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RESEARCH ARTICLE

Evaluation of antioxidant, antimicrobial, and anticancer properties of *Senna tora* (L.) Roxb. Leaf extract

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Abstract

Senna tora is a well-known plant of Fabaceae family. It is common weed found throughout the Indian subcontinent. Traditionally this plant is used for the treatment of leprosy, ringworm, skin and liver disorder. In the present study the antioxidant, antimicrobial and anticancer potential of the *Senna tora* extracts was evaluated. The ethanolic and aqueous extracts of the *Senna tora* leaves was prepared. The phytochemical constitution of both the extract was analysed by liquid chromatography mass spectroscopy (LCMS). The antioxidant potential was checked by DPPH assay and the antimicrobial activity was checked on different gram positive and gram-negative bacteria. MCF-7 cells were used to study the anticancer potential of *Senna tora* extract. Presence of various phytochemicals were noted in LCMS analysis. The ethanolic extract showed better antioxidant and antimicrobial results as compared to the aqueous extracts. The *Senna tora* extracts exhibited cytotoxic potential on MCF-7 cell lines and the IC50 was found to be 22.26 ± 1.23 µg/ml. From the above results it can be concluded that the phytochemicals (flavonoids, alkaloids, saponins, glycosides, fatty acids and etc) present in *Senna tora* leaves extracts are responsible for its antioxidant, antimicrobial and anticancer efficacy. This plant extracts can be used as a suitable drug candidate. However, further in-depth study is required to support this claim.

Keywords: Phytochemical screening, DPPH Assay, antimicrobial, cytotoxic assay.

Introduction

The increases in multidrug-resistant bacteria have increased the need for the development of new and effective antimicrobial agents. There are numerous studies which have reported the antimicrobial activities of various plants (Shereen *et al.* 2024). Just like multi drug resistant bacteria, the menace of cancer is also growing day by day. One out of six women and one out of five men are cancer patients. The high death rates and post-treatment complications in cancer patients are due to the lack of target-specific drugs. Several studies claim that the plant extracts have high antioxidant activities and can successfully decrease the tumour cell mass (Jaber 2024).

Plants have been an essential part of human existence since times immemorial. Humans have used plants as a food and medicines. According to WHO about 80% of human population still depends on plant-based medications. Plants exhibits therapeutic potential due to presence of various phytochemicals such as polyphenols, flavonoids, tannins, saponins, glycosides, alkaloids, resins, volatile oils and etc. (Al Qaisi *et al.* 2024).

Senna tora is a well-known herb found in India and other tropical countries. It is an annual herb with an average size of around 60 to 90 cm; this plant has a very pungent odour. This is a well-documented plant of both the Ayurveda and the Chinese medicinal system (Nkwocha *et al*., 2023a). This plant is known as "*Chakramard*" in Ayurveda, "*Jue Ming Zi*" in Chinese medicinal system, and "*Panwar*" in Unani medicine (Harshal A. Pawar & Priscilla M. D'mello, 2011). In Ayurveda, this plant is used in the form of paste, powder or oil for treatment of inflammation, chronic wounds and ring worms (Srivastava 2017). According to some reports

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the *Senna tora* extract possesses larvicidal activity (Supare and Patil 2015). Various studies have shown that the *Senna tora* extract exhibits various pharmacological activities such as: antidiabetic, antibacterial, antiulcer, anthelminthic, antifertility, antibiofilm, hepatoprotective, and many more (Telrandhe and Gunde 2022). These activities are exhibited by the *Senna tora* extract because of the presence of flavonoids, anthraquinones and glucosides (Jain and Patil 2010). The leaves of *Senna tora* contains kaempferol, chrysophanol, emodin, mannitol, rhein, quercetin, juglanin, stearic, succinic, tartaric acids and etc (Shadab *et al.* 2019).

The present study was designed to determine the antioxidant, antimicrobial and anticancer potential of *Senna tora* leaves extracts.

Materials and methods

Plant collection and authentication

The fresh plants of *Senna tora* were collected from the Hudkeshwar area, Nagpur district of Maharashtra during the month of August to October. The plant was authenticated by Taxonomist, Department of Botany, Rashtrasant Tukadoji Maharaj Nagpur University, and a herbarium specimen was submitted to the Department of Botany (voucher specimen no. 10921).

Preparation of plant extracts

The fresh leaves were washed under running tap water to clear the dust or any other surface impurities. The leaves were shade dried. The dried leaves were grinded to coarse powder using a mechanical blender. The leaf powder (10g) was extracted with ethanol (70%) and distilled water in a Soxhlet extractor for 12-14 hours. The ethanol and aqueous extracts were filtered by using Whatman filter paper and concentrated to dryness using rotary evaporator. The concentrated extract was stored at room temperature for further analysis.

Phytochemical Screening

The phytochemical screening was done to identify the active compounds present in ethanolic and aqueous extracts of *Senna tora*. The presence of alkaloids(Dragendorff's test, Mayer's test), terpenoids(Salkowski test), flavonoids(Shinoda's test, Lead Acetate test), reducing sugars(Benedict's test, Fehling test), tannins(Ferric Chloride test, Lead acetate test), saponins(Froth test), anthraquinone glycoside(Borntrager's test) and C-glycoside(Keller-Kiliani test) was determined in the ethanol and aqueous extracts of *Senna tora* leaves.

Chemo profiling of *Senna tora* leaves extract

The presence of various bioactive compounds in the ethanolic and aqueous extracts of *Senna tora* leaves was identified with the help of liquid chromatography mass spectroscopy (LCMS)

LC-MS Analysis

Metabolite profiling of the crude extract was done using UPLC-QTOF-MS in positive (ESI+) and negative (ESI-) mode. The analysis was done by filtering ethanolic and aqueous whole plant extracts through 0.25 mm polyvinylidene fluoride (PVDF) membrane syringe filters into a 2 ml vial. A sample injection volume of 5 ml was used for chromatographic separation of analytes in ultra-performance liquid chromatography (UPLC) through an RPC-18 column with dimensions of 2.7 mm (particle size), 2.1 mm (internal diameter), 50 mm (length). The analytical run was set at 30 minutes. Gas Temp 350°C, Gas Flow (l/min) 8, Nebulizer (psig) 35, Sheath Gas Temp 350°C, Sheath Gas Flow 11 etc with scan rate 2 spectra/min having Acquisition Mode 60-1700 (m/z) The mobile phase was made up of two solvents: solvent (A) deionized water containing 0.1 % formic acid and solvent (B) acetonitrile. The elution was carried out at room temperature at a flow rate of 0.3 ml/min. The elution profile was isocratic from 0 to 18 minutes 95% (A) and 5% (B), 18 to 25 minutes (5% (A) and 95 % (B), 25 to 25.10 minutes (95% (A) and 5% (B), and 25.10 to 30 minutes (5% (A) and 95 % (B).

Antioxidant Activity (DPPH Scavenging Assays)

With a few modifications, the in-vitro DPPH radical scavenging activity was evaluated using the method published by (Blois, 1958; and Rosidah *et al*., 2008). In the 96 well plates, 10 µl of plant extract solution was combined with 290 µl of 10mg in 1000 mL of DPPH. The plate was then incubated in the dark for 20 minutes. At 517 nm, absorbance was measured. The absorbance of each sample was compared to that of a blank solution (DPPH without sample) to measure its free radical scavenging activity. The positive control was ascorbic acid. The capacity to scavenge the DPPH radical was estimated as a percentage inhibition using the following equation.

DPPH Scavenging Activity %=

Where, OD1 indicates Optical density of control, and OD2 indicates Optical density of test sample.

Antimicrobial activity

The antimicrobial activity of the plant extracts was investigated using the well diffusion method. To establish a homogeneous microbial growth of the plate, bacterial cultures were sub-cultured in Mueller-Hinton Broth medium and incubated for 24 hours at 37°C. Then, the 100 µl cultures were spread out on Mueller-Hinton Agar plates with 2 Gram-positive *Pseudomonas aeruginosa, Staphylococcus aureus*, and 2 Gram-negative *Escherichia coli, Proteus vulgaris* bacterial strains. To investigate the antibacterial activity, 20 µl different concentrations of plant extracts (25, 50, 75, 100 µg/ml), and streptomycin as the positive control and Ethanol and Distilled water were selected as the negative

control. Ultimately, the petri dishes were incubated at 37°C for a whole day. The diameter of the inhibitory zone was measured and allowed researchers to assess the antibacterial efficacy and compare it with the groups in control (Urnukhsaikhan *et al.* 2021).

Cytotoxic assay

Cytotoxicity of the provided samples on MCF-7 (Procured from NCCS Pune) cell line was determined by MTT Assay. The cells (10000 cells/well) were cultured in 96-well plates and incubated (Air-Jacketed CO_2 incubator, Heal Force- HF90) for 24 h in DMEM medium (Dulbecco's Modified Eagle Medium-AT149-1L- HIMEDIA) supplemented with 10% FBS (Fetal Bovine Serum - HIMEDIA-RM 10432) and 1% antibiotic solution (Penicillin-Streptomycin-Sigma-Aldrich P0781) at 37°C with 5% CO_2. The next day, cells were treated from different concentrations of the formulations. Stock solution of samples was prepared in DMSO and further diluted to get different concentrations in incomplete Cell culture Medium (Without FBS). Cells without treatment were considered as Control and cells without MTT were considered as the Blank. After incubation for 24 hours, MTT Solution (5 mg/ml) was added to cell culture and further incubated for 2 h. At the end of the experiment, culture supernatant was removed, and cell layer matrix was dissolved in 100 µl Dimethyl Sulfoxide (DMSO) and read in an ELISA plate reader (iMark, Biorad, USA) at 540 nm. IC_{50} was calculated by using the software Graph Pad Prism

Table 1: List of phytochemicals present in aqueous and ethanolic extracts of the *Senna tora* leaves

Test	*Aqueous Extract*	*Ethanolic Extract*
Alkaloids	+	+
Carbohydrates	+	+
Glucosides	+	+
Saponins	+	+
Phytosterols	+	+
Phenol	+	+
Tannins	+	+
Flavonoids	+	+
Anthraquinones	+	-
Diterpenes	+	+

Results

Phytochemical screening

Qualitative screening of the aqueous and ethanolic extract of the *Senna tora* leaves showed the presence of various phytochemicals listed below (Table 1). Same phytochemicals were present in both the extracts. However, only anthraquinones test was negative in ethanolic extract.

Table 2: List of different phytochemicals present in aqueous extract of the *Senna tora* leaves

Sr. No.	*Groups*	*Compounds*
1	Carbohydrate	Neoglucobrassicin, Ethyl-7-epi-12-hydroxyjasmonate glucoside
2	Amino Acid	Arginyl-Asparagine, Tiopronin, Queuine, 4-Fluoro-L-threonine, alpha-Fluoro-beta-alanine, Indospicine, N2-Acetyl-L-aminoadipate semialdehyde, N-Acetyldjenkolic acid, S-(2-Hydroxyethyl)-N acetyl-L-cysteine
3	Lipids	1-(6Z,9Z,12Z,15Z-octadecatetraenoyl)-2-hexadecanoyl-glycero-3-phosphocholine, 6-Gingerol, Tutin, Alpha,alpha'-Trehalose-6- palmitate, Dihydroxycampesterol
4	Flavonoids	Gallocatechin, Sciadopitysin, 2-(3,5-Dimethoxyphenyl)- 5,6,7,8-tetramethoxy-4H-1-benzopyran-4-one, Camellianin A
5	Carboxylic acid	Doisynoestrol, 2-Hydroxy-cis-hex-2,4- dienoate, Isonicotineamide, Dihydrophaseic acid
6	Alkaloid	Actinidine, Retamine, Podophyllotoxin
7	Fatty acid	2-Oxo-4-hydroxy-5- aminovalerate, Dolichotheline, Heptadecatrienoic acid, Tetrahydrofurfuryl butyrate, Diisopentyl thiomalate, Methyl farnesoate
8	Glycosides	Neolinustatin, Gingerdiol-4'-O-beta-D-glucopyranoside, Xanthotoxol glucoside, Furaneol-4-(6-malonylglucoside)
9	Steroid	17-Hydroxy-3-oxo-17alpha-pregna-1,4-diene-21-carboxylic acid, gamma-lactone, Podecdysone B
10	Terpene	Allamandin
11	Anthraquinone	Hypericin
12	Isoquinoline	4-Hydroxydebrisoquine
13	Terpene glycoside	(4R,5S,7R,11S)-11,12-Dihydroxy-1(10)- spirovetiven-2-one 11- glucoside
14	Sesquiterpene lactone	Taraxacolide-1-O-b-D-glucopyranoside
15	Lactone	D-Homo-A-nor-17a-oxaandrost-3(5)-ene-2,17-dione
16	Sesquiterpenoid	Pentalen-13-al
17	Saponins	Cyclopassifloside II

Table 3: List of different phytochemicals present in the ethanolic extract of the *Senna tora* leaves

Sr. No.	*Groups*	*Compounds*
1	Carbohydrate	6-Chloropurine riboside, Ethyl-7-epi-12 hydroxyjasmonate glucoside, Methyl-7-epi-12- hydroxyjasmonate glucoside, Corchoionol C 9- glucoside, 5-Megastigmen-7-yne-3,9-diol 9-glucoside, xi-3-Hydroxy-5- phenylpentanoic acid O-beta-D-Glucopyranoside
2	Amino Acid	N-Acetyl-leu-leu-tyr-amide, epibatidine, Tiopronin, 4-Fluoro-L-threonine
3	Flavonoid	Sciadopitysin, Naringenin 5-O-glucuronide, Quercetin, Morusin, 6-Gingerol, Valine, Nepitrin
4	Fatty acid	Thiolactomycin, Diisopentyl thiomalate, Panaxytriol
5	Glycoside	3-Ketosucrose, Isorhamnetin 3- rutinoside 4'-rhamnoside, Calotropin, Abscisic alcohol 11- glucoside, Ptaquiloside, Pteroside A, Isopetasoside, Ranunculin, Netilmicin, Iridotrial glucoside, Menthyl beta-D-glucoside
6	Steroid	Fluorometholone 17- acetate, 17[[3-(1- Pyrrolidinyl) propyl] imino] androst-5-en-3beta-ol acetate, Estrone 3-sulfate, Podecdysone B, 3alpha,12alpha-Dihydroxy-5beta-pregnan-20-one diacetate, Diadinoxanthin
7	Terpenoids	Cinnzeylanine, Cincassiol B, Orizabin, Cinncassiol C1, Dihydrophaseic acid, (S)-gamma-Calacorene, Picrasin G, Saussurea lactone, 2-(2-Methylbutanoyl)-9-(3-methyl-2E- pentenoyl)-2b,9a-dihydroxy-4Z,10(14)-oplopadien-3- one, Orizabin, Montanol, Cyclopassifloside II
8	Anthracene	Rheinoside C, Patientoside A
9	Lipid	(22R,23R)-22,23- Dihydroxycampesterol
10	Carboxylic acid	3-[1,4,4-Tris(2-carboxyethyl)-2,3-dioxocyclododecyl propanoic acid
11	Alkaloid	Solanocapsine
12	Quinones	Urdamycinone F
13	L-glutamic acid	5-L-Glutamyl-taurine
14	Isoquinoline	4-Hydroxydebrisoquine
15	Lactone	Taraxacolide 1-O-b-D-glucopyranoside
16	Quinazolines	Aniflorine
17	Isoquinolines	Lotusine
18	Quinolines	Evocarpine

Chemo profiling of *Senna tora* leaves

LCMS

Both the plant extracts were subjected to LCMS analysis and the presence of following compounds was noted. Total 47 compounds were identified in aqueous extract (Table 2) and 61 compounds in ethanolic extract (Table 3).

Antioxidant assay

For this assay, six different concentrations of the plant extracts (100, 200, 400, 600, 800 and 1000 µg/ml) were taken. In this assay it was maximum free radical scavenging activity was seen at the highest concentration, 1000 µg/ml. However, the ethanolic extract showed better free radical scavenging activity as compared to the aqueous extract (Figure 1).

Antimicrobial potential

The antimicrobial potential of both extracts was checked on different strains of gram-positive and gram-negative bacteria. For this assay 5 different concentrations (25, 50, 75, 100 and 1000 µg/ml) of the plant extracts were taken and streptomycin was taken as standard drug. At highest concentration of aqueous extract of maximum zone of inhibition was noted in *Pseudomonas aeruginosa* and minimum zone of inhibition was noted in *Staphylococcus aureus* (fig 2a-2e). At highest concentration of ethanolic extract maximum zone of inhibition was seen in Pseudomonas *aeruginosa,* and the minimum zone of

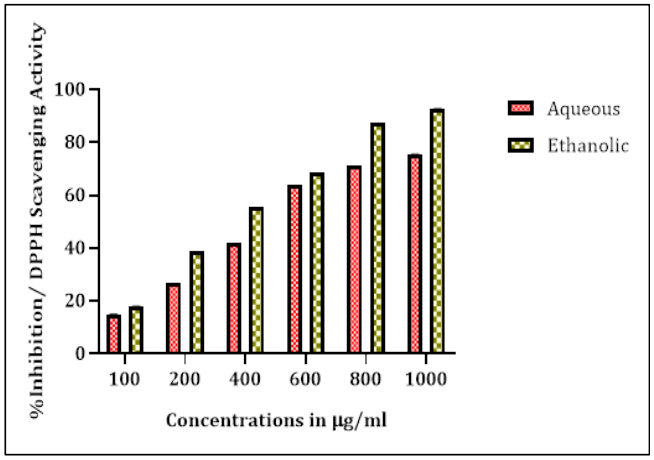

Figure 1: Antioxidant potential of aqueous and ethanolic extract of the *Senna tora* leaves

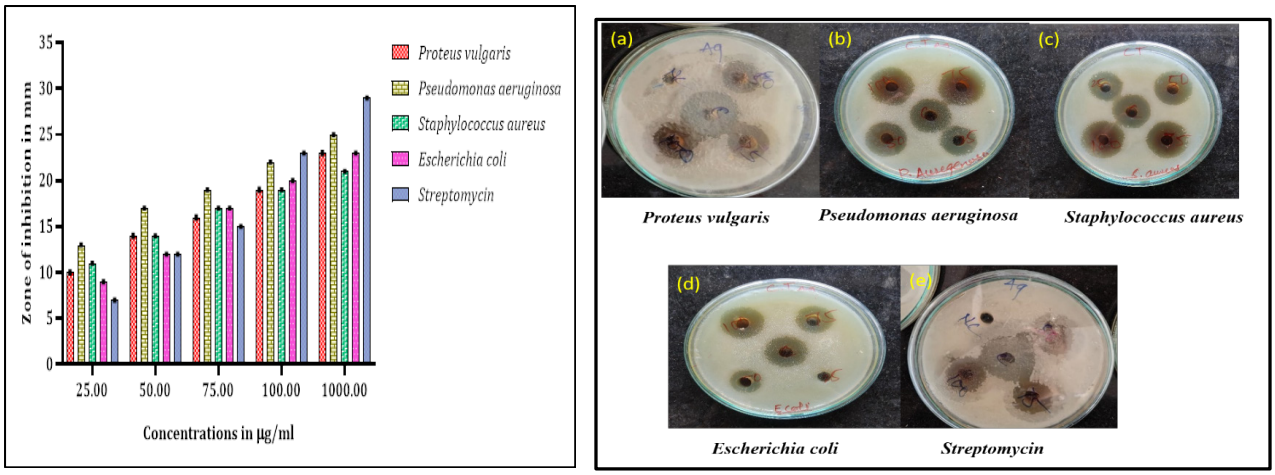

Figure 2: Antimicrobial potential of aqueous extract of the *Senna tora* leaves

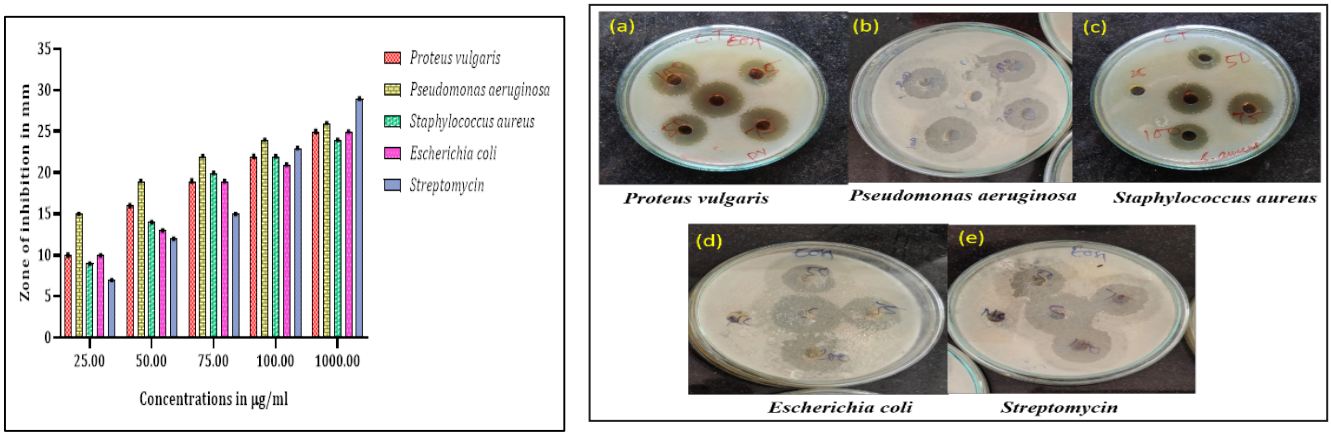

Figure 3: Antimicrobial potential of ethanolic extract of the *Senna tora* leaves

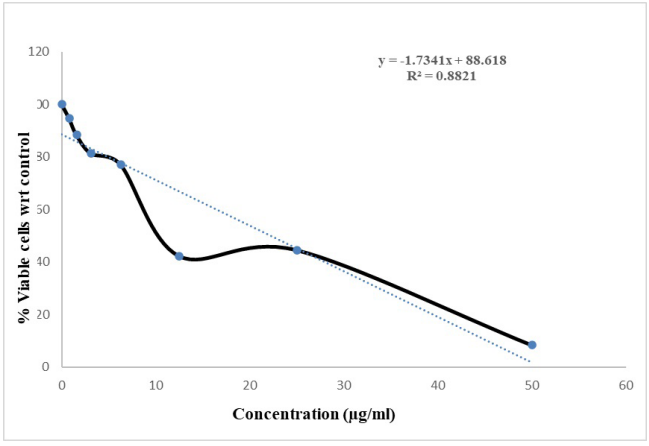

Figure 4: Anticancer potential of ethanolic extract of the *Senna tora* leaves extract

inhibition was seen in *Staphylococcus aureus* (fig 3a-3e). However, the ethanolic extract of *Senna tora* showed better antimicrobial results as compared to aqueous extract.

Cytotoxic assay results

The cytotoxic assay of the ethanolic extract was studied on MCF-7 cell lines. The cell lines were treated with different concentrations of plant extract and the reading were recorded. The IC50 of the ethanolic extract of the *Senna tora* leaves was found to be 22.26 ± 1.23 µg/ml (Figure 4).

Discussion

A wide variety of plant extracts have been screened for their anticancer potential. The plant extracts have shown promising results as anticancer agents due to the presence of various phytochemicals in it (Villacorta *et al.* 2017). In present study we are evaluating the antioxidant, antimicrobial and anticancer potential of *Senna tora* leaves extract. The aqueous and ethanolic extract of *Senna tora* leaves was made and the extracts were subjected to phytochemical analysis. In both aqueous and ethanolic extract various phytochemicals were noted: alkaloids, carbohydrates, glucosides, saponins, phytosterols, phenol, tannins, flavonoids, diterpenes. However, anthraquinones

were only present in aqueous extract. Similar results were reported by (Asba and Meeta 2017). The presence of various phytochemicals was confirmed by liquid chromatography mass spectroscopy (LC-MS). A total 47 compounds were identified in aqueous extract and 61 compounds were identified in ethanolic extract. Most common compounds identified were flavonoids, amino acids, glycosides and alkaloids. The similar findings were reported by (Nkwocha *et al*. 2023) and (Shukla *et al*. 2021). DPPH free radical scavenging activity is an assay that determines the antioxidant potential of plant extracts (Saravanakumar *et al*. 2015). Here in this study, we have determined the antioxidant potential of both aqueous and ethanolic extracts of *Senna tora* leaves. The polyphenolic compounds present in *Senna tora* leaves extracts are responsible for its antioxidant potential (Sirappuselvi and Chitra 2012). Our finding corresponds with the findings of (Chethana *et al*. 2017) and (Kanta Sahu *et al*. 2013) Commelina benghalensis, Senna tora and Bauhinia purpurea are also equally potent. The results show that the ethanolic extract possesses more antioxidant activity than methanol and hexane extract. The phytochemical analysis of leafy vegetables indicates the presence of phenolic contents in different degrees and the positive correlation between antioxidant activity and total polyphenols. *Senna tora* leaves are known for its pharmacological properties (Mazumder *et al*.2005). In present study we have determined the antimicrobial and anticancer efficacy of *Senna tora* leaves extract. The antimicrobial potential of the both aqueous and ethanolic extract was checked on different strains of gram-negative and gram-positive bacteria. Both the extracts showed maximum inhibition on Gram-negative bacteria as compared to Gram-positive bacteria. Our results are in accordance with the results reported by (Chavan *et al*. 2011). The phytochemicals present in *Senna tora* leaves extract have antimicrobial activity as it can degrade the bacterial cell wall thereby killing the bacterial cell (Das *et al*. 2010). The Gram-positive bacteria are more resistant to the antibacterial agent because the Gram-positive bacteria have a thicker peptidoglycan layer as compared to the Gram-negative bacteria (Saravanakumar *et al*. 2015). For further anticancer activity ethanolic extracts was used as the ethanolic extracts showed better results as compared to the aqueous extracts. The anticancer efficacy was checked on MCF-7 cell lines, and the IC50 was found to be 22.26 ± 1.23 µg/ml. The *Senna tora* leaves extract was able to check the growth of the MCF-7 cell line due to the presence of phenolic compounds in the extract. Our results are similar to the results of (El-Halawany *et al*.2007).

Conclusion

Our study compares and demonstrates the antioxidant, antimicrobial and anticancer potential of both aqueous and ethanolic extracts of *Senna tora* leaves. Both the extracts showed antimicrobial efficacy. However, better antimicrobial effect was seen in the groups treated with ethanolic extract of *Senna tora* leaves. The anticancer efficacy of ethanolic extract was checked on MCF-7 cell lines. The ethanolic extract of *Senna tora* leaves possesses anticancer properties. These pharmacological activities could be due to the presence of phenolic compounds in the extract.

In conclusion we in a position to say that the *Senna tora* leaves extract can be used as an antioxidant, antimicrobial and anticancer agent after further in-depth study.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Data availability statement

The data supporting the findings of this study are available from the corresponding authors upon request.

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