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RESEARCH ARTICLE

ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF LEAF EXTRACTS OF SENNA TORA (L.) ROXB.

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Abstract

Plants have always been an important source of medicines since ancient times and seventy percent of the worldwide population still relies on one or other forms of traditional plant based medicine. Plant items have been essential for phytomedicines since days of yore. These can be derived from any part of the plants like bark, leaves, flowers, roots, fruits, seeds, etc. The present exploration has been conducted in the leaf of *Senna tora* performing various phytochemical tests to identify the secondary metabolites present in it such as alkaloids, flavonoids, sugars, glycosides, saponins, steroids, tannins, phenolic compounds, Vitamin C, proteins, amino acids and carbohydrates. The maximum phenolic content was presented in methanol solvents 1.41 ± 0.44 and lowest content was presented in petroleum ether extract 0.17 ± 0.21. Antibacterial activity were estimated and evaluated by using different types of extract against *Escherichia coli, Klebsiella pneumoniae, Pseudomonas stutzeri, Bacillus thuriengensis* and *Staphylococcus*. Among these the maximum antibacterial activity (Zone of inhibition 19.0 mm) shown against *Klebsiella pneumoniae* in the extract of Petroleum ether. The minimum antibacterial activity observed (Zone of inhibition 11.0 mm) against *Staphylococcus ceureus in* extract of Ethanol extract of *Senna tora* (L.) Roxb.

Keywords: *Senna tora* (L.), phytochemical analysis, antibacterial activity, bacterial strains

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Introduction

Several medicinal properties have been attributed to natural herbs. Medicinal plants are the essential provider of imaginative drugs and medical services items (Ivanova *et al.*, 2005). The history of plants used for medicinal purposes is potentially as ancient as human history. Extraction and portrayal of a few dynamic phytocompounds from these green plants have brought forth some high action profile drugs
(Mandal *et al.*, 2007). A developing collection of proof shows that optional plant metabolites assume basic parts in human wellbeing and might be healthfully significant (Hertog *et al.*, 1993). Phytochemical screening of plants has uncovered the presence of various synthetic substances including alkaloids, tannins, flavonoids, steroids, glycosides, saponins and so forth Many plant concentrates and phytochemicals show cancer prevention agent/free extremist searching properties (Larson 1988; Nair *et al.*, 2007). Secondary metabolites of plants serve as defence mechanisms against predation by many microorganisms, insects and herbivores (Lutterodt *et al.*, 1999).

Normally, free radicals of various types are generated at a low level in cells to assist with the regulation of many physiological functions and are extinguished by an integrated antioxidant mechanism in the body. However, when free radicals are created in excess, they may be harmful, leading to inflammation, ischemia, lung damage and other degenerative diseases (Hadi *et al*,. 2000; Cavalcanti *et al*,. 2006). Free extreme responses, particularly with cooperation of oxidative revolutionaries, have been demonstrated to be engaged with numerous natural cycles that cause harm to lipids, proteins, films and nucleic acids, consequently leading to an assortment of sicknesses (Campos *et al.* 2006).

The phenolic compounds are one of the biggest and most omnipresent gatherings of plant metabolites that have a sweet-smelling ring bearing at least one hydroxyl constituents (Singh *et al.* 2007). Phenolic compounds are generally found in the auxiliary results of restorative plants, just as in numerous consumable plants (Hagerman *et al.*, 1998). Various investigations have zeroed in on the natural exercises of phenolic compounds, which are likely cell reinforcements and free extremist scroungers (Cespedes *et al.*, 2008). Several studies have described the antioxidant properties of medicinal plants, foods, and beverages which are rich in phenolic compounds (Krings and Berger 2001). Flavonoids are a wide class of plant phenolics that are known to have a grounded defensive capacity against film lipo-peroxidative harms (Sen *et al*., 2005).

Plant items have been the piece of phytomedicines since days of yore. These can be gotten from any piece of the plant like bark, leaves, blossoms, roots, natural products, seeds, and so on (Gordon and David 2001) that is, any important for the plant might contain dynamic parts. Information on the synthetic constituents of plants is alluring in light of the fact that such data will be of an incentive for the amalgamation of perplexing compound substances. Such phytochemical screening of different plants is accounted for by numerous specialists (Mojab *et al.*, 2003;Parekh and Chanda 2008).

In recent years, the use of secondary plant metabolites (phytochemicals) and Antibacterial efficacy has been actively explored as alternatives to and in combination with antibiotics to cure bacterial infections (Liu *et al.*, 2001). Antioxidants and antimicrobial properties of different extracts from many plants have recently been of considerable concern to both science and the food industry, as their potential use as natural additives has arisen from an increasing tendency to substitute conventional antioxidants and antimicrobials with natural ones (Deba *et al.*, 2008). The cancer prevention agent

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Impacts of plant separates are expected to their polyphenolic materials (Lu and Foo 2001; Murthy *et al.*, 2002). Because such, plants with a high level of polyphenols are more essential than natural antimicrobials (Baravalia*et al*,. 2009).

The scope for the production of antimicrobials from higher plants is rewarding, as it would contribute to the development of phytomedicine for intervention against microbes; as a result, plants are one of the cornerstones of modern medicine for the accomplishment of new concepts (Evans *et al.*, 2002). Plant-based antimicrobials are a vast untapped source of medication. A part of the plant based antimicrobials have tremendous therapeutic promise as they can fulfil the task without any side effects that are often associated with synthetic antimicrobials. Further disclosure of plant-determined antimicrobials is required today (Hussain and Gorsi 2004).

Scientific experiments, chemical studies on the antimicrobial properties of plant components were first reported at the end of the 19th century. It is estimated that today, plant materials are present in, or have provided, 50 percent Western drug models (Robbers *et al.* 1996). Many commercially proven medicines found in modern medicine were originally used in primitive form in conventional or folk healing practises, or for other reasons as indicated.

Senna tora (L.) Roxb. having a place with the family Fabaceae is a native bush of Bangladesh and is broadly disseminated all through the country. A few examinations have been led all through the last decade to research substance and organic properties of *Senna tora*. Antihepatotoxic naphtha-pyrine glycosides were accounted for to be detached from the seeds of *Senna tora*.. Cancer prevention agent properties (Yen and Chuang 2000) and inhibitory impact (Wu *et al.*, 2001) of the concentrate of *Senna tora* have effectively been accounted for. A new report was led by the researchers of the Department of Food Science and Nutrition, Catholic University of Daegu, Korea who inferred that Cassia tora enhancements can assist with further developing serum lipid status in type-II diabetic subjects without critical antagonistic impact (Cho *et al*., 2005). In the new review, evaluating for antibacterial just as pain relieving action of the concentrate of *Senna tora* was directed to offer help for the utilization of this plant as conventional medication. Phytochemical screening gives information on the synthetic constituents of this plant not just for the revelation of new remedial specialists, yet in addition for data in finding new wellsprings of other financial materials. Therefore, in the present study, screening the phytochemical compounds from various extracts of leaves of *Sennatora*(L.) Roxb.; To quantitative Estimation of total Phenolic constituents of leaf of *Senna tora* (L.) Roxb.; and to analysis the Antibacterial activity of various extract of leaf of *Senna tora* (L.) Roxb. have been aimed at and carried out.

Materials and Method

Place of work

The present work has been carried out at PG &Research Department of Biotechnology which is recognized by Sri Vinayaga College of Arts & Science, Ulundurpet.

Collection of Plant

The plant *Senna tora* was collected from near Kunnathur, Ulundurpet during mid-January, 2021 (Plate 1). Then, at that point, the *Senna tora* leaf portions of the plant were cut into little pieces independently and afterward shed dried for around multi week (Plate 1). The plant parts were ground into coarse powder with the assistance of a mechanical processor and the powder was put away in a compartment for extraction.

Preparation of the extract

The powdered plant material of *Senna tora* (L.)Roxb (around 150 g) was taken in a perfect, level packaged glass compartment and absorbed 650 mL of ethanol up to 2 inches height above the test surface as dissolvable can adequately cover the example surface. The compartment with its substance was fixed and saved for a time of about fourteen days going with incidental shaking and mixing. The entire blend was

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then went through a coarse filtration by a piece of perfect, white cotton material. Then, at that point, it was separated through channel paper and after filtration the excess piece of the plant extricate was given for re-extraction for 7 days with one more 150 ml of ethanol. The mixture was again filtered by the same way as previous method. The filtrate (ethanol remove) acquired was dissipated via air provided from a ceaselessly moving electric fan until dried. It delivered a greenish dark kind of build-up of 3.2 g (yield: 2.13%). The greenish dark sort concentrate build up was assigned as rough ethanolic concentrate of the leaf part of *Senna tora*. One gram of re-separated build up was found trailed by the vanishing of residual part. The rough concentrate was then put away in a cool and dry spot ready for considers.

Plate 1. Images **A** shown the collection of *Senna tora*; **B** -*Senna tora* leaf drying by shed method

Qualitative Phytochemical Screening

All the extracts were subjected to preliminary phytochemical screening for the presence of phyto-constituents.

Tests for Carbohydrates (Benedict's test)

Unrefined concentrate when blended in with 2ml of Benedict's reagent and heated up, a ruddy earthy colored hasten shaped which demonstrated the presence of the carbohydrates.

Tests for Amino Acids (Ninhydrin test)

For the analysis of amino acid 3 ml test solution and 3 drops 5% Ninhydrin solution were heated in water bath for 10 min. Noticed for purple or pale blue tone, the presence of shading demonstrates the presence of amino acids.

Tests for Proteins (Biuret test)

3 ml of each test solution was added to 4% NaOH and few drops of 1% CuSO4 solution into separate tubes. The cylinders were noticed for violet or pink shading arrangement.

Tests for Tannins (Ferric Chloride Test)

With 2-3 ml test solution, 5% FeCl3 solution was added for bluish black colour observed indicates the presence of tannins.

Test for Phenolic compounds (Ferric chloride test)

The extract was concentrated to 5 ml with distilled water. To that a few drop of neutral 5% ferric chloride solution was added. A dark green color shows the occurrences of phenolic compounds.

Detection of flavonoids (Lead acetate Test)

The extracts were treated with few drops of 10% lead acetate solution. The development of yellow precipitous established the occurrence of flavonoids.

Tests for Alkaloids (Wagner's test)

2-3 ml extract was taken into separate tubes. To that few drops of Wagner's reagent was added and observed reddish brown precipitate.

Test for saponins (Foam Test)

2 ml of extract taken into test tube, shaken well, when form is appeared, saponins in conforms.

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Tests for Steroids (Salkowski Reaction)

To 2 ml of sample, 2 ml chloroform and 2 ml Concentrated H2SO4 were added and observed chloroform layer for red colour and acid layer for fluorescence.

Test for Glycoside

To 2 ml of plant extract, 1 ml of glacial acetic acid and 5 % ferric chloride was added then a few drops of concentrated sulphuric acid were added. Occurrence of greenish blue colour shows glycosides.

Quantification of Phytochemicals

Estimation of Total Phenolic Content

Total phenolic content in the leaf, stem and root extracts of *Senna tora* was determined by the FolinCiocalteau colorimetric method (Slinkard and Singleton, 1984). For the analysis, 0.5 ml aliauot of sample was added to 0.5 ml of Folin-Ciocalteau reagent (0.5 N) and the contents of the flask were mixed thoroughly. Later 2.5 ml of sodium carbonate (2%) was added, and the mixed was allowed to stand for 30 minutes after mixing. The absorbance was measured at 760 mm in a UV-Visible Spectrophotometer. The all out Phenolic substances were communicated as mg gallic corrosive reciprocals (GAE)/g remove. All the tests were acted in sets of three.

Estimation of Total Flavonoids Content

Total flavonoid content in the leaf, stem and root extracts of *Senna tora* was determined by the colorimetric method. For the analysis, 0.5 ml at a concentration 1ml/ml was taken and the volume was made up to 3ml. Then 0.1ml aluminium chloride (AlCl3) 10 %, 0.2ml of 1M potassium acetate and 5.6ml of distilled water and remains at room temperature for 30 minutes. The absorbance was measured at 420nm. Quercetin was used as standard (1mg/ml). The test solution was vigorously shaken. Absorbance was recorded at 415 nm following 30 minutes of brooding. A standard adjustment plot was created at 415 nm utilizing known grouping of quercetin. The groupings of flavonoids in the test were determined from the alignment plot and communicated as mg quercetin same (QE)/g of test (Aiyegroro and Okoh, 2010). Every one of the tests was acted in sets of three.

Determination of in vitro Antibacterial Activities

Three kinds of circles were ready for antibacterial screening: One gram test removes was disintegrated in 10 mL of ethanol to get ready example arrangement, 0.03 g/10 mL gentamicin standard plate utilized as certain control to guarantee the action of standard anti-infection against the test living beings just as for examination of the reaction created by known antibacterial specialist with that delivered by test tests and third one was a clear example (just ethanol) which was utilized as regrettable control to guarantee that the leftover solvents was not dynamic. Explicit living beings were immunized into recently sanitized supplement agar media, blended completely and moved quickly to the sterile petri dish in an aseptic condition. It was stored in an incubator for about 24 h to allow the proper growth of microbes. Prepared sample solutions were applied to the corresponding cups or holes with the help of a micropipette. The plates were then permitted to remain to diffuse the example arrangement into the anti-toxin medium at room temperature for 2 h. The plates were then brooded at 37°C for overnight. After appropriate hatching, clear zones of hindrance around the place of use of test arrangement were framed. These inhibition zones were measured by slide callipers and expressed in millimetre.

Result

Phytochemical tests

The present exploration has been conducted in the leaf of *Senna tora* performing various phytochemical tests to identify the secondary metabolites present in it such as alkaloids, flavonoids, sugars, glycosides, saponins, steroids, tannins, phenolic compounds, Vitamin C, proteins, amino acids and carbohydrates. The maximum activity was observed in phenolic content solvents. (Table 1).

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Table 1. Phytochemical screening for various extracts of leaf of *Senna tora* (L.) Roxb.

Secondary metabolites	Aqueous	Ethanol	Methanol	Acetone	Ethyl acetate	Chloroform	Petroleum ether
Carbohydrates	-	+	-	+	+	+	+
Amino acids	+	+	+	+	+	+	+
Proteins	+	+	-	+	+	+	+
Vitamin C	-	-	+	+	+	-	+
Tannins	+	+	+	+	+	+	-
Phenolic compounds	-	+	+	-	-	+	+
Flavonoids	+	+	+	+	+	+	+
Alkaloids	-	+	-	+	+	+	+
Saponins	+	+	+	+	+	+	+
Steroids	-	-	+	+	+	-	+
Glycosides	+	+	+	-	-	+	+

Quantitative Estimation of total Phenolic constituents of leaf of *Senna tora* (L.)Roxb.

For quantitative estimation of total Phenolic constituents of leaf of *Senna tora* the solvents such as ethanol, methanol, acetone, ethyl acetate, chloroform and petroleum ether were subjected to phenolic constituents. The maximum phenolic content was recorded in methanol solvents 1.41 ±0.44. Moderate were recorded in chloroform 0.63 ± 0.01. Whereas, ethanol 0.20 ± 0.03 resulted in minimum phenolic content were recorded as summarized in Table 2.

Table 2. Quantitative estimation of total Phenolic constituents of leaf of *Senna tora* (L.)Roxb

S.No	Solvents	Phenolic content
1	Ethanol	0.20 ± 0.03
2	Methanol	1.41 ±0.44
3	Acetone	0.26 ± 0.08
4	Ethyl acetate	0.37 ± 0.23
5	Chloroform	0.63 ± 0.01
6	Petroleum ether	0.17 ± 0.21

Antibacterial activity

Antimicrobial activity of the ethanolic extract of the leaf of the *Senna tora* exhibited maximum zone

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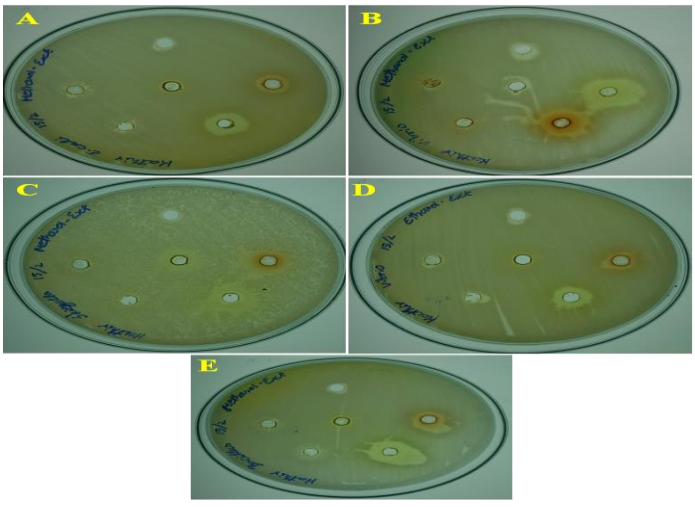
of inhibition in *Klebsiella pneumoniae* and in petroleum ether extract 19.0 mm and a minimum in *Staphylococcus ceureus* and followed by ethanol 11.0 mm were recorded. The maximum zone of inhibition were observed in ethanol extracts of *Klebsillap neumoniae*, *Bacillus thuringiensis* and *Staphylococcus ceureus* 16.0mm, 12.0mm, 11.0mm respectively.

In this experiment, no zone of inhibition was observed in extracts of Aqueous, chloroform, methanol and acetone as shown in Table 3 & Plate 2.

Table 3. Antibacterial activity of various extract of leaf of *Senna tora* (L.)Roxb.

Microorganisms	Aqueous	Ethanol	Methanol	Acetone	Ethyl acetate	Chloroform	Petroleum ether
Escherichia coli	0	0	0	0	0	0	0
Klebsiella pneumoniae	0	16.0 mm	0	0	0	0	19.0 mm
Pseudomonas stutzeri	0	0	0	0	0	0	0
Bacillus thuriengensis	0	12.0 mm	0	0	17.0 mm	0	14.0 mm
Staphylococcus ceureus	0	11.0 mm	0	0	0	0	17.0 mm

Plate 2: Zone of inhibition was observed in the extracts of Aqueous, chloroform, methanol and acetone



Discussion

Recently, both in research and in the food industry, antioxidants and antimicrobial properties of different extracts from many plants have been of great interest, as their potential use as natural additives emerged from an increasing inclination to substitute synthetic antioxidants and antimicrobials with natural ones (Deba*et al.* 2008). In spite of that the secondary plant metabolites of phytochemicals with antibacterial potency have been extensively investigated in the treatment of bacterial infections as alternatives to or in conjunction with antibiotics (Sato *et al.* 1995; Liu *et al.* 2001).

In order to successfully treat infectious diseases, the use of antimicrobial agents is important (Rahman*et al.* 2001). While there are several types of drugs that are commonly used to treat human

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infections, pathogenic microorganisms are constantly developing resistance to these drugs due to the indiscriminate use of antibiotics (Al-Bari *et al.* 2006).

For the use of higher plants and preparations made from them to treat infections is a long-standing practice in a large part of the population, especially in developing countries, where there is reliance on traditional medicine for a variety of diseases (Ahmad and Mohammad 1998). Interest in plants with antimicrobial properties increased due to existing antibiotic-related problems (Pannuti and Grinbaum 1995). A number of researchers have recently documented the antimicrobial effects of different plant extracts on certain pathogens (Parekh and Chanda 2008). However, from our study the reason would be the secondary metabolites present in the leaves of *Senna tora* such as alkaloids, flavonoids, sugars, glycosides, saponins, steroids, tannins, phenolic compounds, Vitamin C, proteins, aminoacids and carbohydrates. From that the various extracts leaf of *Senna tora* were showed as zone of inhibition in the tests of antimicrobial activity.

Conclusion

As apparent from the above conversation, *Senna tora* contains significant synthetic substances that give upon it as a therapeutic specialist which has antimicrobial and pain relieving action. It is apparent from our results and from other workers reports, local use of this plant in various infectious diseases is not at much variance with their antimicrobial properties. Moreover, exhibited potent analgesic activity of the ethanolic extract of *Senna tora* provides support of the claim about leaf part being used as an analgesic in traditional practice. This fact also indicates that the traditional uses of the plant have some scientific basis and therefore, the plant should be thoroughly investigated to fully exploit it's medicinal as well as pharmaceutical potentialities.

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