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Bangladesh J. Sci. Ind. Res. **58(1)**, 45-52, 2023

BANGLADESH JOURNAL OF SCIENTIFIC AND INDUSTRIAL RESEARCH

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Antimicrobial activities of *Cassia sophera* L. extract

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Abstract

This study investigates the antibacterial activities of a variety of extracts from several parts of *Cassia sophera* L. which include the stem, leaf, flower, and seed from n-hexane, chloroform, ethyl acetate, and methanol extract. The disc diffusion method, at a concentration of 300 µg/disc, was employed to test the extracts against several pathogenic microorganisms. Some of the extracts were antibacterial against the bacteria tested, with inhibition zones ranging from 7.0 to 25.0 mm in diameter. In comparison to a streptomycin standard sample, the organic extracts showed almost identical activity in a few cases. The ethyl acetate extract of the seed had the highest activity against *Sarcina sp.* and *Pseudomonas aeruginosa*, with inhibition zones of 25.0 mm and 23.0 mm in diameter, respectively. Methanol extract of the flower was most effective against *Bacillus cereus* (inhibition zone of 18.0 mm), while chloroform extract of the leaf was most effective against *Bacillus cereus* (inhibition zone of 18.5 mm). The result of present research is expressing the high potency of various parts of the plant extracts to stop the growth of bacteria and this extract can be further suggested for medical utilization and could be used as natural antimicrobial source.

Received: 07 November 2022

Revised: 01 February 2023

Accepted: 19 February 2023

DOI: https://doi.org/10.3329/bjsir.v58i1.65115

Keywords: *Cassia sophera* L.; Antibacterial assay; Zone of inhibition; Gram-positive bacteria; Gram-negative bacteria

Introduction

Plants are rich in compounds that could be useful in the development of new therapeutics. Plant antibacterial activity has been linked to a variety of phytochemical compounds that act as secondary metabolites (Cao *et al.* 2010; Sule *et al.* 2011). There is a renewed interest in traditional medicine right now. The widespread belief that "green medicine" is safer and more reliable than expensive synthetic drugs, many of which have negative side effects, has reignited interest in plant-derived drugs. The combined effect of secondary metabolites in a specific plant is taxonomically distinct from other species, which is consistent with the idea that plant medicinal action is unique to a specific plant species (Krishna *et al.* 2015). Medicinal plants have provided modern medicine with a plethora of plant-derived therapeutic agents (Evans, 2000). Medicinal plants are currently gaining a lot of attention around the world for their use, advancement, and preservation.

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Because of their therapeutic properties, approximately 449 plant species in Bangladesh have been identified as medicinal. In Bangladesh, hundreds of conventional medicines, including Ayurvedic and Unani formulations, have been developed. This country has about 400 herbal industries that produce Ayurvedic and Unani medicines and sell herbal products worth five hundred crore taka per year (Farnsworth, 1961). Appropriate scientific assessment of these plants' pharmacological characteristics when used in various formulations holds massive promise for the twenty-first century.

Cassia sophera L. (Caesalpiniaceae), also recognized as "Kasundi" in Bangladesh, is a tropical plant that is native to the subcontinent (Gupta, 2010) . Bangladesh, India, Mauritius, China, East Africa, South Africa, America, Mexico, West Indies, and Brazil are among the Asian countries where it can be found (Khare, 2008). The plant grows in wastelands, along roadsides, and in woodlands. The entire plant is used in traditional folk medicine and has anti-dysenteric, antidiarrheal, and antipyretic effects.

Due to the occurrence of various phytoconstituents, the plant is suitable for treating a variety of ailments and has the potential to provide valuable drugs for human use (Tripathi and Kohli, 2012). Traditional wounds or burns, which may occur due to bacteria or fungi, are treatable by the leaf extract of *Cassia sophera* (Deshpande and Naik, 2016). Although some antimicrobial analyses were carried out different parts of the plant but no systemic researches were done for leaf, flower, seed and stem parts of the plant. Some researchers claimed that *C. sophera* L. is an evolving alternative antimicrobial agent for human uses (Rahman *et al.* 2017; Kirtikar and Basu, 2000). Antibacterial characteristics of herbs are due to various chemical compounds including volatile oil, alcohol, tannins and lipids that are presented in their tissue (Con *et al.* 1998). Previous researchers (Rahman *et al.* 2017) found that ethanol solvent of essential oil *C. sophera* L. leaves has considerable antibacterial activity. Also they found ethanol extract of leaves showed the strongest antibacterial activity against *B. megaterium*. Other researcher's findings (Con *et al.* 1998; Meyer and Afolayan, 1995) were leaf part showed highest zone of zone of inhibition with a diameter 18.5 mm against *B. cerus*. According to Rahman *et al.* 2017, flower part of the plant showed good antibacterial activity against *B. cereus*. Deshpande and Naik, (2016) found that leaf and stem extracts of *C. obtusifolia* in pet ether showed more activity against *E. faecalis*. The leaf extract of *S. sophera* in pet ether showed more sensitivity against *C. albicans*, ethanol extracts against *E. faecalis* and choloroform extracts against *C. albicans*. *C. sophera* stem extracts in pet ether, ethanol, chloroform showed more sensitivity against *E. faecalis.* This is considered as a susceptible agent against the specific bacterial strain. Previous researchers used different bacteria and fungal pathogens in vitro. In our present study various parts of the plant (leaf, flower, seed and stem) from various extract (n- hexane, chloroform, ethyl acetate and methanol) were analyzed with different pathogens (against three Gram-positive bacterial strains: *Bacillus cereus* FM1042, *Bacillus subtilis* FM1057 and *Sarcina sp.* FM1062, as well as three Gram-negative bacterial strains: *Pseudomonas aeruginosa* FM2017, *Salmonella enterica* FM2066, and *Escherichia coli* FM2087). After all Bangladesh is not only geographically but zeologically different from other countries. It is well known that physiological changes do occur in plants due to changes in geographical sites, climatic and environmental conditions which result in the production of nonidentical plant metabolites in this plant grown in different geographical regions. All theses pathogens used in the present study are food borne pathogen. This research is the first reported data for *Cassia sophera* L seed part. As a result, the purpose of this research is to assess the phytoconstituents and the antimicrobial properties of plant extracts. This findings provides an insight into the usage of different parts of the plant in traditional treatment of diseases caused by food born pathogens.

Materials and methods

Chemicals and reagents

Under normal atmospheric pressure, n-hexane, chloroform, ethyl acetate, methanol (Merck, Germany), and other AR-grade chemicals were used to extract plant materials. For antimicrobial activity testing, Luria-Bertani (LB) broth medium, Muller-Hinton agar medium (Oxoid Limited, UK), and other ingredients were used.

Plant materials

Fully matured fresh leaves, flowers, seeds, and stems were collected in June 2019 from Andulbaria village in Jhenidah district, Bangladesh, and identified by a taxonomist at the Bangladesh National Herbarium in Dhaka, where a voucher specimen (No. = 43734) was deposited. The samples were ground into a fine powder and stored in an airtight container in a cool, dark, and dry location with an identification mark for future use.

Preparation of extracts

In brief, 100 g of powdered plant material from each part of the plant is immersed for 5 days at room temperature in suitable solvents of increasing polarity such as n-hexane, chloroform, ethyl acetate, and methanol with occasional shaking, stirring and filtered. A rotary evaporator was used to

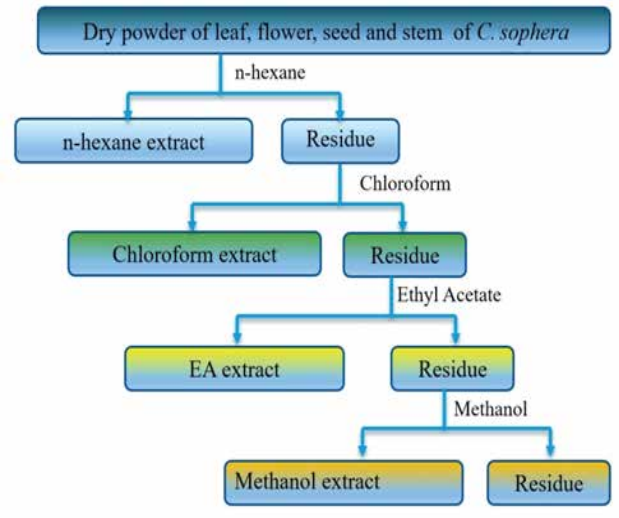
concentrate each filtrate under reduced pressure to obtain a viscous mass. For further analysis, the extracted samples were kept at 4°C.

Cassia Sophera L. Flower

Seed *Stem*

Fig. 1. Different parts of *Cassia Sophera* L.

The flow chart of cold extraction procedure for the preparation of various extracts from different part of the plant are given bellow:



Flow chart for cold extraction

Bacterial activity screening

Antibacterial strains

The antibacterial activity of *Cassia sophera* L. was tested against three Gram-positive bacterial strains: *Bacillus cereus* FM1042, *Bacillus subtilis* FM1057 and *Sarcina sp.* FM1062, as well as three Gram-negative bacterial strains: *Pseudomonas aeruginosa* FM2017, *Salmonella enterica* FM2066 and *Escherichia coli* FM2087. The mentioned six pathogenic microorganisms were collected as pure cultures from the Institute of Food Science and Technology (IFST), BCSIR, Dhaka, Bangladesh. Active cultures were prepared by transferring a loopful of cells from stock cultures to flasks and inoculating them in Luria-Bertani (LB) broth medium for 24 hours at 37°C. Each bacterial strain's culture was kept at 4°C on LB agar medium.

Bacterial Growth Inhibition test of plant extracts

The disc diffusion method (Kirby-Bauer disk diffusion method) (Zaidan *et al.* 2005) was used to test antibacterial susceptibility. Bacterial suspension containing 10^7cfu/ml of bacteria was used on Muller-Hinton agar. The dried extracts were sterilized by filtration through 0.45 μm

Millipore filters (Millipore Corp., Bedford, MA, USA) after being dissolved in the same solvent used for extraction to a final concentration of 30 µg/µl. Following that, the 6mm diameter filter paper discs were coated with 10 µl of 30 µg/µl of organic extracts (300 µg/disc) which were then placed on inoculated Muller-Hinton agar. Negative controls were made using the same solvents as the samples were dissolved in. Streptomycin 10 µg/disc were used as positive control. The plates were incubated for 24 hours at 37°C. The diameter of the zone of inhibition against the bacteria was measured for antibacterial activity.

Statistical analysis

The data represents the mean of three replicates with standard deviation (SD). The results are given in Table II.

Table I. Some Compounds isolated from n-hexane fraction of *Cassia Sophera* L. leaves

Sl. No.	Name of the compound	Molecular Formula	MW
1.	Butanedioic acid, Hydroxyl Diethyl ether (+/-)	$C_8H_{15}O_2$	190
2.	1,2,4- Butanetriol, Triacetate	$C_{10}H_{16}O_6$	232
3.	7- hexadecane (z)-	$C_{16}H_{32}$	224
4.	E-15-Hepatadecenal	$C_{17}H_{32}O$	252
5.	1,2-Benzenedicarboxilic acid, Butyl octyl ester	$C_{20}H_{30}O$	334
6.	3-Eicosene (E)-	$C_{20}H_{40}$	280
7.	10- Heneeicosene (C,T)	$C_{21}H_{42}$	294

Table II. Antibacterial screening of various extracts of flower, leaf, seed, and stem of *Cassia sophera* L.

Part of the plant	Solvent used for extraction	Gram -positive bacteria			Gram -negative bacteria			
		Bacillus cereus	*Bacillus subtilis*	*Sarcina sp.*	*Pseudomonas aeruginosa*	*Salmonella enterica*	*Escherichia coli*	*Mean ± SD*
Stem (A)	n-Hexane	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Chloroform	8.0	0.0	0.0	0.0	0.0	0.0	1.33±3.26
	Ethyl acetate	8.0	0.0	0.0	0.0	10.0	0.0	3.00±4.69
	Methanol	7.0	0.0	0.0	11.0	0.0	0.0	3.00±8.20
Leaf (B)	n-Hexane	13.0	8.0	0.0	0.0	0.0	0.0	3.50±5.65
	Chloroform	18.5	10.0	8.0	8.0	10.0	9.0	10.58±3.98
	Ethyl acetate	10.0	0	10.0	8.0	8.0	8.0	7.33±3.72
	Methanol	8.0	0.0	10.0	10.0	10.0	8.0	7.67±3.88
Seed (C)	n-Hexane	15.0	14.0	12.0	14.0	13.0	13.0	13.50±1.04
	Chloroform	15.0	17.0	14.0	16.0	15.0	16.0	15.50±1.05
	Ethyl acetate	17.0	20.0	25.0	23.0	16.0	20.0	20.17±3.43
	Methanol	18.0	15.0	22.0	21.0	15.0	17.0	18.00±2.97
Flower (D)	n-Hexane	14.0	10.0	10.0	10.0	12.0	13.0	11.50±1.76
	Chloroform	17.0	12.0	12.0	13.0	15.0	14.0	13.83±1.94
	Ethyl acetate	17.0	14.0	12.0	14.0	14.0	15.0	14.33±1.63
	Methanol	18.0	15.0	14.0	17.0	16.0	14.0	15.67±1.63

* Results are expressed as mean ± standard error = No Zone of inhibition

Results and discussion

In vitro antibacterial activity assay

The result of antibacterial activity is given in table I, which clearly shows that all of the extracts from various parts of the plant have antibacterial activity. The presence or absence of an inhibition zone was used to assess the in vitro antibacterial activity of *Cassia sophera* L. extracts (n-hexane, chloroform, ethyl acetate, and methanol) against six pathogenic bacteri-anamed *Bacillus cereus* FM1042, *Bacillus subtilis* FM1057, *Sarcina sp.* FM1062, *Pseudomonas aeruginosa* FM2017, *Salmonella enterica* FM2066, and *Escherichia coli* FM2087. Three of the six bacteria were Gram-positive, while three were Gram-negative.

Fig. 2-5 shows the antimicrobial activity of some plant parts of *Cassia sophera* L. However, the stem extract in n-hexane, ethyl acetate, and chloroform did not show any antimicrobial activity against the other remaining bacteria (*Bacillus cereus* FM1042, *Bacillus subtilis* FM1057, *Sarcina sp.* FM1062, *Salmonella enterica* FM2066, and *Escherichia coli* FM2087).

Chloroform extract of *Cassia sophera* L. leaf was potentially effective, showing an 18.5 mm inhibition zone against *B. cereus* FM1042 (Fig 2). The results showed that various extracts of the leaf are considered antimicrobial agents against *B. cereus.*

The result of ethyl acetate extract of *Cassia sophera* L. seed has considerable antibacterial activity against six pathogenic bacteria, which is shown in Fig 3. The highest antibacterial activity was observed against *Sarcina sp*. FM1062 and *Pseudomonas aureginosa* FM 2017 with an inhibition zone of 25.0 mm and 23.0 mm, respectively.

Methanol extract from the seed of *Cassia sophera* L. was found to have antibacterial property against all the bacterial strains tested. The maximum activity was seen against *Sarcina sp*. FM1062 and *B. cereus* FM1042, with an inhibition zone of 22.0 mm and 18.0 mm respectively.

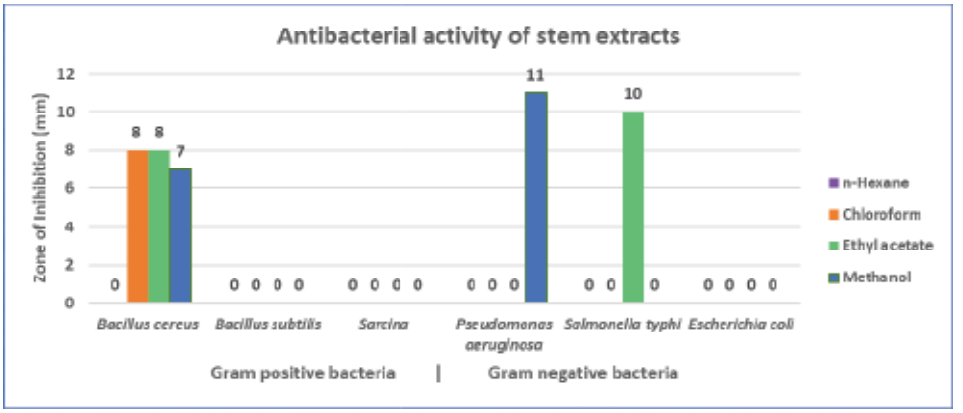


Fig. 2. Antibacterial activity of stem extracts of *Cassia sophera* L. compared with standard streptomycin

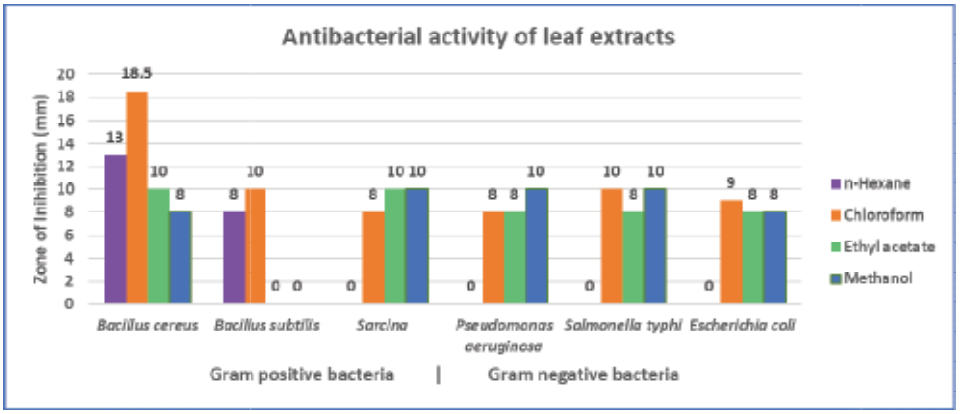


Fig. 3. Antibacterial activity of leaf extracts of *Cassia sophera* L. compared with standard streptomycin

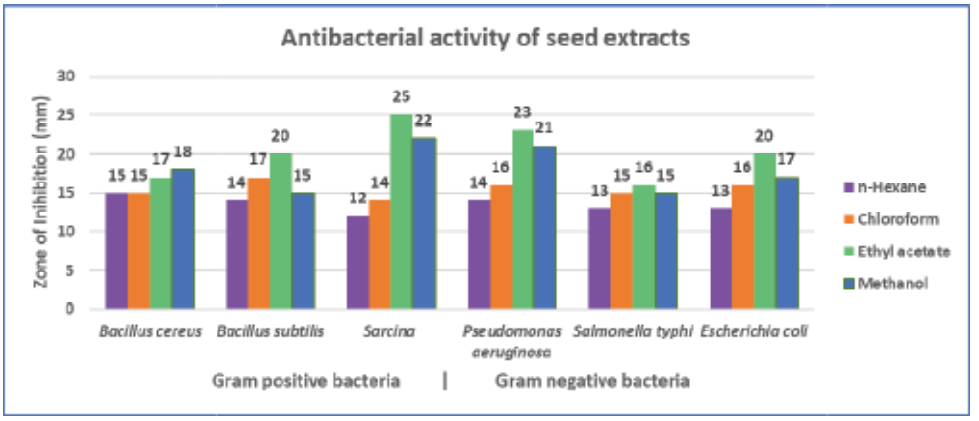


Fig 4. Antibacterial activity of seed extracts of *Cassia Sophera* L. compared with standard streptomycin

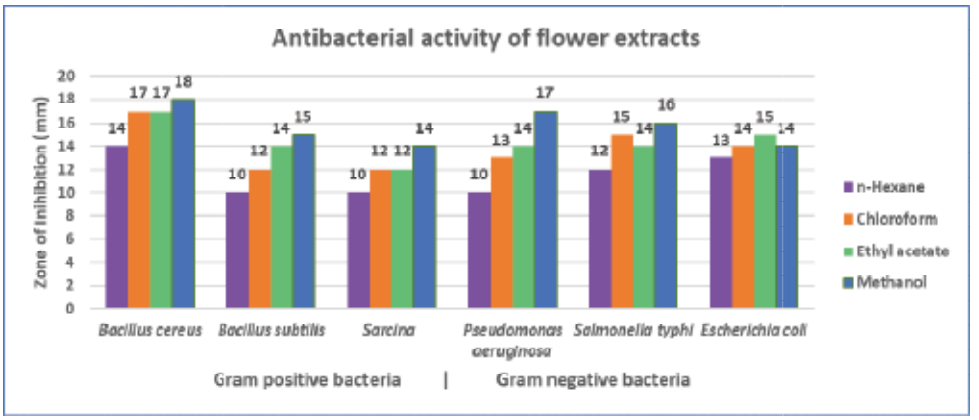


Fig 5. Antibacterial activity of flower extracts of *Cassia sophera* L. compared with standard streptomycin

It was found that flower extract of *Cassia sophera* L. was most effective against *B. cereus* FM1042 in methanol with an inhibition zone of 18.0 mm. The extract was also effective against *Pseudomonas aeruginosa* FM2017 (17.0 mm) and *Solmonella entrerica* FM2066 (16.0 mm), whereas the effectiveness of the extracts was moderate against *E. coli* FM2087 (14.0 mm), *B. subtilis* FM1057 (15.0 mm) and *Sarcina sp.* FM1062 (14.0 mm). Chloroform and ethyl acetate extracts also showed moderate effectiveness. The n-hexane extract was less effective in comparison to other extracts.

Currently, the long-running battle against bacteria and fungi triumphs through the assurance of growing resistance. Nonetheless, advances in medicine result in more patients' being in serious and resistant repressed conditions, necessitating the constant need for new antibiotics and antifungal drugs. As a result, now is an excellent time to discover new microbiological drugs (Mahesh and Satish, 2008). In the therapeutic industry, natural medicines are becoming an increasingly important topic of interest for the preparation of significant remedies (Sule *et al.* 2011). The various extracts of the different part of the plant can be a good option in this direction. From the present study, we find the stem of *Cassia sophera* L. extract in methanol solvent showed a moderately good zone of inhibition against *Pseudomonas aeruginosa* which provide potential antimicrobial agents that may exhibit a variety of therapeutic activities. Studies suggested that this stem extract may be a good source of some antibiotics and bioactive compounds.

Globally, transmissible diseases are the primary causes of sickness and temporariness. Currently, the long-running battle against bacteria and fungi triumphs through the assurance of growing resistance. Nonetheless, advances in medicine result in more patients' being in serious and resistant repressed conditions, necessitating the constant need for new antibiotics and antifungal drugs. As a result, now is an excellent time to discover new microbiological drugs (Mahesh and

Satish, 2008). In the therapeutic industry, natural medicines are becoming an increasingly important topic of interest for the preparation of significant remedies (Sule *et al*. 2011).

The stem of *Cassia sophera* L. extract in methanol solvent showed a moderately good zone of inhibition against *Pseudomonas aeruginosa* FM2017. While the other extracts did not show any antimicrobial activity.

The results from chloroform extracts of *Cassia sophera* L. correlate with the previous study (Rahman *et al.* 2017). It showed that various extracts of the leaf are considered antimicrobial agents against *B. cereus.* Our findings are also similar to those of previous findings (Con *et al.* 1998) and (Meyer and Afolayan, 1995). They described that dichloromethane extract of *Helichrysum aureonitens* is effective against Gram-positive bacteria. In addition, as per correlation of the previous results (Atef *et al.* 2019) discussed in their study that chloroform extract of *Cassia sophera* L. leaf can help to prevent the infection caused by *B. cereus*. n-Hexane extract showed a moderate zone of inhibition against *B. cereus.* No significant results were found against other bacteria.

The highest antibacterial activity was observed for the result of ethyl acetate extract of *Cassia sophera* L. seed against *Sarcina sp*. FM1062 and *Pseudomonas aureginosa* FM 2017. These results by Kirtikar and Basu (2000) demonstrated that ethyl extract of *Cassia sophera* L. seed can help to prevent health-associated infections by *Sarcina sp*. Seed methanol extract of *Cassia sophera* L. was found to have antibacterial property against all the bacterial strains tested. The maximum activity was seen against *Sarcina sp*. FM1062 and *B. cereus* FM1042. However, seed chloroform extract of *Cassia sophera* L. was also found to have good antibacterial activity, whereas n-hexane extract was considered mildly active against bacteria. From these results suggested seed methanol extract showed more potent activity to kill bacterial strains.

It was found that flower extract of *Cassia sophera* L. was most effective against *B. cereus* FM1042 in methanol. The extract was also effective against *Pseudomonas aeruginosa* FM2017 and *Solmonella entrerica* FM2066, whereas the effectiveness of the extracts was moderate against *E. coli* FM2087, *B. subtilis* FM1057 and *Sarcina sp.* FM1062. In summary, it was observed that ethyl acetate, methanol, and chloroform extracts were the most effective against tested bacterial cultures. n-hexane extract was less active against different bacterial isolates.

In recent times, pathogenic organisms have developed resistance to antibacterial drugs (Kebede *et al.* 2021; Atef *et al.* 2019; Ashraf *et al.* 2018). For this reason, it was needed to develop alternative antibiotics from a variety of sources, including medicinal plants. Thus, from the above discussion, it can be said that various parts of *Cassia sophera* L. can be used for medicinal purposes, which are caused by several bacteria (Manandhar *et al.* 2019; Alkmini *et al.* 2021).

Conclusion

Herbal medicines can be a safe alternative to modern medicines. Antimicrobial agents are important to reduce infectious diseases. This study demonstrates that the plant extract of *Cassia sophera* L. possesses antimicrobial activity against pathogenic organisms. The antibacterial properties of the different parts of the plant are observed due to the presence of a variety of bioactive ingredients present in the plant. The results of the present study provide an important basis for the use of the organic solvent extract of *Cassia sophera* L. as a natural antimicrobial agent.

Ethical Approval

It is not applicable

Competing interests disclaimer

The authors have declared that there is no potential conflict of interest, either financial or non-financial.

Acknowledgement

We would like to thank the Bangladesh Council of Scientific and Industrial Research (BCSIR) for funding this study.

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