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Antioxidant and Antibacterial Potential of Different Fractions from Roots of *Eriosema chinense* Vogel

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SKP and SH designed the protocol of the study. Authors SKP and DJ collected, authenticated the plant material and performed the analysis. Authors SKP and MK performed the antibacterial evaluation and drafted the initial manuscript. Author SH managed the analysis and finalized the final drafting of the manuscript. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aim: The roots of the plant *Eriosema chinense* Vogel (Leguminosae-Papilionoideae) is taken in the form of vegetable and is traditionally used for the treatment of diarrhoea by the tribal people of Meghalaya, India. Therefore, the present study is an attempt to assess the antioxidant and antibacterial potential of different fractions from roots of *Eriosema chinense* along with quantitative estimations of phytoconstituents.
Study Design: Extraction, fractionation, analysis and antibacterial evaluation.
Place and Duration of Study: Department of Pharmaceutics, Indian Institute of Technology, Banaras Hindu University, India between April 2012 to October 2012.
Methods: Different fractions i.e**.** aqueous, ethyl acetate, chloroform and hexane fractions were obtained from ethanol extract of roots of *Eriosema chinense* and were subjected to preliminary phytochemical screening and quantification of total phenols, tannins, flavonoid and flavonol. All the fractions were then evaluated for *in-vitro* antioxidant activity by using different models, which includes total antioxidant capacity, assay of reducing power, free radical scavenging activity, nitric oxide scavenging assay, H_2O_2 scavenging activity and scavenging of hydroxyl radical. The study also included assessment of antibacterial activity of all fractions against bacterial strains including those implicated in diarrhoea.

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Results: The chloroform fraction was found to be highly rich in flavonoids and phenols, which was followed by ethyl acetate fraction. In all the tested antioxidant models, chloroform and ethyl acetate fraction demonstrated the highest antioxidant potential as indicative through their IC_{50} values. All the fractions except aqueous fraction depicted a potent antibacterial activity at their respective higher concentration.
Conclusion: The antioxidant and antibacterial potential of *Eriosema chinense* may be attributed to the presence of phenols and flavonoids which plays a significant role in treatment of oxidative stress, cardiovascular arrests, inflammation, cancer and diarrhea.

Keywords: Antimicrobial; diarrhea; Escherichia coli; flavonoids; free radicals; Papilionoideae; roots.

1. INTRODUCTION

The plant *Eriosema chinense* Vogel (Leguminosae-Papilionoideae) is mainly distributed over the Eastern Himalayan region of India and China and is also found in countries like Thailand, Myanmar and Australia. The roots of the plant are eaten raw and in the form of vegetables by the people of Northern Australia and also in Meghalaya (India) [1], while traditionally, it is used by the tribal people of Meghalaya for the treatment of diarrhoea. A decoction prepared from grains is used as astringent, diuretic, tonic, in cold sweats and is used during parturition to promote discharge of the lochia. A decoction of the grains with powdered pepper is given for diarrhea [2-5]. Eight new prenylated flavonoids, khonklonginols A-H, together with six known compounds including five flavonoids, lupinifolinol, dehydrolupinifolinol, flemichin D, eriosemaone A, and lupinifolin, and one lignan, yangambin, have been isolated from this plant. Pharmacological investigations performed on the plant have demonstrated its potential cytotoxic and anti-tubercular activities [6].

In many developing as well as developed countries, chronic diseases, such as cardiovascular diseases and cancer have become leading causes of death. Epidemiological evidence shows that intake of diets which includes mainly fruits and vegetables are associated with reduced risk of these diseases. Fruits and vegetables rich in phenolic and flavonoids components are known to have potent antioxidant properties that decrease oxidative stress in consumers and therefore, are associated with various health benefits [7]. Some flavonoids have been isolated from the plant *Eriosema chinense*. The antioxidant potential of flavonoids has been proven through number of studies [8]. Also, the roots of the plant are traditionally used for the treatment of diarrhoea [2-5]. Literatures have reported that, pathogenic microorganisms play a significant role in causing diarrhoea [9]. *Escherichia coli* Migula is considered to be most common causative agent effecting around 2–5% in developed and 14–17% in developing countries. The other important microorganisms responsible for infectious diarrhoea includes *Campylobacter jejuni* Veron & Chatelain*, Salmonella typhi* Lignieres*, Shigella dysenteriae* Shiga and *Yersinia enterocolitica* Schleifstein & Coleman [10]. Therefore, the present investigation was undertaken to evaluate the antioxidant potential and further, determine the antibacterial efficacy of different fractions of roots from *Eriosema chinense* against various bacterial strains including those implicated in diarrhoea.

2. MATERIALS AND METHODS

2.1 Plant Material and Preparation of Fractions

The roots of *Eriosema chinense* Vogel were obtained from Jowai Area, Jaintia Hills District of Meghalaya (India) in May 2011 and were authenticated by Dr. B.K. Sinha (Scientist C, In charge), Botanical Survey of India, Shillong, India. For future reference, a voucher specimen (COG/EC/14) of the plant has been deposited in Department of Pharmaceutics, Banaras Hindu University, Varanasi, India. The roots of the plant (500 g) were grinded and extracted with ethanol (1.5 L) using Soxhlet apparatus until the whole drug was exhausted. Further, the extract (12.29% w/w) was subjected to fractionation using different solvents and four fractions were obtained which were concentrated and dried in a rotary evaporator under reduced pressure to minimize the residual toxicity. Thus, four fractions from the roots of *Eriosema chinense* i.e. hexane: 2.57% w/w (HFC), chloroform: 22.14%w/w (CEC), ethyl acetate: 9.14% w/w (EAEC) and aqueous: 14.87% w/w (AQEC) were obtained and stored in a desiccator until use.

2.2 Phytochemical Screening and Quantitative Estimations

Preliminary phytochemical screening of all the fractions for the presence of various phytoconstituents such as alkaloids, steroids, glycosides, phenols, flavonoids, carbohydrates, proteins and amino acids was carried out using usual procedures [11]. Total phenolic content in all the fractions from roots of *Eriosema chinense* Vogel was estimated according to the method described by Hagerman et al. [12] using Folin ciocalteau reagent. The reagent causes reduction of total phenols in the sample forming blue colour which gets detected at 765 nm. Total tannins content was also measured by adopting Hagerman et al. [12] method where absorbance was measured at 775 nm. For determining the total flavonoid and flavonol contents in fractions, method proposed by Kumaran & Karunakaran [8] were adopted using aluminum trichloride where absorbance was measured at 415 nm for flavonoid and 440 nm for flavonol.

2.3 *In vitro* Antioxidant Activity

Based upon the results obtained from the quantitative estimations, all the fractions were subjected to *in vitro* antioxidant activity by using different models which includes determination of total antioxidant capacity, assay of reducing power, free radical scavenging activity, nitric oxide scavenging assay, H_2O_2 scavenging activity and scavenging of hydroxyl radical by deoxyribose method [13-15].

2.3.1 Total antioxidant capacity

The total antioxidant capacity of all the fractions was estimated by Phosphomolybdenum method. In this method, 0.3 ml of fractions in methanol (1mg/ml), standard ascorbic acid (25-300 µg/ml) and blank (methanol) were combined with 3 ml of reagent mixture (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) separately and were incubated in a boiling water bath at 95ºC for 90 minutes. After cooling to room temperature, the absorbance of each sample was measured at 695 nm against the blank. The assay involves reduction of the Mo (VI) to Mo (V) by the fractions and subsequent formation of a green phosphate/Mo (V) complex at acidic pH.

2.3.2 Assay of reducing power

Potassium ferricynide method was adopted for performing the assay of reducing power using phosphate buffer (0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN_6)$] (10 g/l). The mixture was then incubated and mixed with trichloroacetic acid (100g/L), centrifuged and supernatant was mixed with 2.5 ml of distilled water and 0.5 ml $Fecl_3$ (1g/L). The standard used in this case was ascorbic acid and phosphate buffer was used as blank solution. Absorbance was measured at 700 nm, where increased absorbance of the reaction mixture indicated stronger reducing power.

2.3.3 Free radical scavenging activity

The free radical scavenging activity of all the fractions was measured by 1, 1-diphenyl-2-picryl-hydrazil (DPPH) method by using 100 µM/ml of DPPH in methanol. The absorbance of the reaction mixture was measured at 517 nm after 30 min. The free radical scavenging activity was calculated using the following equation: DPPH scavenging effect = $[(1-A_1/A_0)] \times 100$ Where A_0 is the absorbance of the blank and A_1 is the absorbance of test sample. Then % inhibition was plotted against respective concentrations used and IC_{50} was calculated by using ascorbic acid as standard.

2.3.4 Nitric oxide scavenging assay

Sodium nitroprusside in aqueous solution at physiological pH was used for carrying out the nitric oxide scavenging assay. This can be determined by the use of the Griess Illosvoy reaction in which 2 ml of 10 mM sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of fractions at various concentrations, incubated at 25ºC for 150 minutes and 0.5 ml was added into 1.0 ml sulfanilic acid reagent (33% in 20% glacial acetic acid) and was again incubated at room temperature for 5 minutes. Finally, 1.0 ml naphthylethylenediamine dihydrochloride (0.1% w/v) was mixed and maintained at room temperature for 30 minutes and absorbance was measured at 546 nm. The reaction spontaneously generates nitric oxide that interacts with oxygen to produce nitrite ions.

2.3.5 Scavenging of hydrogen peroxide

The method described by Jayaprakasha et al. [16] was used for determining the scavenging activity of different fractions of plant extract. A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffered saline (PBS, pH 7.4). To 1 ml of different concentrations of fractions and standards in methanol, 2 ml of hydrogen peroxide solution in PBS was added and finally the absorbance was measured at 230 nm after 10 minutes.

2.3.6 Scavenging of hydroxyl radical by deoxyribose method

For determining the hydroxyl radical scavenging activity of all the fractions different concentrations of fractions were treated with 1 mM $FeCl_3$, 1mM EDTA, 20 mM H_2O_2, 1 mM L-ascorbic acid, and 30 mM deoxyribose, 1 ml of 2.8% (w/v) trichloroacetic acid and 1 ml of 1% (w/w) 2-thiobarbituric acid in potassium phosphate buffer (pH 7.4). The reaction mixture was incubated for 1 hour at 37ºC, and further heated in a boiling water-bath for 15 minutes after addition of 1 ml of 2.8% (w/v) trichloroacetic acid and 1 ml of 1% (w/w) 2-thiobarbituric acid. The colour developed was measured at 532 nm against a blank containing phosphate buffer.

2.4 Antibacterial Activity

Antibacterial activity of different fractions from roots of *Eriosema chinense* Vogel was performed on four reference bacterial strains i.e. *Escherichia coli* Migula (ATCC 25922), *Shigella flexneri* Castellani & Chalmers (ATCC 12022), *Pseudomonas aeruginosa* Schröter (ATCC 27893), *Staphylococcus aureus* Rosenbach (ATCC 25323) and seven clinical bacterial isolates- *Salmonella typhi* Lignieres, *Shigella dysenteriae* Shiga, *Proteus vulgaris* Hauser, *Klebsiella pneumonia* Schroeter & Trevisan, *Shigella boydii* Ewing, *Bacillus cereus* Frankland & Frankland and *Enterococcus faecalis* Orla-Jensen. All the cultures used for the study were obtained from the American Type Culture Collection (ATCC), MTCC, clinical strains preserved at Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India. The antibacterial activity was screened using freshly prepared bacterial broth cultures. The fractions (500 mg) were dissolved in 5 ml of peptone water thus, giving a stock solution of 100 mg/ml. From these stock solution 5 µl was dispensed on a sterile disc of Whatman's falter paper no. 1 of 6 mm diameter for susceptibility testing.

Disc diffusion method was used for assessment of antibacterial activity, where Muller Hinton agar (MHA) plates were used as a nutrient medium. These plates were prepared by pouring 15 ml of molten media into sterile petri plates. Fresh bacterial strains were suspended in sterile saline and a concentration of 107 cfu/ml was achieved. The prepared suspension was then spread on the surface of MHA agar plates which was then allowed to dry for 5 min. Further, different concentrations of fractions to be tested, were put on 6 mm sterile disc of Whatman flter paper no. 1. The discs loaded with the fractions were then placed on the surface of the nutrient medium and the extract was allowed to diffuse for 5 min. Later on, the plates were kept for incubation at 37ºC for 24 hr and further inhibition zones around the discs were examined in triplicate using transparent ruler in millimeters.

The guideline proposed by National Committee for Clinical Laboratory Standards (NCCLS, 2000) was used for determining the MIC of fractions. For determining the MIC of the fractions, they were diluted with equal volume of nutrient broth which was mixed in wells of microtiter plate. In this method, 0.1 ml of standardized inoculums was added in each tube and the plates were incubated aerobically at 37ºC for 18-24 hr. The MIC of a particular fraction may be regarded as the highest dilution of the fractions i.e. lowest concentration producing no visible bacterial growth which was evident through no turbidity compared to the control [9,17]

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening and Quantitative Estimations

Phytochemical screening performed in the present study revealed the presence of flavonoids, phenols, tannins and steroids in all the fractions while alkaloids was found to be absent in aqueous fraction only. The aqueous and ethyl acetate fraction also showed the presence of saponins, carbohydrates, proteins and amino acids (Table 1). The total phenolic content estimated in the present study was reported to be AQEC: 28.19 mg/g, EAEC: 62.56 mg/g, CEC: 71.48 mg/g and HEC: 43.58 mg/g gallic acid equivalent. Total tannin calculated in different fractions was found to be AQEC: 18.26 mg/g, EAEC: 32.33 mg/g, CEC: 41.28 mg/g and HEC: 13.22 mg/g tannic acid equivalent. Total flavonoid quantified was AQEC: 64.26 mg/g, EAEC: 146.38 mg/g, CEC: 203.94 mg/g and HEC: 94.57 mg/g rutin equivalent

while total flavonol reported in all the fractions was AQEC: 4.27 mg/g, EAEC: 9.04 mg/g, CEC: 12.19 mg/g and HEC: 7.89 mg/g rutin equivalent respectively.

Table 1. Preliminary phytochemical analysis of different fractions of *Eriosema chinense* Vogel

Phytoconstituents	Fractions			
	AQEC	EAEC	CEC	HEC
Alkaloids	-	+	+	+
Steroids	+	+	+	+
Anthraquinone glycoside	-	-	-	-
Cardiac glycoside	-	-	-	-
Cyanogenetic glycoside	-	-	-	-
Saponin	+	+	-	-
Phenolic compound	+	+	+	+
Flavonoids	+	+	+	+
Tannins	+	+	+	-
Carbohydrates	+	+	-	-
Proteins	+	+	-	-
Amino acid	+	+	-	-

+ Indicates presence and – indicates absence. In table, AQEC: Aqueous fraction of Eriosema chinense Vogel, EAEC: Ethyl acetate fraction of Eriosema chinense Vogel, CEC: Chloroform fraction of Eriosema chinense Vogel and HEC: Hexane fraction of Eriosema chinense Vogel.

3.2 *In vitro* Antioxidant Activity

Free radicals and reactive oxygen species are involved in the normal physiology of living organisms, which under certain conditions (excess) can induce cellular damage resulting in several human diseases such as cancer, arteriosclerosis, inflammatory disorders as well as in ageing process. Recently, several dietary and herbal products having free radical scavenging potential have gained immense importance in treating such chronic diseases [18]. Therefore, in the present study, based upon the quantitative estimations, radical scavenging activity of different fractions of ethanol extract from roots of *Eriosema chinense* Vogel was studied using well known antioxidant assays.

The total antioxidant capacity of different fractions was determined using the linear regression equation of the calibration curve and was expressed as the number of equivalent of ascorbic acid. Among the tested fractions, CEC depicted the highest antioxidant capacity (97.416±2.166 µg/ml ascorbic acid equivalent) than the other fractions i.e. EAEC: 89.5±2.268, HEC: 76.0±2.32 and AQEC: 44.583±2.455 µg/ml ascorbic acid equivalent respectively. The reducing capacity of a compound may serve as an indicator of its potential antioxidant activity. The reducing capacity of an agent is manly associated with the presence of reductones that exerts antioxidant action by breaking the free radical chain through donation of a hydrogen atom. They also react with certain precursors of peroxide, thus preventing peroxide formation [8]. Our results depicted a concentration dependent reducing power of the tested fractions which was found to be higher in ascorbic acid followed by CEC, EAEC, HEC and AQEC in decreasing order, suggesting a potential antioxidant activity of the plant. This activity may be attributed to various mechanisms, such as prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging [8].

1, 1-diphenyl-2-picryl-hydrazil (DPPH) is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH is a purple colour dye having absorption maxima of 517 nm and its reducing capability is determined by the decrease in absorbance at same wave length induced by antioxidants, which on reaction with a hydrogen donor gets converted into 2, 2-diphenyl-1-picryl hydrazine with a disappearance of purple colour [8]. All the fractions tested in the present study demonstrated a considerable free radical scavenging activity as indicated by obtained IC_{50} values. Standard ascorbic acid was found to have highest IC_{50} value of 77.061±3.303 µg/ml followed by CEC (IC_{50}: 163.128±5.734 µg/ml), EAEC (IC_{50}: 219.85±3.030 µg/ml), HEC (IC_{50}: 247.822±2.093 µg/ml) and AQEC (IC_{50}: 312.127±11.752 µg/ml). Hydrogen peroxide though, itself is not very reactive, but sometimes may lead to cytotoxicity due to generation of hydroxyl radical in the cells. It has also been reported that, naturally-occurring iron complexes inside the cell react with H_2O_2 *in vivo* and generate highly reactive hydroxyl radicals which may attribute to toxic effects [15]. Therefore, its removal is very essential for antioxidant defense mechanism. From the results, it was noticed that all the fractions were capable of scavenging hydrogen peroxide in a concentration dependent manner. Rutin depicted the highest scavenging activity with an IC_{50} value of 87.125±7.961 µg/ml followed by CEC (IC_{50}: 187.661±5.358 µg/ml). The descending order of activity shown by all fractions was as follows: rutin> CEC> EAEC> HEC> AQEC.

Nitric oxide is highly unstable, which on reacting with oxygen molecule produces stable nitrate and nitrite that can be estimated by using Griess reagent. In the present study, CEC demonstrated a highly potent nitric oxide scavenging activity (IC_{50} value 201.827±4.763 µg/ml), whereas aqueous fraction showed the least scavenging activity (IC_{50} value 372.181±9.349 µg/ml). Nitric oxide plays pivotal role in various physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It also act as an effective molecule in diverse biological systems including neuronal messenger, vasodilatation, antimicrobial and antitumor activities. However, excess production of NO may result in several diseases [15]. Iron (II)–dependent deoxyribose damage assay was adopted for determining the effect of all fractions on inhibition potential of hydroxyl radical production. The Fenton reaction generates hydroxyl radicals (OH^-) that degrade deoxyribose using Fe^{2+} salts as an important catalytic component [14]. Oxygen radicals may attack the sugar, which leads to sugar fragmentation. The results revealed a potent hydroxyl radical scavenging activity of all fractions. As seen through the obtained IC_{50} values standard BHT (IC_{50}: 93.973±6.679 µg/ml) showed maximum activity which was followed by CEC (IC_{50}: 154.41±1.435 µg/ml), whereas AQEC depicted the least scavenging activity (Fig. 1).

The overall results depicted a potential antioxidant activity of CEC which was followed by EAEC that may be attributed to the presence of high quantity of phenols, flavonoids and. Tannins. These phytoconstituents prevents damage caused by free radicals such as nitric oxide, hydroxyl radical, and hydrogen peroxide to biological systems in human body. These free radicals combine with DNA nucleotides, thus causing carcinogenesis, mutagenesis, and cytotoxicity. Phytoconstituents such as phenolics, tannins, and flavonoids are strong reducing agents, hydrogen donors, singlet oxygen quenchers, and metal chelating agents that contribute in minimizing the oxidative stress by their scavenging actions due to presence of hydroxyl groups [13].

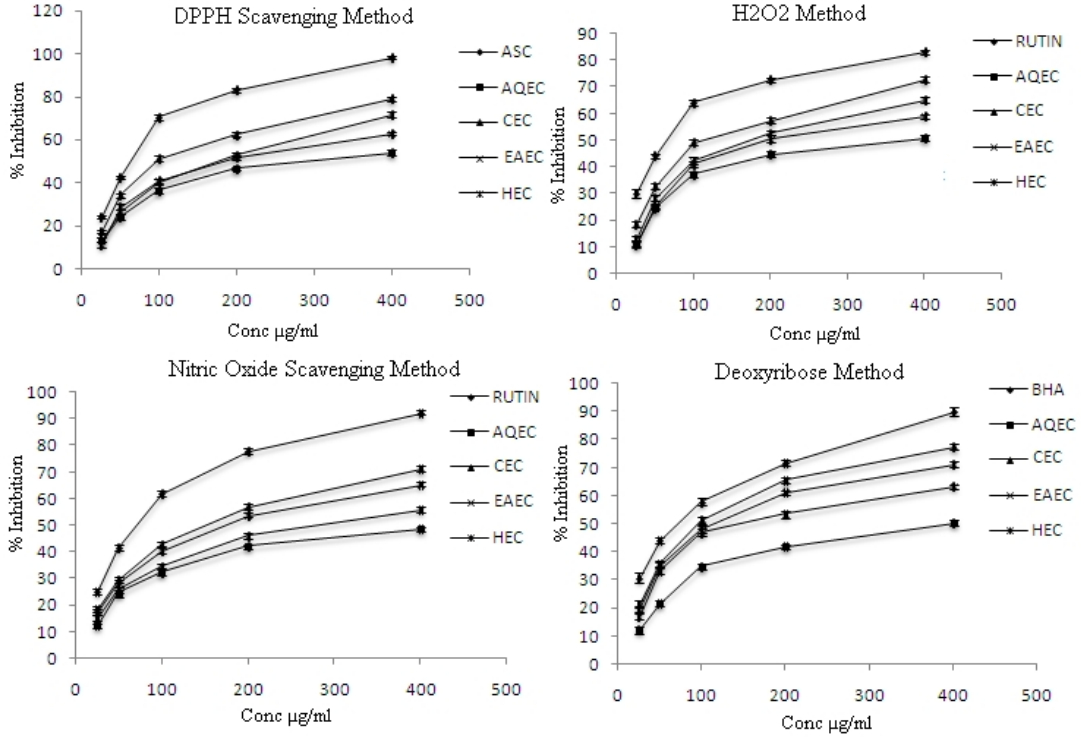

Fig. 1. *In vitro* antioxidant activity of different fractions of *Eriosema chinense* Vogel

Where Asc: Ascorbic acid, BHA: Butylated Hydroxy Anisole, AQEC: Aqueous fraction of Eriosema chinense Vogel, EAEC: Ethyl acetate fraction of Eriosema chinense Vogel, CEC: Chloroform fraction of Eriosema chinense Vogel and HEC: Hexane fraction of Eriosema chinense Vogel.

3.3 Antibacterial Activity

It has been observed that, plants generally survive from microbial attacks through development of various phytoconstituents which include both physical barriers as well as chemical ones, i.e. the presence or accumulation of antimicrobial metabolites [19]. Recently, plants having antimicrobial activities have gained immense importance since people are aware of problems associated with the over-prescription and misuse of traditional antibiotics. However, only approximately 20% of the plants found in the world have been evaluated for pharmacological or biological testing [20].

The roots of the plant *Eriosema chinense* Vogel have been traditionally used for treating diarrhoea. The plant has also been evaluated for potential cytotoxic and anti-tubercular activities [6]. Therefore, the present study deals with assessment of antibacterial activity of different fractions from roots against strains including those implicated for diarrhoea. All the fractions tested excluding AQEC, depicted a potent antibacterial activity against all the bacterial strains (Table 2).

Table 2. Effect of different fractions of *Eriosema chinense* Vogel on zone of inhibition (in mm) and MIC (mg/ml) against different bacterial strains

Strains		BC	SA	EF	SF	ST	SD	PV	EC	KP	PA	SB
Fractions (mg/ml)							**Zone of inhibition (in mm)**					
AQEC		-	-	-	-	-	-	-	-	-	-	-
EAEC	**50**	9.06±0.35	11.9±0.32	7.10±0.26	7.76±0.32	7.16±0.37	7.66±0.34	8.10±0.41	7.63±0.31	-	6.73±0.32	9.06±0.33
	100	12.36±0.28	14.36±0.29	8.73±0.24	10.26±0.29	11.16±0.26	10.86±0.31	11.06±0.32	10.33±0.34	7.66±0.34	10.03±0.38	11.8±0.32
CEC	**50**	8.96±0.33	11.0±0.36	8.0±0.32	7.3±0.40	7.33±0.32	7.30±0.36	8.46±0.42	8.26±0.34	9.70±0.35	9.53±0.33	8.90±0.40
	100	11.23±0.37	18.13±0.27	11.86±0.29	11.26±0.29	11.60±0.25	10.93±0.31	11.76±0.31	12.16±0.31	11.0±0.37	13.16±0.35	13.83±0.29
HEC	**50**	7.46±0.41	11.26±0.34	7.66±0.34	7.03±0.29	5.96±0.33	6.90±0.37	7.76±0.31	8.80±0.32	8.83±0.33	8.73±0.35	8.96±0.38
	100	11.06±0.35	12.93±0.26	12.0±0.32	9.73±0.28	7.23±0.23	8.96±0.34	11.1±0.35	12.86±0.27	10.8±0.32	12.23±0.23	12.16±0.31
Cpr	**0.5**	25.76±0.23	27.96±0.28	30.73±0.24	20.1±0.25	25.9±0.28	23.86±0.26	24.1±0.32	28.76±0.32	28.8±0.45	25.96±0.28	25.73±0.26
MIC (mg/ml)												
AQEC		-	-	-	-	-	-	-	-	-	-	-
EAEC		0.781	0.390	1.562	6.25	3.125	3.125	6.25	1.562	6.25	3.125	0.781
CEC		0.390	0.390	0.781	0.390	3.125	0.781	6.25	0.781	6.25	3.125	0.781
HEC		3.125	1.562	6.25	6.25	6.25	3.125	10	3.125	6.25	6.25	3.125

In table, AQEC: Aqueous fraction of Eriosema chinense Vogel, EAEC: Ethyl acetate fraction of Eriosema chinense Vogel, CEC: Chloroform fraction of Eriosema chinense Vogel, HEC: Hexane fraction of Eriosema chinense Vogel and Cpr: Ciprofloxacin while BC: B. cereus, SA: S. aureus, EF: E. faecalis, SF: S. flexneri, ST: S. typhi, SD: S. dysenteriae, PV: P. vulgaris, EC: E. coli, KP: K. pneumonia, PA: P. aeruginosa and SB: S. boydii.

Moreover, the effect was found to be more prominent in case of Gram positive bacteria compared to Gram negative bacteria. Among the tested fractions, CEC demonstrated the highest activity which was followed by EAEC at their respective higher concentrations which was indicated through the diameter of zone of inhibition. The MIC calculated, showed a wide range of activity of active fractions ranging from 0.390 to 10 mg/ml. Thus, the potential antibacterial activity of active fractions may be attributed to the presence of higher amount of flavonoids and phenols present in the active fractions which has already been reported in previous literatures [21]. It is assumed that, phenols get accumulated in and provide chemical barriers to invading microorganisms. It has been reported that flavonoids especially prenylated flavonoids as reported in *Eriosema chinense* Vogel [6] are highly effective against microorganisms because of the ease with which they penetrate bacterial cells. Studies have reported the antioxidant properties of flavonoids and their ability to reduce the risk of developing cardiovascular disease. They inhibit inflammation by decreasing the release of inflammatory mediators and stabilization of cell membranes and have also found to maintain capillary walls strength thus providing protection against infection [19].

4. CONCLUSION

With a potential antioxidant activity, the fractions have also depicted a potent antibacterial activity against bacterial strains responsible for causing diarrhoea. Pathogenic diarrhoea has become a global health concern in young children, which results in death due to dehydration and malnutrition, while in case of adults; it may lead to nutritional deficiencies and is responsible for around 3.3% of all deaths throughout the world [10,22]. Thus, the study justified the antioxidant and antibacterial potential of roots of *Eriosema chinense* Vogel which can be used as a potential tool in the treatment of disorders associated to oxidative stress, cardiovascular arrests, inflammation, cancer and diarrhoea.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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DECLARATION OF INTEREST

The authors report no conflicts of interest.

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