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EVALUATION OF ANTIDIABETIC ACTIVITY OF *ECHINOCHLOA COLONA* PLANT EXTRACT

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Keywords:

Echinochloa colon , Chromatography, Antiliidemic, Anti diabetic

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ABSTRACT: The current work comprises the assessment of antidiabetic activity of chloroform, ethyl acetate, and ethanol fractions and isolation of some phytoconstituents from chloroform fraction obtained from ethanolic extract of *Echinochloa colona*. The antidiabetic activity was performed using streptozocin induced diabetic model in male Wistar rats (150-200 g). The best restraint results were for metformin, chloroform, ethyl acetate and ethanol fraction was 5.85 ± 0.004 (90.08%), 6.65 ± 0.004 (67.36%), 6.96 ± 0.003 (60.14%) and 7.05 ± 0.004 (58.01%) respectively, comparing controlled diabetic rats which showed a significant reduction of blood glucose level. On total cholesterol level metformin, chloroform, ethyl acetate and ethanol fraction showed decrease in total cholesterol level by 12.16 ± 0.0044 (56.98%), 13.02 ± 0.004 (53.94%), 14.17 ± 0.004 (49.87%) and 16.37 ± 0.004 (42.24%) respectively. Significantly triglyceride was decreased by 10.39 ± 0.0044 (46.49%), 13.16 ± 0.004 (32.23%), 14.06 ± 0.004 (27.60%) and 14.56 ± 0.004 (42.24%) for metformin, chloroform, ethyl acetate and ethanol fraction respectively. Three important phytoconstituents were isolated from chloroform fraction, and structures were elucidated using spectroscopic techniques.

INTRODUCTION: *Echinochloa colona*, regularly known as jungle rice, deccan grass, or awnless farm grass, is a sort of wild grass beginning from tropical Asia. It was some time ago delegated types of Panicum. It is the wild precursor of the developed grain crop *Echinochloa frumentacea*, sawa millet. A few taxonomists treat the two taxa as one animal group, in which case the trained structures may likewise be alluded to as *E. colona* [1, 3].

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Echinochloa Colona Plant Profile:

Kingdom: Plantae
Division: Angiosperms
Class: Monocots
Order: Poales

Family: Poaceae
Genus: *Echinochloa*
Species: *Echinochloa colona*

Botanical Description: *Echinochloa colona* (Poaceae), commonly known as Jungle rice (awnless barnyard grass) in India, is a yearly upright or decumbent, scattering, rooting from the inferior cutline node. It is a terrestrial, tufted, and erect grass propagates vegetatively but mostly by seeds, extensively spread in tropics & subtropics. It is also observed in South-Southeast Asia and tropical Africa. Leaves are spiral; alternatively, sessile linear more than 2 cm long, apex is acute with clasping base and parallel-veined. It culms ascending, or decumbent 10-100 cm long [4].

Pharmacognostic Characteristic:
Macroscopy: Culms are 10-70 cm long, lower nodes are glabrous and upper nodes are glabrous. Sheaths are glabrous, ligules absent, blades are 8-10 cm long and cm^{-3-6} wide. Panicle is cm^{-2-12}, erect, rachises. A primary branch is 5-10 cm, erect otherwise ascending, spike-like, distant, devoid of secondary branches, axes glabrous or sparsely hispid. Spikelet is 2-3 mm, disarticulating at maturity, pubscent to hispid. Lower glumes are as long as spiklets; lower florest are sterile occasionally staminate. Lower lemmas are unawned, upper lemmas are 2.6-2.9 mm. Anthers are 0.7-0.8 mm, caryopses are 1.2-1.6 mm, whitish, and embryos are as long as caryopses. It is distributed in tropical and subtropical areas. It is weedy in North America grown in low-lying, damp to wet, including rice fields. Un branched somewhat widely-spaced panicle branches, which make this one of the easier species of the *Echinochloa colona*.

Traditional Uses: As per the literature, in India, seeds of grass are used to prepare a food dish called khichdi and are consumed during festivals, fasting days. The whole plant is used as fodder by grazing animals, and it cures ingestion. *Echinochloa colona,* is a significant crop. It is a reasonable wellspring of protein, which is exceptionally edible; furthermore, it is a fantastic wellspring of dietary fiber with great measures of solvent and insoluble portions. The starch content is low and gradually edible, which makes the *Echinochloa colona*, a characteristic fashioner nourishment. In the current long periods of expanded diabetes mellitus, *E. colona*, could turn into perfect nourishment.

MATERIALS AND METHODS:

Sample Preparation: *E. colona* samples were collected from a paddy field in Dharmapuri, Tamil Nadu, India province in June 2013 at the flowering stage (red-purple flower). The samples were cleaned and separated into roots and shoots, then air-dried at room temperature and cut into small pieces (5 mm). The *E. colona* samples were extracted using soxhlet extraction methods, previously soaked with n-hexane to remove fatty residue. Then ethanol extract was fractionated (chloroform, acetone, ethyl acetate, ethanol, and methanol) from low to high polarity solvents. The plant parts were extracted using solvent at the rate of 1:5 (w/v) in 1 L beakers and covered with plastic film. The detailed procedure regarding the extraction was described by Gomaa and Abd Elgawad (2012). The beakers were kept in the dark at room temperature for 7 d. The solutions were separated from plant residues and evaporated using a rotary evaporator at 600 °C under reduced pressure. There were six different extracts of *E. colon*a from the shoots and roots using n-hexane, extracts from the shoots and roots using chloroform, acetone, ethyl acetate, ethanol, and methanol.

Column Chromatography: The chloroform fraction of *Echinochloa colona* was subjected to column chromatography using silica gel as it has shown better pharmacological effect comparing to ethyl acetate and ethanol fraction and eluted with solvent mixtures of increasing polarity.

While elution, the chromatographic fractions were collected and monitored on TLC. All the fractions showing a single spot were pulled together, purified, and observed for its R_f value using TLC. The solvents like chloroform, acetone, ethyl acetate, ethanol, and methanol used for separation [5].

Chromatographic Separation of Chloroform Fraction: Slurry of activated silica gel (150 °C for 3 h) was prepared with chloroform, and then the column was packed with slurry. The sample was loaded on the packed silica gel. After stabilization

column was eluted with mobile phase. Fractions were collected and analyzed by TLC.

Preparation of Mobile Phase: All the solvents, chloroform, acetone, ethyl acetate, ethanol, and methanol were distilled and then used for the preparation of the mobile phase. The composition of the mobile phase was made with increasing polarity solvents.

Column Packing: A clean and dry borosil glass column (60 cm, height; 3 cm, diameter) was aligned in a vertical position with the help of clamps attached to metal stand. A piece of cotton soaked in the mobile phase was positioned at the bottom of the column and quietly tamped down by means of a glass rod. The column was then filled about 1/3 volume by the mobile phase. The column was slowly and evenly filled, about 5/6 volumes full with the gradual addition of silica gel slurry. The stopcock was opened to allow the excess mobile phase to drain into the beaker. The side of the column was softly tapped with a cork during the process of packing to compact the silica gel. In the meantime, the stopcock was opened to run down the excess mobile phase. When the packing was finished, the excess mobile phase was drained until it just reaches the top level of silica.

Application of Sample: Weighed quantity of the sample was mixed with 1-2 g of activated silica gel and 3-4 ml of mobile phase to prepare a slurry. The slurry of the sample was added to the top of the packed silica in the column. The stopcock was opened to drain the excess mobile phase until it reaches top level of the sample. A thin disc (column diameter) of filter paper soaked in the mobile phase was placed on top of the bed to prevent disturbing the sample layer after addition of the mobile phase. The column was filled to the top with the mobile phase and allowed to stand for overnight (~24 h) to develop a chromatogram [6, 7].

Anti Diabetic Activity: Anti-diabetic effect of chloroform, ethyl acetate, and ethanol fractions obtained from ethanolic extract of *Echinochloa colona* was performed using streptozotocin-induced diabetic model in Wistar rats. The doses of the fractions were made at 50 mg /mL. All male Wistar rats (150-200 g) were randomly divided into 7 groups, each containing 5 rats [8, 10].

- **Group I:** Normal control (normal saline)
- **Group II:** Normal metformin control (150 mg/kg, *i. p*.)
- **Group III:** Diabetic control (normal saline)
- **Group IV:** Diabetic metformin control (150 mg/kg, *i. p*.)
- **Group V:** Diabetic (chloroform fraction)
- **Group VI:** Diabetic (ethyl acetate fraction)
- **Group VII:** Diabetic (ethanol fraction)

The standard drug and test samples were fed orally with an intragastric tube for 24 h experiment. Diabetes was induced (Group III-VII) by intraperitoneal injection (1 mL/kg) of freshly prepared streptozotocin (45 mg/kg), after baseline glucose was estimated.

After 48 h, blood samples were collected from the tail vein of all rats, and the blood glucose level was estimated. Blood glucose levels above [11]. 1 mmol/L in animals were selected for the studies considering the condition of diabetes was established.

Biochemical Analysis: After treatment, blood samples were collected with the help of disposable syringes from the tail vein of all rats of all groups before and at 0, 1, 2, 3, 6, 10, 16, and 24th h and analyzed for content of blood glucose using Glucometer (Bio Land, Germany). Then all the rats were sacrificed, and approximately 1-2 mL of blood was collected directly from the heart with the help of disposable syringes.

The blood samples were transferred to centrifuge tubes and allowed to centrifuge at 4000 rpm for 10 min; serum was collected used to determine total cholesterol (TC) and serum triglycerides (TG). Serum total cholesterol and triglycerides were estimated at 505 and 546 nm, respectively, using cholesterol oxidase/ p-amino antipyrine (CHO / PAP) method and glycerol 3- phosphate oxidase (GPO) method, respectively according to manufacturer's protocol [11, 13].

Determination of Total Cholesterol: Total cholesterol was determined by reagents kits of Reckon Diagnostics Pvt. Ltd., Baroda.

Method: CHOD-PAP method was used described by Allain *et al.,* 1974. It is an extremely specific

enzymatic colorimetric method for measurements in the visible range (505 nm), well-known for its high flexibility [14, 15].

Test Principle: The esters of cholesterol are hydrolyzed to cholesterol by cholesterol esterase (CE). The cholesterol is then oxidized by cholesterol oxidase (CO) to cholesten 4-en-3-one with the concurrent creation of H_2O_2.

H_2O_2 then reacts with 4-aminoantipyrine (AAP) and phenolic compounds in the presence of peroxidase to give colored complex red at 505 nm (500-540 nm, GREEN filter). The color intensity produced is directly proportional to the concentration of total cholesterol in the test samples.

Cholesterol esters + H_2O/ CE Cholesterol + RCOOH (Fatty acids)

Cholesterol + O_2/CO Cholesterol - 4-en- one + H_2O_2

$2H_2O_2$ + 4- AAP + Phenol POD/ Quinoneimine dye + $4H_2O$

Sample Material: Serum

Procedure: The three tubes were labeled accordingly as blank, standard, and test. 0.01ml standard and serum were added to the corresponding tubes. 1 mL Cholesterol reagent was placed in all tubes, *i.e.* blank, standard, and test, mixed well, and incubated (10 min at 37 °C). Then absorbance was red of test and standard at 505 nm or with green filter against blank reagent.

Calculations:

Cholesterol (mg/dl) = Absorbance in Test × 200 Absorbance in Standard

Determination of Triglycerides: Triglyceride was determined by reagents kits of Reckon Diagnostics Pvt. Ltd., Baroda.

Method: High performance enzymatic GPO-PAP method modified according to Fossati 1982; McGowan *et al.*, 1983.

Test Principle: Triglyceride is hydrolyzed sequentially to Di & Monoglycerides and finally to Glycerol by Lipase. Glycerol kinase (GK) by means of ATP as PO_4 source converts Glycerol to Glycerol-3-phosphate (G-3-Phosphate). G-3-phosphate Oxidase (GPO) oxidised G-3- phosphate to Di-hydroxy acetone phosphate & hydrogen peroxide is formed.

Hydrogen peroxide in presence Peroxidase (POD) oxidised to oxidise 4 amino antipyrine and chlorophenol to a pink coloured complex, which is measured at 546 nm (500-550 nm or with green filter). Absorbance is proportional to Triglycerides concentration.

Triglyceride + H_2O/Lipase Glycerol + Fatty acids

Glycerol+ ATP/GK/Glycerol-3-phosphate + ADP

Glycerol-3-phosphate + O_2 GPO/Di hydroxyl acetonephosphate + H_2O_2

H_2O_2 + 4-aminoantipyrine + Chlorophenol/POD Coloured complex + H_2O

Sample Material Serum

Procedure: 0.05 mL serum, standard, and distilled water were placed in the tubes marked as a test, standard and blank, respectively.

Then working solution 1 mL of was added to every tube, mixed well, and incubated (20 min at 37 °C). After incubation, 1.5 mL of distilled water was added to every tube, mixed well. Absorbance was red of test and standard against the blank at 546 nm (500-550 nm)

Calculations:

Triglyceride (mg/dl) = Absorbancein Test / Absorbancein Standard × 200

Elution: Elution was carried out with the flow rate (1 ml/min). The mobile phase was added at the top of the column from the solvent reservoir, and fractions were collected in an amber colored bottle.

Fractions were concentrated by evaporating at room temperature until volume was reduced to ¼ of the total volume. TLC of concentrated fractions was carried out to detect similarity between the chromatograms of different fractions.

Source	Fraction	Mobile phase	Abbreviation
E. colona	Chloroform	Chloroform: Ethanol 40:60	EC-I
		Chloroform: Ethanol 30:70	EC-II
		Chloroform: Ethanol 10:90	EC-III

Thin Layer Chromatography (TLC): TLC of each fraction was carried out during column chromatography, and the R_f value for EC-I (0.48), EC-II (0.74), EC-III (0.68) was observed.

Characterization of the Isolated Compounds from *E. Colona*:

EC-I: The EC-I fraction was collected at 40:60 in % ratio of (CHC_{l3}: C_2H_5OH). The isolated compound was white amorphous powder. The compound was studied for its qualitative properties and found to be positive with the ferric chloride test, and the phenolic nature of the compound was confirmed. The M.P. of the compound was 145-147 °C.

The UV spectrum showed λ_{max}, 220 nm, Ethanol with typical aromatic bands confirming a substituted aromatic benzoic acid. In the FTIR (KBr, cm^{-1}) signal at 3400 (-OH), 2935 (Ar-H), 1688(-COOH), ^{1}HNMR ($CDCl_3$, 400 MHz) signals at δ 11.80 (-COOH), 6.5 (Ar-H), 5.10 (Ar-OH), 2.33 (Ar-CH_3) and the peak of molecular ion was observed at m/z 184 matching to $C_8H_8O_5$, and main peak at 166 [184-(H_2O)]+ with other fragments at m/z: 120, 84, 42, 27 were observed.

The above spectral data suggested isolated compound is 2, 3, 4-trihydroxy, 6-methyl benzoic acid. The IR, 1HNMR, and MASS spectra were shown in following **Fig. 1** to **3**.

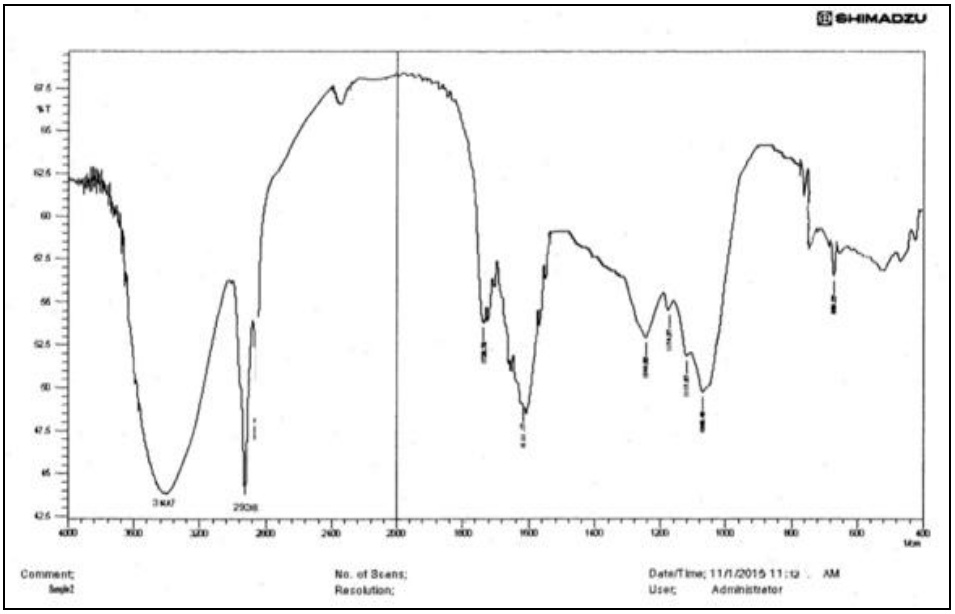

FIG. 1: IR SPECTRA OF EC-I FRACTION ISOLATED FROM *E. COLONA*

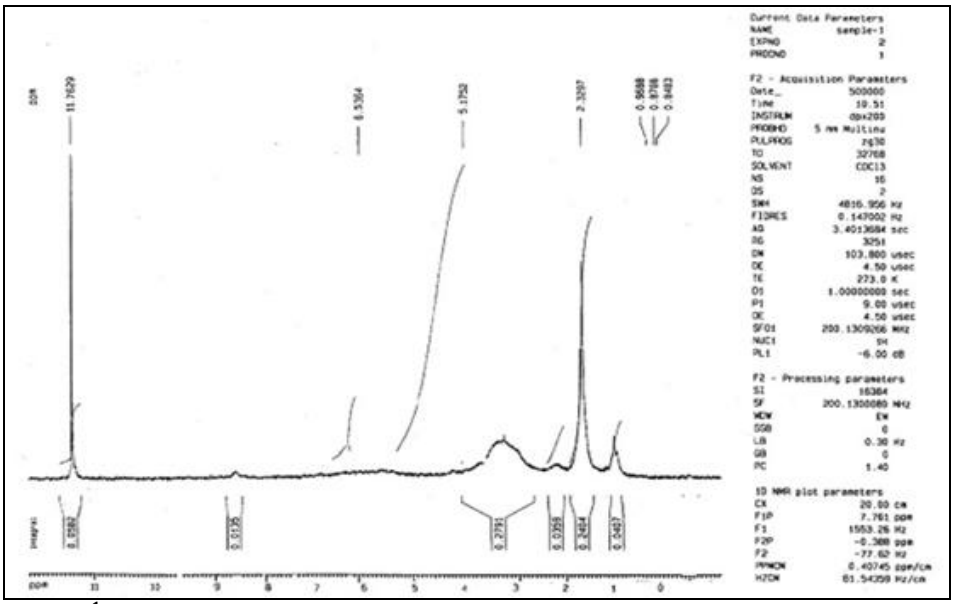

FIG. 2: ^{1}H NMRSPECTRA OF EC-I FRACTION ISOLATED FROM *E. COLONA*

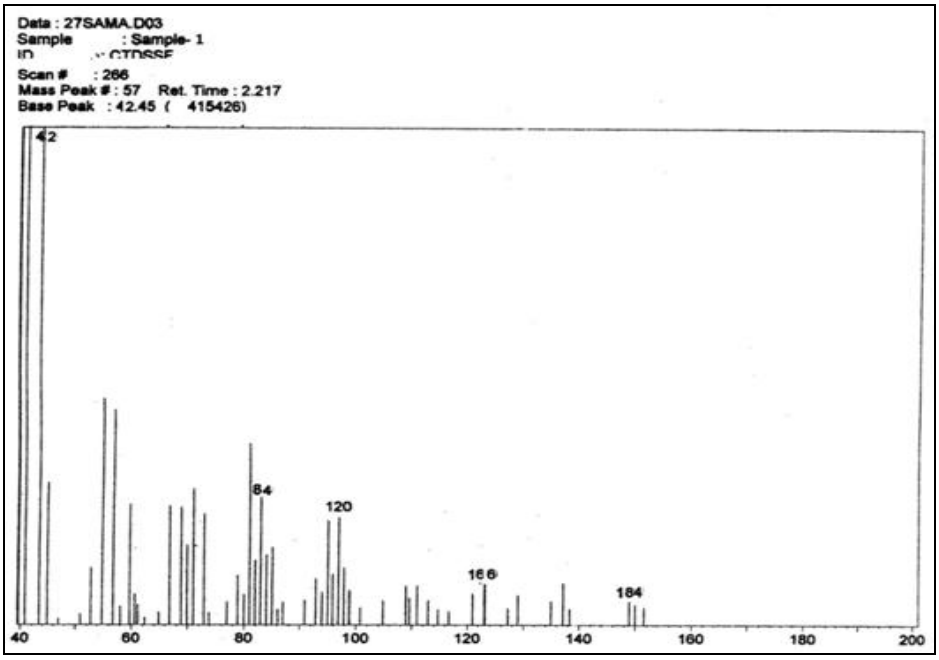

FIG. 3: MASS SPECTRA OF EC-I FRACTION ISOLATED FROM *E. COLONA*

EC-II: The EC-II fraction was collected at 30:70 in % ratio of ($CHCl_3$:C_2H_5OH). The isolated compound was white crystalline powder with a characteristic odour. The compound was studied for its qualitative properties and found to be positive with the Liebermann-Burchard test, and the steroidal nature of the compound was confirmed. The M.P. of the compound was 137-139 °C. UV spectrum showed λ_{max}, 210 nm, ethanol. In the FTIR (KBr, cm^{-1}) 3545 (-OH), 2931 (-CH2), 2860 (-CH), 1637 (-C=C-), 1033 (-C-O), 1HNMR ($CDCl_3$, 400 MHz) signals at δ 1.01, 1.04, 1.06, 1.04, 1.17, 1.21(-CH_3), 1.57, 1.98, 1.13, 1.79, 1.24, 1.27, 1.35, 1.34, 1.25, 1.29, (-CH_2-), 3.25(--CH-), 5.37 (H-cyclohexene) and peak of molecular ion was observed at 414.7 corresponding to $C_{29}H_{50}O$ with other characteristic fragmentations of m/z: 414, 396, 381, 330, 290, 273, 255, 212, 199 and 173 were observed. The above spectral data and those reported in the literature support the proposed structure was β-sitosterol 113, 114, 115. The IR, ^{1}HNMR, and MASS spectra were shown in the following **Fig. 4** to **6**.

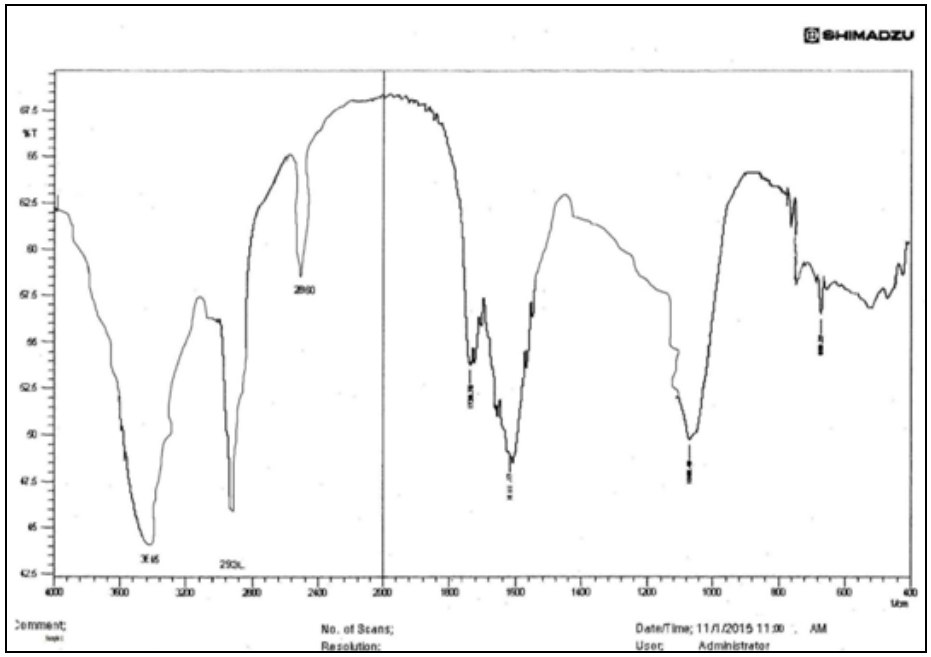

FIG. 4: IR SPECTRA OF EC-II FRACTION ISOLATED FROM *E. COLONA*

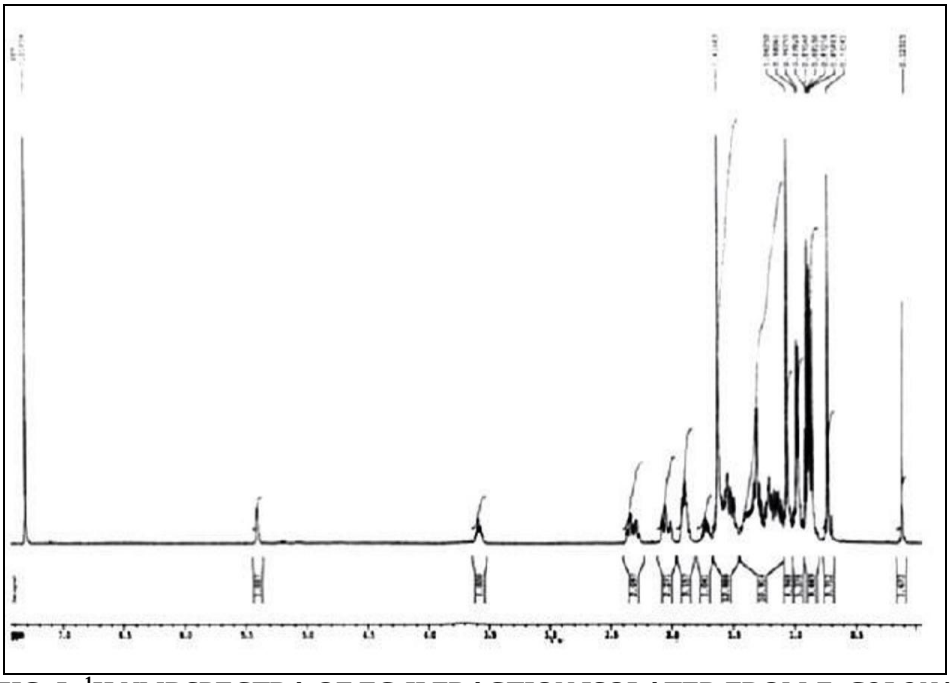

FIG. 5: ^{1}H NMRSPECTRA OF EC-II FRACTION ISOLATED FROM *E. COLONA*

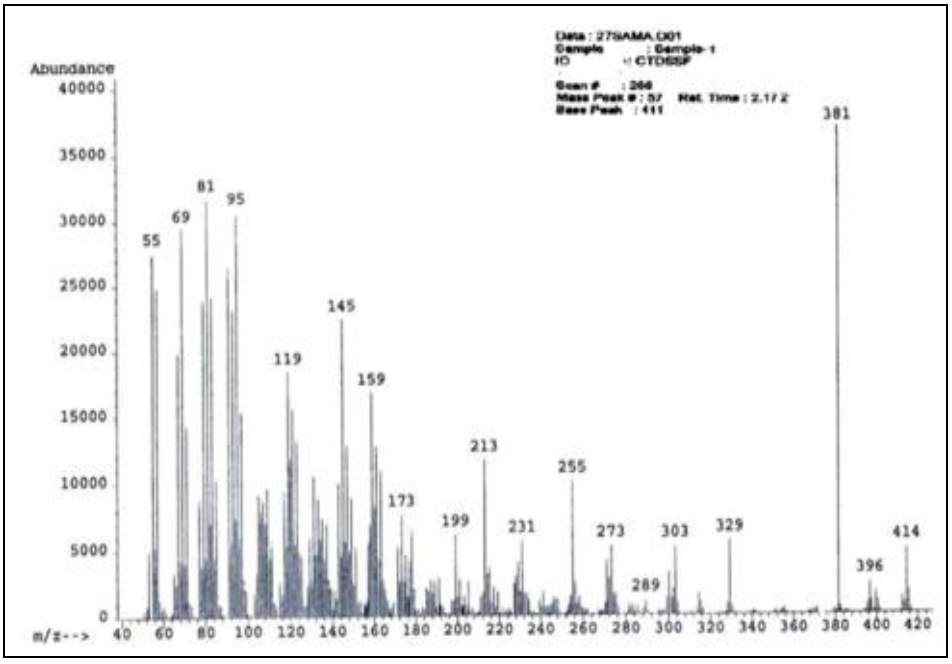

FIG. 6: MASS SPECTRA OF EC-II FRACTION ISOLATED FROM *E. COLONA*

EC-III: The EC-III fraction was collected at 10:90 in % ratio of (CHC_{l3}:C_2H_5OH). The isolated compound was white crystalline powder with a characteristic odour. The compound was studied for its qualitative properties and found to be positive with the ester test, and the ester nature of the compound was confirmed.

The melting point of the compound was carried out and found to be 150- 152 °C. The UV spectrum showed λ_{max}, 226 nm, methanol. In the FTIR (KBr, cm^{-1}) band at 1736 (RCOOR) and 3400 (Ar-OH), 1HNMR ($CDCl_3$, 400 MHz) at δ 1.25 (-CH_3), 4.65 (-CH_2-), 4.58 (Ar-OH) and a peak of molecular ion was observed at m/z 198 correspondings to $C_9H_{10}O_5$ with other fragments at 183, 149 [183-CH_3]$^+$, 129, 111, 97, 83, 69, 57, 43 were observed.

The above spectral data suggested isolated compound was ethyl 3, 4, 5-trihydroxy benzoate. The IR, 1HNMR, and MASS spectra were shown in the following **Fig. 7** to **9.**

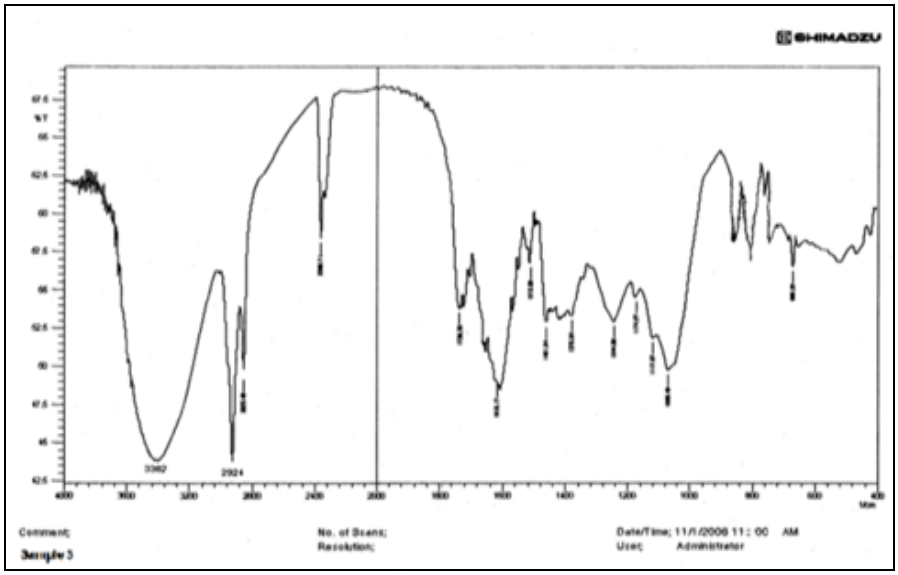

FIG. 7: IR SPECTRA OF EC-III FRACTION ISOLATED FROM *E. COLONA*

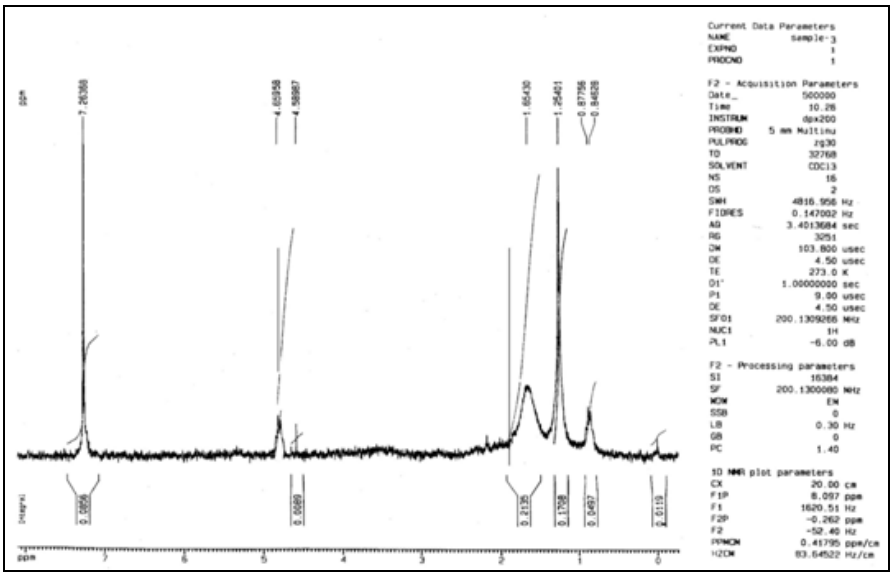

FIG. 8: 1HNMR SPECTRA OF EC-III FRACTION ISOLATED FROM *E. COLONA*

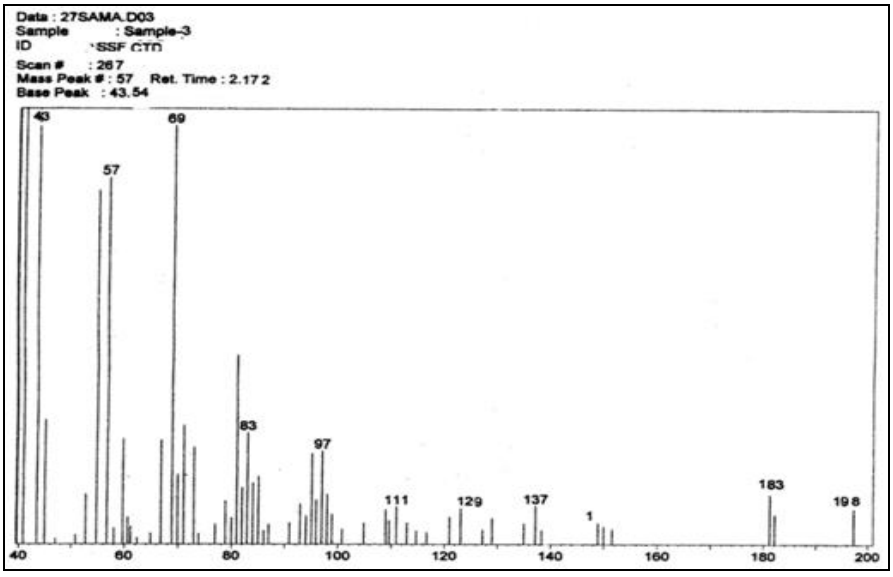

FIG. 9: MASS SPECTRA OF EC-III FRACTION ISOLATED FROM *E. COLONA*

Anti Diabetic Activity: The effects of different fractions (single dose-50 mg/mL) obtained from ethanolic extracts of *E. colonaupon* blood glucose (mmol/L), serum total cholesterol & triglycerides (mmol/L) were investigated within control and streptozotocin induced diabetic rats. Metformin HCl (150 mg/mL) was used as a standard anti-diabetic agent.

Effects of Various Fractions on Blood Glucose: A decrease in blood glucose level was observed in animals treated with different fractions of *E. colonaat* $^{0, 1, 2, 3, 6, 10, 16}$, and 24th h **Fig. 1**.

The blood glucose level was significantly reduced at 24th h of the experiment ($p < 0.01$) for metformin, chloroform, ethyl acetate, and ethanol fraction, it was 5.85 ± 0.004 (90.08%) 6.65 ± 0.004 (67.36%), 6.96 ± 0.003 (60.14%) and 7.05 ± 0.004 (58.01%) respectively comparing controlled diabetic rats **Table 1**.

TABLE 1: EFFECT OF DIFFERENT FRACTIONS OF *E. COLONA* ON BLOOD GLUCOSE IN DIABETIC RATS ON ONE DAY TREATMENT

Groups (Treatment)	Blood glucose levels (mmol/L) at Hours							
	0	1	2	3	6	10	16	24
I	7.25 ±	7.26 ±	7.27 ±	7.28±	7.29 ±	7.30±	7.31±	7.32±
10 ml saline	0.004	0.004	0.004	0.004	0.004	0.002	0.002	0.004
II	7.26±	7.24 ±	7.28±	7.20±	7.19±	6.17±	6.05±	4.13±
150 mg/kg	0.004	0.003	0.004	0.002	0.004	0.004	0.003	0.004
III	11.01±	11.50±	11.95±	12.25±	12.75±	13.10±	13.55±	13.95±
10 ml saline	0.003	0.003	0.004	0.004	0.004	0.004	0.003	0.003
IV	11.12±	10.81±	9.05±	8.75±	6.65±	7.08±	6.16±	5.85±
150 mg/kg	0.003	0.20	0.004	0.004	0.004	0.004	0.004	0.004
V	11.13±	10.61±	10.15±	9.95±	8.85±	7.28±	6.96±	6.65±
50 mg/ml	0.004	0.002	0.008	0.005	0.004	0.004	0.004	0.004
VI	11.13±	10.92±	10.41±	10.31±	9.55±	7.98±	7.86±	6.96±
50 mg/ml	0.004	0.004	0.004	0.004	0.004	0.003	0.002	0.003
VII	11.14±	11.02±	10.91±	10.71±	9.98±	9.07±	7.66±	7.05±
50 mg/ml	0.004	0.004	0.005	0.003	0.004	0.004	0.004	0.004

All values are expressed as mean ± SEM, (n=5) in every group, $p<0.01$, p-values were calculated and compared with control by ANOVA method followed by Dunnett's test

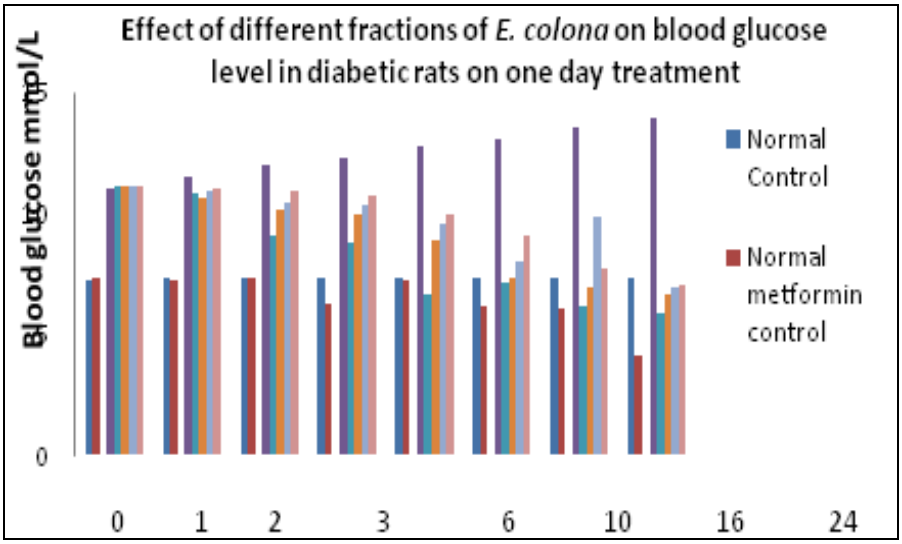

FIG. 10: EFFECT OF DIFFERENT FRACTIONS OF *E. COLONA* ON BLOOD GLUCOSE LEVEL IN DIABETIC RATS ON ONE DAY TREATMENT

Effect of Various Fractions on Total Cholesterol and Triglyceride: After 24 h treatment of different fractions of *E. colonathere* was decreased in total cholesterol and triglyceride on diabetic rats **Fig. 2**. Metformin, chloroform, ethyl acetate and ethanol fraction showed decrease in total cholesterol level by 12.16 ± 0.0044 (56.98%) 13.02 ± 0.004 (53.94%) 14.17 ± 0.004 (49.87%) and 16.37 ±

0.004 (42.24%) respectively, whereas the triglyceride was decreased by10.39 ± 0.0044 (46.49%) 13.16 ± 0.004 (32.23%) 14.06 ± 0.004 (27.60%) and 14.56 ± 0.004 (42.24%) for metformin, chloroform, ethyl acetate and ethanol fraction respectively when compared to diabetic control groups **Table 2.**

TABLE 2: EFFECTS OF DIFFERENT FRACTIONS OF *E. COLONAON* TOTAL CHOLESTEROL & TRIGLYCERIDES IN DIABETIC RAT ON ONE DAY TREATMENT

Parameter (mmol/L)	Groups						
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**
Total Cholesterol	14.37± 0.003	12.24± 0.004*	28.27± 0.004**	12.16± 0.004**	13.02± 0.004**	14.17± 0.004**	16.37± 0.004**
Total Glycerides	12.41± 0.004	10.31± 0.003*	19.42± 0.004*	10.39± 0.004*	13.16± 0.004*	14.06± 0.004*	14.56± 0.004*

All values are expressed as mean ± SEM, (n=5) in every group, *p <0.01, considered extremely significant, p-values were calculated and compared with control by ANOVA method followed by Dunnett's test.

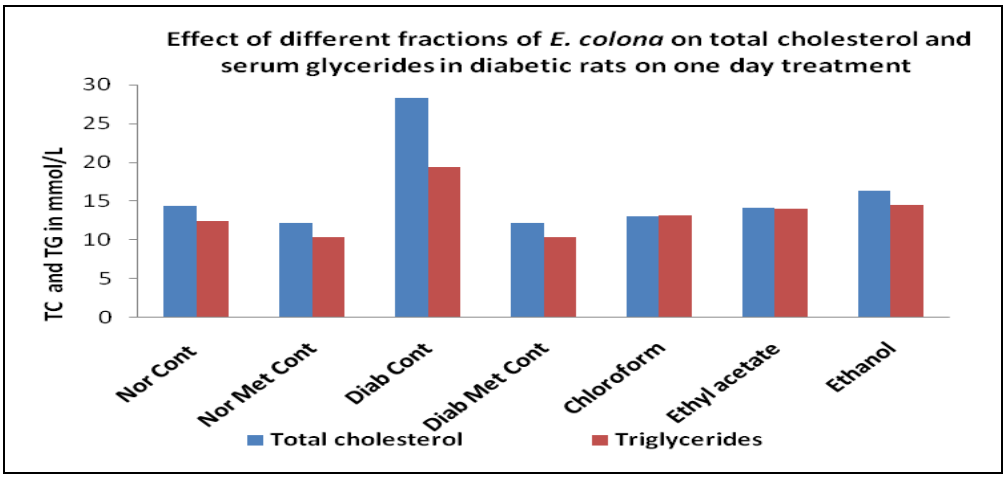

FIG. 11: EFFECTS OF DIFFERENT FRACTIONS OF *E. COLONA* ON TOTAL CHOLESTEROL AND SERUM GLYCERIDES IN DIABETIC RATS ON ONE DAY TREATMENT

CONCLUSION: Given the potent results of the extracts tested in this article against diabetes and the factor responsible for the same (triglycerides). A decrease in blood glucose level was observed in animals treated with different fractions of *E. colona*.

After 24 h treatment of different fractions of *E. colona* there was a significant decrease in total cholesterol and triglyceride on diabetic rats when treated with chloroform fraction. The chloroform fraction exhibiting significant anti-diabetic activity was chromate graphed to isolate three phyto constituents 2, 3, 4-trihydroxy, 6-methyl benzoic acid, β-sitosterol, and ethyl 3, 4, 5-trihydroxy benzoate.

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