.	+

Table 5: Clotting time of blood sampl.e

Sr. No.	Clotting time of blood sample (Control) (min)	Clotting time of blood sample with 0.5 ml extract with variation of fraction of root (Test) (min)			Standard (min)
1.	2:27	100 mg	125 mg	150 mg	5:51
		4:22	5:26	5:49	

Table 6: Formulation of patch.

S. No.	Ingredients	Amount
1.	Polyvinyl Pyrrolidone	2 gm
2.	Polyvinyl Alcohol	2 gm
3.	Propylethylene glycol 400	1 ml
4.	Glycerol	1 ml
5.	Distilled water	20 ml

Table No. 7: Evaluation of patch.

	Characterstics	Result
Organoleptic Characterstics	Appearance	Typical yellow
	Flexibility	Good
	Texture	Smooth
	Shape	Desirable cut in circle
	Diameter	2cm
	Smell	No smell

S. No.	Ingredients	Result
1.	Thickness	0.1cm
2.	Surface pH	7
3.	Folding endurance	Good
4.	Uniformity of wt.	0.13 gm
5.	Percent elongation test	40 %

Fig. 12: Patch formulated.

DISCUSSIONS

Micrometric readings were completed for the plant as Carr's index, bulk density, tapped density, Hausner ratio for flow activities. The angle of repose was to be 38.65^0 it confirms that it has fair flow property. All these properties are shown in table no 1.

The percentage of ash was found to be 9 and their foaming index was less than 100 due to height of foam in every tube was less than 1 cm.

There Fluorescence test were performed using various chemical given above under long, short UV and visible light. These are shown in table no 2.

The organoleptic property of coarse powder was observed. Typically yellowish color, shows characteristic odor having mild bitter taste with rough texture. All these properties are given in table no 3.

In this study we performed the phytochemical test for root extract and we found that presence of flavonoid, phenolic compound which is responsible for activity. These are given in table no 4.

The present study investigated the in vitro effects of *Cynodon dactylon* root extract and its partitioned solvent fractions on blood coagulation of healthy human volunteers. The results shows significant prolongation of the clotting time by the extracts and by the increasing concentration of root fraction like 100, 125, 150 mg. this suggests that clotting time prolongation effect of crude root extract operates optimally within a narrow concentration range; All the fractions significantly prolonged the clotting time beyond the control value. In most cases clotting time at concentration of 100 mg for 3ml were lower than that observed at 150 mg for 3 ml. A comparison of the effect of the different fractions on the clotting time showed that the fraction exhibited the highest anticoagulant activity.

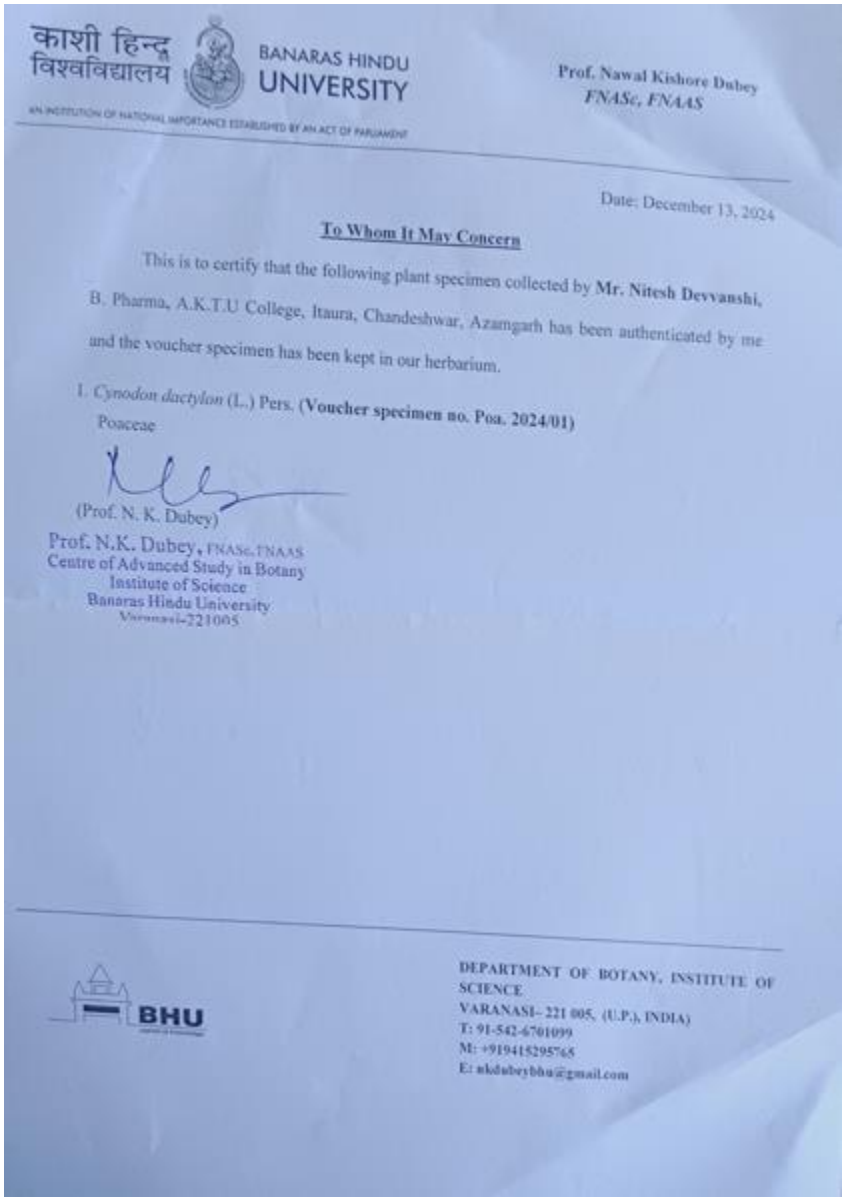
In this research we formulate the patch of *Cynodon dactylon* using polymers, plasticizer etc by the solvent evaporation method and evaluated on some parameters like organoleptic, thickness, pH, folding endurance and percent elongation test was found to be 40 %.

CONCLUSION

The study showed that *Cynodon dactylon* possesses anticoagulant activity which can be exploited in the treatment of blood coagulation disorder. Further in vitro studies are performed their effectiveness with comparison with aspirin tablet. The transdermal patches of *Cynodon dactylon* were formulated successfully by using solvent evaporation method and their evaluation were performed like thickness, pH, folding endurance etc.

Conflicts of interest

The authors declare that there are no conflicts of interest with respect to the authorship and / or publication of this article.



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Prof. Nawal Kishore Dubey
FNASc, FNAAS

Date: December 13, 2024

To Whom It May Concern

This is to certify that the following plant specimen collected by **Mr. Nitesh Devvanshi,** B. Pharma, A.K.T.U College, Itaura, Chandeshwar, Azamgarh has been authenticated by me and the voucher specimen has been kept in our herbarium.

1. *Cynodon dactylon* (L.) Pers. **(Voucher specimen no. Poa. 2024/01)**
Poaceae

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