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Antibacterial activity of saffron stigma and leaf extracts against human pathogenic bacteria

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ABSTRACT

Pathogenic microbes are detrimental to human health. On the other hand, several drugs and antibiotics have already been losing their effectiveness in killing the pathogens. Therefore, it is imperative to explore new drugs using extracts of medicinal plants with improved antimicrobial activity and relatively less side effects. Thus, antibacterial activity of hydroethanolic extracts of saffron leaves and stigma have been studied against 06 Gram-negative and 03 Gram-positive human pathogenic bacterial strains. Herein, antibacterial activity of saffron stigma and leaf extracts were observed in different concentrations (5, 10, and 15 mg/ml) against 09 pathogenic bacterial strains. Results suggested that, both the extracts significantly ($p < 0.05$) retarded the growth of bacterial strains. Further, stigma extract was more effective against *S. flexneri*, *L. monocytogenes*, *S. aureus* and *K. pneumoniae*, where as leaf extract was more effective against the growth of *S. flexneri*, *S. aureus* and *S. pneumoniae*. However, higher concentrations of both the extracts inhibit growth of *P. aeruginosa*. Hence, further research is highly essential relating to the bioactive compound of extracts and its mode of action in inhibiting growth of pathogenic bacteria for drug development.

1. Introduction

Infectious diseases caused by pathogenic microbes such as bacteria, fungi, viruses and parasites are associated with health risks. In the recent decade, the severity of diseases and the pathogenicity of microbes are considered to be a major concern for medical sciences. According to a recent report, most infectious diseases account for nearly 4.3 million deaths in 2016 (World Health Organization, 2019). Now the use of antibiotics has been increased for treatment of various diseases. However, it may kill the infectious and residual microbes inside the body that are not fatal but helpful (Langdon *et al*., 2016). This can also cause serious allergies, vomiting, headache and swelling of the face. Furthermore, some antibiotics and life-saving drugs lose their effectiveness against many diseases as infectious bacteria and other microbes develop resistance to them (Zaman *et al*., 2017; Fair and Tor, 2014). Antimicrobial resistance (AMR) is considered to be a serious concern to public health, as it deals with microbial resistance to antibiotics or any effective treatment previously generated for those microbes. Globally, around 700 thousand deaths per year are due to antimicrobial resistance (Capozzi *et al*., 2019).

The side effects and resistance of microbes to antibiotics led to increased interest in new approaches of using medicinal plants for drug development (Muzaffar *et al*., 2016). Plant metabolites have significant antimicrobial properties and their anti-mutagenic properties prevent mutation in bacteria thereby reducing bacterial antibiotic resistance (Gupta and Birdi, 2017). Nowadays a number of plants have been recognized for their medicinal value and are used as source of various chemical compounds that act against pathogenic microbes. Mostafa *et al*. (2018) reported the antibacterial activity of ethanolic extracts of *Punica granatum*, *Syzygium aromaticum*, *Zingiber officinales, Thymus vulgaris* and *Cuminum cyminum* against

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Staphylococcus aureus, *Bacillus cereus*, *Escherichia coli*, *Salmonela typhi* and *Pseudomonas aeruginosa*. Methanolic extracts of *Oxalis corniculata*, *Artemisia vulgaris*, *Cinnamomum tamala* and *Ageratina adenophora* showed variable antibacterial efficiencies against *Escherichia coli*, *Salmonela typhi*, MDR *Salmonela typhi*, *Klebsiella pneumoniae*, *Citrobacter koseri,* and *Staphylococcus aureus* (Manandhar *et al*., 2019). Gonelimali *et al*. (2018) investigated the antibacterial activity of ethanolic and aqueous extract of *Hibiscus sabdariffa*, *Rosmarinus officinalis*, *Syzygium aromaticum* and *Thymus vulgaris* against some food poisoning bacteria viz., *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enteritidis*, *Vibrio parahaemolyticus* and *Pseudomonas aeruginosa*.

The medicinal saffron plant (*Crocus sativus* L.) is a monocot, sterile, triploid plant, that belongs to the family, Iridaceae and isvegetatively propagated by corms. Saffron stigmas contain more than 150 potential chemical compounds such as carotenoids, precursor compounds of many apocarotenoids such as crocin, picrocrocin, and safranal (Shahi *et al*., 2016). However, recent reports suggested that other parts of the plant (petals, leaves and corms) also contains a number of chemical compounds (Maqbool *et al*., 2022). The medicinal values of many chemical compounds such as anti-inflammatory, anti-depressantant neuro protective, antioxidant & memory enhancing effect, cytotoxic and anti-cancer effect, and antibacterial effect has already been discovered by many researchers (Hosseinzadeh and Younes., 2002; Feizzadeh *et al*., 2008; Zhang *et al*., 2013; Nam *et al*., 2010; Potnuri *et al*., 2018; Papandreou *et al*., 2011; Arzi *et al*., 2018; Samarghandian *et al*., 2013; Aung *et al*., 2007; Nair *et al*., 1995; Pintado *et al*., 2011). Recent studies reported that saffron leaves contain high phenolic compounds, organic acids, naringenin, quercetin and apigenin which has antibacterial activity against different pathogenic bacteria (Jadouali *et al*., 2018; Mykhailenko *et al*., 2021). On account of that, an attempt has been made to evaluate the antibacterial activity of ethanolic extracts of saffron stigma and leaves against nine human pathogenic bacterial strains.

2. Materials and methods

2.1. Collection of plant materials

Saffron corms were collected in the month of June 2022 from the Saffron research station, in Kashmir. The disease-free corms were subsequently soaked in 0.2% of Bavistin fungicide solution and dried for 2-3 h. Then the corms were incubated at 25^{0}C under dark conditions at 85 ± 2% humidity for 3 months (Eftekhari *et al*., 2023). Around 2.5-3 cm larger corms were planted in pots. The pots were kept inside the greenhouse with temperature 20^{0}C in day and 17^{0}C at night and watered every two days. The flowers appeared in the last week of August after the shoot heighted about 1-3 cm. Then the flowers were harvested and the stigma was plucked up followed by shade dried at room condition. The vegetative growth of plants was observed after flowering & the leaves were collected after 45 days and subjected to shade drying followed by oven-drying at 50-60^{0}C for 72 hrs.

2.2. Preparation of ethanol extract of leaves and stigma

Properly dried stigma and leaves were used for the preparation of ethanol extract. Each sample was macerated separately with mixtures of aqueous and ethanol (8:2) at a concentration of 1 gm / 20ml. The ground materials were further centrifuged at 3000 rpm for 15 min, and the supernatants were collected. The process was repeated two times & both the extracts were evaporated and dried under reduced pressure at 40^{0}C using a rotary vacuum evaporator (Fig 1). Crude extracts were freeze-dried and stored at -20^{0}C until further use. The yield percentage (%) of both extracts were calculated by the formula (Mostafa *et al*., 2018).

Yield percentage of extract (%) = Weight of extract after evaporation of the solvent (W_1) X 100 / Dry weight of plant raw sample (W_0)

2.3. Bacterial strain used in this study

The antibacterial activity was investigated against nine bacterial species. Among them, six strains were gram negative (*Shigella flexneri* MTCC 1457, *Escherichia coli* ATCC 25,922, *Salmonella typhimurium* MTCC 3224, *Klebsiella pneumoniae* MTCC 3384, *Pseudomonas aeruginosa* Chl01, *Escherichia coli* K12SMTCC 728) and three were gram positive bacteria (*Listeria monocytogenes* MTCC 1143, *Streptococcus pneumoniae* MTCC 1936, *Staphylococcus aureus* ATCC 25,923) collected from Imgenex India, Bhubaneswar and Environmental microbiology laboratory, Ravenshaw University, Cuttack, Odisha. These bacterial strains cause several diseases in human shown in (Table 1).

2.4. Preparation of inoculums

Bacterial strains were cultured in nutrient broth (LB medium) overnight in an incubator shaker at 37^{0}C. According to 0.5 McFarland standards, each strain culture was adjusted to 108 CFU/ml.

2.5. Antibacterial activity

The in vitro antibacterial activity was carried out by the agar well diffusion method in LB agar plates. The nutrient agar plates were inoculated with 100µl of each microbial suspension and spread uniformly by using a sterile spreader.

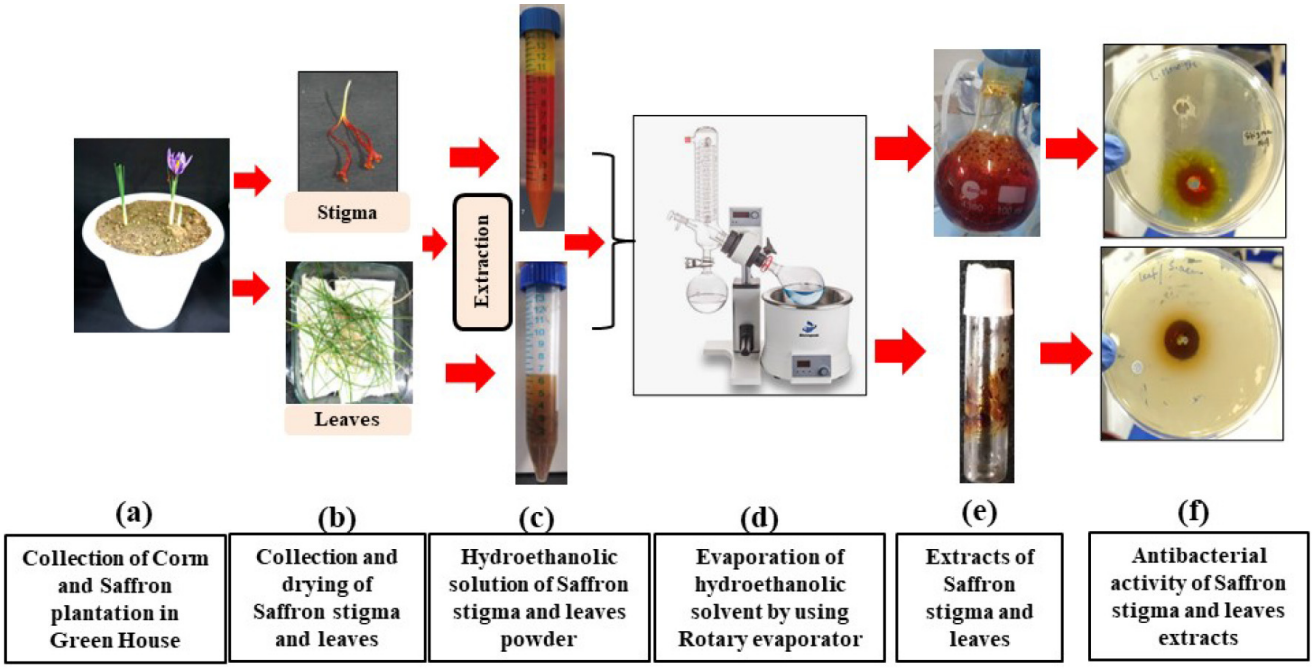

Figure 1: Various steps of hydroethanolic extracts preparation of saffron stigma and leaves to study the antibacterial activity.

Table 1:

List of bacterial disease in human and their causal agent

Bacterial strain name	Gram +/-	Causing disease in human	Reference
Shigella flexineri	Gram (-)	Cause diarrhoea in human	Zaidi and Estrada-Gracia, 2014
Escherichia coli	Gram (-)	Cause bloody diarrhea, urinary tract infections, meningitis etc.	Clements *et al.,* 2012
Listeria monocytogenes	Gram (+)	Causes listeriosis	Jemmi and Stephan, 2006
Staphylococcus aureus	Gram (+)	Cause skin infections	Kobayashi *et al.,* 2015
Streptococcus pneumoniae	Gram (+)	Cause of pneumonia	Weiser *et al.,* 2018
Klebsiella pneumoniae	Gram (-)	Cause pneumonia, urinary tract infections, blood stream infections, sepsis	Bengoechea and Sa Pessoa, 2019
Salmonella typhi	Gram (-)	Causative agent of typhoid fever	Kidgell *et al.,* 2002
Pseudomonas aeruginosa	Gram (-)	Cause nosocomial infections	Fazeli *et al.,* 2012

Then 6 mm diameter of well was prepared using a sterile tip. Stock solutions were prepared by dissolving each extract in DMSO. Then three different concentrations (5, 10, 15 mg/ml) of stigma and leaf extracts were added to the well and incubated at 37°C for 12-18 hrs. Standard antibiotics such as ampicillin and gentamycin were used as positive control while DMSO as negative control. The positive antibacterial activity was recorded on the basis of growth inhibition.

2.6. Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) is the lowest concentration of antibacterial substance that inhibits the visible growth of microorganisms tested (Balouiri *et al.,* 2016). In this study, MICs for leaf and stigma extract were evaluated by the macro broth dilution method (Motamedi *et al.,* 2010). In macro dilution method, concentrations of leaf and stigma extracts ranged from 0 to 50 mg/ml were added to tubes in reference to the concentration of extract responsible for the production of inhibition zones in the antibacterial assay. Each tube containing 1 ml of nutrient medium was inoculated with standardized bacterial suspension adjusted to the 0.5 McFarland scale and incubated at 37°C for 12-18 hrs.

2.7. Statistical analysis

Here, all the experiments were carried out with three replicates, and the data were represented as mean ± standard error of the mean. Results were analyzed through a two-way analysis of variance and the mean was compared by performing Tukey's multiple comparisons test (GraphPad Prism 8.0.1.244). The significant difference was considered at $p< 0.05$.

3. Result and Discussion

3.1. Yield of plant extract

The hydroethanolic extracts of stigma and leaves were harvested from saffron plants (Fig 1). 10 g of leaf and stigma extract of saffron plant sample yielded 4.53 g and 3.12 g of residue from the stigma and leaves extract respectively. So, the yield of stigma extract was comparatively higher than leaf extract.

3.2. Antibacterial activity

Saffron leaves and stigma extract residues were evaluated for antibacterial activity against nine human pathogenic bacterial strains. The antibacterial activity was determined by using agar well diffusion method and effect of both the extracts were observed at concentrations of 5, 10, and 15 mg/ml for all the bacterial strains. The results obtained from this assay revealed that both the extract significantly retarded the growth of bacterial strains (Table 2).

Stigma extract was significantly more effective ($p < 0.05$) against the growth of *S. flexneri, E. coli, L. monocytogenes, S. aureus, S. pneumoniae* and *K. pneumonia* (Fig 2 and 3). The increasing concentration of extracts correspondingly increased the inhibition of bacterial growth. Motamedi *et al.* (2010) also evaluated the antibacterial activity of ethanolic extract of *C. sativus stigma* at a concentration of 50, 100, 200, and 400 mg/ml against *Brucella melitensis*. Effective antibacterial activity of ethanolic extract of stigma (7.5 mg/ml) was observed by against *E. coli* and *S. aureus*, while no antibacterial activity was observed against *K. pneumoniae* (Mir *et al.,* 2011). This may be because of the use of saffron from a different locality. The climatic conditions might involve differences in the effectiveness of saffron extracts against pathogenic bacterial species.

Stigma of saffron is the source of a wide variety of chemical compounds and among them, crocin, picrocrocin, and safranal contribute the important active constituents (Carradori *et al.,* 2016; Shahi *et al.,* 2016; Gohari *et al.,* 2013; Srivastava *et al.,* 2010). Safranal and crocins are volatile and

Table 2:

Antibacterial activity of saffron leaf and stigma hydroethanolic extract against pathogenic bacteria; ND: Inhibion zone Not Determined, SF: *Shigella Flexneri*, EC: *Escherichia coli,* ECK *K12S*: *Escherichia coli K12S,* LM: *Listeria monocytogenes,* SA: *Staphylococcus aureus*, SP: *Streptococcus pneumoniae*, KP: *Klebsiella pneumoniae,* ST: *Salmonella typhi,* PA: *Pseudomonas aeruginosa*

Bacterial strain	Antibacterial activity (mm)					
	Stigma extract	Leaf extract	Stigma extract	Leaf extract	Stigma extract	Leaf extract
	5 mg/ml		10 mg/ml		15 mg/ml	
SF	4.1±0.231^{ab}	6.2±0.265	6.833±0.203^{a}	10.4±0.321^{b}	10.467±0.481	15.333±0.273
EC	3.067±0.145^{a}	ND	5.267±0.176	1.2±0.153	8.467±0.433	3.4±0.1
ECK*K12S*	ND	1.667±0.285	2.067±0.145	4.867±0.176	3.3±0.265^{a}	8.167±0.176
LM	6.267±0.233^{cd}	ND	10.067±0.437^{cd}	ND	15.233±0.260^{cd}	ND
SA	4.367±0.285^{b}	4.067±0.318^{a}	8.033±0.353^{ab}	9.133±0.463^{a}	13.567±0.348^{b}	13.733±0.26^{a}
SP	6±0.404^{c}	4.267±0.233^{a}	9.2±0.346^{bc}	9.7±0.265^{ab}	14.9±0.265^{cd}	14.133±0.26^{a}
KP	6.333±0.41^{cd}	ND	9.433±0.291^{cd}	ND	14.233±0.318^{bc}	ND
ST	1.367±0.176	1.167±0.167	3.433±0.273^{A}	3.1±0.173	4.233±0.176^{aA}	5.867±0.47
PA	ND	ND	ND	ND	ND	ND

Values in the table are means ± Standard deviation of three replicates (n = 3)

Values with different lowercase letter (a-d) in the same columns differ significantly ($p < 0.05$)

Values with same uppercase letter (A) in the same row not significantly differ from each other

water-soluble compounds, thus can easily reach pathogenic bacteria and inhibit their growth (Pintado *et al.,* 2011). The maximum inhibition of bacterial growth by stigma extract was obtained with *L. monocytogenes* (6.267±0.233 mm) and *K. pneumoniae* (6.333±0.41 mm) at a concentration of at 5 mg/ml. Even at higher concentrations of stigma extract (10 and 15 mg/ml), insignificant level of inhibition (3.433±0.273 and 4.233±0.176 respectively) was seen with *S. typhimurium*. *Escherichia coli* K12S showed resistance against saffron stigma extracts at a concentration of 5 mg/ml, while its growth inhibition was observed at higher concentrations of 10 and 15 mg/ml.

The selection of an appropriate solvent also plays a crucial role in extracting compounds of interest from the sample (Truong *et al.,* 2019). Shahidi *et al.,* (2008) reported that polar solvents are best forextraction of effective active constituents from saffron. Many studies reported that, polar solvents such as methanol, acetone, ethyl acetate, ethanol, distilled water, etc. have been used for the extraction. Ethanol has higher polarity than methanol, acetone and ethyl acetate. Methanol also has bleaching properties as it reduces the colouring content of the extract (Sani and Mohseni, 2014). It was also reported that a mixture of aqueous and ethanol is considered as most effective in extracting crocin, picrocrocin, and safranal from saffron (Gazerani *et al.,* 2013). Herein, ethanol was taken as a solvent for extraction of both the saffron stigma and leaf sample.

The leaf extract wassignificantly more effective against the growth of *S. flexneri, S. aureus* and *S. pneumonia* ($p < 0.05$) in comparison to other bacterial growth (Fig 4 and 5). The maximum inhibitory zone at 5 mg/ml of leaf extract was found in S. flexneri (6.2±0.265 mm), followed by *S. aureus* and *S. pneumoniae* formed 4.067±0.318 and 4.267±0.233 mm of inhibitory zone respectively. The minimum inhibition zone was found in *S. typhimurium* (1.167±0.167) and *Escherichia coli* K12S (1.667±0.285 mm). The antibacterial activity of ethanolic extract of leaves at a concentration of 100 mg/ml against *S. aureus* was reported by Okmen *et al.* (2016). However, Vahidi *et al.,* (2002) didn't observe any antibacterial activity against *S. aureus* and *E. coli* in response to ethanolic extract of leaves at 100 mg/ml concentration. Increased concentrations of methanol extracts of leaves showed effective antibacterial activity against *Listeria* spp. (Jadouali *et al.,* 2017). Leaves of Saffron constitute a source of bioactive compounds with different physiological activities and possible applications. Crocus leaves have a higher percentage of protein, lipids, total carbohydrates and total phenolic content than those of the petals. The leaf extract also exhibited higher antioxidant capacity (Jadouali *et al.,* 2017). Mykhailenko *et al.,* (2021) recently reported 16 compounds from saffron leaf extracts among which two major active compounds, mangiferinand isoorientin were found. They also identified some unique compounds such as tectoridin, iristectorigenin B, nigricin, and irigenin in leaves of saffron.

Some bacterial strains such as *L. monocytogenes, K. pneumoniae* and *P. aeruginosa* showed resistance to leaf extract at the supplemented concentrations. However, one bacterial strain *P. aeruginosa* exhibited resistance against stigma extract. Earlier reports suggested that *P. aeruginosa* is resistant to most of the available antibiotics (Tummler, 2019). Mir *et al.* (2011) also observed no antibacterial activity of the ethanolic extract of stigma against *P. aeruginosa*. Whereas, significant antibacterial activity was reported against *P. aeruginosa* at 1000 µg/disk concentrations of petroleum ether and methanolic extracts of stigma respectively (Muzaffer *et al.,* 2016). Mykhailenko *et al.* (2021) recently reported the effect of ethanolic and water extracts of Saffron leaves from Ukraine showed significant antibacterial activity against *Bacillus subtilis, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa* and *Candida albicans.* Jadouali *et al.* (2018) reported that methanolic extract of saffron leaves from Morocco plants did not show antibacterial activity against *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa.* As a result, it may be inferred that saffron plants from different regions may have varying levels of efficiency in reducing bacterial growth.

Significant antibacterial activity was observed by ampicillin and gentamycin against all bacterial strains whereas; DMSO did not interfere with the growth of bacterial strains. As the concentration of extracts increased, antibacterial activity also increased significantly against bacterial strain ($p < 0.05$). Further, similar observations were also reported (Soureshjan and Heidari, 2014; Muzaffar *et al.,* 2016).

3.3. Minimum inhibitory concentration

Minimum inhibitory concentrations (MIC) of plant extract means a minimum concentration of extract to inhibit the growth of bacterial strain (Balouiri*et al*., 2016). MIC of both the extract was measured for all the bacterial strains shown in (Table 3). Low concentrations of stigma extract were determined as MIC against *S. flexneri*, *K. pneumoniae, S. pneumoniae* and *L. monocytogenes* (3.5 mg/ml, 4.5±0.289 mg/ml, 5.333±0.441mg/ml, and 5.5±0.289 mg/ml respectively). The inhibitory effect of leaf extract is most effective against *S. flexneri*, *S. aureus,* and *S. pneumoniae*, so the MICs of the extract are less for these strains (4.5±0.289 mg/ml, 5±0.289 mg/ml, and 5.5±0.289 mg/ml respectively). The MIC of stigma

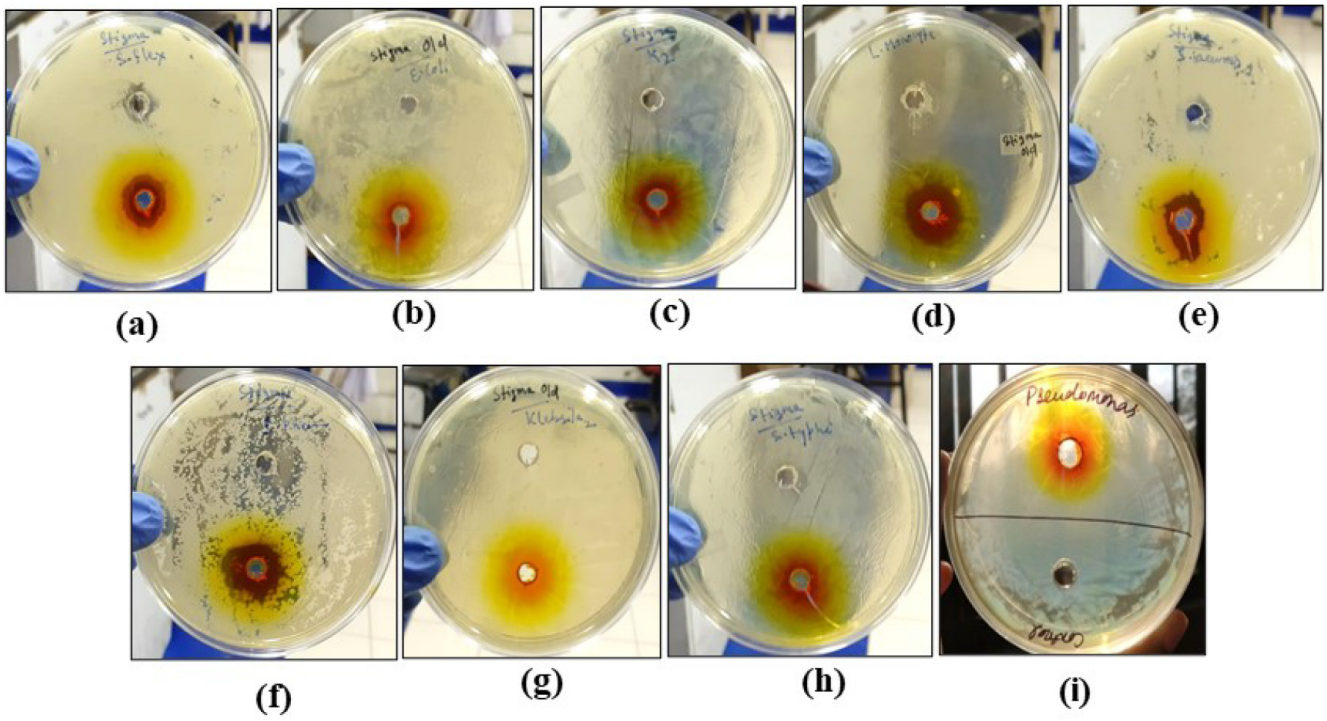

Figure 2: Antibacterial activities of ethanolic extract of stigma using agar well diffusion method showing inhibition zone; (a) *Shigella Flexneri,* (b) *Escherichia coli,* (c) *Escherichia coli K12S,* (d) *Listeria monocytogenes,* (e) *Staphylococcus aureus,* (f) *Streptococcus pneumoniae*, (g) *Klebsiella pneumoniae, (h) Salmonella typhi,* (i) *Pseudomonas aeruginosa*.

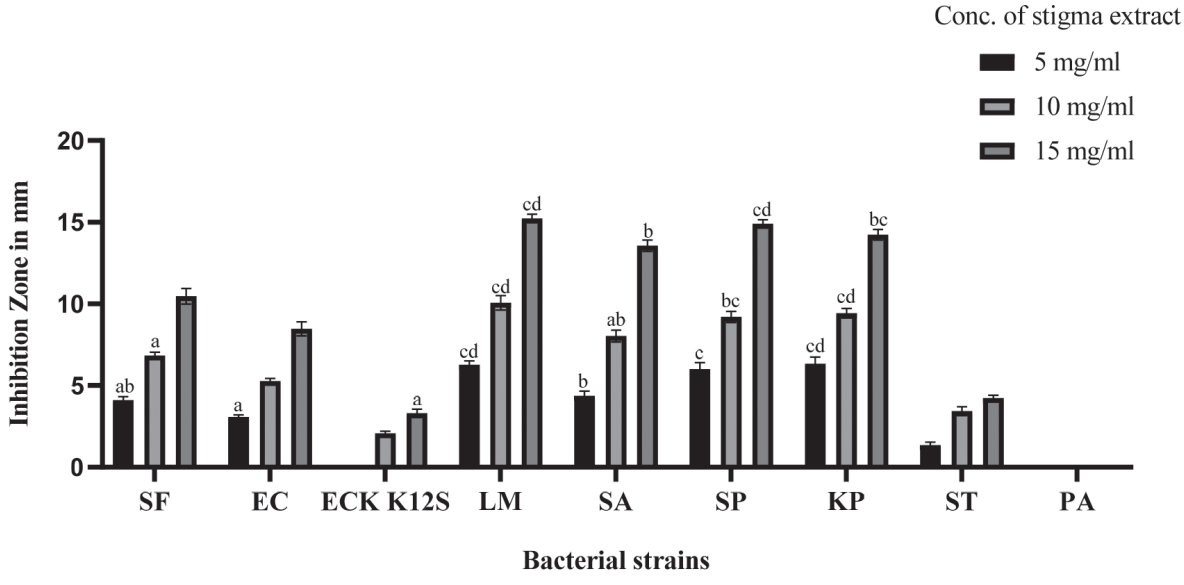

Figure 3: Antibacterial activity by stigma extract; SF: *Shigella flexneri*, EC: *Escherichia coli,* ECK K12S: *Escherichia coli* K12S, LM: *Listeria monocytogenes,* SA: *Staphylococcus aureus*, SP: *Streptococcus pneumoniae*, KP: *Klebsiella pneumoniae,* ST: *Salmonella typhi,* PA: *Pseudomonas aeruginosa*. Values with different lowercase letters (a-d) in the same concentration of extract differ significantly ($p < 0.05$).

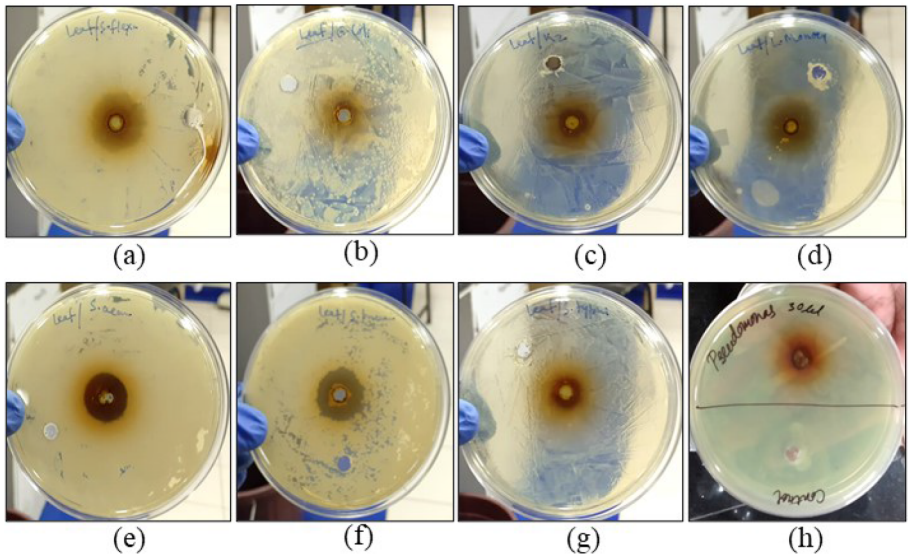

Figure 4: Antibacterial activities of ethanolic extract of leaves using agar well diffusion method showing inhibition zone; (a) *Shigella flexneri,* (b) *Escherichia coli,* (c) *Escherichia coli* K12S, (d) *Listeria monocytogenes,* (e) *Staphylococcus aureus,* (f) *Streptococcus pneumoniae*, (g) *Salmonella typhi, (h) Pseudomonas aeruginosa.*

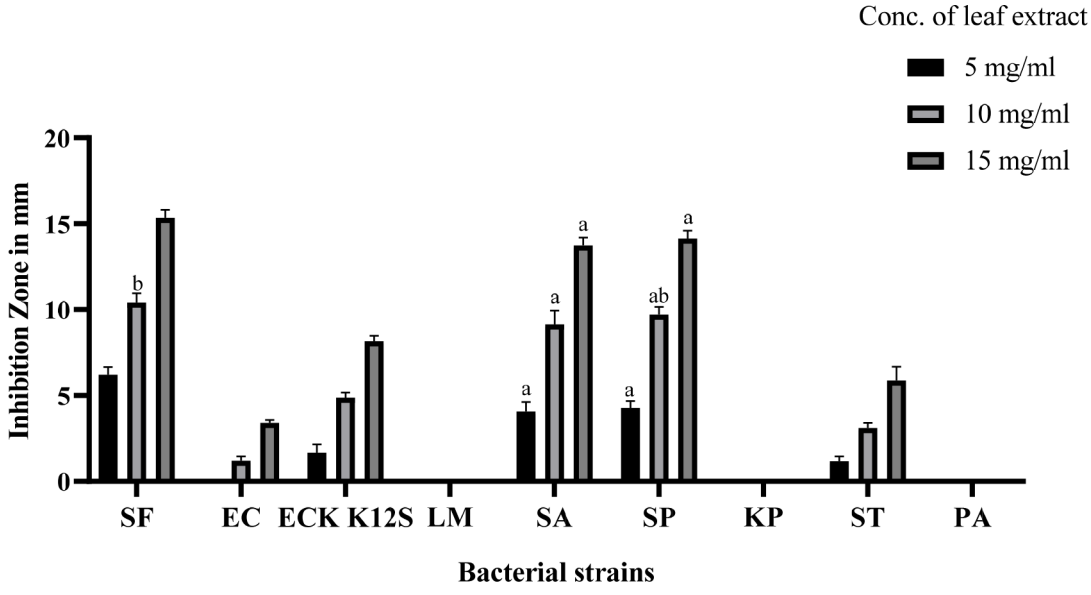

Figure 5: Antibacterial activity of leaf extract; SF: *Shigella flexneri*, EC: *Escherichia coli,* ECK K12S: *Escherichia coli* K12S, LM: *Listeria monocytogenes,* SA: *Staphylococcus aureus*, SP: *Streptococcus pneumoniae*, KP: *Klebsiella pneumoniae,* ST: *Salmonella typhi,* PA: *Pseudomonas aeruginosa*. Values with different lowercase letters (a-d) in the same concentration of extract differ significantly ($p < 0.05$).

extract was found at high concentrations for *P. aeruginosa* (35.500±0.289 mg/ml) whereas, the MIC of leaf extract was observed at high concentrations for *K.pneumoniae, P. aeruginosa*, and *L. monocytogenes* (34.667±0.333 mg/ml, 32±0.289 mg/ml, and 27.5±0.289 mg/ml respectively). Higher concentrations of stigma and leaf extracts were effective in reducing the growth of *P. aeruginosa*. A study reported the MIC of methanolic extract of stigma was 200 ± 0.45, 500 ± 0.45, 300 ± 0.25, and 400 ± 0.15 µg/ ml for *S. aureus*, *E. coli*, *P. aeruginosa* and *S. flexneri* respectively (Parray *et al*., 2015).

Table 3:

Minimum inhibitory concentration of saffron leaf and stigma hydroethanolic extract against pathogenic bacteria; ND: Inhibion zone Not Determined, SF: *Shigella Flexneri*, EC: *Escherichia coli,* ECK*K12S*: *Escherichia coli K12S,* LM: *Listeria monocytogenes,* SA: *Staphylococcus aureus*, SP: *Streptococcus pneumoniae*, KP: *Klebsiella pneumoniae,* ST: *Salmonella typhi,* PA: *Pseudomonas aeruginosa*

Bacterial strain	Minimum Inhibitory Concentration (MIC) (mg/ml)	
	Plant sample ethanolic extract	
	Stigma extract	Leaf extract
SF	3.5±0.00^{a}	4.5±0.289^{a}
EC	6.00±0.289^{bcd}	11.5±0.289
ECK*K12S*	12.667±0.167^{e}	9.5±0.289^{c}
LM	5.5±0.289^{bc}	27.5±0.289
SA	6.333±0.441^{bcd}	5±0.289^{ab}
SP	5.333±0.441^{b}	5.5±0.289^{ab}
KP	4.5±0.289^{a}	34.667±0.333
ST	12±0.289^{e}	8.5±0.289^{c}
PA	35.500±0.289	32±0.289

Values in the table are means ± Standard error of mean of three replicates (n = 3)

Values with different lowercase letter (a-e) in the same columns differ significantly ($p < 0.05$)

4. Conclusion

This study concluded that hydroethanol extract from both saffron stigma and leaf samples have significant anti-bacterial activity against several human pathogenic bacteria. A progressive increase in the concentration of both extracts resulted in a larger inhibition zone. However, the stigma extracts showed effective antibacterial activity by inhibiting the growth of a maximum number of pathogenic bacteria in comparison to the leaf extract. Hydroethanol was also an adequate solvent in extracting important bioactive compounds from saffron samples. Extracts of these two samples (leaf and stigma) of saffron plant can be analysed in future for further assessment of different bioactive compounds that may have applications in various pharmacological industries.

Disclosure statement

All the authors declare that there is no conflict of interest.

Authors contributions

Khirod Kumar Sahoo designed, conceptualized, supervised, and participated in writing the manuscript. Namita Muduli and Purusottam Ojha performed the experiments, analyzed and interpreted the data, and wrote the manuscript. All have given their consent for approval of the final publication of the manuscript.

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