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Research Article

Best from Waste: Bioactivity-guided Formulation Development from a Common Weed - *Commelina benghalensis*

Pooja A. Kansagara*, Devang J. Pandya

Faculty of Pharmacy, RK University, Rajkot, Gujarat, India

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ABSTRACT

Each year, tons of weeds are burned or allowed to dry throughout the world. Despite folklore claims that *Commelina benghalensis* can treat leprosy, fever, snake bites, jaundice, sore throats, headaches, and constipation in both humans and animals, the ubiquitous Indian plant always dies the same way every year. By creating a formulation based on the evaluation of its potential ethnomedicinal properties, the current work attempts to utilize this weed. The complete plant was extracted once at a time using solvents with increasing polarity. Each extract's anti-ulcer and laxative efficacy was investigated using appropriate animal models. The ethnomedicinal claims of the plant were supported by the discovery that the methanolic extract was the most bioactive, followed by the aqueous extract. Important classes of phytoconstituents such as phenolics, alkaloids, saponins, steroids & triterpenoids, flavonoids, and carbohydrates were found by phytochemical screening. From the purified fraction of the most bioactive extract, many chemicals, including stigmasterol and β-sitosterol, were discovered using GC-MS. Using TLC experiments and HPTLC, a chromatographic fingerprint was created. Effervescent granules of the methanolic extract were created and tested on animal models in order to bring the research to the public for their benefit. They were found to be effective as an anti-ulcer and laxative, which was compatible with our goal to create a "Best from Waste" product.

INTRODUCTION

Traditional folkloric medicines play an important role in healthcare systems around the world. World Health Organization states that a medicinal plant is an herbal plant that contains therapeutic phytoconstituents and is also important for synthesizing medicines.[1] *Commelina benghalensis,* Fig. 1, belonging Commelinaceae family, also known as the Benghal dayflower which is a an annual or perennial ethnobotanical world's worst weed[2] inhabitant to tropical Asia and Africa.[3] Which is used to treat, cure or prevent various diseases in India,[5-13] Nepal,[17] China,[14,21] Pakistan,[15-16] Bangladesh,[18,19] and Leshotho.[14,21] *C. benghalensis* can be found on roadways, wasteland, moist grassland, farmlands, home gardens, bushland, tree plantations, river water lands, forest frontiers, and wetlands. Plant augmentation and flowering are best between 20 to 40°C.[12,21-23] The present work attempts to utilize this weed by developing a formulation based on the investigation of its ethnomedicinal claims. The whole plant was successfully extracted by increasing polarity solvents, which served as the test extracts. Various parameters like ulcer index and ulcer protection for anti-ulcer activity and fecal output for laxative activity (± SD) were measured using appropriate animal models (n=6) and analyzed for statistical significance. The methanolic extract was found more effective in both activities, followed by the aqueous extract, thereby supporting the ethnomedicinal claims of the plant. Phytochemical screening revealed the presence of important classes of phytoconstituents like phenolics, alkaloids, saponins, steroids & triterpenoids, flavonoids

***Corresponding Author:** Mrs. Pooja A. Kansagara

Address: Faculty of Pharmacy, RK University, Rajkot, Gujarat, India

Email ✉**:** phsmile12@gmail.com

Tel.: +91-9712974191

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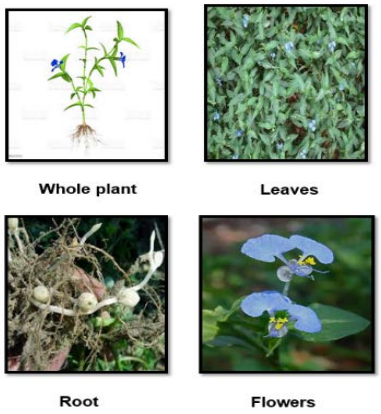


Fig. 1: *C. benghalensis* plant and its different parts

and carbohydrates[10,24-28] have several biological properties which include antioxidant, anti-inflammatory, anti-diarrhea, anti-ulcer.[20,29-31] To accommodate the utility of both these pharmacological actions in a single formulation on the basis of the dose at which it was found to be most effective in the animal models, effervescent granules were prepared, which were also tested on the animal models and found to be equally effective. This bioactivity-guided research can pave the way for isolating bioactive compounds and developing a standardized formulation of *C. benghalensis.* A high percentage of the world population use herbal medicines for primary health care because of their low cost, better acceptability and lesser side effects. This work can give new molecules with anti-ulcer and laxative activities to the pharmaceutical industry & start new thinking for next generation of society with new effervescent granules formulation, which claim "Best from Waste." So, main goal of the present work is "Best From Waste".

Material And Method

Collection and Authentication of Plant

The plant *C. benghalensis* L. was collected in Oct. 2018 from farm backyard of Jamnagar, Gujarat, India. The herbarium of the collected sample was prepared and authenticated by a botanist, Dr. Anjisha Maharshi, School of Science, RK University, Rajkot, Gujarat, India.

Preparation Plant of Extract

Plant extract were prepared by serial extraction method which involves successive extraction with solvents of increasing polarity from non-polar to polar solvents. Petroleum ether > Chloroform > Methanol > Water. Which ensure wide polarity rang of the compound could be extracted.[32]

Preliminary Qualitative Phytochemical Analysis

Preliminary qualitative phytochemical analysis of compounds such as alkaloids, steroids, terpenoids, carbohydrates, flavonoids, tannins and saponins were carried out to identify the secondary metabolites present in the Methanolic extract of the plant. According to Harborne, (1998), the methods were as described below.[33-39,58]

Test for Alkaloids

- *Dragendroff's Test*

Take 2 to 3 mL filtrate add few drops of Dragendroff's reagent if orange brown precipitate found then an alkaloid is present.

- *Wagner's Test*

Mix 2 to 3 mL filtrate add few drops of Wagner's reagent if reddish Brown precipitate found then an alkaloid is present.

- *Hager's Test*

Mix 2 to 3 mL filtrate add few drops of Hager's reagent if yellow precipitate found then alkaloid is present.

- *Mayer's Test*

Mix 2–3 mL filtrate with few drops of Mayer's reagent if precipitate is found then alkaloid is present.

Test for Flavonoid

- *Shinoda Test*

To the extract, add 5 mL 95% ethanol and few drops of concentrated HCl and 0.5 gm magnesium, turning if pink color observed, then flavonoid is present. To small quantity of residue, add few drops of lead acetate solution if a yellow color precipitate is found then flavonoid is present. Adding an increasing amount of NaOH to the residue, yellow color discoloration after the addition of acid confirmed the presence of flavonoids.

Test for Saponins (Foam Test)

The 3 mL of extract was added with a few mL of distilled water to make volume 10 mL. this was agitated for 10 minutes. Formation of foam up to 3 cm indicates the presence of saponins.

Test for Terpenoids

- *Salkowski Test*

To 2 mL of extract, add 2 mL chloroform and 2 mL concentrated H_2SO_4, shake well if the chloroform layer appears brownish red and acid layer shows greenish yellow fluorescence that confirms the presence of phytosterols.

Test for Sterols

- *Libermann-Buchard Test*

Mix 2 mL extract with chloroform. Add 1 to 2 mL acetic anhydride and 2 drops of concentrated H_2SO_4 from the side of test tube if. First red, then blue & finally green color appears that confirms the presence of Steroids.

Test for Tannins & Phenolic

- *Ferric Chloride Test*

To 3 mL of plant extract, a few drops of 0.1% ferric chloride solution were added. The formation of brownish green or a blue-black coloration indicates the presence of tannins/phenolic.

- *Lead acetate Test*

To 3 mL of plant extract, a few drops of lead acetate solution were added. Formation of white precipitate indicates presence of tannins/phenolic.

- *HNO_3 Test*

To 3 mL of plant extract, a few drops of HNO_3 solution were added. Formation of reddish to yellow coloration indicates the presence of tannins/phenolic.

Test for Carbohydrates

- *Molish's Test*

To 2–3 mL aqueous extract, add Molish's reagent, shake it, and add H_2SO_4 from side of the test tube formation of violate ring at the junction of two liquids shows the presence of carbohydrates.

In-vivo Laxative and Anti-ulcer activity

The Pharmacological study was approved by Institutional Animal Ethics Committee (RKCP/Col/RP/20/100) and carried out according to CPCSEA guidelines (n=6).

For Laxative Activity

Male wistar rats (Zydus Cadila Limited, Ahmedabad, India) of weighing 250–300 g were selected for the current study. Animals were fed a standard chow diet and freely available water and maintained under standard conditions of a 12 h dark-light cycle, 60 ± 10% humidity, and a temperature of 21.5 ± 1°C. The animals were acclimatized to laboratory conditions for 1-month before starting the pre-clinical trials. Any rat producing wet faces was rejected. The rats were fasted for 12 hours before dosing but were given water *ad libitum*. Six animals per group were placed in one metabolic cage (each cage is provided with a wire mesh at the bottom and a funnel to collect the urine; stainless-steel sieves are placed in the funnel to retain feces). The normal control group received normal saline (25 mL/kg). The standard control group received 300 mg/kg agar-agar (Pharma Pvt. Ltd.) orally. Two groups of six animals were used for each dose of the test extract. Six animals of the test extract groups received a dose of 200 mg/kg orally, and the remaining Six from each group received a dose of 400 mg/kg body weight.[52] Loperamide (5 mg/kg) was used to induce constipation after 1-hour of administration of each extract. Feces were weighed upto 8 and 16 hours (The results were expressed as the mean (g) of total feces.[53] Results were calculated as Mean ± Standard Deviation (SD). Statistical analysis of control and test data was performed by One-way ANOVA followed by Dunnett's test (Sigmastat software). A probability value of $p < 0.01$ was considered statistically significant.

Methodology

The animals were divided into following groups of six.
Group I (Normal control): Rats received only normal saline (25 mL/kg)
Group II (drug induced disease): Rats received the Loperamide (5 mg/kg)
Group III: Rats received standard agar-agar (300 mg/kg)
Group IV: Prepared in 2 fractions 200, 400 mg/kg, respectively
Group V: Formulation given to the rats in dose of 100 mg/kg

For Anti-ulcer Activity

Male wistar rats (Zydus Cadila Limited, Ahmedabad, India) of weighing 250–300 g were selected for the current study. Animals were fed a standard chow diet and freely available water and maintained under standard conditions of a 12 hours dark-light cycle, 60 ± 10% humidity and a temperature of 21.5 ± 1°C. Coprophagy (and thus re-ingestion of any drug) was prevented by keeping the animals in cages with gratings on the floors. The distribution of animals in the groups, the sequence of trials, and the treatment allotted to each group was randomized. Freshly prepared solutions of drugs or chemicals were used throughout the study. During this time they were kept in cages with mesh at the bottom to prevent Coprophagy. Six animals per group were placed in one cage. Normal control group received normal saline (25 mL/kg) orally. The disease control group received 1-mL 80 % ethanol orally. The standard control group received 300 mg/kg Omeprazole orally. Three groups of six animals were used for each dose of the test extract. Six animals of the test extract groups received a dose of 100 mg/kg orally, six received 200 mg/kg, and the remaining three from each group received a dose of 400 mg/kg body weight.[52] 100 mg/kg prepared formulation 1-hour later, the animals were euthanized with chloroform; the stomachs were excised, cut along the greater curvature, and gently rinsed under tap water. The stomachs were stretched on a piece of foam core mat, mucosal side up.[54,55] All experiments complied with university guidelines for animal experimentation. Throughout the entire study period, the rats were monitored for growth, health status, and food intake capacity to be certain that they were healthy. Utmost care was taken to ensure that animals were treated in the most humane and ethically acceptable manner. The stomachs were removed, opened along the greater curvature, washed with saline, and examined using a 6.4 binocular magnifier. Lesions were assessed by two unbiased observers. The length and breadth of the lesions were measured using Vernier calliper and the ulcer index and ulcer protection percent was calculated as follows:[56]

Calculation of Area

Area of circular lesion= π D2/4
Where D=diameter of stomach mucosa
Every five petechiae were counted as 1 mm^2 area.
Ulcer index
Ulcer index (UI) = 10/X,
Where X = Total area of stomach mucosa/total ulcerated area.
Ulcer protection% Ulcer protection (UP) =[(Disease control mean ulcer index – test mean ulcer index)/disease control mean ulcer index] 100
Results were calculated as mean ± standard deviation (SD). Statistical control and test data analysis was performed by one-way ANOVA followed by Dunnett's test (Sigma-stat software). A probability value of $p<0.001$ was considered statistically significant.

Methodology

The animals were divided into following groups of six.
Group I (control): Rats received only aqueous suspension of 1% CMC vehicle with respect to the individual ulcerogenic procedure.
Group II (drug-induced treatment): Rats received the ethanol for induced ulcer.
Group III: Rats received standard omeprazole (300 mg/kg) 1 h before the ulcerogenic procedure.
Group IV: Prepared in 3 fractions 100, 200, 400 mg/kg, respectively.
Group V: Formulation given to the rats in 100 mg/kg doses.

GC-MS Analysis

Gas chromatography-mass spectroscopy (GC-MS) is a combined analytical technique used to determine and identify compounds present in a plant sample.[59] GC-MS plays an essential role in the phytochemical analysis and chemotaxonomic studies of medicinal plants containing biologically active components.[60] GC-MS analysis of the methanol extract of whole plant was performed using a GCMS-TQ8040. The oven temperature is maintained at 80°C, injection temperature 260°C the carrier gas with a flow rate of 1.46 mL/min. The split sampling technique was used to inject the sample in the ratio of 1:10. Retention indices (RI) of the compounds were determined by comparing the retention times of a series.

Identification of Compound

Each component was identified by comparing its retention index with data in the literature. Mass-spectrum was interpreted using the National Institute Standard and Technology (NIST) database with more than 62,000 patterns.

The spectrum of the unknown components was compared with the spectrum of known components which was stored in the NIST library. The molecular weight, name, molecular formula, RT, peak area%, and pharmacological activity of the components of the test materials was ascertained.

HPTLC Profile (High-Performance Thin Layer Chromatography)

HPTLC studies were carried out following the method[33,57]

Sample Preparation

Methanolic extract and isolated fraction obtained were evaporated under reduced pressure using rotovac evaporator. Each residue were re-dissolved in chromatographic grade 1-mL of methanol and chloroform, respectively which was used for sample application on pre-coated silica gel 60F254 aluminum sheets.

Developing Solvent System

A number of solvent systems were tried, for extract, but a satisfactory resolution was obtained in the solvent system Pet. Ether: Chloroform: Methanol: Formic Acid (3:7:0.5:0.3) (366 nm)

Sample Application

The application of bands of each extract was carried out (8 mm in length and 5, 10, 20 µL successively in concentration) using spray technique. Sample were applied on pre-coated silica gel 60F254 aluminum sheets (5 x 10 cm) with the help of a Linomat 5 applicator attached to CAMAG HPTLC system, which was programmed through WIN CATS software.

Development of Chromatogram

After the application of sample, the chromatogram was developed in twin through glass chamber 10x 10 cm saturated with solvent Pet. Ether: Chloroform: Methanol: Formic Acid (3:7:0.5:0.3) for 20 minutes.

Detection of Spots

The air-dried plates were viewed in ultraviolet radiation to mid-day light. The chromatograms were scanned by densitometer at 366 nm after spraying with anisaldehyde sulphuric acid. The Rf values and finger print data were recorded by WIN CATS software.

Analysis of Phytoconstituents with Standard Using TLC

Silica gel aluminum foil plates of GF 254 (20x20) cm of 0.25 mm thickness were used which activated at 110°C for one hour, and using of different solvents systems to detect stigmasterol and β-sitosterol compounds present in methanolic extract and isolated fraction of *C. benghalensis*.

Different solvent systems were prepared, which placed in a glass tank (22.5 x 22 x 7cm) covered with a glass lid. The atmosphere of the glass tank should be saturated with the solvent vapors before running samples, so part of the inside of tank was lined with filter paper (Whatman No.2) to aid in this saturation process and allowed to stand for 45 minutes before use (12), as well, standard reference

of stigmasterol and β-sitosterol about 10 and 20 µL of methanolic extract and isolated fraction. Methanolic extract and isolated fraction applied on silica gel thin layer chromatography (TLC) coated plates. Extract applied 1-cm above the edge of the chromatographic plates along with the reference standards. By using capillary tubes in form of spots then developed.

Visualization

Visualization was done under UV light or spraying with concentrated H_2SO_4, anisaldehyde sulfuric acid (ANS) reagent, vanillin-sulfuric acid reagent etc. and drying (heat the plate) in the oven at 105°C. till color spots (bands) were visualized and labeled, their retention factors (R_f value) were calculated and compared.[61] The R_f values were calculated according to the following formula:

$$R_f = \frac{\text{Distance from start to center of substance spot}}{\text{Distance from start to solvent front}}$$

Preparation of Herbal Effervescent Granules

Herbal effervescent granules were prepared by wet granulation method. The methanolic extract of *C. benghalensis* (active ingredient) 22.50 gm, sodium bicarbonate 11.5 gm, citric acid 5.5 gm, tartaric acid 5.5 gm, microcrystalline cellulose 3 gm, Jeera oil for flavor 2 mL. The extract was dried in oven at 60°C to constant weight and triturated in a mortar and pestle to make powder then mixed with calculated amount of the other components. The binder was added and formed into a paste and granulated using sieve no. 8. to get granules and these granules were dried in hot air oven at 60°C and then they were packed in air tight container.[62-64]

Evaluation Parameter of Herbal Effervescent Granules

Different parameters are included in the evaluation of Effervescent granules which are mentioned below.[65-73]

Angle of Repose

The fixed funnel method was employed to measure the angle of repose. A funnel was secured with its tip at a given height (h), above a graph paper that is placed on a flat horizontal surface. The blend was carefully pored through the funnel until the conical pile's apex just touches the funnel's tip. The radius of the base of the conical pile was measured.

The angle of repose (θ) was calculated using the following formula: Tan θ = h/r,

W here, θ = Angle of repose, h = Height of the cone, r = Radius of the cone base.

Values for angle of repose ≤ 30° usually indicate a free flowing material and angles ≥ 40° suggest a poorly flowing material

25 to 30 show excellent flow properties

31 to 35 show good flow properties

36 to 40 show fair flow properties

41 to 45 showing passable flow properties.

Bulk Density

15 g powder blend introduced into a dry 100 mL cylinder without compacting. The powder was carefully leveled without compacting and the unsettled apparent volume, Vo, was read.

The bulk density was calculated using the following formula.

$$\rho b = M/Vo$$

Where, ρb = Apparent bulk density, M = Weight of sample, V = Apparent volume of powder

Tapped Density

After carrying out the procedure as given in the measurement of bulk density the cylinder containing the sample was tapped 500 times initially followed by an additional tap of 750 times until the difference between succeeding measurements is less than 2% and then tapped volume, Vf was measured, to the nearest graduated unit. The tapped density was calculated using the following formula in gm/mL.

$$\rho tap = M/Vf$$

Where, ρtap = Tapped density, M = Weight of sample, Vf = Tapped volume of powder

Carr's Index (%)

The compressibility index (Carr's index) is a measure of the propensity of a powder to be compressed. It is determined from the bulk and tapped densities. In theory, the less compressible a material the more flowable it is.

As such, it is measures of the relative importance of inter particulate interactions. Such interactions are generally less significant in a free-flowing powder, and the bulk and tapped densities will be closer in value.

There are frequently greater inter-particle interactions for poorer flowing materials, and a greater difference between the bulk and tapped densities will be observed. These differences are reflected in Carr's Index which is calculated using the following formulas:

$$\text{Compressibility index} = *(\rho tap - \rho b) / \rho tap+ / \times 100$$

Where, ρb = Bulk Density, ρtap = Tapped Density

Hausner's Ratio

Hausner's ratio is an indirect index of ease of powder flow. It is calculated by the following formula.

$$\text{Hausner's Ratio} = \text{Tapped density } (\rho t) / \text{Bulk density } (\rho b)$$

Where ρt tapped density and ρb is bulk density.

Lower Hausner's ratio (<1.25) indicates better flow properties than higher ones, between 1.25 to 1.5 showing moderate flow properties and more than 1.5 poor flow.

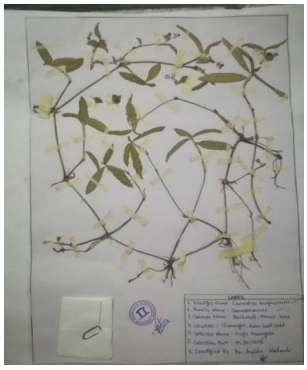


Fig. 2: Herbarium of *C. benghalensis*

Fig. 3: Authentication certificate

Effervescent Cessation Time

The effervescent time of effervescent granules was measured by adding one dose of granules to a glass containing 250 mL of water when a clear solution is obtained the effervescent time will be recorded.

The values of effervescence time were in the range of 80 to 113 sec. The resulted ranges were acceptable for this study according to USP.

Result and Discussion

Authentication was accomplished by prepare a herbarium of the entire plant, as illustrated in Figs 2 and 3. Four distinct extracts were generated by successive extraction from the plant, each with a different yield as shown in Table 1. Among all extracts, methanolic extract contains phytoconstituents such as alkaloid, flavonoid, saponin, tannin/phenolic, and carbohydrates, as shown in Table 2 and Fig. 4. Executing *in-vivo* animal activity, which can considerably display anti-ulcer and laxative pharmacological effect. It was discovered that methanolic extract is the most effective and has significant pharmacological activity such as a laxative and anti-ulcer.

In-vivo Laxative and Anti-ulcer Activity

Table 3 illustrates the laxative activity of aqueous and aqueous fractions of methanolic extract. These data determined that the fecal output of methanolic extract 200 mg/kg at 8 hours and 24 hours shows better pharmacological activity than standard agar-agar (300 mg/kg). Graph 1 depicts a comparison of the laxative potential of several extracts in a loperamide-induced constipation model. The fecal output of standard agar-agar (300 mg/kg) at 8hrs was 5.2 ± 0.1 and at 24 hours was 16.8 ± 0.1, whereas that of aqueous fraction of methanolic extract 200 mg/kg at 8 hours was 9.8 ± 0.1 and at 24 hours was 19.6 ± 0.1, indicating that aqueous fraction of methanolic extract 200 mg/kg showed significant laxative activity.

Table 4 illustrates the anti-ulcer activity of the aqueous extract, aqueous fraction of methanolic extract, and developed formulation. Based on these findings,

Table 1: Yield of Different Extracts of *Commelina benghalensis* L. whole plant

Sr. no.	*Extracts*	*Colour of extract*	*Yield (g)*	*Percentage yield (%w/w)*
1	CBPE[1]	Greenish yellow	3.81	0.38
2	CBC[2]	Greenish yellow	6.66	0.67
3	CBM[3]	Greenish yellow	40.61	4.06
4	CBW[4]	Brown	90.32	9.03

1 CBPE: *C.* benghalensis Petroleum ether extract
2 CBC: *C.* benghalensis Chloroform extract
3 CBM: *C.* benghalensis Methanol extract
4 CBW: *C. benghal*ensis Water extract

Fig. 4: Preliminary Phytochemical Screening of Methanol extract of *C. benghalensis*

Table 2: Preliminary Phytochemical Screening of Methanol and Aqueous extract of *C. benghalensis*

S. No.	*Chemical Constituents/Test*	*Methanol Extract*	*Aqueous Extract*
1)	Test for Alkaloids		
	Dragendroff's Test	+ve	-
	Wagner's Test	+ve	-
	Hager's Test	+ve	-
	Mayer's Test	+ve	-
2)	Test for Flavonoids		
	Shinoda Test	+ve	+ve
	Residue + lead acetate	+ve	+ve
	Residue + NaOH	+ve	+ve
3)	Test for Saponin		
	Foam Test	+ve	+ve
4)	Test for Terpenoids		
	Salkowski Test	+ve	-
5)	Test for Steroids		
	Libermann – Buhard Test	+ve	-
6)	Test for Tannins & Phenolic		
	Fecl3 Test	+ve	+ve
	Lead acetate Test	+ve	+ve
	HNO3 Test	+ve	+ve
7)	Carbohydrats		
	Molish's Test	+ve	+ve

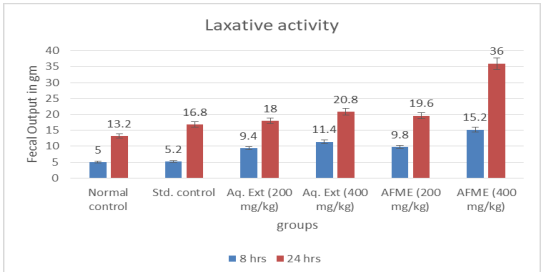
Table 3: Laxative activity of various extracts in Loperamide-induced constipation model[1]

Groups	*Fecal output(g) At 8 hrs*	*Fecal output(g) At 24 hrs*
Normal Control	5 ± 0.1	13.2 ± 0.1
Std (Agar-Agar)	5.2 ± 0.1	16.8 ± 0.1
Aq.Ext. (200 mg/kg)	9.4 ± 0.1	18 ± 0.1
Aq. Ext. (400 mg/kg)	11.4 ± 0.1	20.8 ± 0.1
AFME (200 mg/kg)	9.8 ± 0.1	19.6 ± 0.1
AFME (400 mg/kg)	15.2 ± 0.1	36 ± 0.1

5 Values are expressed as mean ± SD
Number of animal (n) = 6
*p<0.01 (ANOVA followed by student t-test)
Aq. Ex.: Aqueous extract
AFME: Aqueous fraction of Methanolic Extract
Aq. Form.: Aqueous Formulation
AFME Form.: Aqueous fraction of Methanolic Extract Formulation

Table 4: Anti-ulcer activity of various extract and prepared formulation in ethanol induced ulcer model[5]

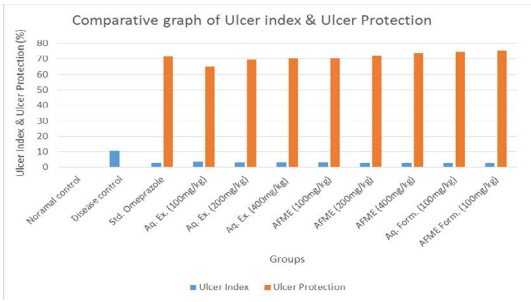
Group	*Ulcer Index*	*Ulcer Protection (%)*
Normal control	0	0
Disease control	10.61 ± 0.1	0
Std. Omeprazole	2.74 ± 0.1	71.53
Aq. Ex. (100mg/kg)	3.72 ± 0.1	64.89
Aq. Ex. (200mg/kg)	3.23 ± 0.1	69.55
Aq. Ex. (400mg/kg)	3.15 ± 0.1	70.3
AFME (100mg/kg)	3.12 ± 0.1	70.59
AFME (200mg/kg)	2.98 ± 0.1	71.91
AFME (400mg/kg)	2.79 ± 0.1	73.7
Aq. Form. (100mg/kg)	2.71 ± 0.1	74.45
AFME Form. (100mg/kg)	2.61 ± 0.1	75.35



*p < 0.01 (ANOVA followed by student t-test)

Graph 1: Comparison of laxative potential of various extracts in Loperamide-induced constipation model

it was determined that methanolic extract 200 mg/kg and developed formulation 100 mg/kg showed greater pharmacological activity than standard omeprazole (300 mg/kg). Graph 2 depicts a comparison of anti-ulcer potential in ethanol-induced ulcers. The ulcer index in the ethanol-induced ulcer model was 2.74 ± 0.1 in the standard



*p < 0.01 (ANOVA followed by student t-test)

Graph 2: Comparison of anti-ulcer potential in ethanol-induced ulcers

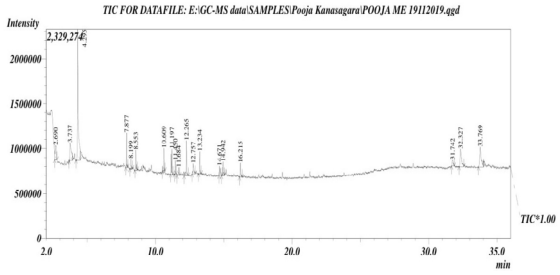


Fig. 5: GC-MS chromatogram of Methanol extract of *C. benghalensis*

omeprazole (300 mg/kg), 2.98 ± 0.1 in the aqueous fraction of methanolic extract (200 mg/kg), and 2.62 ± 0.1 in the developed formulation (100 mg/kg). The ulcer protection of the standard group was 71.53% in the ethanol-induced ulcer model, whereas the ulcer protection of aqueous fraction of Methanolic extract (200 mg/kg) was 71.91% and an aqueous fraction of methanolic extract formulation was 75.35%. Based on the findings, aqueous fraction of methanolic extract (200 mg/kg) and its formulation (100 mg/kg) showed significant anti-ulcer activity.

GC-MS Analysis

The methanolic extract contains 19 bioactive compounds, according to GC-MS analyses. Fig. 5 shows the GC-MS chromatogram, and Table 5 illustrates the bioactivity of all 19 compounds, together with their molecular weight, molecular formula, and compound nature.

HPTLC Fingerprinting data

C. benghalensis showed the best result in the Pet. Ether: Chloroform: Methanol: Formic Acid (3:7:0.5:0.3) solvent system for both methanolic extract and isolated fraction of methanolic extract. The results were shown at 366 nm after scanning and viewing the plates. Fig. 6 shows the HPTLC plate photographs, which reveal that all sample elements were well separated with no tailing or diffuseness. Phytoconstituents were discovered in an HPTLC fingerprinting scanned at 366 nm for methanol extract of *C. benghalensis*. Rf values ranged between 0.06 and 0.99. In various colors, the phytoconstituents of C. benghalensis methanol extract and isolated compound were exhibited on an HPTLC plate. The bands revealed the presence of alkaloids, flavonoids, steroids, terpenoids, and

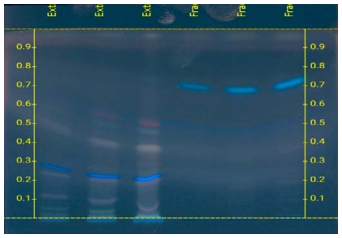


Fig. 6: HPTLC fingerprinting of methanolic extract and isolated fraction of plant

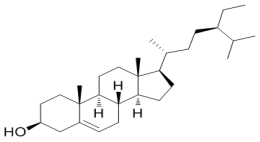


Fig. 8: Stigmasterol

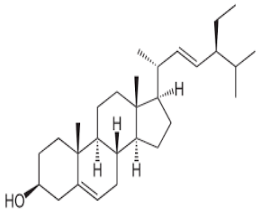


Fig. 9: β-sitosterol

Fig. 7: TLC profile of plant extract and isolated fraction with standard compound

Fig. 10: Herbal effervescent granules of *C. benghalensis*

Table 5: Bioactivity of compounds identified in GC-MS analysis of methanol extract

S. No.	*RT*	*Name of compound*	*Molecular formula*	*Molecular weight*	*Peak area%*	*Bioactivity*	*Compound Nature*
1	2.690	Tridecane	$C_{13}H_{28}$	184	5.39	Antioxidant, Antimicrobial, Anti-inflammatory, Anticancer	Volatile Terpene
2	3.737	Oxygen	O_2	32	7.64	Antimicrobial, Antioxidant	Oxygen
3	4.295	1-Butanol, 3-methyl-, acetate	$C_7H_{14}O_2$	130	30.09	Antimicrobial	Alcoholic, volatile oil
4	7.877	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206	3.17	antioxidant, Antifungal, Harbicidal	Alkaylbenzene Phenol
5	8.199	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl(R)	$C_{11}H_{16}O_2$	180	0.84	Analgesic, antidiabetic, antibacterial, antifungal	Volatile Terpene, Triterpene
6	8.553	n-Pentadecanol	$C_{15}H_{32}O$	228	2.81	Antibacterial	Fatty alcohol
7	10.609	1-Tetradecanol (Myristic alcohol)	$C_{14}H_{30}O$	214	2.01	Antibacterial, Antimicrobial, Antifungal	Fatty alcohol
8	11.197	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268	4.89	Antibacterial , Allelopathic	Diterpenoid
9	11.450	Phthalic acid, butyl tetradecyl ester	$C_{26}H_{42}O_4$	418	1.75	Antioxidant, antibacterial, Adulticidal	Ester of acids
10	11.684	Neophytadiene	$C_{20}H_{38}$	278	1.41	Analgesic, antipyretic, Anti-inflammatory, Antimicrobial, Antioxidant	Sesquiterpenoid
11	12.265	Hexadecanoic acid, methyl ester (methyl Palmitate)	$C_{17}H_{34}O_2$	270	5.88	Antimicrobial, Anti-inflammatory, antioxidant, Antifungal	Fatty acid methyl ester
12	12.757	n-Hexadecanoic acid (Palmitic acid)	$C_{16}H_{32}O_2$	256	2.85	Anti-inflammatory, antioxidant, hypocholesterolemic, nematicide, pesticide, anti-androgenic flavor, hemolytic, 5-Alphareductase inhibitor, potent mosquito larvicide	Fatty acid methyl ester

13	13.234	Behenic alcohol (docosanol)	$C_{22}H_{46}O$	326	2.96	Antiviral, Dermatological antibiotics and chemotherapeutics for dermatological use chemotherapeutics for topical use, Antiinfective	Aliphatic alcohol
14	14.671	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294	2.44	Hepatoprotectiv, Anti-histaminic, Antieczemic , Hypocholesterolemic	Fatty acid methyl ester
15	14.942	Phytol	$C_{20}H_{40}O$	296	3.51	Antimicrobial, anticancer, anti-inflammatory, hepatoprotective, antinociceptive, Diuretic, Antifungal against *S. typhi*, resistant gonorrhea, joint dislocation, headache, hernia, stimulant, Antimalarial	Diterpene alcohol
16	16.215	1-Heptacosanol	$C_{27}H_{56}O$	396	2.51	Nematicidal , anticancer, antioxidant and antimicrobial, Flavor and fragrance agent, cholesterol Lowering, Cytotoxicity, Antithrombotic	Alcohol
17	31.742	Cholest-5-en-3-ol (3.beta.)-, carbonochloridate	$C_{28}H_{45}ClO_2$	448	2.29	Antioxidant, antibacterial	Steroids
18	32.327	Stigmasterol	$C_{29}H_{48}O$	412	7.62	Antimicrobial, anticancer, anti-inflammatory, antioxidant, anticancer, Antiarthritic, Antiasthama, Diuretic	Phytosterol, steroid
19	33.769	(3S,8S,9S,10R,13R,14S,17R)-17-((2R,5R)-5-Ethyl-6-methylheptan-2-yl)-3-methoxy-10,13 -dimethyl-2,3,4,7,8, 9,10,11,12,13, 14,15,16,17-tetradecahydro-1H-cyclopenta [a] phenanthrene	$C_{30}H_{52}O$	428	9.96	Antimicrobial	Triterpenoid saponin

saponins. As a result, the created HPTLC is compatible with the selected solvent system, Rf value, and serves as a better tool for extract standardization. The HPTLC fingerprint of a plant species supports in the precise identification and quality control of a specific plant species and also offers fundamental details regarding the isolation, purification, characterization, and identification of marker chemical compounds of the species. Thus, the current study provides significant information regarding phytoconstituents found in methanol extracts and isolated fractions of *C. benghalensis*, as well as identification, standardization, and quality control of this medicinal plant.

TLC Analysis of Methanolic Extract and Isolated Compound with Reference Standard

According to the research, *C. benghalensis* performed best in the solvent system Toluene: Methanol (9:1) for methanolic extract, isolated fraction, and reference standard (β-sitosterol and stigmasterol). Results were displayed at 254, 366 nm, and sprayed with anisaldehyde sulphuric acid spraying reagent after scanning and examining the plates. The TLC photographs in Fig. 7 showed that phytoconstituents such as β-sitosterol and stigmasterol were clearly separated in all TLC plats after spraying with ANS reagent, with no tailing or diffuseness. The Rf values of all four TLC plats were 0.42. TLC plates revealed a blue color spot of phytoconstituents, indicating the presence of stigmasterol in Fig. 8 and β-sitosterol in Fig. 9 the methanol extract and isolated fraction of *C. benghalensis*.

Herbal Effervescent Granules with Evaluation Parameter:

Herbal effervescent granule was manufactured according to the prescribed formula and can be simply administered by individuals of any age. The granules were dark brown

Table 6: Evaluation parameter of herbal effervescent granules

S. No.	*Parameters*	*Result*	*Flow property*
1	Angle of repose	25.4 ± 0.210	Excellent
2	Bulk density	0.376 ± 0.002	Good
3	Tapped density	0.415 ± 0.006	Good
4	Carr's index	8.94 ± 0.168	Excellent
5	Hausner ration	1.085 ± 0.001	Excellent
6	Effervescent cassation time	101 sec. ± 0.471	Good
7	Color	Dark brown	----
8	Odor	Jeera	-----
9	Appearance	Amorphous granules	-----

in color and had a distinct odor, as seen in Fig. 10. Table 6 illustrates the evaluation parameters of effervescent granules, together with their results and flow property.

Conclusion

This research identified several biologically active secondary metabolites from plant extract, including stigmasterol, β-sitosterol, and others. In an *in-vivo* study, herbal effervescent granules and aqueous fractions of Methanolic extracts with claimed ethnomedicinal applications of *C. benghalensis* showed strong laxative and anti-ulcer properties. Effervescent granules, which revealed simple oral administration to all age groups. The developed granules passed the recognized official monograph tests and were determined to meet BP and IP criteria. These Effervescent granules, made from weeding plants can be utilized as an effervescent medication with a low cost, a rapid absorption rate and the ability to exert the claimed pharmacological activity with the least amount of side effects. It would be great to examine more weeds instead of letting go to waste, more should be researched for their optimum use.

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