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Pharmacological Evaluation of *Lavandula stoechas L.* for Ethanol-induced Gastric Mucosal Ulcer

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1 experiment and compiled the data.
7 histopathological results were descripted
3, 4 analyzed the data,
3 manuscript was written
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ABSTRACT

Background*:* The constituents of *Lavandula stoechas L.* possess antioxidant properties that help in protecting the mucosal cells from oxidative damage and speed up the healing process however, its role in the treatment of ethanol-induced peptic ulcers is not clear.

Objectives: We aimed to evaluate the pharmacological potential of *Lavandula stoechas L.* extracts for anti-ulcer activity, and compare with the standard drugs and to explore novel treatment for peptic ulcer.

Methods: We evaluated anti-ulcer potential of plant extract in ethanol-induced ulcer model in rats. Omeprazole and ranitidine were standard drugs. After 5 h of disease induction, animals were sacrificed to get tissues for histological evaluation and ulcer index was measured. While the antimicrobial potential of *Lavandula stoechas L.* aqueous and methanolic extracts was evaluated against different bacterial stains using standard antibiotic discs. Qualitative phytochemical and GCMS analysis were performed to identify novel constituents.

Results: The methanolic extract of *Lavandula stoechas L*. showed antimicrobial activity against *Proteus Mirabilis* while the GCMS based analysis revealed the presence of 10 phytochemicals including camphor (antimicrobial agent). The aqueous extract showed significant anti-ulcer activity in ethanol-induced gastric ($P < 0.001$) and duodenal ($P < 0.01$) ulcers when compared with controls. Aqueous and methanolic *Lavandula stoechas L.* extracts showed strong free radical scavenging activity.

Conclusion: *Lavandula stoechas L.* extract possess antimicrobial and anti-ulcer activity in alcohol-induced ulcer model in experimental animals.

Keywords: Antimicrobial and anti-ulcer activity, Bacterial spectrum, Free radicals, *Lavandula stoechas L.*, Gas chromatography

INTRODUCTION

Gastric ulcer is a common gastrointestinal disease that affects appoximately 5% of people globally [1]. An imbalance between the potential harmful (acid, pepsin, bile, drugs) and the protective (mucus & bicarbonate secretion, prostaglandins, nitric oxide) factors is the main cause of gastric ulcer [2]. Excessive alcohol intake, caffeine, tobacco, as well as stress may also lead to gastric ulcer [3]. The motility and the absorption capacity of gastrointestinal tract may be compromised upon prolong exposure to alcohol. The severity of ulcer can be accessed on the basis of ulcer index or score calculated as percentage of the ratio of ulcerated area to total surface area of the glandular stomach [4]. Pharmacological treatment options for gastric ulcer include H2-receptor blockers (ranitidine), proton-pump inhibitors (PPIs, i.e. omeprazole) and mucosal protecting agents (i.e. sucralfate) [5]. The adverse effects such as drug allergy, collageneous colitis and interstitial nephritis associated with commonly used anti-ulcer agents, urge the need of new therapeutic strategies for peptic ulcer [6].

A number of studies have been conducted on naturally occurring medicinal plants containing essential ingredients that may be useful ailment for gastric ulcer [7]. Genus *Lavandula* belongs to Lamiaceae family with most important species including *Lavandula stoechas L.* and Spike Lavender (*Lavandula Spica L.*). Francesca Algierion and her colleagues evaluated the anti-inflammatory potential of *Lavandula stoechas L.* extracts in colitis model in rats. The results showed that *Lavandula stoechas L.* hydroalcoholic extract possessed antioxidant and intestinal anti-inflammatory activities and therefore, can improve the healing process as well as intestinal epithelial barrier [8].

Oral administration of ethanol to experimental animals induced gastrointestinal lesions [9, 10]. The process of ethanol-induced injury is not fully known however; it causes a disturbance in gastric mucosal integrity through exfoliation of cells and thus, may lead to gastric mucosal bleeding [11, 12]. Neutrophils are recruited to the site of injury lead to ROS production that results in oxidative stress to mucosal cells and ulcer. Therefore, the use of antioxidants might be helpful in protecting the mucosal cells from oxidative damage and speeding up the healing process [12]. The constituents of *Lavandula stoechas L.* include camphor, linalool, linalyl acetate, 1-8 cineole and γ-terpinene while its antioxidant potential is found to be comparable with vitamin E as reported in previous studies [13]. In addition, literature showed that methanolic extract of *Lavandula stoechas L.* (800 mg/kg/p.o.) significantly ($P < 0.001$) reduced MDA (malondialdehyde) levels while increased SOD (superoxide dismutase) and catalase levels in scopolamine-induced-amnesic animal model [14]. Many studies has been conducted on antioxidant enzymes like catalase, MDA, SOD and 1,1-diphenyl-2-picryl-hydrazyl (DPPH)) for anti-ulcer activity of various plants [15].

To the best of our knowledge, *Lavandula stoechas L.* has not been evaluated for its possible role in the treatment of ethanol-induced peptic ulcers. Therefore, we aimed to evaluate and compare the pharmacological potential of *Lavandula stoechas L.* extracts for anti-ulcer activity with the standard drugs and to explore novel treatment for peptic ulcer.

MATERIAL AND METHODS

Preparation of *Lavandula stoechas L.* extracts

Maceration method [16] was used to prepare *Lavandula stoechas L.* extracts. *Lavandula stoechas L.* powder (500 grams from arial parts) was separately added to 1 L of each ethanol and distilled water. The mixtures were preserved at research laboratory for 14 days and the soaked powder was stirred and degassed, twice daily. After maceration, we filtered the mixtures through linen cloth, followed by filtration with Whatman filter paper (0.45µm). The solvents were evaportaed at 50 ^{o}C using a hot-air oven until the extract dried.

Preparation of agar plates and microbial suspensions for antimicrobial activity

Muller Hinton Agar (Biomark® Laboratories India, lot # 0419/0087) media was autoclaved, 20 ml was added equally in each petry dish and then cooled at room temperature. The cultures of bacteria were donated by the Microbiology laboratory, The University of Lahore. These cultures were previously characterized as *Proteus, E.coli, Klebsiella, Micrococcus luteus, Bacillus cereus* and *Salmonella typhi.* Agar plates were innoculated with different bacterial strains and antimicrobial activity was performed using solutions (1%, 0.5%, 0.25%, & 0.125%) of dry extracts powder dissolved in Dimethyl Sulfoxide (DMSO). The antimicrobial activity was

performed by the well diffusion method [17]. Solutions of extracts were introduced into the wells and the agar plates were kept at 37 °C overnight in a laminar safety hood.

Qualitative phytochemical analysis

The reagents (Wagner, Hager, Dragendorff, Mayer, Godine) were prepared for the qualitative phytochemical analysis of *Lavandula stoechas L.* [18]. Tests were performed for glycosides (Borntrager & Keller kiliani test) [19], alkaloids [20], tannins [21], flavonoids [20], saponins [21], carbohydrates [21], proteins and terpenoids [21] by following previously published methods.

GCMS analysis

We used GCMS based method for qualitative analysis of phytochemicals present in ethanolic and water extracts of *Lavandula stoechas L.* [22]. The sample was washed with de-ionized water, shade-dried for 10 days, powdered and stored in zipper plastic bags for further processing. The dried powder (100 g) was dipped in ethanol (1 L, 95%) for 3 days followed by filtration using Whatmann filter paper. The filtration process was repeated again and the filtrate was concentrated at 40°C and the resulting extracts were kept at 4°C.

The GCMS, consisted of pumps (Agilent Technology, USA, Model; 7890A), spectrophotometer (Agilent Technology, USA, Model; 5975C) and column (HP-5MS, 30 m x 250 µm x 0.25 µm, Agilent Technology, USA). Ethanol was used as solvent and reagent in this procedure (BDH, UK, 10107 7Y) Following GCMS parameters were used: oven program; 60°C for 0 minute then 10°C/minute to 300°C for 4 minutes, run time; 29 minute, heater; on, temperature program; 280°C for 0 minute, inert gas; helium (99.99%), flow; 1 ml/minute, acquisition mode; scan, scan mass range; 50-650, solvent delay; 4 minute, EMV mode; relative, relative voltage; 59 and resulting EM voltage; 1306. The identification of compounds was done on the bases of Wiley & NIST libraries and the comparison of retention time peaks.

Experimental animals

Male albino rats were randomly grouped (5 in each group), kept under controlled environment (temperature 24 ± 2 °C, humidity 45-55% and 12 h light/dark cycle) and fed with *ad libitum diet* [23]. All protocols were followed according to the agreement of the Institutional Animal Ethics Committee, University of Lahore, Lahore, Pakistan (IREC-2018-FEB-49).

Gastroprotective potential of *Lavandula stoechas L.* extract

Solutions of omeprazole (4 mg/ml) and ranitidine (25 mg/ml) were made in distilled water. Animals were kept on fasting overnight and orally received following different treatments : group I and II (normal and disease control respectively), group III; omeprazole (20 mg/kg) [24], group IV; ranitidine (30 mg/kg) [25] and group V; *Lavandula stoechas L.* extract (300 mg/kg) [26]. After 1 h, disease (gastric mucosal ulcer) was induced in animals (group II-V) by oral administration of 1 ml of ethanol (80%) [27]. After 5 h, animals were anesthetized using xylazine (10 mg/kg, intraperitoneal) & ketamine (100 mg/kg, intraperitoneal) [28] and sacrificed to obtain the stomach. Ulcerative lesions were measured by planimetry the ulcer area was calculated [29]. Gastric contents were collected, stomachs were washed with normal saline and preserved for morphological examination. For histological examination, excised stomachs were fixed with formalin (10 %), dehydrated and cleared using paraffin & xylene. Then the tissues were cut into slices (4-5 mm), stained using hematoxylin & eosin (H & E) dyes and examined under microscope [29].

Antioxidant activity of *Lavandula stoechas L.* extract

In-vivo antioxidant potential of *Lavandula stoechas L.* extract has already been reported in literature [29]. For *In-vitro* antioxidant activity, the extract and ascorbic acid was separately dissolved in DMSO (1 mg/mL). DPPH solution (1 ml, 0.1 mM in DMSO) was added to 3 ml of test samples in various concentrations (10 - 100 µg/ml). The mixture was incubated (30 min, room temperature) and absorbance was recorded at 517 nm.

Statistical analysis

As the data was normally distributed with a bell shaped histogram so, the sum results were expressed by means ± SEM (standard error of mean). Parametric data were assessed by one-way ANOVA (analysis of variance) followed by Dunnett's test. Graphics and statistical hypothesis testing were done using Graph Pad Prism version 5.0 and IBM SPSS version 19. Values $P < 0.05$ were considered as statistically significant, $P < 0.01$ were considered as

very significant and those with $P < 0.001$ were regarded as highly significant.

RESULTS

Antimicrobial activity with ethanolic extract

Ethanolic extract (1%) of *Lavandula stoechas L.* showed antimicrobial activity against *Proteus mirabilis* as evident by 3 mm inhibitory zone using augmentin (amoxicillin 20 µg + clavulanic acid 10 µg) as standard disk (Figure **1a**, Table **1**). The ethanolic extract did not show any antimicrobial activity against *M. luteus* and *E.coli* (using gentamycin 10 µg as standard disk) (Fig **1b-c**). No antimicrobial activity was found against *Klebsiella* using augmentin (amoxicillin 20µg + clavulanic acid 10 µg) as standard disk (Fig. **1d**) and against *B. cereus* & *S. typhi* using Ciprofloxacin 5 µg as standard disk (Fig. **1e-f**). Antimicrobial activity of *Lavandula stoechas L.* aqueous extract was determined against different bacterial species using standard disks in same experimental settings as used for ethanolic extracts (Fig. **2a-f**). No antimicrobial activity was seen against any of the bacterial species used in the experiment (Fig. **2a-f**).

Table 1. Antimicrobial activity of *Lavandula stoechas L.* ethanolic and water extracts at 1%, 0.5%, 0.25% and 0.125% dilutions in Dimethyl Sulfoxide (DMSO).

Microorganism	Ethanolic extract					Aqueous extract				
	Crude Extract	1%	0.5%	0.25%	0.125%	Crude Extract	1%	0.5%	0.25%	0.125%
Proteus	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
M. Luteus	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
E. coli	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Klebsiella	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
B. Cereus	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
S. typhi	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

+ve → Antimicrobial activity is present, -ve → Antimicrobial activity is not present

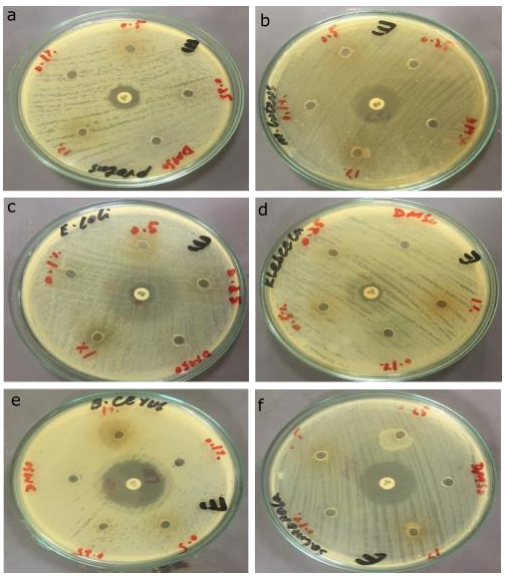

Figure 1. Antimicrobial activity of ethanolic extract from *Lavandula stoechas L.* against different bacterial species: **a;** using augmentin, antimicrobial activity was found positive against *Proteus Mirabilis*, **b & c;** using gentamycin, no antimicrobial activity was found positive against *M. Luteus* and *E.coli,* **d;** using augmentin, no antimicrobial activity was found positive against *Klebsella*, **e & f;** using ciprofloxacin, no antimicrobial activity was found positive against *B. Cereus* and *Salmonella Typhi.*

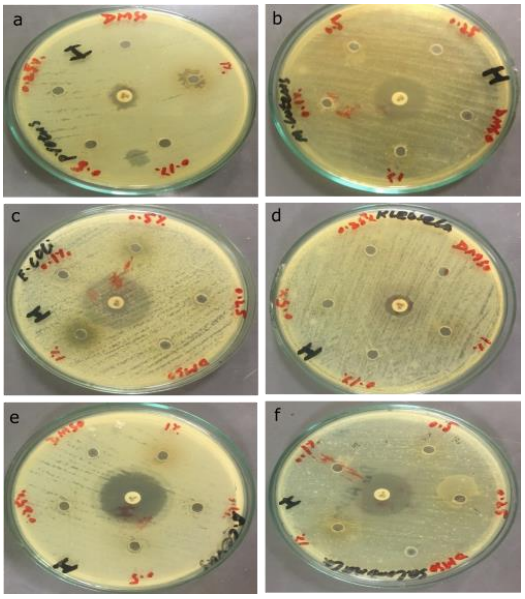

Figure 2. Antimicrobial activity of *Lavandula stoechas L.* aqueous extract against different bacterial species: **a;** using augmentin, no antimicrobial activity was found positive against *Proteus Mirabilis*, **b & c;** using gentamycin, no antimicrobial activity was found positive against *M. Luteus* and *E.coli,* **d;** using augmentin, no antimicrobial activity was found positive against *Klebsella*, **e & f;** using ciprofloxacin, no antimicrobial activity was found positive against *B. Cereus* and *Salmonella Typhi.*

Table 2. Phytochemicals identified in GCMS analysis.

Sr #	Retention time (min)	Identified compound	Mol. formula	Mol. wt
1	6.39	Cyclohexanone,2-methyl-5-(1methylethenyl) also known as camphor	$C_{10}H_{16}O$	152
2	11.025	4,7,10-Hexadecatrienoic acid, methyl ester	$C_{17}H_{28}O_2$	264
3	12.247	9-octadecenoic acid , 2-hydroxy-3-[(1-oxooctadecyl)oxy]propyl ester	$C_{39}H_{74}O_5$	622
4	12.498	9-octadecenoic acid-2-(9-octadecenyloxy)ethyl ester	$C_{38}H_{72}O_3$	576
5	13.170	6,9,12,15-docosatetraenoic acid, methyl ester	$C_{23}H_{38}O_2$	346
6	16.177	2,4,6,8,10-tetradecapentaenoic acid,9a-acetoxy-1a,1b,4,4a,5,7a,7b,8,9,9a-decahydro-4a,7b-dihydroxy-3-(hydroxymethyl)-1,1,6,8tetramethyl-5-oxo-1Hcyclopropa[3,4]benz[1,2-e]azulen-9yl ester(limonoic acid)	$C_{36}H_{46}O_8$	606
7	19.429	9,12-octadecadienoic acid, methyl ester(methyl linoleate)	$C_{19}H_{34}O_2$	294
8	19.538	10-octadecenoic acid, methyl ester(methyl octadec-9-enoate)	$C_{19}H_{36}O_2$	296
9	19.959	Heptadecanoic acid,16-methyl-,methyl ester(methyl stearate)	$C_{19}H_{38}O_2$	298
10	20.991	9-octadecenoic acid, 2-hydroxy-1,3-propanediyl ester	$C_{39}H_{72}O_5$	620

Qualitative phytochemical analysis of *Lavandula stoechas L.*

The results of chemical tests of *Lavandula stoechas L.* ethanolic extracts revealed that it contained saponins, glycosides, phenols, tannins and terpenoids. The results of GCMS based qualitative analysis showed that 10 different phytochemicals were identified in the ethanolic extract as summarized in Table **2**. Fig. **3** depicts the GCMS spectrum of phytochemicals identified in the qualitative analysis. Of the 10 identified molecules, one was cyclohexanone, 2-methyl-5-(1methylethenyl) which is commonly known as camphor.

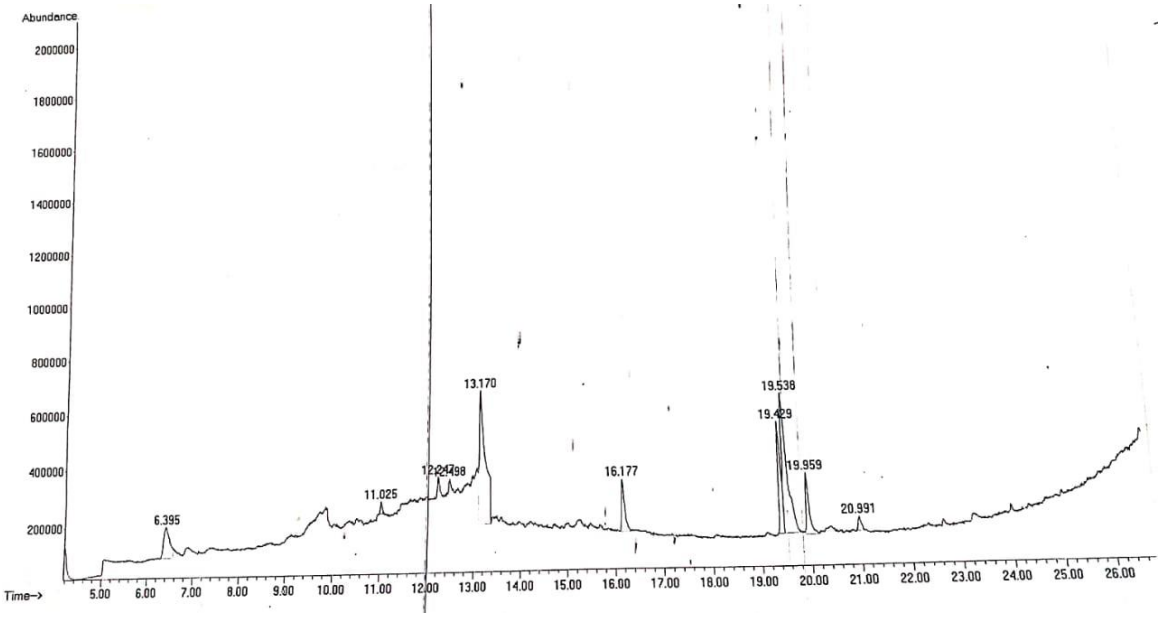

Figure 3. GCMS spectrum of phytochemicals identified in the qualitative analysis. The retension time mentioned on each peak corresponds to one phytpchemical.

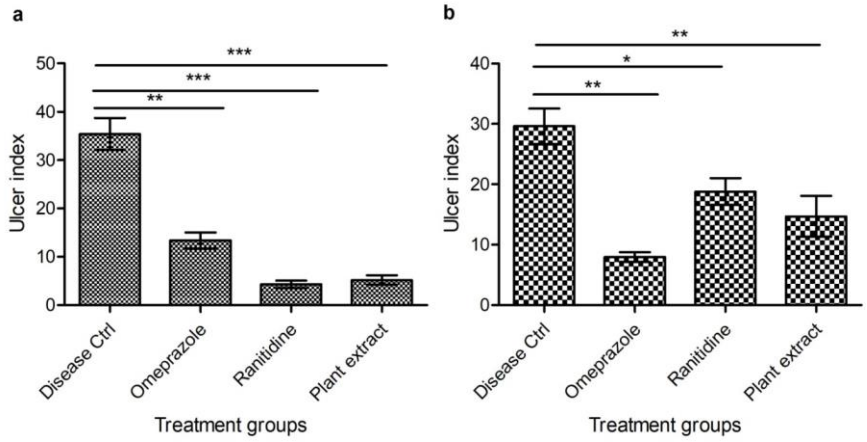

Figure 4. Effects of *Lavandula stoechas L.* aqueous extract on ulcer index: **a;** gastric ulcers, **b**; duodenal ulcers in ethanol-induced ulcer model of experimental rats. Values are expressed as Means ± SEM, n=5; One way ANOVA followed by Dunnett's multiple comparison test was used; *** = $p < 0.001$ (highly significant results); ** = $p < 0.01$ (very significant) & * = $p < 0.05$ (significant).

Gastroprotective effects of *Lavandula stoechas L.* extract

Oral administration of ethanol successfully induced the peptic ulcer in animals. Oral administration of *Lavandula stoechas L.* aqueous extract caused a significant reduction in ulcer index for both gastric ($P < 0.001$) and duodenal ulcers ($P < 0.01$) as compared to disease control as shown in Fig. **4a-b**. Administration of standard drugs; omeprazole and ranitidine also caused marked reduction in gastric and duodenal ulcers ($P < 0.05$).

The histological examination of rat stomach and duodenum revealed that the intraperitoneal administration of ethanol caused disruption in the mucosal epithelium as shown Fig. **5c-d**. Oral administration of plant extract markedly improved the mucosal integrity both in animal stomach and duodenum (Fig. **5e-f**). Moreover the standard drugs; omeprazole and ranitidine also caused significant improvement in gastrointestinal epithelial structure as depicted in Fig. **5i-j**.

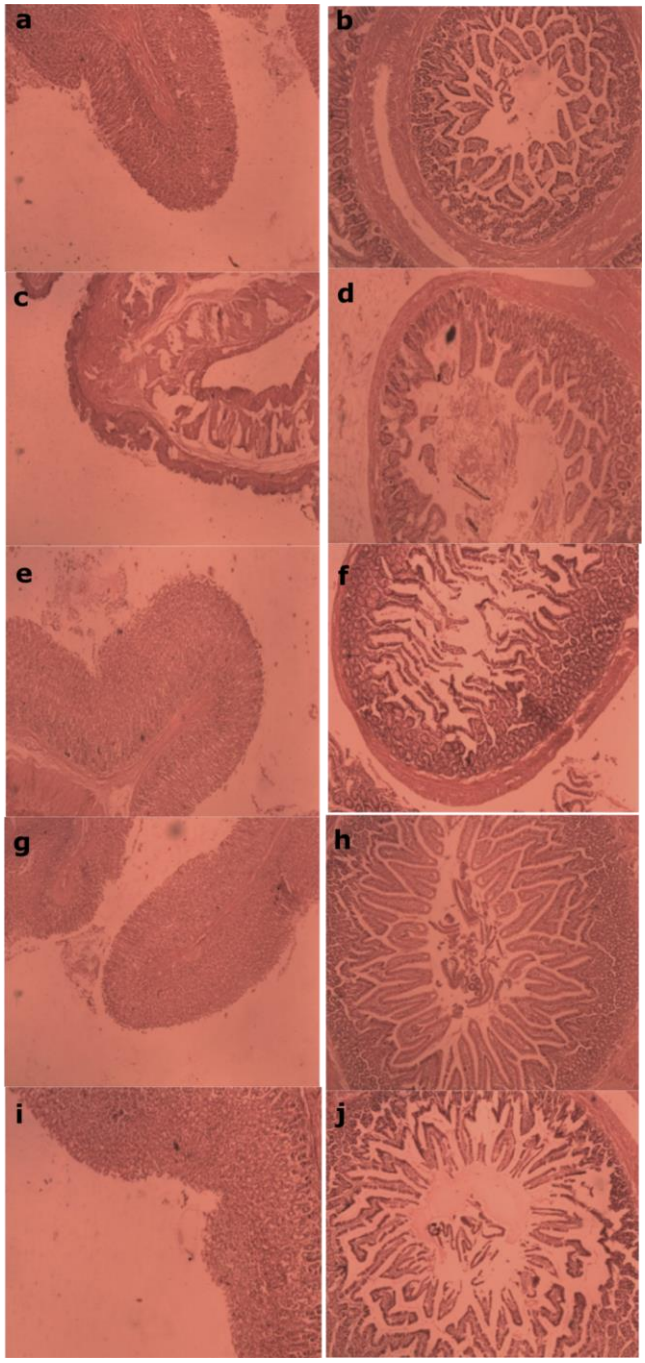

Figure 5. Histological examination of stomach and duodenum respectively from: **a-b**; Normal control group, **c-d**; Ethanol treated rats resulting in disruption of epithelium, **e-f**; Omeprazole treated rats resulting in normalization of epithelium, **g-h**; Ranitidine treated rats resulting in normalization of epithelium, **i-j**; Rats treated with *Lavandula stoechas L.* aqueous extract showing complete normalization of epithelial architecture.

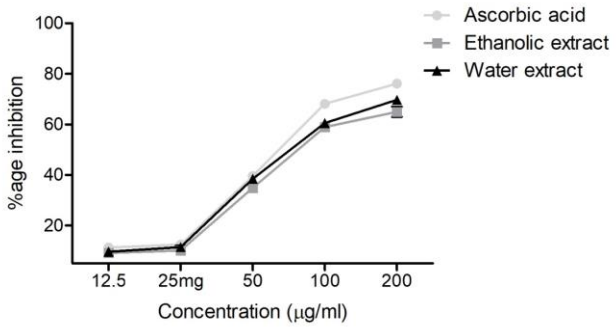

Figure 6. Showing mean % inhibition of ascorbic acid and *Lavandula stoechas L.* aqueous & methanolic extracts by using DPPH method at various concentrations. X-axis shows different concentration (µg/mL) and y-axis shows % inhibition. Values are expressed as Means ± SEM; n=5.

In-vitro antioxidant activity of *Lavandula stoechas L.* extract

In-vitro free radical scavenging activity of *Lavandula stoechas L.* aqueous and methanolic extracts was determined in context of their ability to reduce the DPPH, a stable free radical using ascorbic acid as reference standard. Both the aqueous and methanolic extracts showed strong concentration dependent DPPH reducing activity (Fig. **6**).

DISCUSSION

Peptic ulcer disease is characterized by the erosion of gastric and duodenal mucosa. It affects 5% of the world population with continuously increasing disease incidence every year. The etiology of the peptic ulcer disease is not completely understood however; alcohol, smoking, stress and nutritional deficiencies are major contributors to the disease [30]. *The use of H_2 receptor antagonist* (H_2RAs) and PPIs h*ave significantly decreased the incidence of ulcerative complications [31] however; both may cause* adverse effects (drug allergy, collageneous colitis and interstitial nephritis) [32]. A number of studies have been conducted on naturally occurring medicinal plants containing essential ingredients that can be used in the treatment of peptic ulcer. *Lavandula stoechas L.* is a medicinal plant and its hydroalcoholic extract possessed antioxidant and intestinal anti-inflammatory activity thus, can improve the healing process as well as intestinal epithelial barrier [33]. The present study was conducted to investigate the anti-ulcer potential of *Lavandula stoechas L.* extracts

in animal model of ethanol-induced peptic ulcer and to explore novel treatment strategies for peptic ulcer. Phytochemical analysis, antimicrobial and free radical scavenging activity of *Lavandula stoechas L.* extracts were also evaluated.

Antimicrobial activity of *Lavandula stoechas L.* extract against various periodontal bacterial strains was evaluated by well diffusion method. The ethanolic extract of *Lavandula stoechas L.* showed antimicrobial activity against *Proteus mirabilis* as confirmed by (3 mm) zone of inhibition while it was completely ineffective against *B. cereus, E.coli, Klebsiella, M. luteus*, and *S. typhi* (Fig. **1**). Moreover, the water extract of the *Lavandula stoechas L.* did not show any antimicrobial activity against any of the bacterial species used (Fig. **2**). The results of antimicrobial activity, obtained in the present study, were consistent with existing literature for instance the bacteriostatic activity of essential oil of the *Lavandula stoechas L.* has already been reported against periodontal bacteria [34].

The phytochemical analysis of *Lavandula stoechas L.* ethanolic extract performed in the present study revealed that it contains saponins, glycosides, steroids, phenols, tannins, and terpenoids (Table **1**). In order to investigate the possible phytochemicals responsible for antimicrobial activity of ethanolic extract, GCMS based assay was performed which showed that ethanolic extract contained 10 different novel constituents (Table **2**). Of these 10 constituents, one was cyclohexanone,2-methyl-5-(1methylethenyl) which is commonly known as camphor. The results of phytochemical analysis correlated well with previously reported studies such as gas chromatographic analysis of *Lavandula stoechas L.* extract revealed the presence linalyl acetate, γ-terpinene and camphor [35]. According to literature, antipruritic and antimicrobial activity of camphor has been well documented. So, it is conceivable the antimicrobial activity of *Lavandula stoechas L.* extract against *Proteus mirabilis* might be due to camphor.

In the present investigation, oral administration of ethanol successfully induced the peptic ulcer in experimental rats [35]. However, oral treatment of rats with *Lavandula stoechas L.* prior to induction of disease protected the animals against both ethanol-induced gastric ($P < 0.001$) and duodenal ($P < 0.01$) ulcers as compared to disease control (Fig. **4a-b**). In addition, the standard drugs; i.e. omeprazole and ranitidine also protected the animals against peptic ulcer. The exact mechanism how ethanol causes gastric lesions is not fully understood [9, 10] however, ethanol is reported to produce a disturbance in gastric mucosal barrier via exfoliation of cells [11, 35] followed by the recruitment of inflammatory cells to the site of injury and production of reactive oxygen species (ROS). As ROS and other mediators of inflammation may lead to oxidative damage [12, 36] therefore, by scavenging free radicals, antioxidants might prove as useful gastroprotective agents. In the present study, both the aqueous and methanolic *Lavandula stoechas L.* extracts showed strong free radical scavenging activity as determined by DPPH method (Fig. **6**) which was comparable with that of ascorbic acid (reference standard). It is conceivable that the gastroprotective effects of *Lavandula stoechas L.* aqueous extract were due to its free radical scavenging activity.

The above discussed gastroprotective effects were also supported by the histological examination of stomach and duodenum derived from animals. Ethanol treated rats showed necrosis of gastric epithelium, manifested by disappearance of nuclei and aggregation of inflammatory cells as compared to control group, that might be due to the formation of free radicals and oxidative stress induced by ethanol (Fig. **5c-d**) and this was quite consistent with the available literature [37]. In addition, treatment of the animals with *Lavandula stoechas L.* aqueous extract lead to restoration of gastric epithelium integrity (Fig. **5i-j**) which further confirmed the gastroprotective effects which were comparable with standard drugs (omeprazole & ranitidine). Our results were also comparable with previous reports of other natural gastroprotective agents i.e. *Acanthus ilicifolius, Anogeissus latifolia, Berberis vulgaris & Argyreia speciose etc* [38]. Based upon the findings of the present investigation, it is quite evident that *Lavandula stoechas L.* extract possessed antimicrobial activity against *Proteus mirabilis* and also proved effective against ethanol-induced ulcers in animal model.

CONCLUSION

It is concluded from the study that *Lavandula stoechas L.* methanolic extract posses antimicrobial activity against periodontal bacteria like *Proteus mirabilis* which might be due to the presence of camphor as confirmed by the GCMS based

phytochemical analysis. The aqueous extract of *Lavandula stoechas L.* also showed gastroprotective potential in ethanol-induced gastric and duodenal ulcer model of experimental animals as confirmed by histomorphological changes, those might be due to its strong free radical scavenging activity. In traditional medicine, bacterial spectrum is a step towards the utilization of plant extracts against specific bacterial infections. So, *Lavandula stoechas L.* containing pharmaceutical dosage forms need to be developed and tested against *Proteus* infection and peptic ulcer disease in animal models. Moreover, the efficacy of *Lavandula stoechas L.* against other bacterial strains still needs to be tested in future studies.

LIMITATION

Due to the limited financial resources and geographical area, the findings of this study could not be generalized. Therefore, there is a need to validate these findings on a larger scale before entering into preclinical trials. The literature available about other phytochemicals, identified in the GCMS spectrum, is very limited so, these compounds need further testing and evaluation.

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