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Research article

Comparison of piperine content, antimicrobial and antioxidant activity of *Piper chaba* root and stem

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ABSTRACT

Piper chaba (locally known as "Choi Jhal") is used traditionally as spices and folk medicine in different parts of Bangladesh. One of the most important bioactive compounds in this plant is piperine. In this study, the amount of piperine in *P. chaba* root and stem was investigated and the optimal solvent for piperine extraction at room temperature was also studied. High performance liquid chromatography (HPLC) was operated using a reverse phase column where methanol and water (70:30) were used as mobile phase. The detection was performed using photo diode array (PID) detector at a wavelength of 345 nm. The standard piperine showed linearity between 0.005 % and 0.04 % and the correlation co-efficient found for the linearity was 0.9933. The percentage of relative standard deviation (RSD) for both retention time and peak area were less than 2.0 %. The theoretical plate number (N) $>$ 3000 and a tailing factor (T) $<$ 1.5 were found in the acceptable range. The recovery percentage (%) of standard piperine was 99.16 %. Low value of co-efficient of variation and standard deviation are recognized for high precision of the method. The highest amount of piperine was found in root extracted with methanol (MR) amounting to 1.75 % in the root powder. The maximum amount of piperine in the stem was 1.59 % when extracted with methanol (MS). The piperine quantification in other extract like n-hexane root (HR), ethyl acetate root (ER), n-hexane stem (HS), ethyl acetate stem (ES) were 0.76 %, 1.69 %, 0.33 % and 1.46 % respectively. Methanol has given the highest yield of piperine compared to ethyl acetate and n-hexane for both root and stem. The developed method was simple, rapid, economic and validated in terms precision, accuracy and recovery. This selective method is found to be repeatable, accurate and successfully utilized for the Piper extract in marketed and pharmaceutical samples with well chromatographic conditions. The ethyl acetate extract of root and stem showed promising DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging activity with an IC_{50} value of 39.62 $\pm$ 0.95 μg/mL and 43.85 $\pm$ 1.50 μg/mL respectively. The study reports potential antibacterial activity and antifungal activity of *P. chaba* root and stem extracts. These outcomes revealed that different extracts of *P. chaba* may be used as natural preservatives.

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1. Introduction

A medicinal plant is a species which contains chemical substances that are applicable for therapeutic purposes. Plants have been used in traditional folk medicine for several thousands of years [1]. These medicinal plants are considered as more recognized and accepted to be more effective than synthetic drugs. Another benefits of herbal medicines are that they are cheap and available. In addition, plant-based herbal medicines are biodegradable. It is observed that these medicines have been used by about 80 % of rural people [2]. The current problems of medicinal plant preparations are include contamination with environmental and biological pollutants, adulteration with other unknown species and the unsustainable harvest resulting in qualitative and quantitative variations in bioactive chemical compounds [3]. In human pathogenic microorganisms, antimicrobial drug resistance increased due to extensive use of commercial antibacterial drugs used in the treatment of contagious diseases [4]. In this context, it is the duty of scientists to search for antimicrobial drugs, especially from natural sources like plants. Conessine, reserpine, piperine, and berberine are used as plant-based antimicrobial agents [5].

Piper chaba Hunt. *(P. chaba)* recently accepted taxon *Piper retrofractum* Vahl is locally called Choi Jhal and a member of Piperaceae family. It is a climbing, glabrous shrub found in different parts of India, Malaysia and Bangladesh. It is a creeper plant which spreads on the land. It may also grow around large trees. The leaves of the plant are oval-shaped and about 2–3 inches (5.1–7.6 cm) long. The flowers are monoecious and blossom during the monsoon. The fruit looks elongated shape and grows up to 3 inches (7.6 cm) long. The fruit is red while ripening and dark brown or black when in dry state [6]. In Bangladesh it grows in plenty in the southern part especially in Khulna, Jashore, Bagerhat and Satkhira regions [7,8]. It is generally used as spices in meat curry and other dishes [8]. Piperine has been accepted as a main alkaloid which is responsible for the pungency of *P. chaba*, along with isomer of piperine named chavicine [4,9]. The piperine (1-piperoylpiperidine) (structure 1) is a nitrogenous pungent material belonging in the fruits, roots and stem of *P. chaba* and other piper species [9,10]. Hans Christian Orsted isolated piperine in 1819 [11]. It is used in traditional folk medicine in different forms and also used as an insecticide [12]. Its pungency was quantified as 100000–200000 scoville unit [13]. It has been reported that piperine accelerates the bioavailability of some therapeutic drugs and phytochemicals, like curcumin, through inhibiting their metabolism [14,15]. It has showed antipyretic, anti-inflammatory and CNS depressant activity [16]. It is also used extensively in pain management, influenza, fever and for treating rheumatism arthritis [17] addition to appetie simulation, salivation and enhancement of blood circulation [18]. Piperine also is known to enhance the bioavailability of antitubercular drugs in human when applied together [19]. Piperine enhances bioavailability of rifampicin in patients of pulmonary tuberculosis. By adding piperine the dose of rifampicin was reduced [20]. Piperine has exhibited various biological activities such as anti-depressant activity [21], anti-inflammatory [22,23], anti-arthritic effects [23], antibacterial activity [24], anti-oxidative effect [25] and anticancer activity [26, 27].

In Piperaceae family, the piperine content is varied from plant to plant [28]. The quantitative amount of piperine can be affected by variations of conditions of cultivation such as weather, rain, drying conditions and area [29]. The production of secondary metabolites in medicinal plants is highly influenced by different factors like uv radiation, heavy metal, air pollution [30–32]. Therefore, it is time to standardize the pharmacological active plants quantitatively used all over the earth widely. Piperine is poorly soluble in water (approximately 40 mgL-1 but is readily soluble in organic solvents like n-hexane, chloroform, dichloromethane, ethylacetate and methanol. Among these solvents chloroform is more suitable solvent for extraction of piperine but it is one of the carcinogenic solvent [33]. As chloroform is toxic, an effort has been made to choose a solvent with a similar polarity index to chloroform. The solvent polarity index of chloroform and ethyl acetate are 4.1 and 4.4 respectively [34]. Three solvents like n-hexane (non-polar), ethyl acetate (moderately polar) and methanol (polar) were used for comparing a wide range of organic solvents like from non-polar to polar solvents. In the current investigation, several solvents were tested for extracting piperine and then, HPLC-PDA was used for quantitative estimation of the amount extracted. Several methods have been established to estimate piperine using HPLC [4,9,35–39].

To the best of our knowledge, no HPLC analytical method for piperine estimation from *P. chaba* root and stem has been published anywhere. As a part of our present research work is to develop a simple, precise HPLC method by using low-cost solvent water and methanol as a mobile phase for quantification of piperine in cultivated *P. chaba* root and stem to study the distribution of this compound in stem and root of the plant. In the current study, several solvents were used to extract piperine from the root and stem of *P. chaba* at room temperature followed by quantitative determination of piperine using HPLC-PDA technique, in aim to find the optimal extracting solvent. The propsed HPLC-PDA could be used routine quality control for piperine containing material. Additionally, various solvent extracts extracted from the root and stem of *P. chaba* would be explored for their antimicrobial, antifungal and DPPH scavenging activities.

2. Materials and methods

2.1. Solvents and reagents

The Analytical grade n-hexane was a product of Merck (Darmstadt, Germany), methanol was purchased from Fisher Scientific Co. (Leicestershire, UK), ethyl acetate from Sigma (Steinheim, Germany), HPLC grade methanol and water were products of Daejung (Korea) and were used without any further purification. The standard piperine and DPPH were bought from Tokyo Chemical Industry (Japan). BHT (tert-butyl-1-hydroxytoluene) was purchased from Scharlab S.L. (Spain). Potato dextrose agar and Mueller–Hinton agar were products of HiMedia Laboratories Pvt. Ltd., India.

2.2. Plant materials and extraction

The stem and root of *P. chaba* were collected from Khulna region of Bangladesh and washed with water to remove mud and dust particles. The sample was identified by Dhaka University Salar Khan Herbarium, Department of Botany, University of Dhaka, Dhaka-1000, Bangladesh (Voucher no.: DUSH 110827). Then it was chopped into smaller pieces by cutter. The plant was first dried in air for 3–4 days. Then they were dried at 40 °C in an electric oven (Hot Air Sterilizer, Model: YCO-010, Gemmy Industrial corporation). The dried plant was grind to make powder of mesh size 100 by a grinder (Model: MX-AC400, Power: 220–240 V, Materials: stainless steel, Panasonic). The powder was stored in an air tight bottle for extraction. The stem and root powder (20 g each) were soaked separately with n-hexane (200 mL), ethyl acetate (200 mL) and methanol (200 mL) at room temperature in an air-tight container for 72 h, with occasional shaking. This process was repeated for three times and combined each extract separately. The extract was filtered through a Whatmann filter paper (no. 1) and concentrated using a rotary evaporator at 40 °C. All analytical conditions were kept same for each extract.

2.3. Free radical scavenging activity

The antioxidant capacity of *P. chaba* root and stem extracts was quantified using the DPPH assay method [40]. The antioxidant activity was analyzed from the bleaching effect of purple colored DPPH radical solution in methanol by the root and stem extract as compared with standard tert-butyl-1-hydroxytoluene (BHT) by UV–visible spectrophotometer at wavelength 517 nm. Various concentrations of each extract were prepared by methanol. 2 mL of every sample solution was mixed with 3 mL of DPPH solution (20 μg/mL) and was kept for half an hour incubation period. Free radical DPPH inhibition percentage (I%) was calculated as of the following:

$$\text{Inhibition percentage (I\%)} = \left(1 - \text{Abs}_{\text{sample}} \,/\, \text{Abs}_{\text{blank}}\right) \times 100$$

where, U_{blank} is the absorbance of the DPPH solution in methanol (negative control). The antioxidant capacity has been quantified in terms of IC_{50} value, which is the amount of the sample needed to decrease 50 % of DPPH free radical. IC_{50} was calculated from the graph inhibition percentage (I %) vs. sample concentration. Low IC_{50} value means that the sample is potent at low concentrations.

2.4. Antimicrobial activity assay

Agar disc diffusion method was applied to assess the antimicrobial activity of the root and stem extracts of *P. chaba* [41,42]. For the broth culture of the tested component, Mueller Hinton Agar (MHA) for bacteria and Potato Dextrose Agar (PDA) for fungi were utilized as the media. After solidification of the MHA and PDA media we tested them for contaminations by keeping in incubation for 24 h. *P. chaba* root and stem extracts were inoculated on the incubated media in an even fashion by using previously sterilized cotton bar. Incubation period at 37 °C for the discs with applied extract was 24 h for antibacterial experiment and at 26 °C for antifungal experiment was 48 h. All the discs containing 300 μg of crude extracts (both root and stem) with 50 μL volume in DMSO was injected. On the other hand, in case of positive control test, standard amphotericin B and ceftriaxone solutions in DMSO containing 50 μg each were placed per disc for antifungal and antibacterial activity respectively.

Efficacy of the plant extracts were assessed against different bacterial and fungal strains, namely 2-g positive such as *Staphylococcus aureus* and *Bacillus megaterium*, 2 g-negative *Escherichia coli* and *Salmonella typhi* bacteria, and two fungal strains *Aspergillus niger* and *Trichoderma harzianum* were used in the present research.

2.5. Instrumental apparatus and analytical conditions

HPLC (Shimadzu, Prominence-i LC-2030C 3D Plus, Japan), Auto sampler, variable wave length programmable photo diode array (PID) detector (Shimadzu) was used for the analysis of piperine. The data was acquired on the Lab solution administrator data system (Japan) and Shimadzu Shim-pack GIST C18 column (250 mm × 4.6 mm, 5 μm) (Japan) was used. Injections were carried out using a 20 μL loop at room temperature and the flow rate was 1 mL/min. Mobile phase (Methanol: Water = 70:30) was filtered through 0.45 μm millipore nylon filter and degassed by sonication for 30 min. The mobile phase elution was isocratic and runtime was 15 min. The column temperature was maintained at 27 °C and detection was carried out at wavelength 345 nm 10 μL of respective sample was injected each time.

2.6. Preparation of stock and sample solutions

The standard piperine solution was made by dissolving 5.0 mg piperine in 10 mL volumetric flask using HPLC grade methanol as solvent to get 0.5 % (1.75 mM) piperine solution. The stock solution was diluted to obtain 0.005 %, 0.01 %, 0.015 %, 0.02 % and 0.04 %, standard solutions. 0.02 % sample solution was prepared by dissolving 10 mg of n-hexane, ethyl acetate and methanol extract of root and stem in HPLC grade methanol of a 50 mL volumetric flask and sonicated for half an hour. The flask was made up to 50 mL by HPLC grade methanol and then filtered by using high-pressure vacuum pump through 0.2 μ membrane filter and the clear solutions were used for HPLC fingerprint analysis.

2.7. Method validation

The feasibility and acceptability of the developed HPLC method is determined by suitability of the HPLC system. The outcomes of various system suitability parameters namely retention time (RT), peak area, tailing factor (TF) and theoretical plate (TP) count are demonstrated for acceptable range. For the acceptance of the method, the tailing factor must not exceed 1.5 and the theoretical plate count must be greater than 3000. Also, the relative standard deviation (RSD) must be less than 2.0 % [43]. The system was validated for accuracy, specificity, precision using piperine standard solutions.

The sharp and prominent peak was obtained for piperine as shown in Fig. 1. The data obtained from the system suitability parameters study demonstrated the method was suitable to perform further analysis of piperine in different formulations and it is applied to analyze root and stem extracts of *P. chaba*.

2.7.1. Range of linearity: The standard calibration curve was demonstrated using five standard concentrations 0.005 %, 0.01 %, 0.015 % 0.02 % and 0.04 % of the tested compound piperine. The developed method shows linearity between concentrations and peak areas. The regression analysis data revealed that the developed method was linear with the different range of concentrations (0.005–0.04 %) of piperine, which were quantified at 345 nm.

2.7.2. Precision: The precision is the extent of closeness of agreement between the numbers of measurements acquired from numbers of samples of the same sample under the definite steps. The precision of the instruments was tested by repeatedly injecting and analyzing (n = 5) 0.02 % standard piperine solution. The outcomes are represented in terms of relative standard deviation (RSD). The values for percentage RSD values less than 2 % for both retention time and peak area indicating precision assays satisfy acceptance criteria and indicated the precise characteristic of the developed method. The suitability test would be accepted when the RSD values of these parameters were less than 2 %.

2.7.3. Accuracy (recovery): Accuracy of the method was determined by spiking the *P. chaba* root extract with known amount of standard piperine solution. The average percentage of recovery was calculated. Recovery test was performed at three root extract such as n-hexane extract, ethyl acetate extract and methanol extract. The mean percent recoveries were in the close to 100 for piperine, which indicates that the developed method was applicable for broad scale of sample investigation.

3. Results and discussion

3.1. Method validation

3.1.1. Range of linearity

In order to investigate the area count and linearity achieved for these solutions, standard piperine solutions at various concentrations of 0.005 %, 0.01 %, 0.015 %, 0.02 %, and 0.04 % were taken into consideration. The results are shown in Table 1. Piperine exhibited good linearity with a correlation coefficient of 0.9933 within the range of 0.005 %–0.02 % for concentration. The findings indicated that the relative standard deviation of retention time is 0.045 %.

3.1.2. Precision of the method

To find out the precision of the applied method was demonstrated through five injections of a single sample solution and calculated the relative standard deviation (RSD) (Table 2).

At an optimal wavelength of 345 nm, the obtained HPLC chromatogram using 0.02 % standard piperine revealed a mean area of 5241042 in Table 2 at a mean retention time of 8.578 min shown in Fig. 2. The relative standard deviation of peak area and retention time 0.273 % and 0.064 % respectively.

3.1.3. Recovery of the method

The mean recovery value of standard piperine was 99.16 %. The recovery of the method is shown in Table 3.

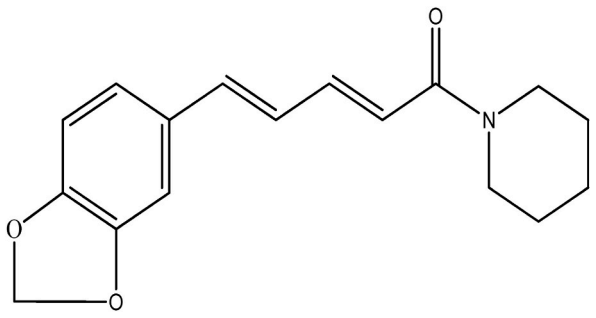


Fig. 1. Structure of piperine.

Table 1
Determination of range of linearity for standard piperine.

Sl. No.	Concentration of piperine (%)	Retention time (min.)	Peak area	Number of theoretical plates (TP)	Tailing factor (TF)
1	0.005	8.576	1305030	7419	1.12
2	0.01	8.577	2685815	7450	1.13
3	0.015	8.579	4947378	7370	1.15
4	0.02	8.577	5291549	7527	1.14
5	0.04	8.570	11152121	7359	1.17

Table 2
System suitability parameters (precision) for standard piperine.

Sl. No.	Concentration of piperine (%)	Retention time (min.)	Peak area	Number of theoretical plates (TP)	Tailing factor (TF)
1	0.02	8.577	5291549	7527	1.14
2	0.02	8.574	5227783	7461	1.14
3	0.02	8.578	5215266	7468	1.14
4	0.02	8.576	5257483	7450	1.14
5	0.02	8.588	5213130	7475	1.14

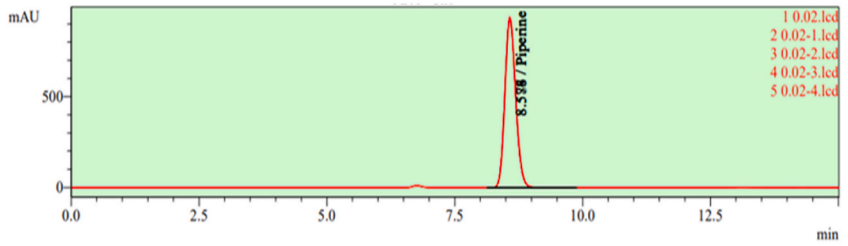


Fig. 2. HPLC chromatogram of standard piperine.

Table 3
System suitability parameters (accuracy or recovery) for standard piperine.

Root sample	Amount of sample taken (A), mg	Amount of piperine present in A (B), mg	Amount of piperine added to A (C), mg	Total amount of piperine taken (T), mg	Amount of piperine obtained (O), mg	Recovery (O/T × 100) %
HR	100	35.65	10.00	46.65	46.31	99.27
ER	100	39.36	10.00	39.36	38.98	99.03
MR	100	21.98	10.00	31.98	31.72	99.19

3.2. Assay of piperine

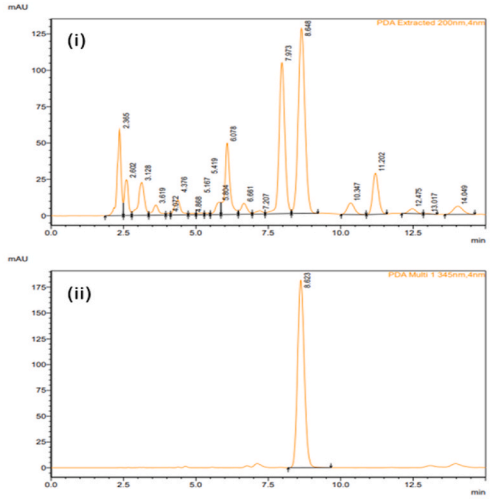
The HPLC chromatograms of root of and stem of *P. chaba* were observed at a retention time between 8.586 and 8.629 min that matched with corresponding standard piperine utilizing a wavelength of 345 nm (Fig. 3) (see Table 4). The distinction in retention time of peak of piperine may be due to the presence of other chemical components. Piperine content in root and stem extract of *P. chaba* samples were quantified using the equation obtained from piperine standard calibration curve.

The piperine content in root extract like HR, ER and MR were determined as 0.76 %, 1.69 % and 1.75 % respectively. The piperine content in root extract such as HS, ES and MS were quantified as 0.33 %, 1.46 % and 1.59 % respectively (Fig. 4).

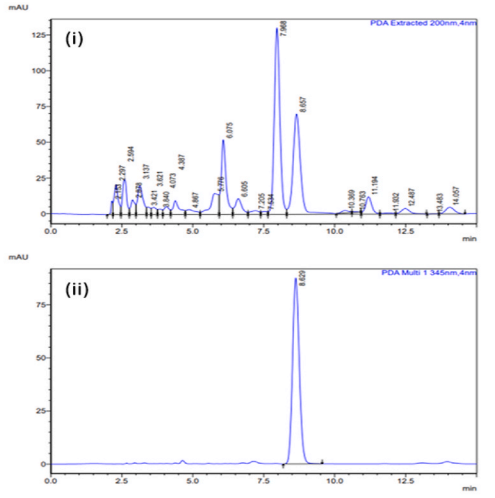
The quantitative evaluation of piperine was found greater in root than stem in every case of each solvent extract. From literature, it is seen that piperine was more soluble in chloroform solvent but chloroform is toxic solvent. As solvent polarity index of chloroform and ethyl acetate are near to each other, in present research ethyl acetate was used as alternative of chloroform. In both case root and stem the highest amount piperine was quantified when the sample was extracted with methanol. Methanol is used as optimum solvent in the proposed method to extract piperine as a marker compound from Piper species.

3.3. DPPH free radical scavenging activity

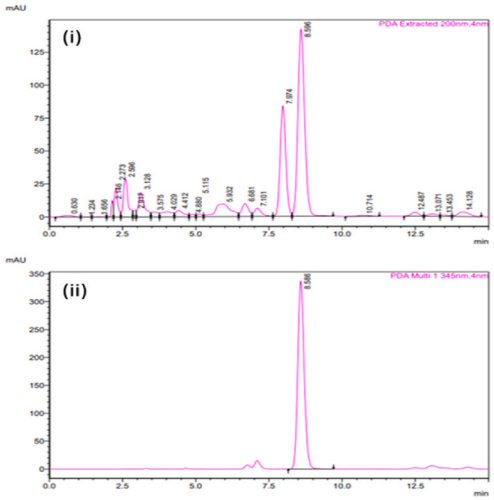
The different extracts obtained from the root and stem of *P. chaba* were tested for their antioxidant activity using DPPH radical scavenging assay method. As compared to the positive control BHT (IC_{50} 16.71 ± 0.31 μg/mL), the ER extract of the stem had the highest DPPH scavenging activity with an IC_{50} value of 39.62 ± 0.95 μg/mL (Fig. 5). In this present study, IC_{50} value of ethyl acetate



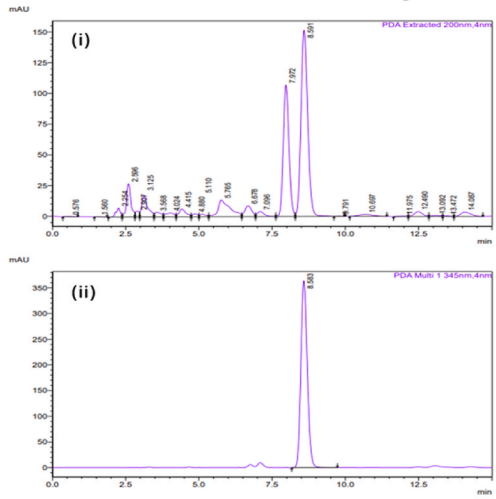
(a). HPLC chromatogram of n-hexane extract of root (HR); (i) Full chromatogram, (ii) Extracted chromatogram for piperine



(b). HPLC chromatogram of n-hexane extract of stem (HS) ; (i) Full chromatogram, (ii) Extracted chromatogram for piperine



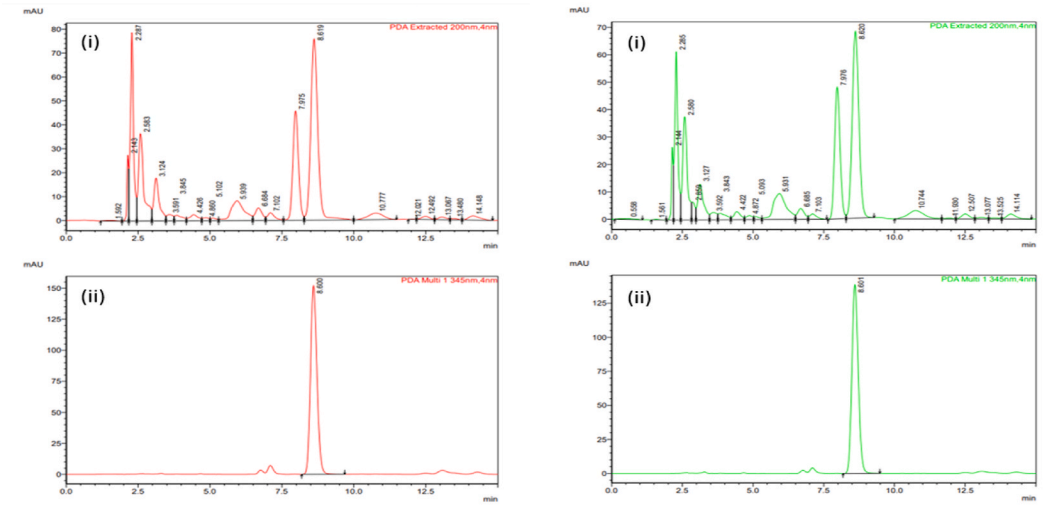
(c). HPLC chromatogram of ethyl acetate extract of root (ER) ; (i) Full chromatogram, (ii) Extracted chromatogram for piperine



(d). HPLC chromatogram of ethyl acetate extract of stem (ES) ; (i) Full chromatogram, (ii) Extracted chromatogram for piperine

Fig. 3. HPLC chromatogram of a) n-hexane extract of root (HR), b) n-hexane extract of stem (HS), c) ethyl acetate extract of root (ER) d) ethyl acetate extract of stem (ES), e) methanol extract of root (MR), f) methanol extract of stem (MS) of *P. chaba* by using PID detector
Fig. 3 (a). HPLC chromatogram of n-hexane extract of root (HR); (i) Full chromatogram, (ii) Extracted chromatogram for piperine. Fig. 3 (b). HPLC chromatogram of n-hexane extract of stem (HS); (i) Full chromatogram, (ii) Extracted chromatogram for piperine. Fig. 3 (c). HPLC chromatogram of ethyl acetate extract of root (ER); (i) Full chromatogram, (ii) Extracted chromatogram for piperine. Fig. 3 (d). HPLC chromatogram of ethyl acetate extract of stem (ES); (i) Full chromatogram, (ii) Extracted chromatogram for piperine. Fig. 3 (e). HPLC chromatogram of methanol extract of root (MR); (i) Full chromatogram, (ii) Extracted chromatogram for piperine. Fig. 3 (f). HPLC chromatogram of methanol extract of stem (MS); (i) Full chromatogram, (ii) Extracted chromatogram for piperine.

extract of stem was found 151.35 ± 2.66 μg/mL. Ethyl acetate extract of *P. chaba* stem was explored by Rahman et al. with an IC_{50} value 312.56 μg/mL [44]. For most of the plants external factors like temperature, light, soil water and salinity can affect growth and development of the plants, synthesize of the secondary metabolites and variations are found on phytochemicals [45]. The IC_{50} value may be deviated as the IC_{50} value depends on the phytochemicals present in the plant.



HPLC chromatogram of methanol extract of root (MR) ; (i) Full chromatogram, (ii) Extracted chromatogram for piperine

HPLC chromatogram of methanol extract of stem (MS) ; (i) Full chromatogram, (ii) Extracted chromatogram for piperine

Fig. 3. (*continued*).

Table 4
DPPH scavenging activity (mean ± SD, n = 3) of standard BHT and n-hexane, ethyl acetate and methanol extracts of *P. chaba* root and stem.

HS	HR	ES	ER	MS	MR	BHT
2344.16 ± 7.28	2182.52 ± 6.13	43.85 ± 1.50	39.62 ± 0.95	151.35 ± 2.66	70.46 ± 1.35	16.71 ± 0.31

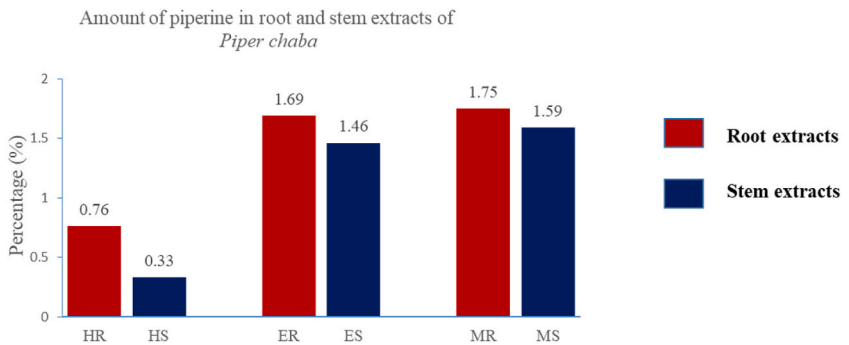


Fig. 4. Comparison of piperine content in n-hexane, ethyl acetate and methanol extracts of root and stem of *P. chaba* using different solvents.

3.4. Antimicrobial activity

The antibacterial and antifungal activity of *P. chaba* root and stem extracts were assessed by agar disc diffusion technique and are summarized in Table 5.

3.4.1. Antibacterial activity

Antimicrobial screening was carried out with a concentration of 300 μg/disc in each case for sample and 50 μg/disc for standard antibacterial drug ceftriaxone (Fig. 6).

Of all tested extracts HS showed the highest antibacterial activity against *Salmonella typhi* having zone of inhibition 25 mm. In general, all extracts had Inhibition zones ranging between 7 and 25 mm, against the tested bacterial species (Fig. 7). Different extracting solvents affects the chemical composition of the obtained plants extracts and consequently affect their observed antibacterial potentials.

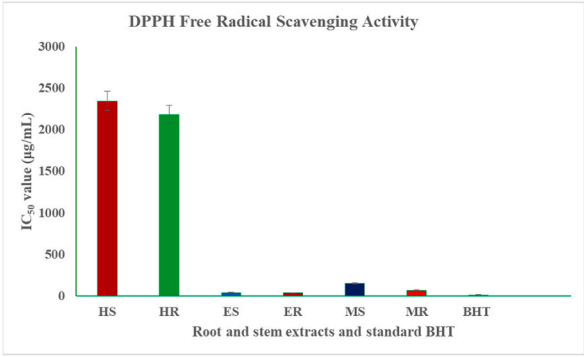


Fig. 5. DPPH free radical scavenging activity of n-hexane, ethyl acetate and methanol extracts of root and stem of *P. chaba* using BHT as a standard.

Table 5
Zone of inhibition (mm) against gram positive bacteria (+), gram negative bacteria (−) and fungi of n-hexane, ethyl acetate and methanol extracts of root and stem of *P. chaba* and standard antibiotic drug (ceftriaxone) and standard antifungal drug (amphotericin B).

Test Microorganisms	HS	HR	ES	ER	MS	MR	Ceftriaxone	Amphotericin B
Staphylococcus aureus (+)	16	16	17	10	10	18	41	
Bacillus megaterium (+)	17	18	22	20	21	17	44	
Escherichia coli (−)	14	15	14	16	7	15	39	
Salmonella typhi (−)	25	16	17	16	18	15	30	
Trichoderma harzianum	12	11	15	16	15	18		10
Aspergillus niger	13	16	16	13	16	13		22

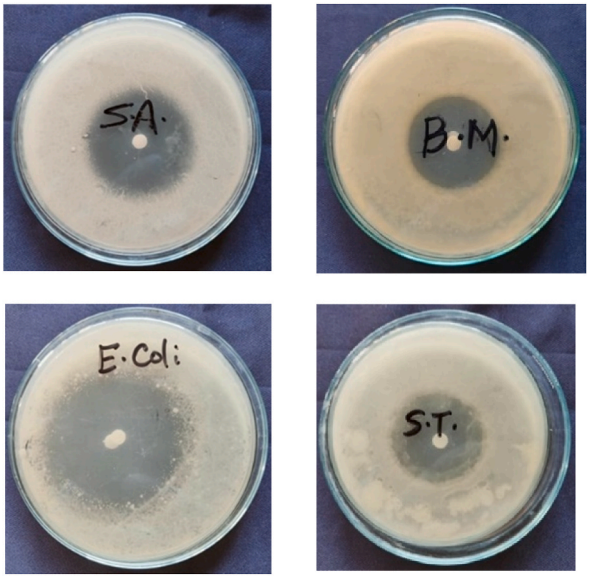


Fig. 6. Inhibited zone shown by standard antibiotic drug ceftriaxone against *Staphylococcus aureus* (SA), *Bacillus megaterium* (BM), *Escherichia coli* (*E. coli*) and *Bacillus megaterium* (BM) bacterial strains.

3.4.2. Antifungal activity

The antifungal assay result of *P. chaba* root and stem extracts with a concentration of 300 µg/disc in each case for sample and 50 µg/disc for standard antifungal drug amphotericin B is summarized in Table 5.

ME and different fractions of this medicinal plant moderately inhibited the fungi *T. harzianum* and *A. niger* (Fig. 8). MR had the highest inhibition (18 mm) against *T. harzianum*.

The root and stem extracts of *P. chaba* have promising antioxidant activity and potential antimicrobial activity. The plants having good antioxidant and antimicrobial activities are probable source of natural preservatives for food [44]. This results revealed that different extracts of *P. chaba* may be used as natural preservatives. The bioactive compounds present in plants are responsible for

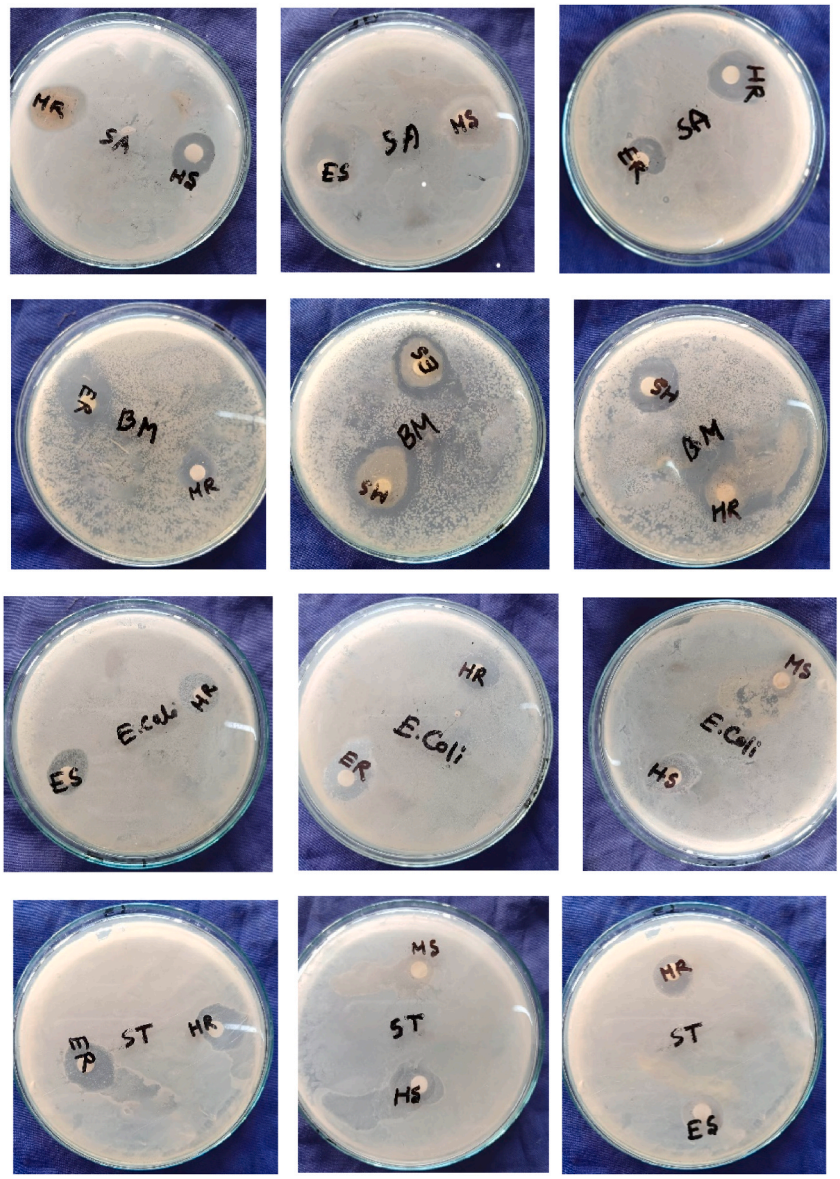


Fig. 7. Inhibited zone shown by n-hexane, ethyl acetate and methanol extracts of root and stem of *P. chaba* against four bacterial strains.

showing antimicrobial activity because it contains alkaloids, terpenoids, flavonoids, steroids, saponins and tannins [46,47]. The present research of comparison of the antimicrobial property of *P. chaba* root and stem is studied for the first time.

4. Conclusion

The assay of piperine in *P. chaba* extract is very simple, cost-effective, sensitive and rapid. This developed RP-HPLC method was reversed-phase, isocratic and validated for the evaluation of purity and assay of piperine in pharmaceutical. Additionally, due to the absence of acid in the mobile phase and the use of widely available solvents, such as methanol and water, ensure that the suggested procedure will prolong the column time and HPLC system. The statistical analysis also showed that the method is linear, accurate, precise and specific for the analysis of piperine. The method has been proved to be selective and stable. This study reveals that the highest amount of piperine was found by methanol extraction for both root and stem. Root has more piperine content than stem. It may be occurred for the production of derivative of piperine in the stem. The ethyl acetate extract of root and showed highest DPPH free radical scavenging activity having IC_{50} value of 39.62 $\pm$ 0.95 μg/mL. Root has more antioxidant property than stem among each extract. Both root and stem have good antimicrobial property. *Piper chaba* is an important medicinal plant and its further assessment is necessary, which can provide help in the discovery of new antibiotic drug development in the market.

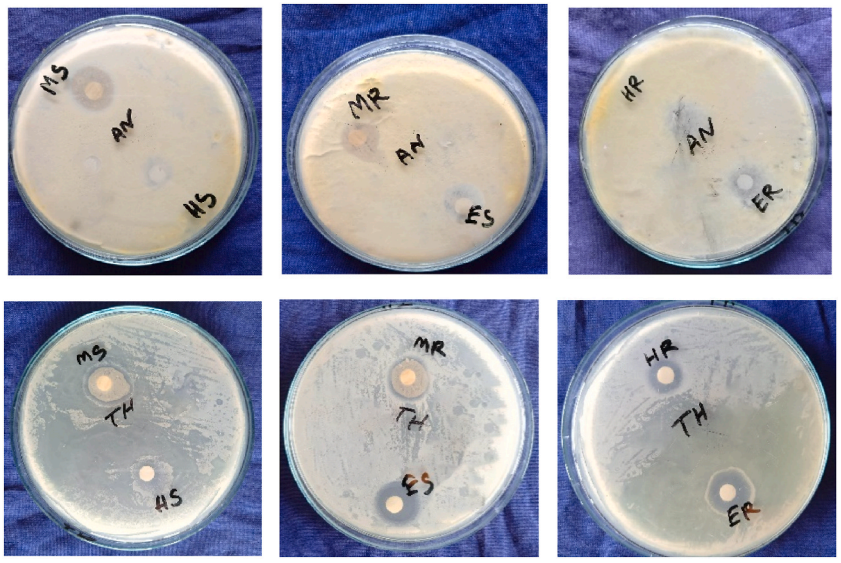


Fig. 8. Inhibited zone shown by n-hexane, ethyl acetate and methanol extracts of root and stem of *P. chaba* against *Aspergillus niger* (AN) and *Trichoderma harzianum* (TH) fungal strains.

Data availability statement

The data included in this article will be made accessible on request from the corresponding author (MM: mzaman_103@chem.kuet.ac.bd).

CRediT authorship contribution statement

Md Rubel Al-Mamun: Writing – original draft, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Md Maniruzzaman:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Md Mizanur Rahman Badal:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Md Aminul Haque:** Writing – review & editing, Methodology, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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