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FORMULATION AND EVALUATION OF HERBAL GEL OF CYNODON DACTYLON [DURVA] FOR WOUND HEALING

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Abstract

The plant cynodon dactylon is a medicinal plant having family poaecae is used in clinical practice and having high wound healing activity,antioxidant activity,have been reported in persistent finding till date. This plant is very familiar to us and available in all places of the world. There are many reports. There are many reports revealing pharmacological potential of cynodon dactylon extract but only few literature reports highlight the coversion of these extract in suitable dosage form. The juice of plant is also used astringent and used in treatment of cuts and wounds.The wound healing activity was described earlier in an ayurvedic formulation [durva – ghrita] contains cynodon dactylon but the significant data and the correct justification was not clarified that how it affects on wound healing.Wound healing property of the extract of cynodon dactylon was evaluated by using herbal gel for wound healing activity. The gel preparation having higher patient compliance and helps in reducing dose of drug. The formulation was evaluated for spreadability,extrudability,and viscosity in vitro drug release study.

Keywords :- cynodon dactylon, Bermuda grass , herbal gel, carbapol 940.

INTRODUCTION

Nowadays about 80% population of world depend on the traditional medicines for various skin diseases. Recently the traditional use of plants for wound healing has received attention by the scientific community.(37)Approximately one-third of all traditional medicines in use are for the treatment of wounds and skin disorders, compared to only 1-3 % of modern drugs. (38)

Medicinal plants are rich in several potential drugs and it holds healthier and harmless alternate to synthetic drugs. (3) Different parts such as leaf, root, stem, fruit, seed, and park are used to obtain several phytochemical constituents. In addition, medicinal plants are rich in biologically active compounds and play an important role in drug discovery. Extracts of medicinal plants are useful in the treatment of several health problems such as bacterial infections (4), ulcers (5), arthritis (6) and inflammatory (7).
Cynodon dactylon is commonly known as “Aruvaum pullu” (Tamil), “Doob” (Hindi) and “Garike hullu” (Kanada), Dhoorva (Marathi), “Garike and Thella gariki”
(Telulgu) and is termed as a creeper in India (8). The English name of Cynodon is Bermuda grass (9) and belongs to family of Poaceae. It is native to East Africa, Asia, Australia and southern Europe. Cynodon is a weed and has been found to possess various potential medicinal properties (10). The plant is traditionally used as an agent to control diabetes in India (11). The extract of C. dactylon leaf has been reported to be anti-diabetic (12, 13, 14), antioxidant and hypolipidemic efficacy (1), healing of minor injuries (15), immunomo-dulatory and hepatic antioxidant (17) activities.

The aqueous fluid extract of C. dactylon rhizome is used for diuretic, anti-emetic, purifying agent and dysentery (18,19,20). The plant extract also has significant application in dropsy and secondary syphilis wounds (21), and cardio protective (22).

In a recent study, the extracts of C. dactylon had also been reported to be effective for antimicrobial activity against bacterial pathogens and fungus [23]. Based on the aforementioned comments, it is not surprising that the pharmacological benefits of C. dactylon have been attracting great interest. Therefore, the present review has been detailed updates of the phytochemical and pharmacological properties of C. dactylon as well as its miscellaneous uses.

Phytochemical properties :-

Several compounds have been identified and quantified from different morphological parts of the C. dactylon. The plant contains proteins, carbohydrates, minerals and other compounds like terpenoids, vitamin C, palmitic acid and alkaloids [24]. Green grass contains (dry matter basis) 10.47% crude protein, 28.17% fiber and 11.75% of total ash [25]. Other important phyto-constituents reported from this plant were Flavonoids: apigenin, luteolin, orientin and vitexin[26,27]; carotenoids: beta-carotene, neoxanthin, violaxanthin [28], phenolics [29], phytosterols, glycosides, saponins [30] and volatile oils [31]. Chemical structures of flavonoids (Figure 1) and carotenoids, phytol, tricosane.

Gas chromatography-mass spectrometry (GC-MS) analysis of *C. dactylon* leaves contained glycerin (38.49%), 9, 12-octadecadienoyl chloride, (Z, Z)-(15.61%), hexadecanoic acid, ethyl ester (9.50%), ethyl α-D-glucopyranoside (8.42%), linoleic acid ethyl ester (5.32%) and phytol (4.89%) as well as other bioactive compounds were reported by (32,33) isolated major constituents such as tricosane (22.05%), 1, 2-propanediol (20.30%), 3- benzyloxy-1, 2 diacetyl (12.62%) and other 7 minor constituents in ethanolic extracts of C. dactylon. Hydroalcoholic extract of *C. dactlyon* was found to contain 22 compounds in total, mainly hexadecanoic acid, ethyl ester (17.49%), D-mannose (11.48%) and linolenic acid, ethy ester (11.28%). In addition, hydro-quinone (69.49%), furfural (6.0%) and levoglucosenone (2.72%) were found to be the richest constituents among the 20 characterized constituents from phenolic extracts [34].

Skin play role as a barrier to water and various pathogens. But wound and injuries destroy this barrier that normally prevents invasion of bacteria ,fungi, and viruses. Skin is often injured by wounding or physical trauma, and certain damage to the skin initiate a series of complicated and well orchestrated events of repair processes ending with complete reestablishment of integrity of damage tissue and restoration of this functional barrier.In some critical conditions like large full thickness skin defects ,complete reepithelization takes long time.

The process of wound healing involving the different variety of the biological responses such as acute inflamation,cellular proliferation and a contraction of the collagen lattice formed.[39,40] the healing process is starts with clotting process of blood and at the end it completes with remodeling of the cellular layers of skin. The appropriate methods for wound healing is essential for the restoration of the damaged tissue anatomical continuity and disturbed functional status of theskin. Research on wound healing agents is one of the developing areas in india,china and all over the world. Many medical practitioners across the world having valuable information of many plants for treating wound and burns.[41]For this reason traditional plants based remedies are back and find increasing theie application as source of direct therapeutic agents. among numerous species of plants growing in india ,doob grass or durva or taxonomically the cynodon dactylon family poaceae occupies its unique place and key position in ethnomedicinal practices and traditional medical(Ayurvedic,unani ,nepales and chinese) knowledge system. The herbal preparations of this grass are being based on traditional wisdom. [42]

Traditional Uses of Cynodon dactylon :

It is used in the treatment of cancer, cystitis, headache, hysteria, asthma, tumors, stones, eye disorders, weak vision, pain, inflammation and grippe in children. Cynodon dactylon is mixed with sugar to treat the case of urine retention.

Taxonomical classification of cynodon dactylon :-

Kingdom : plantae
Division : magneliophyta
Class : liliopsida
Order : cyperales
Family : poaceae
Genus : cynodon
Species : cynodon dactylon

According to ayurveda, India's traditional pharmacopoeia, cynodon plant is the bitter, heating ,pungent ,fragrant, appetizer, vulnerary, anthelmintic, antipyretic, alexiteric. It help in destroying breathing problems, useful in leucoderma, bronchitis, piles, asthma, tumors, and enlargement of spleen. In homeopathic system of medicine it helps to treat all types of bleeding and skin troubles [43]

The physicians have a wide choice for treatment from solid dosage to semisolid dosage to liquid dosage formulation. Among the topical formulation clear transparent gels have widely accepted in both cosmetics and pharmaceuticals.

The "gel" was introduced in the late of 1800 to name some semisolid preparations according to pharmacological, rather than molecular composition.[45] The u.s.p defines that the gels are a semisolid system consisting of dispersion made up of either small inorganic particle or large organic particle enclosing and interpenetrated by liquid. [46,47,48,)
The inorganic particle from a three dimensional "house of cards" structure.

Several drugs derived from plants are well known for increasing healing different types of wound.traditionally the leaves of cynodon dactylon are used for cuts and wound.

Standardization of cynodon dactylon leaf :-

Standardization of herbal drug means confirmation its identity,quality and purity.

1]Morphological studies : evaluation of size, shape, colour, odour, taste, and particular characteristics like texture etc.

2] determination of ph :- a] ph of 1% solution
b] ph of 10% solution

3] loss on drying :- determination of moisture content

4] determination of Ash value :- a] determination of total ash value
b] determination of acid soluble ash value
c] determination of water soluble ash value

5]Determination of foaming index : for the presence of saponins

6] determination of swelling index :- for the determination of mucilage content.

Material and method : -

Materials :-

Cynodon dactylon leaf	Api
Petroleum ether	Defatting agent
Ethanol	Extracting solvent
Carbapol 940	Gelling agent
Propylene gycol	Plasticizer
Methyl paraben	Preservative
Propyl paraben	Preservative
Glycerin	Solvent
Triethanolamine	Maintain the ph
Water	solvent

Apparatus :-

Conical flask 50 ml,pipette 10ml, glass beaker 50ml, 100ml,1000ml, aluminium foil tube 10 ml

Instruments :-

Uv visible spectrophotometer, weighing balance, magnetic stirrer, homogenizer, Brookfield viscometer, USA franz diffusin cell, refrigerator, incubator, waterbath and oven.

Collection of plant material :-The whole plant of cynodon dactylon were collected from the botanical garden of SBSPMS's college of B pharmacy Ambajogai Dist. Beed, Maharashtra, India in January 2023. It was cleaned and dried at room temperature in shade and keep away from direct sunrays**.**

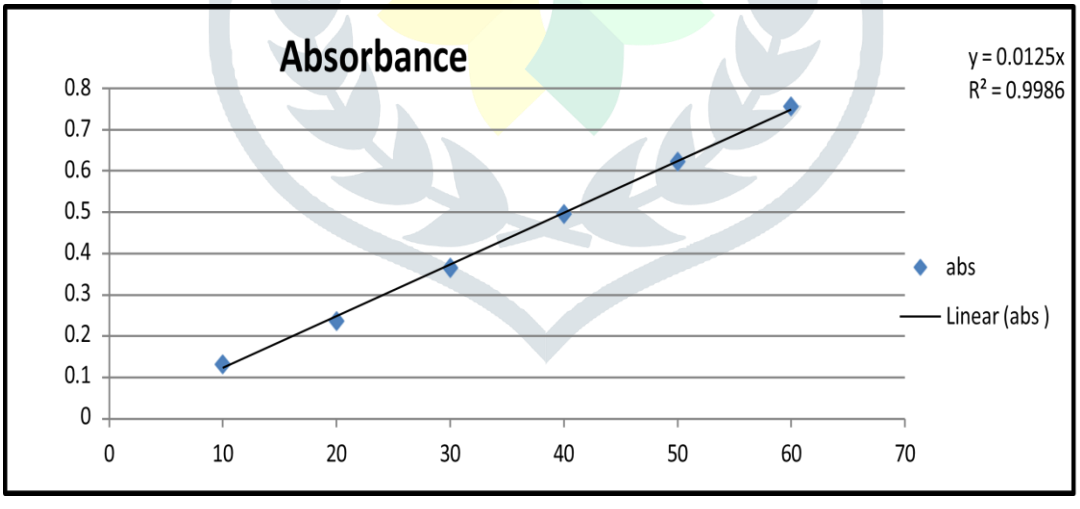
Authentication of plant :- The plant authentication was done by the head of department of botany, yogeshwari science college ambajogai.

Preparation of extract :-The whole plant of cynodon dactylon were collected and dried in the shade and then pulverized in grinder. The drug is powdered and was utilized for the extraction.then material was passed through 120 meshes to remove fine powder and coarse powder was used for extraction. A soxhlet extraction method is used for extraction.

Preliminary phytochemical screening :- Phytochemical screening was carried out according to standard methods. The extract showed that the presence of flavanoids,glycosides,carbohydrates, alkaloids,
Phenolic compounds, tannins, fixed oil, etc. Above constituents present in selected plant cynodon dactylon.

Estimation of UV Spectroscopy-

Calibration of cynodon dactylon leaf extract :-



Formulation of gel :-

	F1	F2	F3	F4
Carbapol 940	0.15gm	0.3gm	0.6gm	0.6gm
Cynodon dactylon extract	3gm	3gm	3gm	3gm
Methyl paraben	0.3gm	0.3gm	0.3gm	0.3gm
triethnolamine	0.1ml	0.1ml	0.1ml	0.1ml
water	Quantity sufficient	Quantity sufficient	Quantity sufficient	Quantity sufficient

Procedure of formulation of gel :-

Appropriate quantity of gelling agent was soaked in water for a period of 2 hours. gelling agent then neutralized with Triethanolamine with stirring . Then specified amount of drug was in appropriate and reweighted amount Propylene glycol and glycerin solvent blend was transferred the container And agitated for 20 min . the dispersion was then allowed to hydrate and Swell for 60 min. Final adjust the pH with 98% Triethanolamine. PH value Approximately reached (6.8-7). During Ph adjustment ,the mixture was stirred Gently with a spatula until homogenous gel was formed .all the samples were allowed to equilibrate for at least 24 hours at room temperature .

Physical examination :-

The prepared gel are examined for the colour ,homogeneity, spreadability, ph, consistency and microbial growth and viscosity. The ph is measured by each sample at room temperature using digital ph meter and the spreadability is measured by the using two horizontal plate (20cm.)

Drug content studies :-

The 100 gm drug sample is dissolve in 100ml of methanol and then this solution is transferred in volumetric flask and stirring for 2 hrs at 250 rpm ,then filter the solution to remove the undissolved residues and determine spectrophotometrically for the drug content.

Viscosity measurement :

Brookfield viscometer is used for the viscosity measurement. The spindle used are helipackt spindle set (Lv-3). The spindle are rotated at 30 rpm at specific temp. for the viscosity studies of various formulation revealed that formulation f2 is better to compare to other from among all developed formulation f2 shows better drug diffusion did good reheological property so it was concluded that formulation f2 has best viscosity. Hence f2 has better result as compare to other batches.

Homogeneity :- Narrow transparent glass filled with gel and observed under light to check for any lumps or practical. this are the visual observation was to find out the homogeneity.

Consistency :- The consistency of the cynodon dactylon gel from which was evaluated by conical projection

In - Vitro diffusion study :-

The semi-permeable membrane of eggs is in between the outer calcified shell and the inner contents like albumin and yolk. The shell was removed chemically by placing the eggs in 2M HCl for an overnight. This resulted in the complete decalcification of the egg and then it was washed with distilled water Carefully with a sharp pointer a hole was made on the top so that the contents squeeze out completely from the decalcified egg. The membrane was rinsed with distilled water and stored in refrigerator. The egg membrane was clamped between the donor and the receptor chamber of the Franz diffusion cell with an effective permeation area of 1.76 cm2 and a receiver cell volume of 8 mL. PBS containing 20% ethanol was used as the receptor solution and incubated at 37 ± 0.2 °C using a water bath with a magnetic stirrer at 500 rpm. Preparation of standard solutions A stock solution is prepared using an analytical balance (1 mg/ml) that is 100 mg of Cynodon dactylon gel is dissolved in 1000ml of phosphate buffer pH 6.8. Different working standard namely 5µg/ml , 10 µg/ml, 15 µg/ml, 20µg/ml and 25µg/ml was prepared by appropriate dilutions. Absorbance of those solutions is measured. Calibration Curve For the calibration curve, accurately weighed of Cynodon dactylon gel was transferred to a 100 ml volumetric flask and dissolved in a mixture of buffer. From this solution, other solutions with concentrations of different µg ml were obtained by diluting adequate amounts in triplicate.

The cumulative amount of drug permeated through a unit area of skin was plotted against time. Steady state flux values (Jss) were calculated from the slope of the linear portion of the plot. The obtained Jss was then used to calculate the apparent permeability coefficient (Papp) by

use of the following equation:

Papp(cm/s)=Jss/Cd, where Jss is the observed flux rate at steady-state (µg/s) and Cd (µg/ml) was concentrations of solution in donor chamber.

Result And Discusion :-

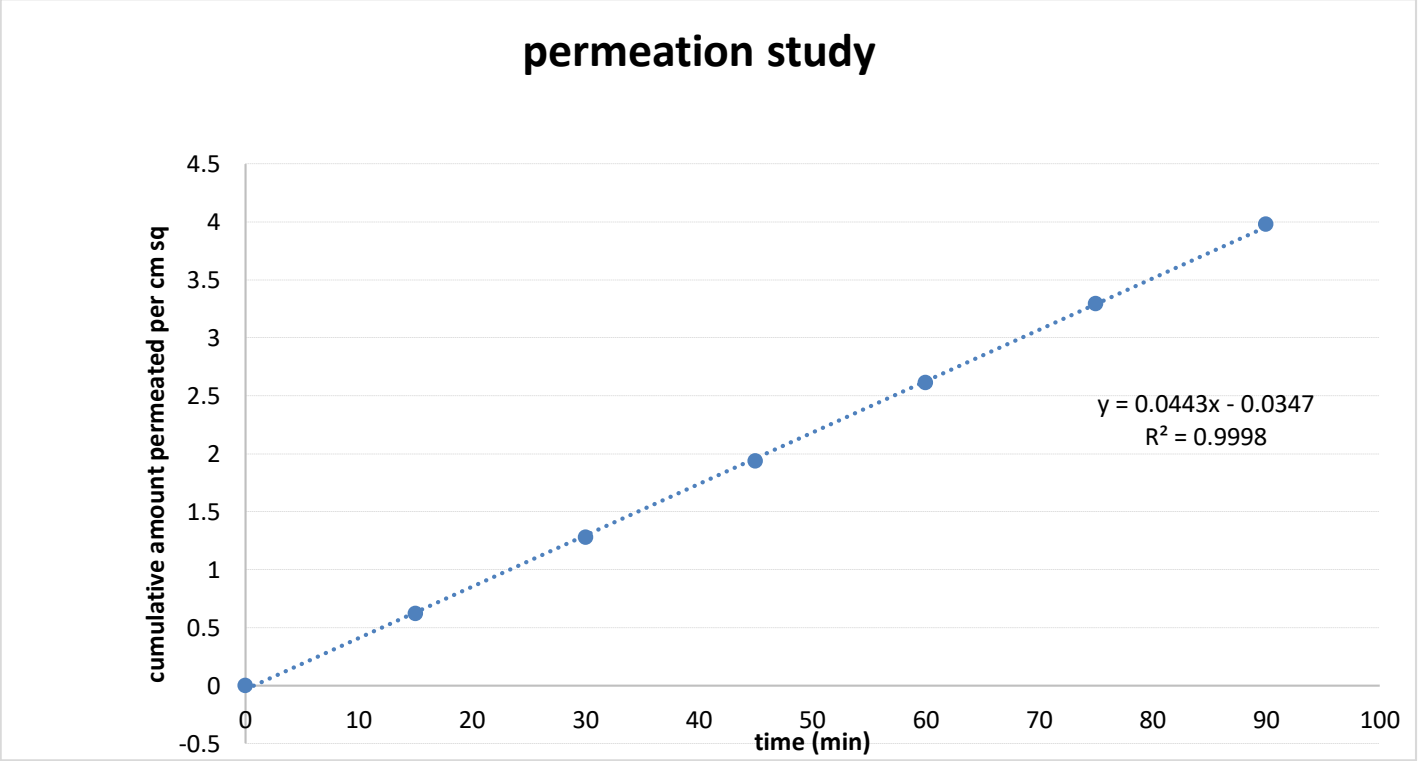
Sr.no	PH	Spreadability (g/cm/s)	Homogeneity	Viscosity
1	6.7	21.80	Good	1600
2	6.4	20.83	Good	1450
3	6.7	22.70	Good	1120
4	6.3	20.75	Good	750

Table:- Physical evaluation values for cynodon Dactylon gel formulation

8. MEAN CUMULATIVE PERCENTAGE DRUG RELEASE :-

Time (min)	Absorbance	conc. (µg/ml)	Dilution factor	concentration in receptor(8ml)mg	cumulative amount (mg)	cumulative amount permeated per cm sq
0	0	0	0	0	0	0
15	0.1763	13.66666667	136.6666667	1.093333333	1.093	0.621022727
30	0.1862	14.43410853	144.3410853	1.154728682	2.24772868	1.277118569
45	0.1868	14.48062016	144.8062016	1.158449612	3.406178295	1.935328576
60	0.1919	14.87596899	148.7596899	1.190077519	4.596255814	2.611508985
75	0.1936	15.00775194	150.0775194	1.200620155	5.796875969	3.293679528
90	0.194	15.03875969	150.3875969	1.203100775	6.999976744	3.977259514

dilution	10	
area of cell diffusion	1.76	
slope	0.0129	
flux	0.387	conc. Of drug taken = 10 mg
permeability coefficient	0.1732	



Conclusion :-

Cynodon dactylon is extremely useful in ayurvedic, unani and siddha medicine. It has significant role in wide variety of disease and disorder. It is found all over the year and very easily. Different types of therapeutic action shows the medicinal value of Cynodon dactylon. Different types of phytochemical like proteins, minerals, carbohydrates, vitamin C, terpenoids, alkaloids and palmitic acid, Flavonoids: apigenin, orientin, letuolin, and vitexin found from different parts of the plant. Biologically active phytoconstituent of the cynodon dactylon are effective against the wound healing and are of higher importance once the product are made easily available.

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