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Phytochemical Investigation of Eleusine Indica for In-Vivo Anti-Hypertensive Activity

A. V. Desai, V. M. Patil, S. S. Patil, V. A. Kangralkar
Department of Quality Assurance Techniques,
Ashokrao Mane College of Pharmacy, Peth-Vadgaon,
Kolhapur, Maharastra, India 416112.
abhidesai532@gmail.com

ABSTRACT: *Eleusine indica L. Gaertn* also known as wire grass belonging to family *poaceae*. The plant is used for deforming, eliminate or reduce cough and lung troubles, dysentery, heart problems and high blood pressure, spleen and liver complaints, bladder and kidney stone and in case of sprains, dislocation of bones and lumbago. The plant contains cyan genetic glycoside, albuminoids, starch, fatty oils, phenol compounds and flavonoids. The present investigation was carried out to isolate, purify and characterize active constituents from the whole plant of *Eleusine indica L. Gaertn*. Powdered material of whole plant of *Eleusine indica L. Gaertn* was taken for the preparation of extracts using soxhlet apparatus. The various photochemical tests were performed for alkaloids, glycosides, phenol, flavonoids etc. TLC and HPTLC were done for isolation of compounds. The isolated compounds were further characterized by FTIR, Ultraviolet spectroscopy and GC-MS. The anti-hypertensive activity was evaluated by using *in-vivo* model.The ethanolic extract of *Eleusine indica L. Gaertn* significantly inhibited the hypertension. Thus ethanolic extract of whole plant of *Eleusine indica L. Gaertn*have significant anti-hypertensive activity.

Keywords: Eleusine indica L. Gaertn, Poaceae, HPTLC, FTIR, GC-MS, anti-hypertensive activity.

I. INTRODUCTION

Hypertension is reported to be the fourth contributor to premature death in developed countries and the seventh in developing countries. Recent reports indicate that nearly 1 billion adults (more than a quarter of the world's population) had hypertension in 2000, and this is predicted to increase to 1.56 billion by 2025. Earlier reports also suggest that the prevalence of hypertension is rapidly increasing in developing countries and is one of the leading causes of death and disability. [1]

The World Health Organization (WHO) defines traditional medicine as: "the health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral-based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to diagnose, treat and prevent illnesses or maintain well-being. [2] ***Eleuscine indica L. Gaertn*** belonging to the family ***poaceae*** is annual or short-lived perennial tufted grass, branching from the base. The ascending culms, 5 to 60 cm high, are sometimes compressed while the leaf blades are flat or sometimes folded, 15 – 30 cm long and 4 -6 cm wide. Eleusine species the grass is used in folklore medicine by the Ibibio's of Niger Delta region of Nigeria in the treatment of malaria related fever, diabetes, stomach disorders and infections.[3] Hypertension is the most common cardiovascular disease and is a major public health issue in developed as well as developing countries. Symptoms of high blood pressure are headaches, heart palpitations, catching your breath after exertion, fatigue, flushed face, blurry vision, nosebleeds, strong need to urinate often, ringing in ears and dizziness. These symptoms can be resolved by herbals. Herbs do not cause side effect like weakness, tiredness, drowsiness, impotence, cold hands and feet, depression, insomnia, abnormal heartbeats, skin rash, dry mouth, dry cough, stuffy nose, headache, dizziness, swelling around eyes, constipation or diarrhoea, fever or anaemia alone and associated with pressure medicines. 100% natural herbs are completely safe. Herbals won't interfere with medications including diuretics, blood thinners, β-blockers and calcium channel blockers. [4]

II. MATERIAL AND METHOD

A. Collection, authentication and drying of plant material

Eleusine indica (L.) Gaertn is annual plant grows in rainy season so the whole plant was collected in the month of August (2016) from Krishna riverside near Nag thane, Tal-Palus, Dist-Sangli. Maharashtra, India. After collection the plant material was identified, confirmed and authenticated. Then drying of plant material was done using shade drying and crushed in an electrical grinder and then powdered.

B. Preparation of plant extract

The powdered material was subjected for extraction with various solvent in soxhlet apparatus for 24 hrs based on increasing polarity in order of petroleum ether (60-80^{0}c) for

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defeating, chloroform, methanol and ethanol separately. All the extracts were concentrated and the solvent was evaporated in order to get dry extracts. The percentage yield of various extracts was calculated by following formula-

% Yield = Weight of extract (g)/ Weight of dry powder (g) x 100

C. Phytochemical screening

It was carried out by using standard chemical tests**. [5]**

- *Isolation and characterization of individual compounds*

After development of chromatogram the plates were allowed to dry. Distance travelled by solute and solvent were marked and measured and their Rf values were calculated. Further from the data obtained from Rf values of each extract the individual spots were isolated from the TLC and HPTLC plates. After drying of the plates each individual spot was scrapped from the plate by using sharp pointer. Each spots were collected in different test tubes according to their Rf values and they were further analyzed for their identity by using analytical method such as FTIR, UV-Spectrophotometer and GC-MS.

Table 1: Solvent systems for TLC and HPTLC of different extracts

Sr. No.	Extract	Solvent systems
i.	Ethanol	a) Ethyl acetate : Acetic acid : Formic acid : Water (100 : 11 : 11 : 27) b) Ethyl acetate : Formic acid : Water (82 : 9 : 9)
ii.	Methanol	
iii.	Chloroform	

D. Selection of animals

Albino Westar rats of either sex (n=6) of weighing 200-250 g were used for the present study. The animals were procured from animal house, Tatyasaheb Kore College of Pharmacy, Warananagar, Maharashtra. The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±20˚C and relative humidity of 30 – 70 %. A light and dark cycle was followed. All animals were fed on standard balance diet and provided with water *ad libitum.*

All the experimental procedures and protocols used in study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) of Tatyasaheb Kore College of Pharmacy, Waranagar, Maharashtra and care of laboratory animals was taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) Ref. No. TKCP/2017/12/03.

E. Evaluation of In-vivo anti-hypertensive activity

The in vivo anti-hypertensive activity was evaluated using adrenalin induced rat by non-invasive tail-cuff method. [6]

F. Method to induce hypertension by adrenaline

Rats were anaesthetized with diethyl ether and 0.1 ml of adrenaline was injected into rats by intra-peritoneal (I.P) injection using a 1 ml disposable syringe for 10 consecutive days to induce hypertension. To confirm the induction of hypertension, different hemodynamic parameters were measured by using Non-invasive tail cuff method with CODA Non-Invasive Blood Pressure System by Kent Scientific Corporation. (Appasaheb Birnale College of Pharmacy, Sangli, Maharashtra.)

Table 2: Trial design

Group	Receiver	Dose
Normal control	Dimethyl sulfoxide (DMSO) as a vehicle	1% v/v
Disease control	Adrenaline	0.5 mg/kg/100 µL, i.p
Standard	Enalapril inj.	48 mg/kg, i.p.
Test P	Ethanolic extract (P) of Eleusine indica (L.) Gaertn	400 mg/kg, oral
Test Q	Methanolic extract (Q) of Eleusine indica (L.) Gaertn	400 mg/kg, oral
Test R	Chloroform extract (R) of Eleusine indica (L.) Gaertn	400 mg/kg, oral

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After administration of dose to animals, blood pressure was measured by Non-invasive Tail cuff. The rat was kept in strainer and the tail cuff was applied on the tail of rat for determination of blood pressure. Normal blood pressures of all threats were recorded as baseline blood pressure. After that the animals were treated with respective treatment and again blood pressure was recorded as after drug treatment. The blood pressures SBP (systolic blood pressure), DBP (Diastolic blood pressure),were displayed on monitor were recorded. To evaluate anti-hypertensive effect of drugs, adrenaline was injected after 5 minutes. Again the blood pressure was recorded and the difference between baseline blood pressure and blood pressure after adrenaline treatment were calculated and compared.

Statistical analysis

To check the significance of data Bonferroni's test was carried out

III. RESULT AND DISCUSSION

Table 3: Percentage yield of various extracts

Sr.No.	Extract	Yield(% w/w)
1	Ethanol	4.06
2	Methanol	4.18
3	**Chloroform**	**2.68**

Table 4: Photochemical tests for different extracts of *Eleusine indica L. Gaertn*

Test	Ethanol	Methanol	Chloroform
A. Test for carbohydrates			
I. Molish test	+	-	-
II. Fehlingsolution Test	-	-	-
III. Benedict's test	-	-	-
B. Test for proteins			
I. Biuret test	-	-	-
II. Millon's test	-	-	-
C. Test for amino acid			
I. Ninhydrine test	+	+	-
D. Test for steroid			
I. Libermann Burchard test	+	+	-
E. Test for glycosides			
I. KellerKillani test	+	+	+
II. Foam test	+	+	-
III. Guignard reaction or sodium picrate test	-	-	-
F. Test for flavonoids			
I. Sulphuric acid test	+	+	-
G. Test for alkaloids			
I. Dragendroff's test	+	+	-
II. Mayer'stest	+	-	+
III. Hager test	+	+	+
IV. Wagner's Test	+	+	-
H. Test for tannins and phenolic compounds	+	-	-

(+) indicates presence, (-) indicate sabsence.

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- *Chromatographic analysis of different extract by TLC and HPTLC*

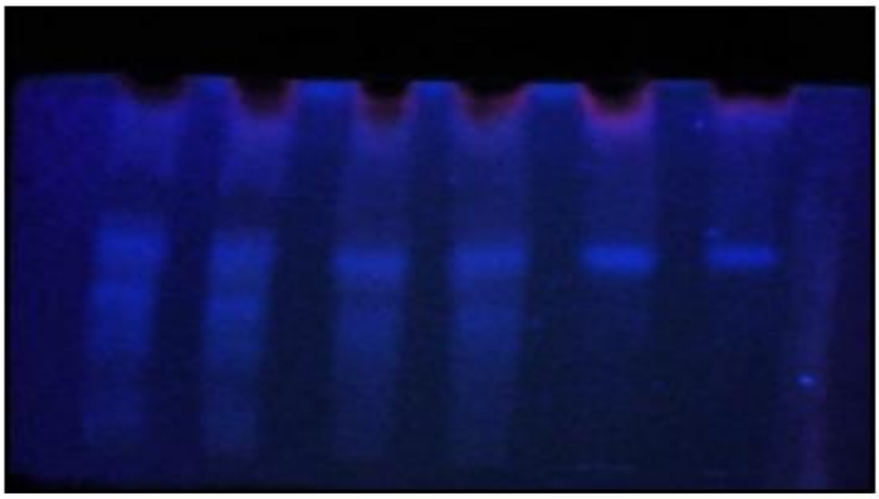

Fig. 1: TLC profile of Ethanol (P), Methanol (Q) and Chloroform (R) extract

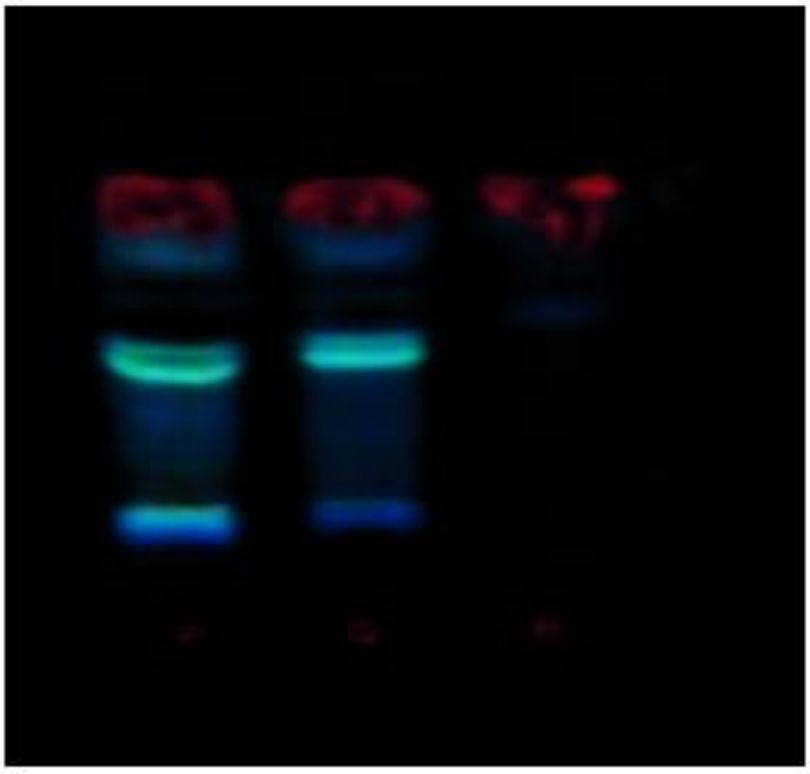

Fig. 2: HPTLC profile of Ethanol (P), Methanol (Q) and Chloroform (R) extracts.

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Table 5: TLC and HPTLC profile of different extracts

Sr. No.	Spot	R_f value
1	A	0.60
2	B	0.48

- *Characterization of isolated compounds by Spectral analysis*

a) Compound A

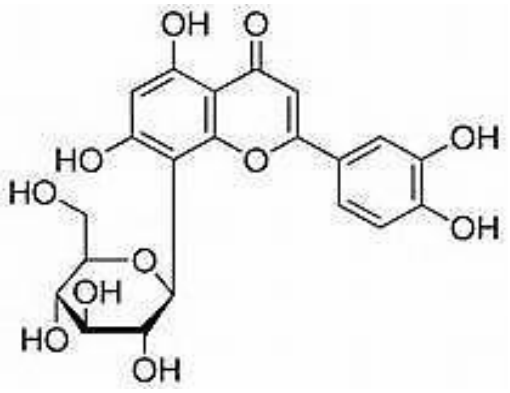
Table 6: Interpretation of FTIR spectra of isolated compound (A) of *Eleusine indica L. Gaertn*

Sr.No.	Observed Value (cm^{-1})	Indication
1	2854.474	$C\text{-}H_{str}$
2	1598.406	$C{=}C_{str}$
3	1359.796	O-H Aromatic
4	1225.433	C=O Aromatic ketone
5	1068.382	O-H Aliphatic
6	922.636	$C\text{-}O_{str}$
7	1016.123	$C\text{-}C_{str}$

Inter pretationof UV-visible spectra of isolated compound (A)of *Eleusine indica L. Gaertn*
Isolated compound A shows maximum Wavelength (λ max) 256 nm and absorbance 0.818

Interpretation of GCMS spectra of isolated compound (A) of *Eleusine indica L. Gaertn*
Isolated compound A shows mass peak (m/z) are 430,401,284,185,143

Identified compound (A):
From above data of spectral analysis compound A of *Eleusine indica L. Gaertn* was found to be **Orient in**



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b) Compound B

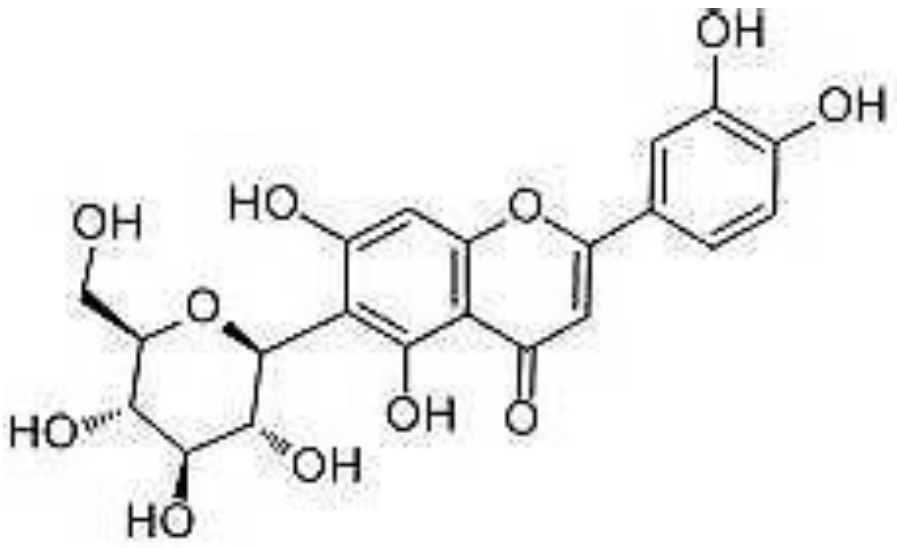
Table 9: Interpretation of FTIR spectra of isolated compound (B)of *Eleusine indica L. Gaertn*

Sr.No.	Observed Value (cm^{-1})	Indication
1	3347.311	$O\text{-}H_{str}$
2	2923.954	$C\text{-}H_{str}$
3	1714.390	$C{=}O$ Aromatic ketone
4	1580.567	$C{=}C_{str}$
5	1394.586	O-H Aromatic
6	1042.406	O-H Aliphatic
7	1259.709	$C\text{-}O_{str}$
8	877.299	$C\text{-}C_{str}$

Inter pretationof UV-visible spectra ofisolated compound (B)of *Eleusine indica L. Gaertn*
Isolated compound B shows maximum Wavelength (λ max) 272 nm and absorbance 0.558

Interpretation of Mass spectra of isolated compound (B) of *Eleusine indica L. Gaertn*
Isolated compound B shows mass peak (m/z) are 419, 283, 269, 185, 143

Identified compound B:
From above data of spectral analysis compound B of *Eleusine indica L. Gaertn* was found to be **Isoorientin**



Evaluation of *In-vivo* anti-hypertensive activity
Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP)were measured in different groups before and after treatment using tail-cuff apparatus.

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Table 12: Systolic blood pressure in adrenaline induced hypertensive rats.

Group	Before treatment SBP (mmHg)	After adrenalin SBP (mmHg)	Induction of SBP (mmHg)	% inhibition in SBP
Normal control	130.5	-	-	-
Disease control	132	164.67	32.67	-
Enalapril	132	164.67	32.67	18.25
P	130.5	163.17	32.83	15.94
Q	131.83	164.83	33	12.39
R	130.5	162.83	32.33	5.43

Table 13: Diastolic blood pressure in adrenaline induced hypertensive rats.

Group	Before treatment DBP (mmHg)	After adrenalin DBP (mmHg)	Induction of DBP (mmHg)	% inhibition in DBP
Normal control	92.67	-	-	-
Disease control	94.33	126.67	32.33	-
Enalapril	94.67	127.5	32.83	23.29
P	92.5	125.33	32.83	21.19
Q	93.67	126.5	32.83	15.67
R	93	125.67	32.67	6.40

Induction of SBP and DBP as well as % inhibition in SBP and DBP were measured which indicated antihypertensive action of Ethanol (P), Methanol (Q) and Chloroform (R) extracts of *Eleusine indica L. Gaertn* herb which was compared with disease control group.

Table 14: Induction and % inhibition of systolic blood pressure and diastolic blood pressure in adrenaline induced hypertensive rats.

Group	Induction of SBP	% Inhibition in SBP (%)	Induction of DBP	% Inhibition in DBP (%)
Normal control	-	-	-	-
Disease control	32.67±0.56	-	32.33±0.61	-
Enalapril	32.67±0.88***	18.25±0.76***	32.83±0.91***	23.29±0.64***
P	32.83±0.83***	15.94±0.43***	32.83±0.95***	21.19±0.75***
Q	33±1.13***	12.39±0.49***	32.83±0.91***	15.67±0.78***
R	*32.33±0.88***	*5.42±0.21***	*32.67±0.61***	*6.50±0.38***

n = 6; results were shown as mean ± SEM
Data was analysed by one way ANOVA (Bonferroni's test)
In induction, *** indicate significant difference in the data compared to normal control group and the level of significance was $p<0.0001$ ≈ highly significant.

In inhibition, *** indicate significant difference in the data compared to disease control group and the level of significance was $p<0.0001$ ≈ highly significant.

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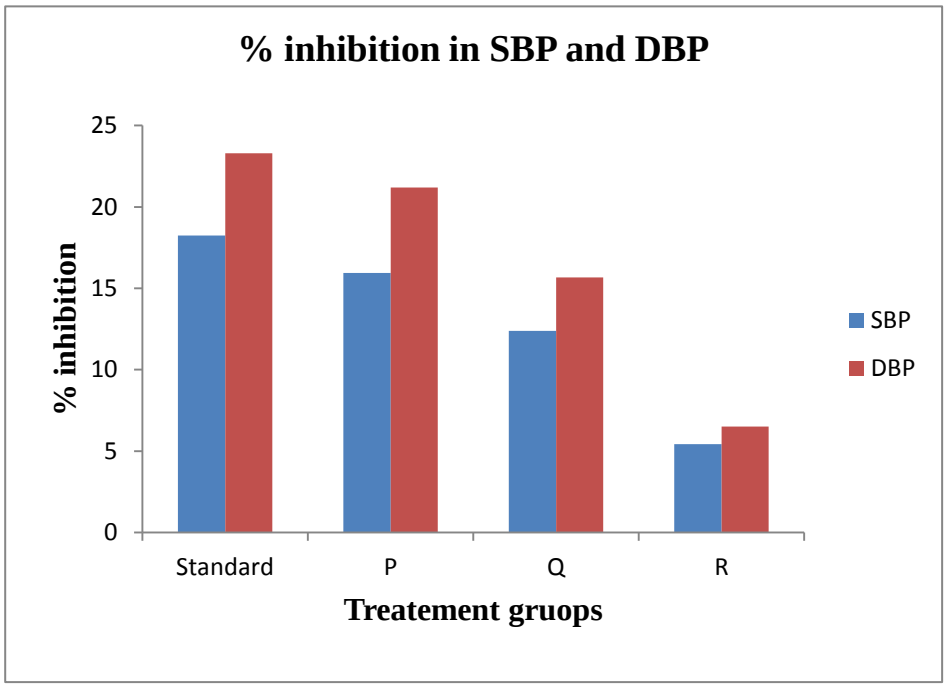

Fig. 9: % Inhibition in systolic blood pressure and diastolic blood pressure

% inhibition in SBP and DBP in disease control, standard (Enalapril) and test groups (*Eleusine indica L. Gaertn*) P, Q and R were measured. % inhibition in SBP of standard, P, Q and R group was found to be 18.25±0.76,15.94±0.43,12.39±0.49,5.42±0.21 and % inhibition in DBP was found to be 23.29±0.64,21.19±0.75, 15.67±0.78, 6.50±0.38 respectively. % inhibition in SBP and DBP in disease control, standard (Enalapril) and test groups (*Eleusine indica L. Gaertn*) P, Q and R were significantly increase as compared to disease control group which indicate that standard and test drugs decrease in systolic blood pressure and diastolic blood pressure. It may be suggested that the plant constituents might be responsible, at least in part, for the observed antihypertensive activity and that they may act individually or synergistically. Previous studies have also demonstrated that there are several compounds which could be responsible for the plants antihypertensive effects such as flavonoids, glycosides and phenolic compounds.

IV. CONCLUSION

The present study deals with the extraction, isolation, molecular characterization of secondary metabolites and pharmacological evaluation. Characterization of isolated compounds was done by TLC, HPTLC, UV visible spectroscopy, FTIR and GC-MS. Constituent's orientin, and disorienting has been isolated from *Eleusine indica L. Gaertn* plant.

The plant *Eleusine indica L. Gaertn* seems to be a promising candidate with respect to its anti-hypertensive activity and may be used as adjuvant to dietary therapy and drug treatment for controlling hypertension. For evaluation of anti-hypertensive activity *in-vivo* tail-cuff method with adrenalin induced hypertensive rats model was used. Hypertension was significantly inhibited by ethanol extract. On the other side methanol extract satisfactorily inhibited the hypertension and chloroform extract weakly inhibited the hypertension.

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