

# Evaluation of Antiulcer and Antioxidant Activity of *Barleria gibsoni* Dalz. Leaves

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## ABSTRACT

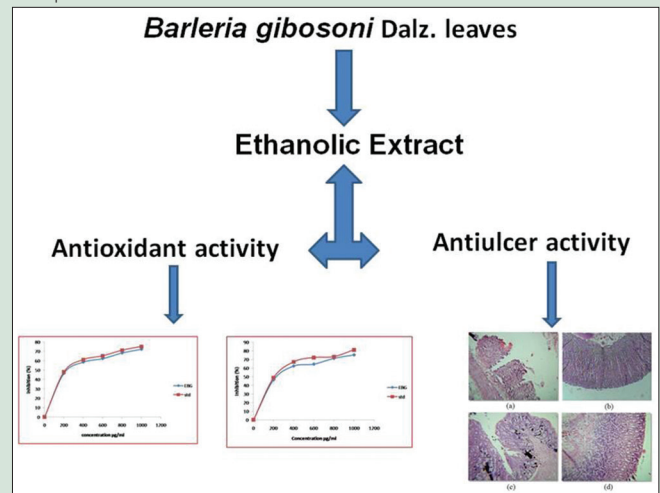
**Background:** Peptic ulcer is a digestive disorder most commonly found in clinical practice. Given the many side effects of modern medicine, the initial acquisition of fewer side effects, and medication of indigenous drugs, it should be considered as a better alternative for the treatment of peptic ulcer. **Objective:** To assess antiulcer and antioxidant activity of ethanol extract of *Barleria gibsoni* (EBG) Dalz. leaves in ulcer-induced rats and *in vitro* antioxidants method, respectively. **Materials and Methods:** Ethanol EBG was screened for antiulcer activity in pylorus ligation-induced ulcer models in Wistar rats. *In vitro* antioxidant activity of the extracts was tested using 2,2-diphenyl-1-picrylhydrazyl (DPPH), nitric oxide (NO) radical scavenging activity. Total phenol and flavonoid content in the extracts were determined spectrophotometrically. **Results:** Oral administration of ethanol extract of leaves at doses of 250, 500 mg/kg p.o. reduced significant gastric lesions induced by pylorus ligation-induced ulcer as compared to standard omeprazole (20 mg/kg p.o.). The  $IC_{50}$  values were found to be 150  $\mu$ g/mL in leaves extract. The ethanol extracts showed good antioxidant capacity in DPPH radical scavenging assay and NO radical scavenging activity when compared to standard. The total phenolic content using Folin-Ciocalteu reagent estimated in 1 mg of leaves extracts was 368  $\mu$ g and 481  $\mu$ g with gallic acid equivalent and also the total flavonoid content found to be 240 and 410  $\mu$ g, respectively, with quercetin equivalence. **Conclusion:** These findings suggest that the leaves of *B. gibsoni* possessed antiulcer potential and antioxidant compared to standard. This is the first ever report of antiulcer and antioxidant activities in *B. gibsoni* (*Acanthaceae*).

**Key words:** *Acanthaceae*, *Barleria gibsoni*, gallic acid, L-ascorbic acid, pylorus ligation-induced ulcer model, quercetin

## SUMMARY

- *In vivo* antiulcer and *in vitro* antioxidant activity of *Barleria gibsoni* was evaluated.
- Soxhlet extraction was carried out and extracts were subjected to qualitative phytochemical analysis. Extract obtained by Soxhlet showed higher total phenolic and flavonoid contents.
- EBG showed DPPH and Nitric oxide scavenging activity indicating its strong antioxidant potential.
- On pylorus ligation-accumulated secretions and the related ulcers confirm gastric acid output to be the basic cause of gastric ulcers. Ethanol extract of leaves attenuated the gastric volume, free acidity, total acidity and ulcer index thus showing the anti-secretory mechanism.
- The results of the histopathological investigation of *Barleria gibsoni* leaves

for antiulcer effects using pylorus ligation induced ulcer model in rats laid credence to traditional use of the plant leaves in ulcer treatment. The ethanol extract of leaves demonstrated increase in percentage preventive index compared to omeprazole respectively. From the present study results reveals the antiulcer activity of ethanol extract leaves which is comparable to that of Omeprazole.



**Abbreviations Used:** EBG: Ethanol extract prepared from the leaves of *B. gibsoni*; ROS: Reactive Oxygen Species, DPPH: 2,2-diphenyl-1-picrylhydrazyl, NO: Nitric Oxide,  $IC_{50}$ : The half maximal inhibitory concentration, m: mg, DNA: deoxyribonucleic acid, GAE: Gallic acid equivalence,  $AlCl_3$ : Aluminium chloride

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## INTRODUCTION

Gastric hyperacidity is a very common global problem affecting millions of people worldwide due to an imbalance between aggressive and protective factors.<sup>[1]</sup> The gastric mucosa is continuously exposed to potentially injurious agents such as acid, pepsin, bile acids, bacterial products (*Helicobacter pylori*), and drugs. The current treatment of peptic ulcer is mainly done with  $H_2$  receptor antagonists, proton pump inhibitors, and antimuscarinics. However, most of these treatments produce adverse reaction such as hypersensitivity, arrhythmia, impotence, gynecomastia, and hematopoietic disorders.<sup>[2]</sup>

Antioxidants apparently protect the living system from oxidative insults, which is a hallmark feature of cancer, cardiovascular disease,

and diabetes.<sup>[3]</sup> This oxidative damage is caused by reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, hydroxyl,

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and nitric oxide (NO) radical.<sup>[4]</sup> These ROS accumulations lead to damage to crucial biomolecules such as nucleic acids, lipids, proteins, polyunsaturated fatty acids, carbohydrates, and DNA in living system also directly stimulate histamine release from mast cells.<sup>[5]</sup> Most of the antioxidants present in vascular plants such as Vitamin C and E, carotenoids, flavonoids, and tannins.<sup>[6]</sup> The naturally polyphenolic compounds, especially flavonoids have been largely studied for their strong antioxidants capacity.<sup>[7]</sup>

*Barleria (Acanthaceae)* is a large genus with about 230 species of herbs and shrubs distributed chiefly in the tropical and subtropical parts of the world. About thirty species occur in India, many of which are known for their ornamental and/or medicinal value. Some of the important species of this genus are *Barleria prionitis*, *Barleria greenii*, *Barleria albostellata*, *Barleria cristata*, *Barleria strigosa*, *Barleria tomentosa*, etc., In some *Barleria* species, biological activities such as anti-inflammatory, analgesic, antileukemic, and hypoglycemic have been reported.<sup>[8]</sup>

Therefore, current research is focused on the discovery of natural antiulcer and antioxidant compounds from the plants for new and safer treatment options, with fewer side effects. Thus, the objective of the present investigation was to evaluate *in vivo* antiulcer and *in vitro* antioxidant activity of *Barleria gibsoni* leaves extract (EBG).

## MATERIALS AND METHODS

### Collection of the plant samples

The fresh leaves of the *B. gibsoni* were collected during the month of May-June when flowering, from Satara region, Maharashtra, India. The plant authenticated by Botanical survey of India, Pune, Maharashtra, India. A voucher specimen (BSI/WRC/Tech/2013/FAT 01 dated December 27, 2013) has been deposited at the herbarium of the same place for further reference.

### Chemicals

Ethanol, methanol, gallic acid, Folin-Ciocalteu reagent, potassium ferricyanide, trichloroacetic acid, ferric chloride, ferrous chloride, sodium nitroprusside, sulfanilamide, o-phosphoric acid, naphthylethylenediamine dihydrochloride, sodium carbonate, sodium dihydrogen phosphate dihydrate, disodium hydrogen phosphate dodecahydrate, aluminum chloride, quercetin, L-ascorbic acid, and 2,2-diphenyl-1-picrylhydrazyl (DPPH), were obtained from HiMedia Laboratory and Research Lab, India. All the chemicals used in this study including the solvents were of analytical grade.

### Animal selection

The experiments were carried out on Wistar strain male adult rats, aged 12–15 weeks and weighing 120–150 m. The animals were housed in colony cages and maintained under standard environmental conditions: 25°C ± 2°C temperature, 12:12 h light: dark cycle, and 45–55% relative humidity, with free access to food and water *ad libitum*. All experiments were carried out during the light period (08.00–16.00 h). The Institutional Animal Ethics Committee approved the protocol of the study (BVCPK/CPCSEA/IAEC/02/01 dated 07/02/2014).

### Extract preparation

The collected fresh matured leaves of *B. gibsoni* were washed with tap water, air-dried at room temperature for 2–3 weeks at 35–40°C, and then reduced to coarse powder. One hundred grams powdered leaves was obtained after defatted with petroleum ether and successively extracted with ethanol using Soxhlet apparatus subsequently 12.0 g of extracts was obtained.

## Phytochemical screening

Phytochemical screening was carried out to identify the secondary metabolites present in defatted ethanol EBG.<sup>[9,10]</sup>

### Estimation of total phenolic content

The total phenolic content of the EBG was determined using gallic acid equivalence as described previously.<sup>[11]</sup> An aliquot (1 ml) of extracts and standard solution of gallic acid was added to 25 ml of volumetric flask, containing 9 ml of distilled water. Reagent blank was prepared using distilled water. One milliliter of Folin-Ciocalteu phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% sodium carbonate solution was added to the mixture. The volume was then made up to the mark.

After incubation for 90 min at room temperature, the absorbance against the reagent blank was determined at 760 nm on spectrophotometer with a preprepared gallic acid calibration curve from 0.69 µg to 3.01 µg of standard gallic acid.

### Determination of total flavonoid content

The content of total flavonoid in ethanol EBG leaves was assessed using aluminum chloride colorimetric method.<sup>[12]</sup> The plant extract samples were mixed with 1.5 ml of methanol, 0.1 ml 10% AlCl<sub>3</sub>, 0.1 ml of 1M potassium acetate, and 2.8 ml distilled water. It was incubated at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm using ultraviolet spectrometer. The calibration curve was prepared by preparing standard quercetin solution at concentration 20–100 µg/ml in methanol.

### 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity

The ability of EBG to scavenge DPPH radical was assessed with modification.<sup>[13]</sup> An aliquot of the EBG, 200–1000 µg/ml was mixed with 3 ml DPPH solution (0.5 mmol/l in methanol), and the resultant absorbance was recorded at 517 nm after 30 min incubation at 37°C. The standard drug ascorbic acid was used. The percentage of scavenging activity was derived using the following formula,

$$\text{Percentage of inhibition (\%)} = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100$$

Where,

A control-absorbance of DPPH; a sample-absorbance reaction mixture (DPPH with sample).

### Nitric oxide radical scavenging activity

NO radical scavenging of EBG was carried out as previously described.<sup>[14]</sup> Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates NO, which interacts with oxygen to produce nitrite ions and can be determined by the use of the Griess-Ilosvay reaction. Two milliliters of 10 mM sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of EBG at various concentrations and the mixture incubated at 25°C for 150 min. From the incubated mixture, 0.5 ml was taken out and added into 1.0 mL sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. Finally, 1.0 ml naphthylethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min before measuring the absorbance at 540 nm was measured with a spectrophotometer. The NO radicals scavenging activity was calculated.

### Pyloric ligation in rats

Animals are randomized into four groups ( $n = 6$ ). Control group was received distilled water orally while omeprazole, in the dose of 20 mg/kg

was administered orally for the second group and served as a reference group for ulcer protective studies; third and fourth groups received EBG in a dose of 250 and 500 mg/kg, respectively. After 45 min of EBG and omeprazole treatment, pyloric ligation was done by ligating the pyloric end of the stomach of rats of respective groups under ether anesthesia at a dose of 35 mg/kg of body weight. Ligation was done without causing any damage to the blood supply of the stomach. Animals were allowed to recover and stabilize in individual cages and were deprived of water during postoperative period. After 4 h of surgery, rats were sacrificed and ulcer scoring was done. The ulceration in gastric mucosa was measured and scored.<sup>[15]</sup> The ulcer index, the percentage ulcerated surface, and the percentage of inhibition were estimated as described below.

A score for the ulcer was studied similar to pyloric ligation-induced ulcer model. Scoring of ulcer was made as follows; normal stomach (0), red coloration (0.5), spot ulcer (1), hemorrhagic streak (1.5), ulcers (2), and perforation (3); mean ulcer score for each animal will be expressed as ulcer index.

### Measurement of gastric acidity

One milliliter of the total centrifuged gastric contents from each pylorus ligated rat was analyzed for hydrogen ion concentration by titrating against 0.01N solution of NaOH. The experiment was done in triplicate.

Acidity = volume of g NaOH × normality of NaOH × 100/0.1 mEq/L

### Histopathological evaluation

The stomachs tissues were fixed using 10% buffered formalin and were processed using a tissue processor. The processed tissues were embedded in paraffin blocks and about 5 μm thick sections were cut using a rotary microtome. These sections were stained with hematoxylin and eosin using routine procedures. The slides were examined microscopically for pathomorphological changes such as congestion, hemorrhage, edema, and erosions using an arbitrary scale for the assessment of severity of these changes. The gastric tissue samples were fixed in neutral buffered formalin for 24 h. Sections of tissue from stomachs were examined histopathologically to study the antiulcerogenic activity of *B. gibsoni*.<sup>[16]</sup>

### Statistical analysis

Statistical analysis was performed using one-way ANOVA and significance of difference between the treatments was accepted at the level of significance  $P < 0.05$ . Data were expressed as mean ± standard error of the mean.

## RESULTS

### Phytochemical screening

The ethanolic EBG leaves revealed the presence of alkaloids, saponins, terpenoids, flavonoids, steroids, cardiac glycosides, tannins, aminoacids, and proteins [Table 1].

### Estimation of total phenolic content

The total phenolic content in *B. gibsoni* ethanolic extract of leaves was obtained [Figure 1]. The gallic acid linear curve obtained using the

$y = 0.002x$  ( $R^2 = 0.993$ ). Using this gallic acid standard curve, the total phenolic content in *B. gibsoni* leaves was found to be 310 μg/mL. It shows the *B. gibsoni* leaves contained a high phenolic content which may be attributed to its high total antioxidant activity.

### Determination of total flavonoid content

The total flavonoid (mg/mL) content was obtained using the regression calibration curve  $y = 0.000x - 0.004$ ,  $R^2 = 0.9955$  [Figure 2] using quercetin equivalent and the crude EBG leaves contain 260 μg/mL. High amount of flavonoids was found in EBG.

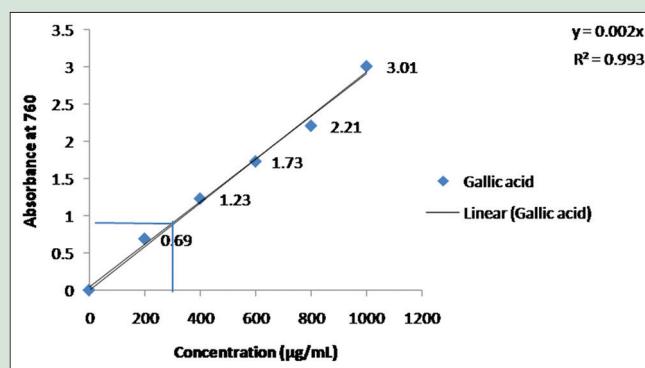


Figure 1: Total phenolic content

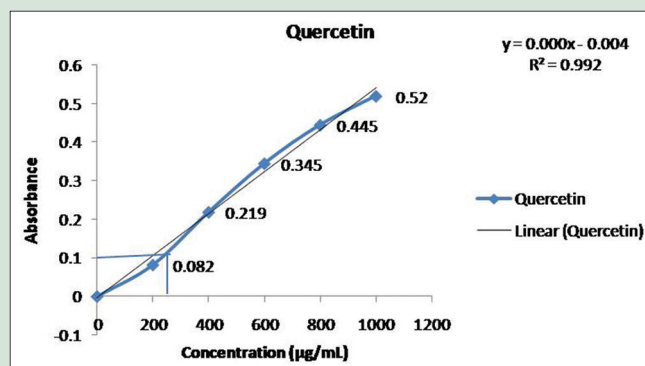


Figure 2: Total flavonoid content

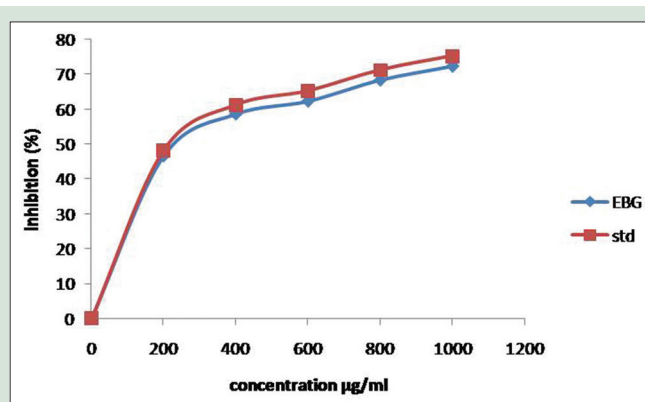


Figure 3: 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity

Table 1: Phytochemical screening of *B. gibsoni*

Extracts	Major Secondary Metabolites present								
	AL	SA	TER	FLA	S	CG	T	AA	P
Leaves	+	-	+	+	+	+	+	+	+

Alkaloids - AL, Saponins - SA, Terpenoids - TER, Flavonoids - FLA, Steroids - S, Cardiac glycosides - CG, Tannins - T, Aminoacids - AA and Protein - P.

## 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity

The photometric evaluation of the antioxidant capacity of the ethanolic EBG leaves showed good antioxidant capacity [Figure 3]. A significant decrease was observed in the DPPH radical activity due to the scavenging ability of the extracts.

The  $IC_{50}$  value of the ethanol EBG leaves and standard antioxidant (ascorbic acid) was found to be 180 and 170  $\mu\text{g}/\text{mL}$ , respectively. A lower  $IC_{50}$  value indicates a higher free radical scavenging activity. The ability of DPPH radical scavenging was higher in the EBG even significantly lower than standard ascorbic acid thus proving strong antioxidant potential of *B. gibsoni*.

## Nitric oxide radical scavenging activity

NO radical scavenging activity of EBG was performed by the formation of NO using sodium nitroprusside. Sodium nitroprusside acts as a major source of NO radicals. EBG scavenges the NO formed from sodium nitroprusside by inhibiting the chromophore formation, hence absorbance decrease as the concentration increased. The  $IC_{50}$  value was found to be 220  $\mu\text{g}/\text{ml}$  of EBG [Figure 4].

## Pyloric ligation-induced gastric ulcer

In pyloric ligation-induced ulcer model, oral administration of leaves in two different doses showed significant reduction in ulcer index, gastric volume, free acidity, and total acidity as compared to the control group, whereas omeprazole as reference standard drug showed a significant reduction in ulcer index, gastric volume, free acidity, and total acidity [Table 2].

## Histopathological evaluation

Histopathological changes on pylorus ligation model showed the degeneration, hemorrhage, and edematous appearance of the gastric tissue, whereas EBG (500 mg/kg) and omeprazole (20 mg/kg) treated

groups showed regeneration and prevents the formation of hemorrhage and edema and it was shown in Figure 5.

## DISCUSSION

Ulcer is formed due to pylorus ligation which can lead to the accumulation of gastric juice in the stomach, damaging the balance of aggressive and protective factors. On pylorus ligation, accumulated secretions and the related ulcers confirm gastric acid output to be the basic cause of gastric ulcers.<sup>[16]</sup> EBG attenuated the gastric volume, free acidity, total acidity, and ulcer index thus showing the antisecretory mechanism.

The results of the histopathological investigation of *B. gibsoni* leaves for antiulcer effects using pylorus ligation-induced ulcer model in rats laid credence to the traditional use of the plant leaves in ulcer treatment. The ethanol EBG leaves demonstrated decreasing oxidative stress and increasing percentage preventive index compared to ascorbic acid and omeprazole, respectively. From the present study, ethanol EBG results reveal that antioxidant and antiulcer activity is equipoint to that of omeprazole. The isolation of bioactive compounds from *B. gibsoni* will definitely serve as good phytotherapeutic agent, and it may be beneficial in the management of ulcer.

Nowadays, most of the people include natural antioxidants as nutraceuticals or as food additives. Many medicinal plants contain higher phenolic compounds such as flavonoids, monophenols, and polyphenols.<sup>[17-19]</sup> The polyphenolic compounds are directly correlated

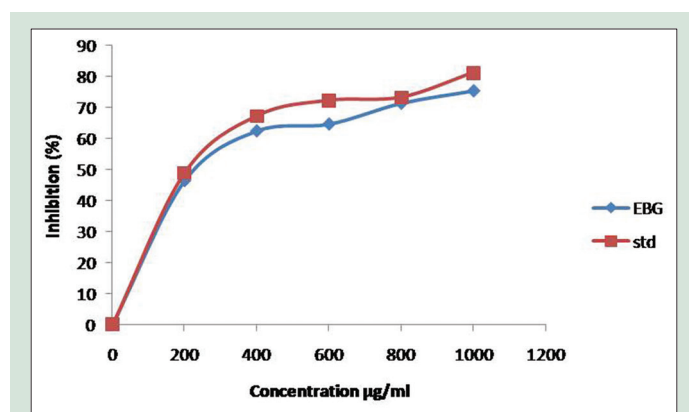


Figure 4: Nitric oxide radical scavenging activity

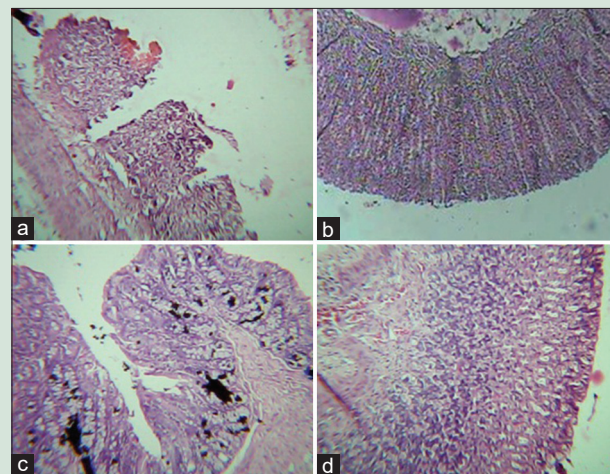


Figure 5: Antiulcer activity of ethanol extract obtained from *Barleria gibsoni* leaves: Photomicrograph of rat stomach mucosa section was showing (a) mucosal epithelium in control group; (b) normal mucosal epithelium with no ulcer in omeprazole-treated animal (c) normal mucosal epithelium with mild regeneration and prevents formation of hemorrhage in ethanol extract of *Barleria gibsoni* 250 mg/kg treated animal; (d) showing normal mucosa with regeneration and prevents formation of hemorrhage ethanol extract of *Barleria gibsoni* 500 mg/kg treated animal

Table 2: Effect of *B. gibsoni* leaves extracts on various parameters in pyloric ligation induced gastric ulcers

Group	Treatment	Dose (mg/ml)	Ulcer index	pH of gastric juice (ns)	Vol. of Gastric juice in ml/100g body wt.	Free acidity meq/ltr/100gm	Total acidity meq/ltr/100g
I	Control	1	14.8±0.36	3.48±0.31	4.25±0.17	67.17±0.72	138.1±0.72
II	Omeprazole	20	4.4±0.40**	4.75±0.19	2.38±0.14	32.8±0.50**	66.76±1.42**
III	EBG leaves	250	11.7±0.36**	4.06±0.28	4.26±0.12***	47.8±0.72**	76.76±1.64**
IV	EBG leaves	500	10.7±0.36**	4.1±0.34	3.90±0.12***	37.8±0.72**	71.96±1.51**

Values are express as mean±SEM of 6 observations, statistical dunnet test comparisons to control as follows: significant at ns (Non significant) \*\*\* $P<0.001$ , \*\* $P<0.01$ , \* $P<0.05$  compared to control group.

with the antioxidant ability. The plant derived antioxidants, especially polyphenols and flavonoids have been used to treat various disease such as cancer, diabetic, aging, and prevention of cardiovascular disease.<sup>[20]</sup>

The DPPH free radical scavenging activity of the ethanolic EBG Dalz. leaves possess ability to scavenge DPPH free radicals as equal to the standard antioxidant L-ascorbic acid. By conversion of the unpaired electrons to paired electron by hydrazine due to the hydrogen donating ability of the extract.<sup>[21]</sup>

NO is a potent pleiotropic mediator of physiological processes such as neuronal signaling, smooth muscle relaxant, regulation of cell-mediated toxicity, and inhibition of platelet aggregation. It is a diffusible free radical which plays many roles as an effectors molecule in diverse biological systems including vasodilatation, antimicrobial, neuronal messenger, and antitumor activities.<sup>[22]</sup> Although NO and superoxide radicals are involved in host defense, overproduction of these two radicals contributes to the pathogenesis of some inflammatory diseases.<sup>[23]</sup> EBG was found to be potent NO scavenger and thus may have potential pharmaceutical and nutraceutical application.

## CONCLUSION

The results of this investigation showcase the potential antiulcer and antioxidant activities of ethanol EBG. However, more work is required for the isolation and characterization of the active principles responsible for these activities.

## Financial support and sponsorship

Nil.

## Conflicts of interest

There are no conflicts of interest.

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