Exploring seeds of *Cassia tora*, a wild herb for its biological potential as antibacterial and antioxidant agent

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Abstract
Plants show medicinal properties due the presence of variety of chemical compounds in them. These are safe and affordable sources of medicines. In this study a wild herb was selected to study its biological potential. Methanol extract, ethyl acetate extract and petroleum ether extract of the seeds of plant Cassia tora were prepared using Soxhlet extraction method. Extractive yield of the methanol extract was found to be highest. These extracts were screened for the presence of phytochemical constituents in them and were then evaluated for their antioxidant activity via DPPH assay.

Using the agar well diffusion method, its antibacterial potential was assessed against both Gram positive and Gram negative bacteria. It was concluded that the all the seed extracts of this plant showed excellent antioxidant and antibacterial activity.

Keywords: Extracts, *Cassia tora*, Antioxidant, Antibacterial.

Introduction

Antioxidants are essential for shielding the body from oxidative stress brought on by free radicals, which are unstable molecules rich in electrons that can harm cells and cause chronic illnesses including cancer, heart disease, neurological disorders etc.[4,28] In order to prevent oxidation, to extend shelf life and to boost the nutritional content of food, synthetic antioxidants like butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been widely utilised in the food and pharmaceutical industries. But excessive and incorrect use of these synthetic antioxidants is linked to health risks[35].

Studies have shown that these compounds can have carcinogenic effects and may disrupt endocrine function, leading to potential long-term health problems[33]. An increasing amount of research indicates that natural antioxidants are safer and more beneficial for human health[27]. Therefore, it becomes essential to explore the natural antioxidants that can be used over synthetic ones.

Plants produce wide variety of secondary metabolites that have numerous pharmacological activities and are used in traditional and modern medicine. Some secondary metabolites, like vitamins and antioxidants, are important for human nutrition and health. These are naturally occurring compounds that the human body is accustomed to processing. They often work synergistically, meaning they enhance each other's effectiveness when consumed together. For instance, the combination of vitamin C and E can provide greater protection against oxidative damage than either antioxidant alone[6].

Herbal plants have been used for centuries for their medicinal properties including their role as antioxidants and antibacterial agents as well. These natural compounds can be effective against a range of bacteria. The complex mixture of bioactive compounds in plants can reduce the likelihood of bacteria developing resistance. In contrast, bacteria can quickly develop resistance to synthetic antibiotics, which usually have a single mode of action[26,32]. Therefore, the development of affordable, safe and natural medications is also desperately needed. The use of herbal plants as antibacterial agents has gained considerable interest, particularly due to the rise of antibiotic-resistant bacteria. Herbal plants contain a wide variety of bioactive compounds that can exert antibacterial effects and they often come with fewer side effects compared to synthetic antibiotics[3].

In this study, one such herbal plant, *Cassia tora* from the genus Cassia was explored for its biological potential. *Cassia tora* is a kind of plant in the Fabaceae family that grows easily in desert places during the rainy season. This species is wild. In the arid soil of the western tropics, this plant grows well. This is an annual herbaceous plant that resembles a little shrub and can grow up to a height of one metre. Yellow blooms with five petals are paired and placed on the leaf axils. Pods are sickle-shaped, 8–12 cm long and slightly flattened. Each rhombohedral pod contains 30 to 50 seeds. Ethnobotanical study of the herb suggests that *C. tora* preparations have been used as laxatives, as well as a treatment for rheumatic disease and other skin conditions[2].

It has been discovered that the *C. tora* leaf extract exhibits strong hepatoprotective and inflammatory properties[22]. Roasted *Cassia tora* seeds can be used in place of coffee seeds and have also been used as a colouring agent and diuretic. Seeds have high protein content and are therefore used to feed dehydrated birds and animals. The primary phytochemical compounds found in this plant include Qurcetin, Emodin, Physcion, Rhien, Alaternin and Chrysoobtusin etc. Table 1 details their various actions which include hepatoprotective, anthelmintic, fugicidal and

neuroprotective and many other properties[7,10,14,18,15,24,25,29,34,37]. Current study reveals the type of phytochemicals present in the seed part of plant and evaluating its petroleum ether extract, ethylacetate extract and methanol extract for their antioxidant and antibacterial potential.

Material and Methods

Soxhlet apparatus was used for preparation of different seed extracts. DPPH was purchased from Sigma Aldrich, India and was used without further purification. From the Department of Biosciences, Microbial Biotechnology Laboratory, H. P. University Shimla, two pure cultures of two different kinds of bacteria were obtained. The antibacterial activity of different plant extracts was investigated using these two bacterial strains: Gram negative strain, *Escherichia coli* and Gram positive strain *Staphylococcus aureus*. The plant under examination is a wild species that can be easily found in the Himachal Pradesh, India, near the Shivalik foothills at latitude 30.92 and longitude 76.83. Dr. Suman Rawat from the Department of Botany, H.P. University Shimla, identified the plant.

Table 1
Showing Phytoconstituents of seed extracts of *Cassia tora*

S.N.	Secondary metabolite	Extract	Pharmacological activity
1.	Obtusifolin-2-glucoside, chryso-obtusin-6-glucoside and norrubrofusarin-6-glucoside (Anthraquinone glycosides)[24]	Ethylacetate	Antitumour promoting activity
2.	questin and chryso-obtusin (Anthraquinones)[24]	Chloroform fraction	
3.	Questin and 2-Hydroxyemodin -1-methyl ether(Anthraquinones)[15]	Methanol	Inhibitory property against angiotensin converting enzyme.
4.	Two phenolic triglucosides, torachrysone 8-O-[b-D-glucopyranosyl(1→3)-O-b-D-glucopyranosyl(1→6)-O-b-D-glucopyranoside] (1) and toralactone 9-O-[b-D-glucopyranosyl-(1→3)-O-b-D-glucopyranosyl-(1→6)-O-b-Dglucopyranoside] (2)[10]	Ethanol	Estrogenic activity
5.	chrysophanol, isochrysophanol and aloe-emodin (anthraquinones)[7]	Ethyacetate	Immunostimlating activity
6.	Emodin, Physcion and Rhein(Anthraquinones)[18]	Chloroform fraction	Fungicidal activity
7.	Alaternin (Anthraquinone)[24]	Methanol	Strongest peroxynitrite scavenging activity
8.	Phenolicglycosides (rubrofusarin triglucoside, nor-rubrofusarin gentiobioside, demethylflavasperone gentiobioside,torachrysone gentiobioside, torachrysonetetraglucoside,torachrysone apiglucoside, torachrysone, toralactone, aloe-emodin, rhien and emodin[14].	Methanol	Antibacterial
9.	1,3,8-Trihydroxy-6-methyl-9,10-anthracenedione (Emodin)[36]	Methanol	Antioxidant
10.	Anthraquinone glycosides: 1-[(β-d-glucopyranosyl-(1 → 3)-O-β-d-glucopyranosyl-(1 → 6)-O-β-d-glucopyranosyl)oxy]-8-hydroxy-3-methyl-9,10-anthraquinone, 1-[(β-d-glucopyranosyl-(1 → 6)-O-β-d-glucopyranosyl-(1 → 3)-O-β-d-glucopyranosyl-(1 → 6)-O-β-d-glucopyranosyl)oxy]-8-hydroxy-3-methyl-9,10-anthraquinone and 2-(β-d-glucopyranosyloxy)-8-hydroxy-3-methyl-1-methoxy-9,10-anthraquinone[28]	Methanol	Hepatoprotective
11.	Naphtho-pyrone glycosides: 9-[(β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl)oxy]-10-hydroxy-7-methoxy-3-methyl-1H-naphtho[2,3-c]pyran-1 -one (5) and 6-[(α-apiofuranosyl-(1→6)-O-β-D-glucopyrano-syl)oxy]-rubrofusarin (6), together with cassiaside (3) and rubrofusarin-6-β-gentiobioside[34]	Methanol and chloroform	Hepatoprotective

Collection of the herb: Herb is in the full bloom in the month of September and October. It reaches maturity in the month of October and is ready for harvest (Fig. 1). The seed pods were manually opened, gathered, cleaned to remove any remaining dust and then allowed to air dry for 15 days in the shade at room temperature. Seeds were protected from the sun's rays to maintain the purity of their chemical composition.

Preparation of different seed extracts of the plant: Shade dried seeds (500g) were made in to a coarse powder with the help of pestle and mortar and subjected to extraction using Soxhlet extractor. The coarse powder of seeds was packed in Soxhlet apparatus. This device is made up of a bulb condenser at the top and a round-bottom flask at the bottom that is fitted with a glass extractor. Seed powder is placed within the thimble in between two plugs of cotton. The round bottom flask contains an extracting solvent, 2-3 boiling chips were added to this and heated on a water bath. Extraction process was run for three days for 6 hrs each day with different solvents like petroleum ether, ethylacetate and methanol successively to get the extracts. The solvent vapour condenses and gathers inside the extractor as it ascends to the condenser.

Mass transfer occurs as the solvent comes into touch with the powdered seeds, also allowing phytoconstituents to be leached out of the seeds as the condensate passes through them. Collected extracts were concentrated and dried under reduced pressure using rotator evaporator, labeled and stored at 4^{o}C until used. These three extracts, petroleum ether, ethylacetate and methanol were subjected to phytochemical screening using standard procedures and all the extracts were tested for their antimicrobial and antioxidant potential. This technique separates the phytoconstituents based on their respective solubilities in different polarity solvents as in fig. 2.

Qualitative phytochemical investigations: Following established methods[1], qualitative phytochemical studies were performed on petroleum ether extract, ethylacetate extract and methanol extracts. All the extracts around 5ml were completely dissolved in 50ml of their parent solvents and are labeled as stock solution for the phytochemical screening.

Antioxidant study (DPPH assay): The DPPH method was used to examine the free radical scavenging activity of the seed extracts, namely the methanol, ethyacetate and petroleum ether extracts. A minor change was made to the previously published protocol[11]. A DPPH solution at 0.3 mM was made in methanol. The three extracts were simultaneously produced as a stock solution in methanol at concentrations ranging from 50 ug to 250 ug. 3 mL of the plant extract was combined with 1 mL of this DPPH solution, mixed well and left in the dark for half an hour. At 517 nm, the absorbance was measured using a spectrophotometer. The following formula was used to determine the DPPH radical's capacity: Percentage of DPPH scavenged = absorbance control-absorbance test/absorbance control X100.

Antibacterial study: All three of the plant's seed extracts were tested for antibacterial activity using the agar well diffusion method[1]. *Escherichia coli* and *Staphylococcus aureus*, two types of bacteria that are both Gram positive and Gram negative, were procured from the Microbial Biotechnology Laboratory of the Department of Biosciences at H. P. University, Shimla. These bacterial strains were exposed to ampicillin and gentamycin as positive controls respectively. The bacterial cultures were prepared on agar medium. They were then put inside the Petri dishes that had been cleaned and allowed to dry. Following drying, 6 mm wells were created, with plant extracts serving as the negative control and antibiotics serving as the positive control.

Fig. 1: Plant *Cassia tora* and its seeds

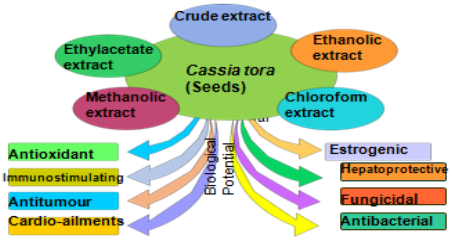

Fig. 2: Biological applications of the plant *Cassia tora* reported in literature.

Results and Discussion

Extractive yield: Seeds of *C. tora* were harvested during the month of October, cleaned, dried and crushed coarsely. Three plant extracts were prepared using seeds of the plant. Concentrated petroleum ether extract, ethylacetate extract and methanol extract weighed 8.04g, 6.14g, 28.58g respectively (Fig.3). It was observed that proportion of the compounds in methanol extract was higher than other extracts (Table 2). High yield of methanol extract indicates that it is rich in polar compounds which leached into methanol.

Phytochemical investigation: These seed extracts viz. petroleum ether extract; ethylacetate extract and methanol extract were subjected to phytochemical screening i.e. qualitative analysis of the plant. Qualitative investigation of *Cassia tora* seed extracts was found to be rich in alkaloids, flavonoids, phenols, tannins, saponins and pholobotannins and anthraquinones in methanol extract[20] whereas above mentioned phytochemicals were absent in petroleum ether extract (Table 3.) Ethylacetate extract of the plant shows the presence of alkaloids, flavonoids, phenols and terpenoids only (Fig. 4).

Antioxidant activity: Antioxidant activity of prepared extracts was evaluated using DPPH essay. A reference sample of ascorbic acid was utilized to compare the antioxidant potential of prepared seed extracts of the plant (Fig. 5) (Table 4). Studies report that DPPH ^{0} (2, 2-Diphenyl-1-picrylhydrazyl radical) due to the presence of three aromatic rings is relatively stable free radical. It can easily accept an electron or H radical to become stable diamagnetic molecule[5]. Bioactive molecules present in plant extracts are capable of donating hydrogen atoms which changes DPPH (purple) to their non radical form (yellow) which can be measured spectrometrically.

$DPPH^0 + AH \text{ -----} \rightarrow DPPHH + A^0$

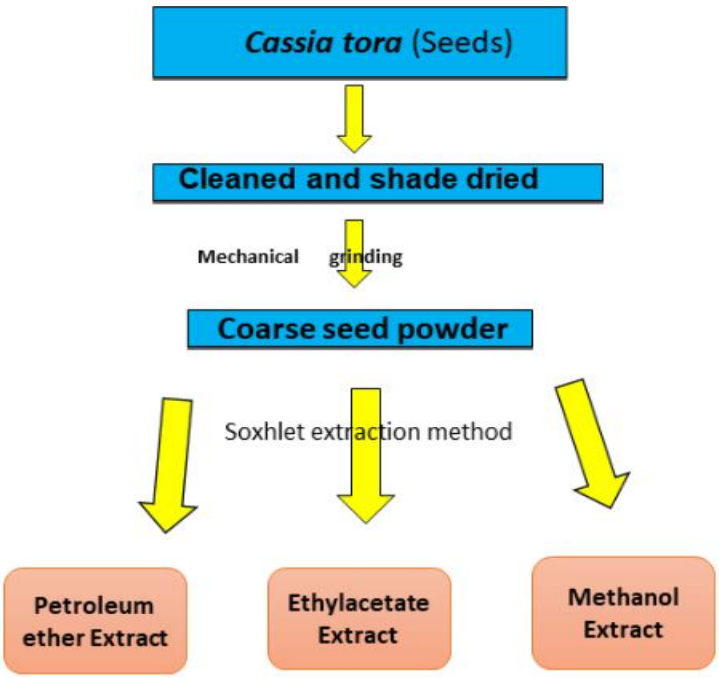

Fig. 3: Graphical representation of the extraction process

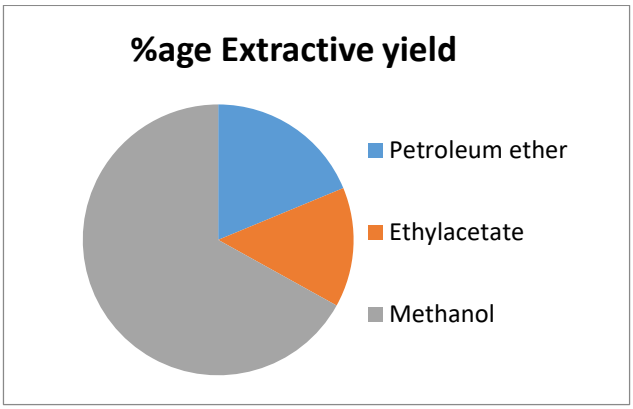

Fig. 4: Extractive yield of various extracts of seeds of *Cassia tora*

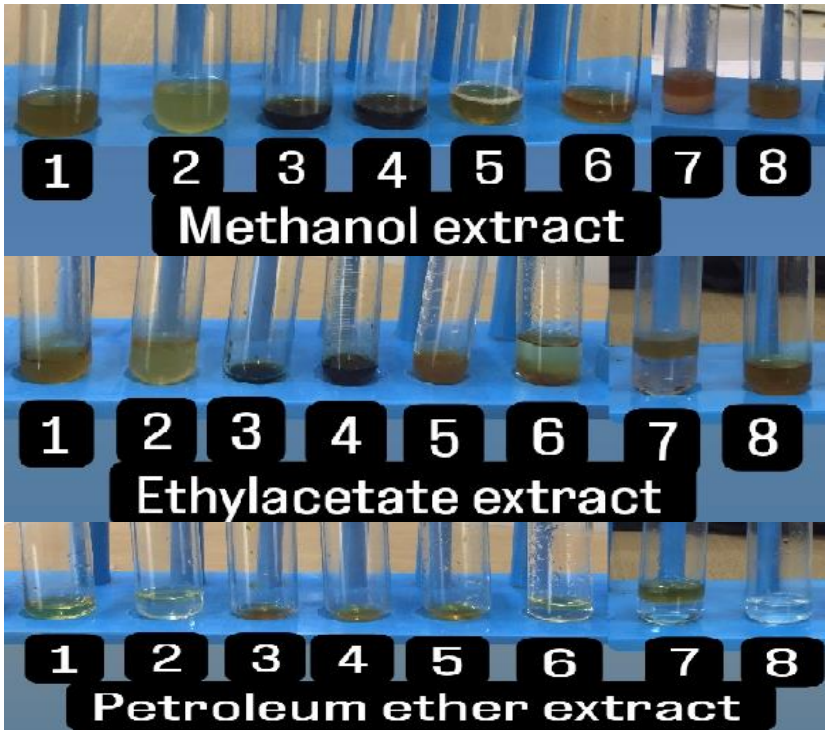

Fig. 5: Phytochemical investigations of the Methanol extract, Ethylacetate extract and Petroleum ether extract.

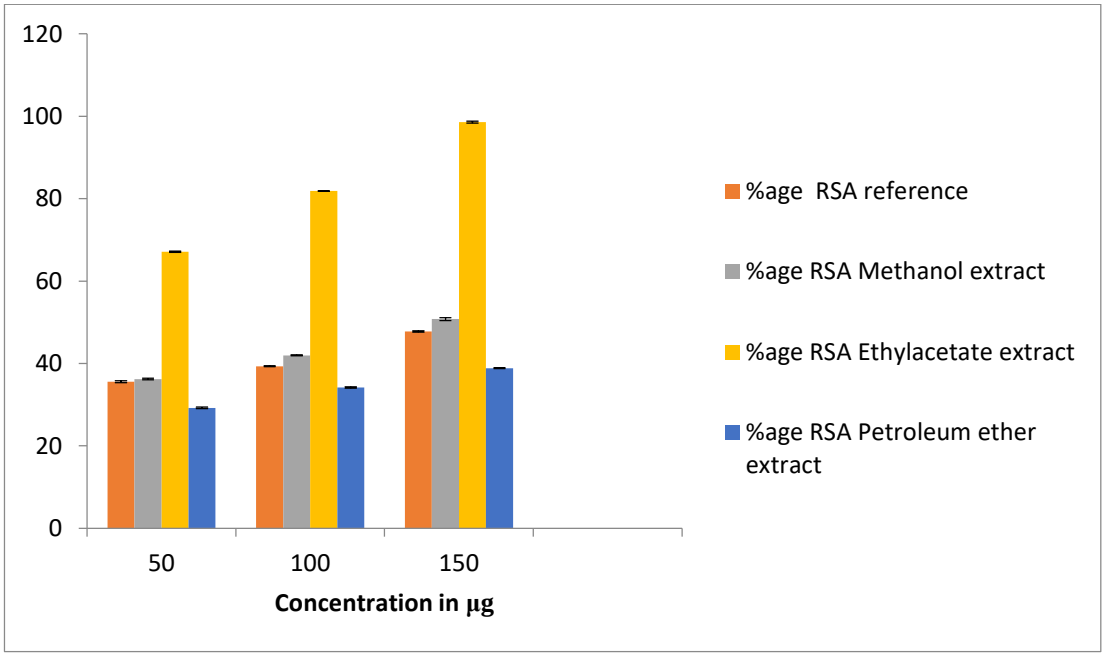

Fig. 6: Radical scavenging activity of different seed extracts of the plant *Cassia tora*.

It was observed that all the three plant extracts show good antioxidant activity. Ethylacetate extract showed maximum percentage of radical scavenging activity. Dose affects radical scavenging activity.

As the concentration of plant extract is raised, the percentage of increased radical scavenging activity also rises[23]. When an antioxidant donates an electron to a free radical, it forms a more stable, less reactive product[16].

Studies on the structure-activity relationship demonstrate that the presence of substituents like -OH inside these biomolecules is what gives them their antioxidant action. Increased hydrogen donor capacity will result in increased antioxidant activity[17].

Phenols and flavonoids inactivate the free radical species according to the hydrogen transfer mechanism and forms products that are less reactive. During this process, there is homolytic cleavage of O-H bond. Its polyphenols are more effective reducing agents than monophenols. These polyphenols have more electron-withdrawing groups which boost their antiradical potency[21]. Present study shows that ethylacetate extract shows highest antioxidant activity

suggesting that antioxidant activity is affected by the solvent used[38]. Polar biomolecules are more active in polar media[37].

Antibacterial activity: The value of zone inhibition shown in the table indicates that all *Cassia tora* plant's seed extracts have varying degrees of efficacy in suppressing the development of harmful bacteria (Fig. 6 and table 5).

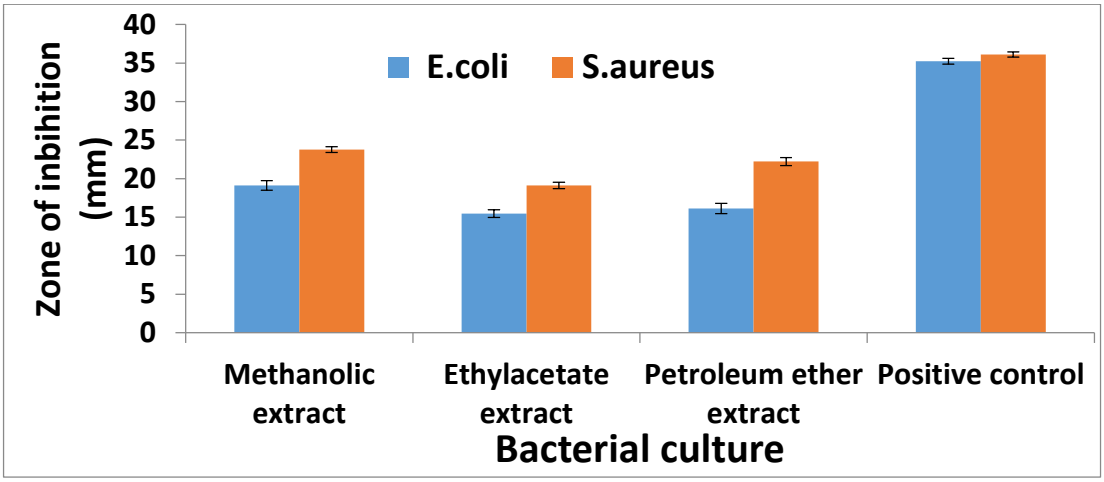

Fig. 7: Antibacterial potential of different seed extracts of the plant *Cassia tora*.

Table 2
Showing %age yield of the extracts

Extracts	Petroleum ether	Ethylacetate	Methanol
%age yield	1.60	1.22	5.71

Table 3
Showing Phytochemical screening of the seed extracts of plant *Cassia tora.*

Phytochemicals	Petroleum Ether extract	Ethylacetate extract	Methanol extract
Alkaloids	-	+	+
Flavonoids	-	-	+
Phenols	-	+	+
Tannins	-	-	+
Saponins	-	-	+
Terpenoids	-	+	-
Pholobotannins	-	-	+
Anthraquinones	-	-	+

Table 4
Showing percentage Radical scavenging activity of the extracts

Extracts	%age radical scavenging activity (50ug/ml)	%age radical scavenging activity(100ug/ml)	% age radical scavenging activity(150ug/ml)
Petroleum ether extract	29.23±0.34	34.19±0.15	38.86±0.45
Ethylacetate extract	67.13±0.45	81.86±0.24	98.56±0.32
Methanol extract	36.23±0.36	41.96± 0.23	50.77±0.05
Reference	35.56±0.23	39.34±0.04	47.76± 0.25

Table 5
Showing antibacterial activity of different plant extracts of Cassia tora

S.N.	Type of plant extract	Zone of inhibition value in mm *E.coli*	Zone of inhibition value in mm *S.aureus*
1	Methanolic	19.12±0.62	23.77±0.38
2	Ethyl acetate	15.45±0.51	19.11±0.42
3	Petroleum ether	16.12±0.68	22.22±0.51
4	Positive control	35.23±0.37	36.11±0.35

Certain phytochemicals found in plants such as tannins, flavonoids and phenols, can stop a variety of microbes from growing and functioning[8,30]. Polyphenols play an important role in defensive system of plants. These polyphenols stop the growth and activity of many type of microorganisms[9]. Relative toxicity towards microorganisms is affected by the position and number of hydroxyl groups on the phenol group.

With increase in hydroxylation, toxicity increases[31]. Here in the current study, extracts of different polarity show significant antibacterial potential. Plant extracts have a major impact on the cell membranes of GPB and GNB. A plausible process could involve modifications to the bacterial membrane[13]. An additional finding is that plant extracts have a higher inhibitory effect on *S. aureus* (GPB) compared to *E. coli* (GNB)[19]. The existence of an outer membrane encircling the GNB cell membrane could be the cause of this increased resistance[12].

Conclusion

The growing awareness of the potential health risks associated with synthetic antioxidants, coupled with the numerous benefits of natural antioxidants, underscores the need to prioritize natural sources. By consuming a diet rich in fruits, vegetables, nuts and seeds, individuals can enhance their antioxidant intake in a safe and effective manner. Additionally, the food and pharmaceutical industries should focus on developing and utilizing natural antioxidants to promote better health outcomes and sustainability. Plants have potential to be used as medicines as they contain a diverse spectrum of chemical components. They contain secondary metabolites that have medicinal characteristics.

Plant, *Cassia tora* is a wild herb easily available in North western parts of the country. Seed part of the herb was explored for the presence of phytochemical constituents. Different seed extracts prepared, were examined for the presence of phytochemical constituents qualitatively and quantitatively and hence were evaluated for their antioxidant and antibacterial potential. It was observed that due to the presence of significant amount of variety of phytochemical constituents, the seed extracts showed excellent antioxidant and antimicrobial potential. This herb has potential to cure many diseases as reported in past. This is wild, edible and easily available herb and can be explored more for its pharmacological potential. Emphasizing natural antioxidants over synthetic ones is a step towards a healthier and more environmentally responsible future.

References

1. Anita, Anjali, Awasthi A., Thakur V., Kaur M. and Sharma P., Green synthesis, characterization and antibacterial activity of Tin (IV) oxide nanoparticles using root extract of Cassia tora, Mater Today Proc. **(2023)**

2. Asolkar L.V., Kakkar K.K. and Chakre O.J., Second supplement to glossary of Indian Medicinal Plants, PID, CSIR, New Delhi, 180-181 **(1992)**

3. Blumenthal K.G., Peter J.G., Trubiano J.A. and Phillips E.J., Antibiotic allergy, *The Lancet*, **393(10167)**, 183-198 **(2019)**

4. Bocci V. and Valacchi G., Free radicals and antioxidants: how to reestablish redox homeostasis in chronic diseases?, *Current Medicinal Chemistry*, **20(27)**, 3397-415 **(2013)**

5. Brand-Williams W., Cuvelier M.E. and Berset C., Use of a free radical method to evaluate antioxidant activity, *LWT - Food Sci Technol*, **28(1)**, 25–30 **(1995)**

6. Chen X., Li H., Zhang B. and Deng Z., The synergistic and antagonistic antioxidant interactions of dietary phytochemical combinations, *Critical Reviews in Food Science and Nutrition*, **62(20)**, 5658-5677 **(2022)**

7. Chung H.S., Anthraquinones with Immunostimulating Activity from Cassia tora L., *Preventive Nutrition and Food Science*, **10(3)**, 267-271 **(2005)**

8. Cushnie T.T. and Lamb A.J., Antimicrobial activity of flavonoids, *International Journal of Antimicrobial Agents*, **26(5)**, 343-356 **(2005)**

9. Dilsha Davis and Narasimhan S., Detrimental Effects of Lithium on in vitro Seedlings of Pea (Vigna radiata), *Res. J. Biotech.*, **19(1)**, 87-90 **(2024)**

10. El-Halawany A.M., Chung M.H., Nakamura N., Ma C.M., Nishihara T. and Hattori M., Estrogenic and anti-estrogenic activities of Cassia tora phenolic constituents, *Chemical and Pharmaceutical Bulletin*, **55(10)**, 1476-1482 **(2007)**

11. Ghanimi R., Ouhammou A., El Atki Y., Bouchari M.E. and Cherkaoui M., The antioxidant activities of ethanolic, methanolic, ethyl acetate and aqueous extracts of the endemic species, Lavandula mairei Humbert (a comparative study between cold and hot extraction), *Ethiopian Journal of Health Sciences*, **32(6)**, 1231-1236 **(2022)**

12. Gyawali R., Hayek S.A. and Ibrahim S.A., Plant extracts as antimicrobials in food products: Mechanisms of action, extraction methods and applications, Handbook of Natural Antimicrobials for Food Safety and Quality, **49**, 49-62 **(2015)**

13. Hartmann M., Berditsch M., Hawecker J., Ardakani M.F., Gerthsen D. and Ulrich A.S., Damage of the bacterial cell envelope by antimicrobial peptides gramicidin S. and PGLa as revealed by transmission and scanning electron microscopy, *Antimicrobial Agents and Chemotherapy*, **54(8)**, 3132-3142 **(2010)**

14. Hatano T., Uebayashi H., Ito H., Shiota S., Tsuchiya T. and Yoshida T., Phenolic constituents of Cassia seeds and antibacterial effect of some Naphthalenes and anthraquinones on methicillin-resistant Staphylococcus aureus, *Chemical and Pharmaceutical Bulletin*, **47(8)**, 1121-1127 **(1999)**

15. Hyun S.K., Lee H., Kang S.S., Chung H.Y. and Choi J.S., Inhibitory activities of Cassia tora and its anthraquinone constituents on angiotensin-converting enzyme, *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, **23(2)**, 178-184 **(2009)**

16. Jovanovic S.V., Steenken S., Hara Y. and Simic M.G., Reduction potentials of flavonoid and model phenoxyl radicals, Which ring in flavonoids is responsible for antioxidant activity?, *Journal of the Chemical Society, Perkin Transactions 2*, **1(11)**, 2497-2504 **(1996)**

17. Karagoz A., Artun F.T., Ozcan G., Melikoglu G., Anıl S., Kultur S. and Sutlupınar N., *In vitro* evaluation of antioxidant activity of some plant methanol extracts, *Biotechnology & Biotechnological Equipment*, **29(6)**, 1184-9 **(2015)**

18. Kim Y.M., Lee C.H., Kim H.G. and Lee H.S., Anthraquinones Isolated from Cassia tora (Leguminosae) Seed Show an Antifungal Property against Phytopathogenic Fungi, *J Agric Food Chem*, **52**, 6096-6100 **(2004)**

19. Lee N.H., Lee S.M., Song D.H., Yang J.Y. and Lee H.S., Antimicrobial effect of emodin isolated from *Cassia tora* Linn. seeds against food-borne bacteria, *Journal of Applied Biological Chemistry*, **56(3)**, 187-189 **(2013)**

20. Lohar D.L., Chawan D.D. and Garg S.P., Phytochemical studies on Cassia species of Indian Arid Zone, *Current Science*, **44(2)**, 676 **(1975)**

21. Mahdi-Pour B., Jothy S.L., Latha L.Y., Chen Y. and Sasidharan S., Antioxidant activity of methanol extracts of different parts of Lantana camara, *Asian Pacific Journal of Tropical Biomedicine*, **2(12)**, 960-965 **(2012)**

22. Maitya T.K., Mandal S.C., Mukherjee P.K., Saha K., Dass J., Saha B.P. and Pal M., Evaluation of hepatoprotective potential of Cassia tora leaf extract, *Nat. Prod. Sci*., **3(2)**, 122-126 **(1997)**

23. Molinu M.G., Azara E., Barberis A. and Sanna D., Reaction time and DPPH concentration influence antioxidant activity and kinetic parameters of bioactive molecules and plant extracts in the reaction with the DPPH radical, *Journal of Food Composition and Analysis*, **35(2)**, 112-119 **(2014)**

24. Park Y.B., Isolation and Identification of Antitumor Promoters from the Seeds of *Cassia tora*, *J Microbiol Biotechnol*, **21**, 1043-1048 **(2011)**

25. Park T.H., Kim D.H., Kim C.H., Jung H.A., Choi J.S., Lee J.W. and Chung H.Y., Peroxynitrite scavenging mode of alaternin isolated from Cassia tora, *Journal of Pharmacy and Pharmacology*, **56(10)**, 1315-1321 **(2004)**

26. Qadri H., Shah A.H., Ahmad S.M., Alshehri B., Almilaibary A. and Mir M.A., Natural products and their semi-synthetic derivatives against antimicrobial-resistant human pathogenic bacteria and fungi, *Saudi Journal of Biological Sciences*, **29(9)**, 103376 **(2022)**

27. Sarkar A. and Ghosh U., Natural antioxidants-The key to safe and sustainable life, *Int. J. Latest Trends Eng. Technol*, **6(3)**, 460-466 **(2016)**

28. Stohs S.J., The role of free radicals in toxicity and disease, *Journal of Basic and Clinical Physiology and Pharmacology*, **6(3-4)**, 205-228 **(1995)**

29. Sui-Ming W., Wong M.M., Seligmann O. and Wagner H., Anthraquinone glycosides from the seeds of Cassia tora, *Phytochemistry*, **28(1)**, 211-214 **(1989)**

30. Talib W.H. and Mahasneh A.M., Antimicrobial, cytotoxicity and phytochemical screening of Jordanian plants used in traditional medicine, *Molecules*, **15(3)**, 1811-1824 **(2010)**

31. Urs N.V.R.R. and Dunleavy J.M., Enhancement of the bactericidal activity of a peroxidase system by phenolic compounds (Xanthomonas phaseoli var. sojensis, soybeans), *Phytopathology*, **65**, 686–690 **(1975)**

32. Varela M.F., Stephen J., Lekshmi M., Ojha M., Wenzel N., Sanford LM., Hernandez A.J., Parvathi A. and Kumar S.H., Bacterial resistance to antimicrobial agents, *Antibiotics*, **10(5)**, 593 **(2021)**

33. Wang W., Xiong P., Zhang H., Zhu Q., Liao C. and Jiang G., Analysis, occurrence, toxicity and environmental health risks of synthetic phenolic antioxidants: A review, *Environmental Research*, **201**, 111531 **(2021)**

34. Wong S.M., Wong M.M., Seligmann O. and Wagner H., New Antihepatotoxic Naphtho-pyrone Glycosides from the Seeds of Cassia tora1, *Planta Medica*, **55(3)**, 276-280 **(1989)**

35. Xu X., Liu A., Hu S., Ares I., Martínez-Larrañaga M.R., Wang X., Martínez M., Anadón A. and Martínez M.A., Synthetic phenolic antioxidants: Metabolism, hazards and mechanism of action, *Food Chemistry*, **353**,129-488 **(2021)**

36. Yen G.C. and Chuang D.Y., Antioxidant properties of water extracts from Cassia tora L. in relation to the degree of roasting, *Journal of Agricultural and Food Chemistry*, **48(7)**, 2760-2765 **(2000)**

37. Yen G.C., Chen H.W. and Duh P.D., Extraction and identification of an antioxidative component from Jue Ming Zi (Cassia tora L.), *Journal of Agricultural and Food Chemistry*, **46(3)**, 820-824 **(1998)**

38. Zhenbao J., Fei T., Ling G., Guanjun T. and Xiaolin D., Antioxidant properties of extracts from juemingzi (Cassia tora L.) evaluated *in vitro*, *LWT-Food Science and Technology*, **40(6)**, 1072-1077 **(2007)**.

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