PHARMACOGNISTICAL, PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF *Hydrolea Zeylanica* (Hydrophyllaceae) AND *Echinochloa Colona* (Poaceae)

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INTRODUCTION

In common usage today, many phytochemicals are associated with health benefits with a long history and still continues as medicines. Many, though not all, of these materials are classified as secondary metabolites. This terminology suggests, often incorrectly, that they are not essential for the normal growth, development or reproduction of the plant. The use of natural products as medicinal agents presumably predates the earliest recorded history as the earliest humans used various, but specific plants to treat illness. Records from as early as 2700 B.C. from China, traced to the Emperor Shennung, indicate the usefulness of plants for treating disease, and the Ebers papyrus, written in about 1550 B.C., includes many of the plants used in Egyptian medicine. Theophrastus (370-285 BC) began the scientific classification of plants, and Dioscorides De Materia Medica (77 AD) reported the uses, medicinal and otherwise, of over 600 plants. Ibn al-Baitar (1197-1248) listed over 1400 drugs and medicinal plants in his Corpus of Simples. In Europe, after the tenth century, much of the medicinal lore was based in the church, particularly the monastic orders, but by the 1500’s, after the invention of the printing press, herbals available to the general public were popular, particularly in England. By the late 1700’s, studies like William Withering’s An Account of the Foxglove and its Medicinal Uses (1785) began to appear. These were based on case histories and described specific doses and gave administration instructions for herbal remedies. In the United States, before the advent of specific pharmaceuticals, herbal medicine was relied upon to treat many illnesses. Development of drugs based on natural products has had a long history in the United States, and in 1991, almost half of the best selling drugs were natural products or derivatives of natural products. There has recently been a resurgence of interest in herbal remedies, and a Reuters/Zogby poll in 2000 showed that 40 % of people in the U.S. had tried herbal remedies. In 1998, the U.S. market for natural supplements was over $12 billion in sales and increasing by as much as 10 % per year. Herbs such as St.
John’s Wort, ginkgo, echinacea, and ginseng are among the most popular herbs. In 1999, echinacea was reported to make up 38% of the U.S. market, with ginkgo a close second at 34%. The efficacy of these herbs is being investigated in many laboratories, and efforts are also being made to isolate and identify any active constituents.

Natural products, as the term implies, are those chemical compounds derived from living organisms, plants, animals, insects, and the study of natural products is the investigation of their structure, formation, use, and purpose in the organism. Drugs derived from natural products are usually secondary metabolites and their derivatives, and today those must be pure and highly characterized compounds. Until the late 1800’s, organic chemistry was almost exclusively the study and use of natural products. The purpose of these compounds in the organisms and their formation was little understood or investigated, primarily due to the lack of appropriate techniques and structural theory. The natural products that were studied and used tended to be the compounds that occurred in the largest amounts, mostly in plants, and were most easily isolated in a pure, or sometimes not very pure form by techniques such as simple distillation, steam distillation, or extraction with acid or base. Originally teas or decoctions (aqueous extracts) or tinctures or elixirs (alcoholic extracts) were used to prepare and administer herbal remedies, these were usually the starting points for isolation work. We now employ different solvents, e.g., ethanol to extract, hexane to concentrate non-polar constituents, methanol to concentrate polar constituents, and modern isolation techniques include all types of chromatography, often guided by bioassays, to isolate the active compounds. Up until the 1950’s, the structures of natural products, when determined, were determined by degradative techniques, and a structure was not proven until the compound had been synthesized in a definite manner. Now, structures are elucidated primarily by spectroscopic techniques, and the stereochemistry is an important feature of the structure.
Echinochloa colona (EC) of Poaceae family is distributed in tropics and sub tropics, including India, Southeast Asia and tropical Africa, commonly known as known as Jungle rice in India, Jangali Chawal (Hindi), Borur (Marathi), Othagaddi (Telgu) and Kaddu (Kanada) 5. It is an annual erect or decumbent or spreading, rooting from the lower cut-line nodes. It is a terrestrial, tufted and erect grass. Leaves are alternate spiral, sessile linear, more than 2 cm long/wide, apex acute, base clasping, parallel-veined. It is Annual, culms ascending, or decumbent; 10-100 cm long 6. In India seeds of plant are used to prepare a food dish called khichdi and consumed during festival and fasting days. The whole plant is used as fodder by grazing animals and it cures ingestion 7,8.

Hydrolea zeylanica (HZ) of Hydrophyllaceae family is distributed throughout India (Kerala, Maharashtra) mostly in open wet places, often common in rice paddles as a weed. The plant is native to temperate and tropical Asia and Australia, commonly known as Koliary (Hindi), Popti (Marathi), Vellel (Tamil) 9. It is an annual marsh herb usually decumbent, 15-50 cm long, leaves 2.5-6.5 cm long pointed at both ends, lanceolate or linear lanceolate, acute, glabrous, flowers numerous, in racemes, short, lateral branches, corolla blue with darker veins, deeply divided, 5-6 mm long, capsule 4 mm long, ovoid, oblong, enclosed in the enlarged, persistent, sepals 10.

In the traditional system of medicine the plant is used for different types of diseases and health problems. The roots are reported to be effective in wounds, diabetic carbuncle and fistula 11. A paste of whole plant with coconut oil is applied on minor cuts, wounds and boils as antiseptic for quick relief 12, 13.

Hydrolea zeylanica was found to contain 18.3 dry matter, 2.2 quercetin, 7.9 kaempferol, and 10.1 total flavonoids (mg/100g) 14. Leaf and twigs of Hydrolea zeylanica is used by various tribes of Southern Assam as antidiabetic remedy 15. The leaves of plant are used by the folklore of Andhra Pradesh, India for ulcer treatment 16. The plant is used as wound healing remedies also 17.
AIMS AND OBJECTIVE

This project is aimed with identifying & isolating phytochemicals present in the plants and evaluation of some pharmacological properties with the following specific objectives,

a) To carry out the extraction and fractionation of phytochemicals.

b) To carry out the preliminary phytochemical analysis of different fractions obtained from *Hydrolea Zeylanica* and *Echinochloa Colona*.

c) To evaluate the fractions obtained from ethanolic extract of *Hydrolea Zeylanica* and *Echinochloa Colona* for antidiabetic, anti ulcer and wound healing activity using appropriate animal models and antioxidant activity using *in vitro* models.

d) To isolate and elucidate the structures of isolated compounds from the most promising fractions of *Hydrolea Zeylanica* and *Echinochloa Colona* using IR, NMR and Mass spectrometry.
PLAN OF WORK

A) Selection of plant (2 plants were selected for further study)
- Authentication and Standardization of selected plant materials
  a) Ash Value: Total, acid-insoluble and water soluble
  b) Loss on drying (LOD)
  c) Extractive value
- Permission to carryout animal experiments

B) Extraction (Extract of each plant material)
  a) Fractionation (Increasing polarity solvents)
  b) Phytochemical evaluation (All fractions)
  c) Acute and sub acute toxicity study (Each plant extract)
  d) Pharmacological screening (Selected fractions)
    1) In vitro Antioxidant activity
    2) Antidiabetic activity (Streptozotocin induced)
    3) Anti ulcer activity (Pyloric ligation model)
    4) Wound healing activity
      a) Guinea pig punch wound model
      b) Wound assay
      c) Chick chorioalantoic membrane model

C) Chromatographic separation of fraction with pronounced Pharmacological activity
  a) Column chromatography
  b) Thin layer chromatography

D) Characterization of isolated compounds
  a) IR
  b) NMR
  c) Mass Spectrometry
MATERIALS & METHODS

Plant Material:
The plant material of *Hydrolea Zeylanica* and *Echinochloa Colona* were collected from Dharmapuri, Tamilnadu, India in June 2013 and was identified and authenticated by J. Jayanthi., Botanical Survey of India, Pune (Voucher No. BOIVS/B3/B4/BS/WRC/Tech/2013).

PHYSICAL EVALUATION OF POWDERED DRUG
The shade dried whole plant of *Hydrolea Zeylanica* and *Echinochloa Colona* was coarsely powdered and evaluated for its physicochemical properties such as ash value, total ash, acid insoluble ash, water soluble ash and loss on drying.

PREPARATION OF PLANTS EXTRACT
The whole plant of *Hydrolea Zeylanica* and *Echinochloa Colona* were cleaned, dried in shade and powdered by a mechanical grinder. 5 kg powder of each plant was macerated with n-hexane for 24 h and then extracted with ethanol using Soxhlet apparatus for 72 hrs. The ethanolic extract was then concentrated to dryness at room temperature (35-40°C) so as to obtain dry extract.

Preparation of fractions of *E. colona* and *H. zeylanica*
The ethanolic extract of *Hydrolea Zeylanica* and *Echinochloa Colona* was dried in air; resinous mass was discarded and subjected to further fractionation. The fractionation was carried out successively using petroleum ether, n-hexane, chloroform, ethyl acetate and ethanol to obtain various fractions. All fractions were pooled, transferred to previously weighed petri dish and evaporated to dryness at room temperature (35-40°C) so as to obtain dried fractions. After complete drying, the petri dish was weighed again. The yield of fractions was calculated as percent yield.
The yield of different fractions of *Echinochloa Colona* was 2.96%, 3.25%, 7.52%, 11.26%, and 2.75% for petroleum ether, n-hexane, chloroform, ethyl acetate and ethanol respectively. The yield of differ-
ent fractions of *Hydrolea Zeylanica* was 4.20%, 4.15%, 8.26%, 13.20%, and 4.90% for petroleum ether, n-hexane, chloroform, ethyl acetate and ethanol respectively. Fluorescence analysis of all the fractions was carried out.

**PHYTOCHEMICAL ANALYSIS**

Test solutions of petroleum ether, n-hexane, chloroform, ethyl acetate and ethanol fraction were prepared in corresponding solvents in concentration 100 mg/ml and phytochemical analysis of all fractions of *Hydrolea Zeylanica* and *Echinochloa Colona* was carried out.

**Animals:**

The antidiabetic and anti ulcer activity was performed using male albino wistar rats (150-250g), whereas wound healing activity was carried out using male guinea pigs (300–325g). Acute and sub acute toxicity study was performed using healthy female wistar rats (150-200 g). On arrival, all the animals selected for the corresponding study, were placed randomly in polypropylene cages (six per cage) with paddy husk as bedding and kept in well ventilated animal house (12 hr light/dark cycles) at 27± 5 0, relative humidity 50% ± 5, fed with standard diet, water and *ad libitum*. Animals had free access of water filtered through Aqua guard® and standard pellet animal diet (Chaken oil Mill, Pune; India) *ad libitum*. On the day of experiments, animals were food deprived at 06:00 h and placed in the experimental room at 08:00 h. All experimental protocols were approved by the Institutional Animal Ethical Committee (IAEC).

**ACUTE ORAL TOXICITY STUDIES**

A single dose of ethanolic extract of *Hydrolea Zeylanica* and *Echinochloa Colona* were made at 450, 1500 and 5000 mg/kg. The three female rats each sequentially were treated with a single dose of 450, 1500 and 5000 mg/kg of ethanolic extract orally at intervals of 48 h and observed individually for acute toxicity signs, survival or death and behavioral changes 1 h post dosing and at least once daily for 14
days. For the above study OECD guidelines for the testing of chemicals/section 4, no. 423 was followed.

**EXPERIMENTAL PROTOCOL**

1. **In Vitro Antioxidant Activity:**

   a) **Reducing power assay**

   The reducing power assay was performed for the fractions of *Hydrolea Zeylanica* and *Echinochloa Colona*. The concentrations of fractions of chloroform, ethyl acetate and ethanol were made (25, 50, 75 and 100 mg/mL in phosphate buffer pH 6.6). Likewise standard ascorbic acid solution was prepared. To the above each sample, 2 mL of potassium ferricyanide (10 mg/mL) was added and incubated at 50°C for 20 min followed by addition of 2 mL of trichloroacetic acid (100 mg/L) and were centrifuged at 3000 rpm for 10 min. A volume of 2 mL from each upper layer of the solution was collected and mixed with 2 mL of distilled water and 0.4 mL of 0.1% (w/v) fresh neutral ferric chloride. After 10 minutes, the absorbance was measured at 700 nm using UV spectrophotometer. Higher reducing power was indicated by increase in absorbance of the reaction mixture. The concentrations of standard ascorbic acid was prepared and treated similarly. Reducing power was given in ascorbic acid equivalent (ASE mL⁻¹).

   b) **2, 2- Diphenyl-1-picrylhydrazyl assay**

   The free radical scavenging activity of fractions of *Hydrolea Zeylanica* and *Echinochloa Colona* was performed and measured as a decrease in the absorbance of methanolic solution of DPPH. The stock solution of DPPH was prepared by dissolving 33 mg in 1L methanol and stored at 20°C until necessary. The absorbance of stock solution was obtained 0.47 at 517 nm using UV spectrophotometer. The concentrations of fractions of chloroform, ethyl acetate and ethanol were made (25, 50, 75 and 100 mg/mL in methanol). Likewise standard ascorbic acid solution was prepared (25-100 mg/mL). A 5 mL of DPPH stock solution was added to 100 μL of above each sample. After shaking the reaction mixture well, it was incubated in the dark for 30 min at room
temperature and then absorbance was taken at 517 nm along with the standard ascorbic acid. The control was prepared devoid of any sample as above. Percent inhibition by sample was calculated in comparison with vehicle control.

c) Nitric oxide radical assay

The fractions of chloroform, ethyl acetate and ethanol of *Hydrolea Zeylanica* and *Echinochloa Colona* were prepared (25, 50, 75 and 100 mg/mL in phosphate buffer pH 7.4). Likewise standard ascorbic acid solution was prepared (25-100 mg/mL). Sodium nitroprusside was added (5 µM) in each tube; and incubated at 25°C for 5 hrs. Control experiments without sample were carried out with identical conditions. After 5 hrs, 1mL of incubated solutions were removed and diluted with 1 mL of Griess reagent. The absorbance of the color developed after diazotization of nitrite with sulphanilamide and its coupling with napthylethylenediamine hydrochloride was observed at 546 nm using UV spectrophotometer. Scavenging of Nitric oxide was calculated as “inhibition percentage” in comparison with vehicle control.

2. Antidiabetic activity

*Streptozotocin induced diabetes in wistar rats*

Antidiabetic activity of chloroform, ethyl acetate and ethanol fractions obtained from ethanolic extract of *Hydrolea Zeylanica* and *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. 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) and Group VI: Diabetic (ethanol fraction). The standard drug and test samples were fed orally with intra gastric tube for 24 h experiment. Diabetes was induced (Group III-VII) by intra peritoneal injection (1mL/kg) of freshly prepared Streptozoto-
cin (45 mg/kg), after base line glucose was estimated. After 48 h blood samples were withdrawn from the tail vein of the rats and the blood glucose level was estimated. Animals with blood glucose levels above 11.1mmol/L were selected for the study considering the condition of diabetes was established.

After treatment, blood samples were collected with the help of disposable syringes from tail vein of each rat of all groups before and at 0, 1, 2, 3, 6, 10, 16, and 24th h and analyzed for blood glucose content using Glucometer (Bio Land, Germany). Then all the rats were sacrificed, about 1-2 mL blood was collected from the heart directly 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 to determine total cholesterol and serum triglycerides at 505 and 546 nm respectively.

**Biochemical Evaluation**

Following parameters were evaluated using various Diagnostic kits.

1. Serum Total Cholesterol (Reckon Diagnostic kit)
2. Serum Triglycerides (Reckon Diagnostic kit)

**3. Anti ulcer activity**

**Pyloric ligated induced ulcers in albino wistar rats:**

Anti ulcer activity of chloroform, ethyl acetate and ethanol fractions obtained from ethanolic extract of *Hydrolea Zeylanica* and *Echinochloa Colona* was performed. The doses of the fractions were made at 50, 75 and 100 mg/mL each. Male albino wistar rats (200-250 g) were randomly divided into 12 groups each containing 6 rats and treated for 7 days. Group I: Normal control (normal saline), Group II: Pyloric ligation control (normal saline), Group III: Pyloric ligation Ranitidine control (50 mg/kg p. o.), Group IV-VI: treated with chloroform fraction (50, 75 and 100 mg/kg), Group VII-IX: treated with ethyl acetate (50, 75 and 100 mg/kg), Group X-XII: treated with ethanol fraction (50, 75 and 100 mg/kg).
After the completion of the treatment, the rats were kept for fasting for 24 h with free access to water. Then after, abdomen was opened and pylorus was ligated under light ether anesthesia avoiding damage to the blood vessels. After the 4 h of ligation stomach was dissected out and abdominal wall was closed with sutures and stomach contents was collected to determine gastric volume, pH, free acidity, total acidity and ulcer index.

a) Estimation of Gastric Volume:
The gastric contents were collected into a measuring cylinder and volume of gastric contents was measured.

b) Determination of pH:
An aliquot of 1ml gastric juice was collected and diluted with 1ml of distilled water and pH of the solution was measured using pH meter.

c) Estimation of Free and Total Acidity:
The gastric content was collected and centrifuged at 2000 rpm for 10 min. 1 ml from each supernatant liquid obtained was withdrawn with the help of pipette and diluted with distilled water up to 10 ml and then was titrated against previously standardized 0.01 N NaOH using Topfer’s reagent as indicator, titration was allowed to continue till orange color is obtained. The consumed volume of NaOH solution was taken as corresponding to the free acidity. The titration was continued with the addition of 1 % phenolphthalein indicator till pink color was obtained. The volume of NaOH solution was noted and the sum of the volume of NaOH solution consumed was considered for total acidity. The free and total acidity is expressed as mEq/L and was calculated using formula,

\[
\text{Acidity} = \frac{\text{Vol. of NaOH} \times N \times 100}{0.1} \text{ mEq/L}
\]

d) Estimation of Gastric Ulcerative Index:
The stomach was opened along the greater curvature and washed with normal saline and then ulcerative index was calculated by mounting the stomach on flat wooden plate using formula,

\[
\text{Ulcer Index} = \frac{10}{X}
\]
Where X was total mucosal area/total ulcerated area.

% Ulcer protection was calculated using following formula,

\[
\% \text{ Ulcer Protection} = \frac{\text{Ulcer index of control group} - \text{Ulcer index of treated group} \times 100}{\text{Ulcer index of control group}}
\]

4. Wound healing activity

a) Guinea pig punch wound model

The wound healing activity of the fractions was performed using guinea pig punch wound model. All the fractions of chloroform, ethyl acetate and ethanol were mixed with ointment base to prepare 1% ointment of each fraction. All fifteen animals were divided into 5 groups, each containing three animals. Group I: Vehicle control (ointment base), Group II: Standard (Povidone- Iodine ointment), Group III: treated (chloroform fraction), Group IV: treated (ethyl acetate fraction), Group V: treated (ethanol fraction).

The dorsal surface of the animals was shaved; sterilized (70% alcohol) and four cutaneous circular wounds of full thickness, completely transdermal of 8 mm diameter were made with the help of a biopsy punch. Thiopentone sodium (25 mg: kg, i. p.) was used to produce anesthesia to carry out all surgical procedures. Each fraction was applied topically on wounded area for 7 days. All the animals were allowed to recover and were housed in standard experimental conditions supplied with standard diet. After the completion of the treatment the wound healing was assessed by measuring area of wound, collagen estimation and tensile strength in healing tissue.

Area of wound:

On 7th and 10th day, the surface area of the healing wound was measured by tracing the boundary of wound which was still open using semi-transparent paper and wound area was calculated using a graph paper.

Collagen estimation:

On 7th day, collagen was estimated by determining hydroxyproline content from wound tissues, a basic bio constituent of collagen; in-
volved in healing of tissue. The tissues taken from wound were dried in a hot air oven at 60–70°C to constant weight and then were hydrolyzed using 6 N HCl at 130°C for 4 h in sealed tubes. The hydrolysate so obtained was neutralized to pH 7.0 and then chloramine-T was added to the above and subjected to oxidation for 20 min. after 20 min. the reaction was terminated by addition of 0.4 M perchloric acid and then Ehrlich reagent was added (60°C), color was produced which was then measured at 557 nm using UV spectrophotometer.

**Tensile strength:**

After the completion of 7 day treatment of wounds, on the 7th day the animals were anaesthetized and healing tissue along with normal skin at two ends was pooled and tensile strength was measured using tensile testing machine. The excised tissues from treated and control animals were cut out of 8 mm width and 20 mm length and loaded between the upper and lower holder of the machine. The total breaking load was measured in Newtons and the tensile strength was calculated by using following equation,

\[
\text{Tensile strength} = \frac{\text{Total breaking load}}{\text{Cross-sectional area}}
\]

**b) In-vitro wound assay**

The chloroform, ethyl acetate and ethanol fractions obtained from ethanolic extract of *Hydrolea Zeylanica* and *Echinochloa Colona* were assessed for in-vitro wound assay. The doses of the fractions were made at 50, 100, 150 and 200 μg/mL. The 42 embryos were selected and incubated for 11 days at 37°C (80% relative humidity) to mature chorioallantoic membrane (CAM) completely. On the 12th day of incubation, the outer shell was sterilized with 75% ethanol applying to the surface. All the 42 embryos were divided into 14 groups each containing 3 embryos. Group I: Treated with standard diclofenac sodium (50 μg/mL), Group II: Control (normal saline), Group III-VI: treated with chloroform fraction (50, 75, 100 and 200μg/mL), Group VII-X: treated with ethyl acetate fraction (50, 75, 100 and 200μg/mL), Group XI-XIV: treated with ethanol fraction (50, 75, 100 and 200μg/mL).
All the eggs were kept under aseptic conditions. A tiny hole was made carefully in the egg shell with a needle and a small window of the shell was cracked open to expose the inner shell membrane. 0.5-1 ml of sterile saline solution was transferred to the inner shell membrane to make it translucent then the layer was peeled to visualize the CAM which was then pulled using sterile forceps and an excision wound of approximately 3 mm diameter was created in the CAM layer by using a small dissecting scissor. The discs saturated with chloroform, ethyl acetate and ethanol fraction (50, 75, 100 and 200 μg/mL) along with standard diclofenac sodium (50 μg/mL) were then placed on the CAM of the embryos labeled with the corresponding concentrations and controls. The window of the egg shell was covered with para film and then all eggs were incubated at 37°C (80% relative humidity). Wound closure was measured on alternative days of 5 day observation period after wounding as percentage wound contraction using following formula,

\[
\text{Percent Wound Contraction} = \frac{\text{Initial wound size} - \text{Specific day wound size}}{\text{Initial wound size}} \times 100
\]

c) **In-vitro chick chorioallantoic membrane model**

The angiogenic property of the fractions was assessed using *in-vitro* chick chorioallantoic membrane model (CAM) \(^{36}\). Nine day-old 39 fertilized chick eggs were selected and incubated at 37°C and (80% relative humidity) in an incubator for 72 h., the shells of the eggs were wiped with 70% ethanol to avoid infections. All the 39 fertilized chick eggs were divided into 13 groups each containing 3 embryos. Group I: Control treated (normal saline), Group II-V: treated with chloroform fraction (10-40 mg/disk), Group VI-IX: treated with ethyl acetate (10-40 mg/disk), Group X-XIII: treated with ethanol fraction (10-40 mg/disk). After incubation of all fertilized chick eggs, a small window of 1.0 cm\(^2\) was made in the shell of eggs. The membrane was felled by drilling a small hole at the air space and air was removed out using a rubber
bulb. The window was opened and a sterile disk of methylcellulose loaded with different concentrations (10–40 mg/disk) of chloroform, ethyl acetate and ethanol fraction was placed in at the junction of two big vessels. The window was then resealed by tape and all eggs including control (normal saline) containing disks without test compounds were incubated at 37°C in a in an incubator for 72 h. After 72 h the eggs were opened and new vessel formation if any was observed and compared with control.

**STATISTICAL ANALYSIS**

All the biochemical and experimental data obtained were expressed as mean ±SEM. Difference between the groups was statistically determined by analysis of variance (ANOVA) with Dunnett’s test multiple comparisons test, analyzed by graph pad Instat software version 3.01, Graphpad Software, CA, USA. The p < 0.05 = * and p < 0.01 = ** were considered as significant.

**CHROMATOGRAPHIC SEPARATION**

**Column chromatography:**

The chloroform fraction of both *Hydrolea Zeylanica* and *Echinochloa Colona* were 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 single spot were pulled together, purified and observed for its Rf value using TLC 37. The solvents such as chloroform, acetone, ethyl acetate and ethanol were used for separation.

**Thin Layer Chromatography (TLC):**

The slurry was prepared by suspending silica gel G in distilled water (1:2). Measured amount of slurry was put on the clean and dry glass plate, which was kept on a level surface. The plate was then tipped back and forth to spread the slurry uniformly over the surface. The plates were dried in air for 30 min and then in oven at 110°C for
another 60 min for the activation of adsorbent layer. A spot of sample was applied on the starting line, which was parallel and about 10 mm above the lower edge, with help of glass capillary. Sample spots were allowed to dry at room temperature. Sufficient quantity (3-4 ml) of mobile phase was poured into the chamber. To achieve saturation, chamber was closed and allowed to stand for 15-20 min. The plate was placed as nearly vertical as possible into the chamber, ensuring that the points of application were above the surface of the mobile phase. Chamber was closed and mobile phase was allowed to ascend to specified distance. Plate was removed, position of mobile phase front was marked and mobile phase was allowed to evaporate at room temperature. Plate was observed in the daylight, under UV light and then in iodine chamber. After each observation the central points of spots appeared on chromatogram were marked with needle. Retention factor (Rf) was calculated by formula: - \( R_f = \frac{A}{B} \) where, \( A \) = Distance between point of application and central point of spot of material being examined and \( B \) = Distance between point of application and the mobile phase front.

**CHARACTERIZATION OF ISOLATED COMPOUNDS**

All fractions showing single spot on TLC plate were pulled together, purified and observed for \( R_f \) value. The isolated compounds were characterized with the aid of UV, IR, 1HNMR and MS\(^{38, 39}\).
RESULTS

Ash value:
Ash values of powdered drug of both the plants were estimated. Total ash, acid insoluble ash, water soluble ash and loss on drying were found to be 11.88, 1.14, 4.48 and 8.46 respectively for *E. colona*, whereas it was 15.49, 3.13, 6.35 and 9.50 for *H. zeylanica* respectively.

Extractive value:
Percent yield by different solvent fractionation was calculated. The extractive value (%) of petroleum ether, hexane, chloroform, ethyl acetate and ethanol fraction was 2.96, 3.25, 7.52, 11.26 and 2.75 respectively for *E. colona*, whereas it was 4.20, 4.15, 8.26, 13.2 and 4.90 for *H. zeylanica* respectively.

Phytochemical investigation:
Preliminary Phytochemical analysis revealed the presence of different phytochemicals in different extracts of *E. colona* and *H. zeylanica*. *E. colona* mainly showed the presence of flavones as major constituents of petroleum ether fraction whereas hexane fraction was rich in alkaloids and steroids. Most of the phytoconstituents of *E. colona* were found to be present in chloroform fraction including alkaloids, tannins & phenols, flavones and steroids. Ethyl acetate and ethanol fraction showed positive tests for carbohydrates and glycosides. Proteins and amino acid was found to be present in chloroform, ethyl acetate and ethanol fraction.

Different fractions of *H. zeylanica* were also tested for various phytoconstituents. Tests revealed the presence of flavones & flavonoids in the petroleum ether fraction. Hexane fraction of *H. zeylanica* possessed alkaloids and steroids while chloroform fraction showed positive tests for alkaloids, tannins & phenols, flavones, steroids, proteins and amino acid. Ethyl acetate and ethanol fraction was also rich in carbohydrates, glycosides, proteins and amino acids.
**Acute toxicity assessment**
Acute toxicity studies revealed the non toxic nature of ethanolic extract of both the plants. There were no lethality or toxic reactions found at any stage of the study period. All the animals were alive, healthy and active during the observation study for the given dose, so the doses were fixed for pharmacological study.

**Evaluation of *In-vitro* antioxidant activity**
Antioxidant activities of chloroform, ethyl acetate and ethanol fractions obtained from ethanolic extract of *E. colona* and *H. zeylanica* were carried out using reducing power assay, 2, 2- Diphenyl-1-picrylhydrazyl assay and Nitric oxide radical assay. DPPH free radical scavenging assay and nitric oxide radical assay was performed using ascorbic acid as standard reference. The inhibition of DPPH and nitric oxide free radical was calculated as percent inhibition. The exposure of above fractions showed good antioxidant activity.

**Evaluation of *in-vitro* antioxidant activity by reducing power assay:**
It is found that the reducing power of all the fractions of *E. colona* was increased with increasing concentration. The absorbance of standard ascorbic acid was 0.51 (±0.05), 0.70 (±0.06), 0.81(±0.02), and 1.41(±0.04), at 25, 50, 75 and 100 mg/mL (p < 0.01) concentration respectively whereas, it was 1.08 (±0.03), 0.89 (±0.02) and 0.92 (±0.02) for chloroform, ethyl acetate and ethanol fractions respectively at 100 mg/mL. The increase in absorbance suggested that chloroform fraction has shown higher reducing power as compared to ethyl acetate and ethanol fractions.

Chloroform, ethyl acetate and ethanol fractions of *H. zeylanica* were also evaluated for reducing power property, where the absorbance of standard ascorbic acid was 0.59 (±0.01), 0.70 (±0.01), 0.89 (±0.008) and 1.21 (±0.12) at 25, 50, 75 and 100 mg/mL (p < 0.01) concentration respectively whereas, it was 0.96 (±0.03), 0.65 (±0.01) and 0.57 (±0.017) for chloroform, ethyl acetate and ethanol fractions respective-
ly at 100 mg/mL. The increase in absorbance suggested that chloroform fraction has shown higher reducing power as compared to other.

**Evaluation of *in-vitro* antioxidant activity by 2, 2- Diphenyl-1-picrylhydrazyl assay:**
The percent inhibition by different fractions of *E. colona* was observed. It was 41.49 (±0.31), 63.64 (±0.02), 77.53 (±0.06) and 91.22 (±0.46) for ascorbic acid at 25, 50, 75 and 100 mg/mL respectively, whereas, it was 69.06 (±0.33), 59.79 (±0.48) and 64.81 (±0.52) for chloroform, ethyl acetate and ethanol fractions respectively at 100 mg/mL. Chloroform fraction has shown better inhibition of DPPH free radical as compare to other at the same concentration. The IC$_{50}$ values of standard ascorbic acid, chloroform, and ethyl acetate and ethanol fractions were 58, 62, 72, and 71 respectively.
The percent inhibition by different fractions of *H. zeylanica* was 41.11 (±0.64), 67.67 (±0.20), 73.84 (±0.89) and 83.62 (±0.48) for ascorbic acid at 25, 50, 75 and 100 mg/mL respectively, whereas 68.32 (±0.70), 58.27 (±0.67) and 46.23 (±0.31) for chloroform, ethyl acetate and ethanol fractions respectively at 100 mg/mL. The IC$_{50}$ values of standard ascorbic acid and chloroform, ethyl acetate and ethanol residues were 65.78, 85.01, 97.22 and 150 respectively.

**Evaluation of *In-vitro* antioxidant activity by nitric oxide radical assay:**
The percent inhibition by different fractions of *E. colona* was observed. It was 34.39 (±0.21), 55.54 (±0.01), 67.43 (±0.05) and 79.12 (±0.45) for ascorbic acid at 25, 50, 75 and 100 mg/mL respectively, whereas 69.03 (±0.33), 59.89 (±0.46) and 59.71 (±0.52) for chloroform, ethyl acetate and ethanol fractions respectively at 100 mg/mL. Chloroform fraction has shown better inhibition of nitric oxide radical as compare to other at the same concentration. The IC$_{50}$ values of
standard ascorbic acid, chloroform, ethyl acetate and ethanol fractions were 55.12, 65.41, 72.57 and 77.51 mg/mL respectively.

The percent inhibition by different fractions of *H. zeylanica* was 41.71 (±0.70), 59.48 (±0.33), 68.77 (±0.87) and 82.75 (±0.82) for ascorbic acid at 25, 50, 75 and 100 mg/mL respectively, whereas 70.70 (±0.52), 60.35 (±1.71) and 42.73 (±2.27) for chloroform, ethyl acetate and ethanol fractions respectively at 100 mg/mL. The IC₅₀ values of standard ascorbic acid, chloroform, ethyl acetate and ethanol residues were 78.16, 86.50, 89.16, and 115.5 respectively.

**Evaluation of antidiabetic activity induced by streptozotocin in male wistar rats**

The effects of different fractions (single dose-50 mg/mL) obtained from ethanolic extracts of *E. colona* and *H. zeylanica* on the blood glucose (mmol/L), serum total cholesterol (mmol/L) and serum triglycerides (mmol/L) levels were investigated in the control and streptozotocin induced diabetic rats using metformin HCl (150 mg/mL) as standard antidiabetic agent.

**Effect of various fractions on blood glucose:**

Decrease in blood glucose level was observed in animals treated with different fractions of *E. colona* at 0, 1, 2, 3, 6, 10, 16, and 24th hours. The significant reduction in blood glucose level at 24th hour 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 in comparison to controlled diabetic rats.

Animals treated with different fractions of *H. zeylanica* caused significant reduction in blood glucose level at 24th hour of the experiment (*p*<0.05), for metformin, chloroform, ethyl acetate and ethanol fraction, it was 6.05±0.008 (82.31%), 6.35±0.008 (75.11%), 8.95±0.003 (24.58%) and 8.11±0.004 (38.22%) respectively in comparison to controlled diabetic rats.
**Effect of various fractions on total cholesterol and triglyceride:**
After 24 h treatment of different fractions of *E. colona* there was decrease in total cholesterol and triglyceride on diabetic rats. 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 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 when compared to diabetic control groups.

There was decrease in total cholesterol and triglyceride on diabetic rats after 24 h treatment, when treated with different fractions of *H. zeylanica*. Metformin, chloroform, ethyl acetate and ethanol fraction showed decrease in total cholesterol level by 12.06±0.004 (62.85%), 13.06±0.004 (59.77%), 20.07±0.004 (38.18%) and 23.10 ±0.004 (28.85%) respectively, whereas triglyceride was decreased by 10.33 ±0.004 (43.76%), 11.76±0.004 (35.98%), 13.56±0.004 (26.18%), and 16.06±0.004 (12.57%) for chloroform, ethyl acetate and ethanol fraction respectively comparing to diabetic control groups.

**Evaluation of anti ulcer activity induced by pyloric ligation in albino wistar rats**
The effects of different fractions dosed at 50, 75 and 100 mg/mL obtained from ethanolic extracts of *E. colona* and *H. zeylanica* on the gastric volume, pH, free acidity, total acidity and ulcer index parameters were investigated in the control and pyloric ligated induced ulcers in albino wistar rats using ranitidine (50 mg/kg, p. o.) as standard antisecretory agent.

When anti ulcer effect of different fractions of *E. colona* was studied; gastric volume, pH, free and total acidity in pyloric ligated control group was found to be 2.95±0.89, 2.26±0.14, 67.51±0.39, 105.2±0.4, and 5.34±0.4 respectively, whereas it was 1.62±0.33, 6.13±0.13,
27.00±0.36, 56.20±4.05 respectively for pyloric ligated ranitidine control group with 1.20±0.30 ulcer index.

The pH of chloroform fraction at the dose of 100 mg/kg was found to be 5.88±0.16 with significant reduction in gastric volume (1.58±0.38), free acidity (27.20±0.4 mEq/L), total acidity (49.59±3.46 mEq/L) ulcer index (1.5±0.27) and increase in ulcer inhibition (71.91 %). The ulcerative index for ethyl acetate and ethanol fraction was 2.15±0.39 and 2.19±0.4 with ulcer protection of 59.73% and 57.86% respectively at 100 mg/kg.

The anti ulcer effect of different fractions of *H. zeylanica* was studied. There was accumulation of gastric acid (2.83 mL) in pyloric ligated control group. The pH, free and total acidity estimated in PL control group was 2.3±0.10, 61.10±2.55 and 101.50±2.11 mEq/L with 4.67±0.36 ulcer index respectively, whereas it was 6.1±0.10, 29.10±2.34 and 52.30±3.69 with 1.37±0.31 ulcer index respectively for pyloric ligated ranitidine control group.

The pH of chloroform fraction at the dose of 100 mg/kg was found to be 5.8±0.13 with significant reduction in gastric volume (1.63±0.37), free acidity (31.01±1.63 mEq/L), total acidity (52.91±3.70 mEq/L) ulcer index (1.67±0.33) and increase in ulcer inhibition (64.23 %). The ulcerative index for ethyl acetate and ethanol fraction was 2.11±0.38 and 2.42±0.31 with ulcer protection of 54.81% and 64.01% respectively at 100 mg/kg.

**Evaluation of wound healing activity**

The wound healing effect of different fractions of *E. colona* and *H. zeylanica* was studied using guinea pig punch wound model dosed at 1 % concentration of different fractions, *in-vitro* wound assay using embryos with complete matured chorioallantoic membrane (50, 100, 150 and 200 μg/ml) and *in-vitro* chick chorioallantoic membrane model (10–40 mg/disk) to assess angiogenic activity.
Evaluation of wound healing activity using guinea pig punch wound model:

In this model the wound healing was assessed as decrease in wound area (mm²), estimation of hydroxyproline content (mg/g tissue) a major determinant of collagen content and tensile strength (N/cm²) of the healing tissue.

When wound healing effect of different fractions of *E. colona* was studied, chloroform fraction at 1% concentration was able to decrease wound area by 14.8 (51.53%) as compare to vehicle control (ointment base) and standard (Povidone iodine ointment) 8.5 (72.22 %) measured on 10th day. There was 62.03±0.56 (15.96%) and 11.03±1.16 (17.22 %) increase in hydroxyproline content and tensile strength with chloroform treatment as compare to vehicle control. The ethyl acetate and ethanol fraction was able to decrease wound area by 28.3 (7.51 %) and 28.2 (7.84 %). The hydroxyproline content and tensile strength for ethyl acetate fraction was 53.33±0.57 (2.25 %) and 10.43±1.09 (12.46 %) respectively whereas, there was no increase in hydroxyl proline content observed for ethanol fraction, but tensile strength was found to be 09.63±1.09 (5.19 %) for ethanol fraction.

The effect of different fractions of *H. zeylanica* was also studied for wound healing activity. Chloroform fraction at 1% concentration was able to decrease wound area by 12.73 (68.73%) as compare to vehicle control (ointment base) 40.72 and standard (Povidone iodine ointment) 9.67 (76.25%) measured on 10th day. There was 75.53±0.31 (19.06 %) and 15.06±0.87 (26.09 %) increase in hydroxyproline content and tensile strength with chloroform treatment as compare to vehicle control 61.13±0.58 and 11.13±0.58 respectively. The ethyl acetate and ethanol fraction was able to decrease wound area by 18.33 (54.98%) and 22.55mm² (44.62%). The hydroxyproline content and tensile strength for ethyl acetate fraction was 69.38±0.54 (11.89%) and 13.79±1.12 (19.28%) respectively whereas, it was 62.94±0.28 (2.87%) and 12.53±1.09 (11.17%) for ethanol fraction.
Evaluation of wound healing activity using in-vitro wound assay:
In the in vitro wound assay, among different fractions of *E. colona*, chloroform fraction at maximum concentration (200μg/ml) was able to contract the wound by 1.1±0.10 (40.67 %) as compared to standard diclofenac sodium 0.5±0.03 (85.33%) at 50 μg/ml. Whereas ethyl acetate and ethanol fraction at same concentration was able to contract the wound by 1.6±0.10 (26.47%) and 1.5±0.10 (30.61%) respectively.

The chloroform fraction of *H. zeylanica* at 200μg/ml produced 1.8±0.11 (77.77 %) wound contraction as compared to standard diclofenac sodium 1.5±0.11 (81.70%) at 50 μg/ml whereas; ethyl acetate and ethanol fraction at same concentration was able to contract the wound by 2.4±0.28 (70.37%) and 2.8±0.23 (65.43%) respectively.

Evaluation of wound healing activity using in-vitro chick chorioallantoic membrane model:
In the in-vitro chick chorioallantoic membrane model, among the of fractions of *E. colona*, chloroform fraction at 10, 20, 30 and 40 mg/disk concentration was able to form 7, 8, 11 and 14 new blood vessels as compared to control which was found to be 4 in this model whereas, it was 1 each for ethyl acetate and ethanol fraction at 40 mg/disk.

The chloroform fraction of *H. zeylanica* at 10, 20, 30 and 40 mg/disk concentration was able to form 2, 4, 6 and 12 new blood vessels as compared to control which was found to be 4 in this model whereas, it was 3 and 2 for ethyl acetate and ethanol fraction at 40 mg/disk respectively.

CHROMATOGRAPHIC SEPARATION

Column chromatography:
The chloroform fraction of both *E. colona* and *H. zeylanica* was subjected to column chromatography and eluted with solvent mixtures of increasing polarity. While elution, various fractions of 20 ml each was collected and monitored on TLC. On the basis of similarity in the $R_f$
value, appearance of color in daylight, UV (254 and 366 nm) and exposure to iodine vapors, the fractions were combined to get pooled fraction EC-I, EC-II, EC-III, HZ-I, HZ-II and HZ-III, all these fractions were obtained by eluting column with mobile phase, Chloroform: Ethanol (40:60), Chloroform: Ethanol (30:70), Chloroform: Ethanol (10:90), Chloroform: Methanol (30:20), Chloroform: Ethanol (30:70) and Chloroform: Ethyl acetate (20:90) respectively.

**Thin Layer Chromatography (TLC):**
TLC of each fraction was carried out during column chromatography and The Rf value for EC-I (0.48), EC-II (0.74), EC-III (0.68), HZ-I (0.64), HZ-II (0.48) and HZ-III (0.89) was observed.

**CHARACTERIZATION OF ISOLATED COMPOUNDS**
The structure of the isolated compounds was established using various spectroscopic techniques such as UV visible spectroscopy, Fourier transform infrared spectroscopy (FTIR), Proton nuclear magnetic spectroscopy (1HNMR) and Mass spectrometry (MS) and comparing the spectroscopic data with previous published data.

**EC-I:** The EC-I fraction was collected at 40:60 in % ratio of (CHCl₃:C₂H₅OH). The isolated compound was white amorphous powder. The compound was studied for its qualitative properties and found to be positive with ferric chloride test and phenolic nature of the compound was confirmed. The melting point of the compound was carried out and found to be 145-147°C. The UV spectrum showed λmax, 220 nm, ethanol with typical aromatic bands confirming a substituted aromatic benzoic acid. In the FTIR (KBr, cm⁻¹) signal at 1688 (-COOH), 2935 (Ar-H), 3400 (-OH), 1HNMR (CDCl₃, 400 MHz) signals at δ 11.80 (-COOH), 6.5 (Ar-H), 5.10 (Ar-OH), 2.33 (Ar-CH₃) and molecular ion peak at m/z 184 corresponding to C₈H₈O₅ and main peak at 166 [184-(H₂O)]⁺ 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.
**EC-II:** The EC-II fraction was collected at 30:70 in % ratio of (CHCl₃:C₂H₅OH). The isolated compound was white crystalline powder with characteristic odor. The compound was studied for its qualitative properties and found to be positive with Liebermann-Burchard test and steroidal nature of the compound was confirmed. The melting point of the compound was carried out and found to be 137-139°C. UV spectrum showed λmax, 210 nm, ethanol. In the FTIR (KBr, cm⁻¹) 3545 (-OH), 2931 (-CH₂), 2860 (-CH), 1637 (-C=C-), 1033 (-C-O), 1HNMR (CDCl₃, 400 MHz) signals at δ 1.01, 1.04, 1.06, 1.04, 1.17, 1.21(-CH₃), 1.57, 1.98, 1.13, 1.79, 1.24, 1.27, 1.35, 1.34, 1.25, 1.29, (-CH₂-), 3.25(--CH-), 5.37 (H-cyclohexene) and molecular ion peak at 414.7 corresponding to C₂₉H₅₀O 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 supports the proposed structure was β-sitosterol ⁴⁰, ⁴¹, ⁴².

**EC-III:** The EC-III fraction was collected at 10:90 in % ratio of (CHCl₃:C₂H₅OH). The isolated compound was white crystalline powder with characteristic odor. The compound was studied for its qualitative properties and found to be positive with ester test and 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 λmax, 226 nm, methanol. In the FTIR (KBr, cm⁻¹) band at 1736 (RCOOR) and 3400 (Ar-OH), 1HNMR (CDCl₃, 400 MHz) at δ 1.25 (-CH₃), 4.65 (-CH₂-), 4.58 (Ar-OH) and molecular ion peak at m/z 198 corresponding to C₉H₁₀O₅ with other fragments at 183, 149[183-CH₃]+, 129, 111, 97, 83, 69, 57, 43 were observed. The above spectral data suggested isolated compound was ethyl 3, 4, 5-trihydroxy benzoate.

**HZ-I:** The HZ-I fraction was collected at 30:20 in % ratio of (CHCl₃:CH₃OH). The isolated compound was white needle shaped crystal with characteristic odor. The compound was studied for its qualitative properties and found to be positive with Liebermann-Burchard test and steroidal nature of the compound was confirmed. The melting point of the compound was carried out and found to be
138-140°C. UV spectrum showed λmax, 276 nm, methanol. In the FTIR (KBr, cm⁻¹) 3525.02 (-OH), 3242.35 (-CH=CH-), 3079.95 (-C-H-), 2936.00 (-CH-), 1615.18 (-C=C-), 1468.32 (-CH₂)n, 1370.05 [-CH₂(CH₃)₂], 1020.50 (-C-O), 830.25 cm⁻¹ (C-H vibration of unsaturated part), 1HNMR (400 MHz, CDCl₃) δ: 0.77560, 0.84281, 0.92660, 1.05401, 1.25401, 1.54300 (CH₃), 3.4013 (-CH-), 5.4300 (H-cyclic), 5.1809, 5.0092 (-CH=CH-) and molecular ion peak at m/z 427[M]⁺ corresponding to C₂₉H₄₈O with other characteristic fragmentations at m/z: 412[CH₃]⁺, 394[H₂O]⁺, 308[C₆H₁₄]⁺, and 264[C₃H₈]⁺. The above spectral data was compared with reported data and suggested that isolated compound was Stigmasterol⁴³,⁴⁴.

HZ-II: The HZ-II fraction was collected at 30:70 in % ratio of (CHCl₃:C₂H₅OH). The isolated compound was white crystalline powder with characteristic odor. The compound was studied for its qualitative properties and found to be positive with ferric chloride and phenolic nature of the compound was confirmed. The melting point of the compound was carried out and found to be 278-280°C. UV spectrum showed λmax, 365 nm, methanol. In the FTIR (KBr, cm⁻¹) 3445, 3330 (Ar-OH), 2955, 2930- (Ar-CH), 2860, 2620, 1668 (-C=C-), 1615 (-C=C-), 1579, 1510 (Ar-C=C), 1450, 1409 (-C=C-), 948, 820, 1120 (substituted ring), 1HNMR (CDCl₃, 400 MHz) δ: 5.1752 (Ar-OH), 5.9688(Ar-OH), 7.3252 (Ar-OH), 6.8766 (Ar-H), 12.7041, 9.4066, 14.7629(cyclic-OH) and molecular ion peak at m/z 286.72[M]⁺ corresponding to C₁₅H₁₀O₆ and other fragments at m/z 258 [H₂O], 241[C₂H₂], 213[C₃H₈O], 185[H₂O], 167 [C₂H₄O], 123[CH₄O], 91[CH₄O], and 58. The above spectral data was compared with reported data and suggested that isolated compound was Kaempferol⁴⁵,⁴⁶.

HZ-III: The HZ-III fraction was collected at 20:90 in % ratio of (CHCl₃:CH₃CH₂COOCH₃). The isolated compound was white needles with characteristic odor. The compound was studied for its qualitative properties and found to be positive with sodium bicarbonate test and acidic nature of the compound was confirmed. The melting point of the compound was carried out and found to be 187-189°C. UV spec-
trum showed $\lambda_{\text{max}}$, 310 nm, methanol. In the FTIR (KBr, cm$^{-1}$) 3391.5 (Ar-OH), 2830.6 (=CH), 2560.3 (-CH=CH-), 1655.2 (-C=C-), 1599 (-CH$_2$), 1509.1, 1465, 1425, 1320, 1HNMR (CDCl$_3$, 400 MHz) $\delta$: 6.58 (2H, -C=C-), 6.9 (2H, -C=C-), 5.20 (Ar-OH), 9.17 (CH=CH), 12.76 (OH-carboxylic) and molecular ion peak at 163.04 corresponding to C$_9$H$_8$O$_3$ with other fragments at 119 [CO$_2$]$^+$, 91[C$_2$H$_4$], 63 [C$_2$H$_4$]. The above spectral data was compared with reported data and suggested that isolated compound was p-Coumaric acid.$^{47,48}$
CONCLUSION

The present study concludes that the different fractions obtained from ethanolic extract of *Hydrolea Zeylanica* and *Echinochloa Colona* showed the presence of various phytochemicals. In particular the chloroform fraction of *Hydrolea Zeylanica* and *Echinochloa Colona* showed better antioxidant, antidiabetic, anti-ulcer and wound healing activity; this could be a result of the presence of diverse phytochemicals which are responsible for the different pharmacological activities of the drugs.

The chloroform fraction of both the plants was chromatographed to separate the bioactive constituents. Three compounds were isolated from each plant. *Echinochloa Colona* was found to contain 2, 3, 4-trihydroxy, 6-methyl benzoic acid, β-sitosterol and ethyl 3, 4, 5 trihydroxy benzoate. Stigmasterol, Kaempferol and p-Coumaric acid were isolated from *Hydrolea Zeylanica*. All the compounds were characterized by various spectroscopic techniques. The spectral and physical data of isolated compounds were matched with earlier reports. In conclusion *Hydrolea Zeylanica* and *Echinochloa Colona* may be composed of principal source of presumed bioactive compounds which may be responsible for many of the pharmacological properties.
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