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In vitro antioxidant and antimicrobial potential of *Sterculia urens* Roxb. root extract and its bioactive phytoconstituents evaluation

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Abstract

Background: The plant *Sterculia urens* Roxb. of Malvaceae family is comparatively understudied. Genus *Sterculia* is widely recognized by its phytomedicinal and ethnomedicinal attributes. The study is aimed to evaluate the qualitative analysis, Fourier transform infrared (FT-IR), thin layer chromatography (TLC), total phenolic content (TPC), antioxidant and antimicrobial activities of the crude hydro-methanolic extract of *S. urens* root.

Results: The antioxidant activity, antimicrobial assay for clinical isolates, and TPC were measured by DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity, agar well diffusion method, and Folin–Ciocalteu assay respectively. Hydro-methanolic extract confirmed the presence of alkaloids, flavonoids, tannins, phenols, saponins, steroids, and glycosides as primary and secondary metabolites, which was later confirmed by TLC. FT-IR spectroscopy revealed the presence of alkanes, alkenes, alkyl halides, halogen compounds, primary alcohol, tertiary alcohol, aldehyde, aromatic amine, secondary amines, amide group, and carboxylic acid. The crude extract was composed of a significant quantity of total phenolic content with 705 ± 0.40 mg GAE/g. Synergistically, the IC_{50} value of the crude extract and ascorbic acid was found to be $27.055 \mu\text{g/ml}$ and $37.244 \mu\text{g/ml}$, respectively, which suggests that root extract possesses strong antioxidant properties. The majority of the microbial strains exhibited varying degrees of sensitivity to the root extract with a notable inhibitory effect against *Escherichia coli*, *Klebsiella pneumoniae*, and *Penicillium glaucum*.

Conclusion: The findings of this analysis suggest that the hydro-methanolic extract from *S. urens* root exhibit antioxidant activity quantified by its ability to scavenge DPPH; antimicrobial activity displayed appreciable microbial sensitivity. These properties are associated with the presence of high phenolic content, different secondary metabolites, and their functional groups. The results are suggestive that *S. urens* root is rich in bioactive compounds, which serve as a novel natural source for potential therapeutic applications.

Keywords: Antimicrobial, Antioxidant, Phytochemistry, Root extract, *Sterculia urens*

Statement of novelty

Sterculia urens Roxb. is highly exploited for karaya gum due to commercial demand but remained unexplored for its pharmacological values. This work shows various secondary metabolites, phenolic content, antioxidant,

and antimicrobial potential of *S. urens* root. This primary information introduces a new arena for the discovery of bioactive ingredients useful for the manufacturing of new drugs.

Background

Every medicinal plant's remedial effects are associated with their distinct chemical properties; hence, they are the 'chemical factory' which yields compounds like

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saponins, terpenes, alkaloids, lactones, oleoresins, glycosides, resins, and essential or fixed oils [1]. For all living organisms, metabolism is necessary, and plants have two types called primary metabolites and secondary metabolites [2, 3]. Although secondary metabolites are reported lower than primary metabolites, they have considerable biological activities [4]. For that reason, the most promising source of novel biologically active substances that protect human from various diseases is medicinal plants [5].

Moreover, concerns about the adverse health effects are growing due to the industrial use of synthetic products. It has been shown that some synthetically manufactured antioxidants, including dibutylhydroxytoluene and butylated hydroxyanisole, promote carcinogenic activity [6, 7], while naturally obtained phenolic antioxidants are shown to manifest a variety of health-promoting biological activities. Since the last few years, FT-IR has played a crucial role in scientific research; most recently, spectroscopy has made considerable advances in the area of clinical assessment and arose as one of the key tools for biomedical claims. Research on many different natural tissues utilizing spectroscopic techniques, such as FT-IR spectroscopy, has been adapted [8].

Natural products derived from plants such as terpenoids, alkaloids, and flavonoids have gained significant exposure for the past few years due to their various pharmacological properties, including antimicrobial, antioxidant, and anticancer activities [9]. Antioxidants are natural or artificial substances capable of preventing or limiting certain kinds of cell injuries. Antioxidants often are found in several plants produces, especially vegetables and fruits, and also readily available as food supplements [10]. Adequate intake of dietary antioxidants can boost defence against free radicals, as the plant-based natural antioxidants can scavenge nocuous free radicals generated in the human body. It is also suggested that the intake of antioxidant-rich foods plays a vital and beneficial role in preventing or postponing the onset of degenerative diseases [11]. Recently, high levels of bioactive compounds with good antioxidant potential have been found in the roots [12, 13].

The discovery of novel drugs from plants was encouraged by the resistance developed in pathogenic microbes against conventional antibiotics and oxidative stress produced by free radicals. A significant number of plants are recorded to possess antimicrobial and antioxidant properties [14]. Since ancient times, plants have been used to produce a range of chemical substances with medicinal value, including antidiabetic, anticancer, antimicrobial, antioxidant, and anti-inflammatory. The World Health Organization (WHO) also considers plants to be the ultimate source of numerous drugs and advocates the

continued use of these herbal medicines to treat various microbial and non-microbial diseases [15].

Moreover, various plant genera are extensively explored for phytochemicals with regard to their ethnomedicinal properties. One of those genera includes *Sterculia* representing 200 species, predominantly distributed across tropical and subtropical regions. Plants which belong to this genus contain significant to the low amounts of flavonoids, terpenoids, phenolic acids, phenylpropanoids, alkaloids, and other metabolites, including carbohydrates, lipids, lignans, and lignin [16]. One such species of these genera is *Sterculia urens* Roxb. (Fig. 1) commonly known as 'gum karaya'; it is one of the critical commercial trees of India [17]. Earlier, it was kept under the family Sterculiaceae but currently belongs to the subfamily Sterculioideae and classified under family Malvaceae [16, 18]. In India, it is distributed across the sub-Himalayan range, Rajasthan, Madhya Pradesh, Andhra Pradesh, Maharashtra, Uttar Pradesh, Kerala, and Gujarat [19, 20]. It offers numerous applications in food, dairy, beverages, cosmetic, textile, oil, and pharmaceutical sectors [18]. Therefore, this experiment is aimed to investigate the phytochemical profiles, including total phenolic content, thin-layer chromatography assay, and FTIR screening, along with antioxidant and antimicrobial activities of the *S. urens* root extract.

Methods

Collection and authentication

Sterculia urens Roxb. (gum karaya) was collected by the first author from Kansarvid, Banaskantha district, Gujarat, India, in August 2019 (latitude 24.26° N and longitude 72.51° E); immediately, its identification and pre-treatment were carried out. The plant material was authenticated, and the herbarium specimen was deposited with reference ID (GUSU-01).

Plant material

The root samples appeared reddish within the cortex region (Fig. 2) and washed twice using deionized water (Milli-Q Millipore, 0.054 mS/cm) to clean soil and dirt. The root sample was slightly scraped with a scalpel blade (No. 24), cut into pieces with 2.5 cm length each, sun-dried for 7 days, ground to a fine powder, and then stored for further analysis at - 20 °C.

Preparation of plant extract

The Soxhlet extraction method was used to prepare plant extract; 90% hydro-methanolic (1:9 v/v) was used as an extracting solvent. This fresh solvent and solid residues (solid-to-solvent ratio, 1:10 g/ml) were placed in a 22 mm × 80 mm extraction thimble [DWK Life Sciences (Wheaton), Mumbai, India] and extracted at 65 °C with 90 ml methanol and 10 ml distilled water (D. W.) for 18 h with 4–5 cycles/h. The collected extracts were filtered

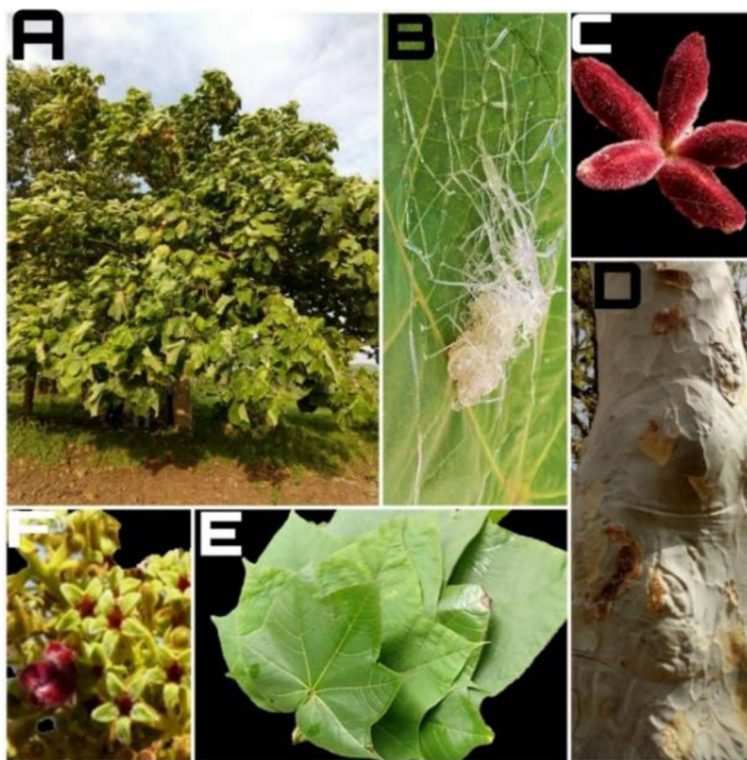


Fig. 1 *Sterculia urens* Roxb. **a** Tree. **b** Karaya gum. **c** Fruit. **d** Bark. **e** Leaf. **f** Flowers

with Whatman No. 1 filter paper, and the extra solvent was evaporated by rotary evaporator at reduced pressure for 30 min (IKA-RV10 Digital V, Karnataka, India) at a controlled temperature of 40 °C and stored for further analyses at 4 °C. Finally, the yield value of crude hydro-methanolic extract was calculated by the given standard formula [21].

$$\text{Yield(\%)} = \frac{\text{Final weight of extract obtained}}{\text{Initial weight of extract}} \times 100$$

Reagents and chemicals

The DPPH and the Folin–Ciocalteu phenol reagent were purchased from Sigma-Aldrich Co., Germany. Sodium carbonate (Na_2CO_3) ethanol and gallic acid and were obtained from Himedia, India. The majority of the chemicals (AR: analytical grade) sourced in the present study were procured locally (India).

Bioassays

Preliminary phytochemical screening

Qualitative phytochemical analysis of crude extract determines the presence of compounds like sterols, glycosides, saponins, tannins, flavonoids, phenols, resins, and alkaloids using the standard protocol. The qualitative phytochemical screening of crude extracts was done using the methods described by the various workers [22, 23].

Thin layer chromatography

Dried root material was mixed with MeOH (100 mg/ml), vortexed (1.5 min), and centrifuged (10 min at 10,000 rpm). After removing the supernatant, the residues were reintroduced thrice to obtain a concentrated sample. Mobile phase for elution of phenolics (toluene to acetone; 9:1 v/v),



Fig. 2 Morphology of *Sterculia urens* Roxb. roots

alkaloids (toluene to diethylamine to methanol; 8:1:1 v/v), flavonoids (glacial acetic acid to ethyl acetate to formic acid to water; 0.5:10:0.5: 1.3 v/v), steroids (butanol to methanol to water; 16:4:0.2 v/v), and Tannins (methanol to chloroform to water; 35:65:10 v/v) were considered. The sample was employed in TLC plates (200 μm layer thick, 10 \times 10 cm silica gel-60, mean particle size of 10–12 μm , precoated with UV254 and aluminum-backed) for phenolics, alkaloids, flavonoids, steroids, and tannins. They were sprayed with staining reagents including alcoholic ferric chloride, Dragendorff reagent, 10% methanolic sulfuric acid, anisaldehyde sulfuric acid, and vanillin respectively. Different solvent systems revealed various visible bands. Besides, invisible bands on TLC plates in daylight were screened using ultraviolet light.

Fourier-transformed infrared spectra

For FTIR analysis, lyophilized extracts were used, because it increases the intensity of spectral bands as it removes water and methanol interference. FTIR spectra were recorded on Bruker alpha eco atr, Optics, Germany, in conjunction with an attenuated total reflectance (ATR), incorporated with a reflection crystal (ZnSe). The spectra were achieved at 20 °C with an average of 128 scans, ranging frequency from 3500 to 500 cm^{-1} , processing software OPUS (v.5.5, Bruker Optics, Germany) for a quick scan. For background, the air spectrum was recorded and subtracted spontaneously with suitable software [24].

Total phenolic content

For measuring total phenolic content (TPC) of samples, the Folin–Ciocalteu assay was followed. At this method, solutions of 7.5% w/v, Na_2CO_3 , and 10% v/v Folin–Ciocalteu reagent (Sigma-Aldrich Co., Germany) were formulated. Then, 0.75 ml of Folin–Ciocalteu reagent was added to 100 μl extract and was stored in the dark (5 min, 20 °C). Next, 7.5 w/v of 0.75 ml Na_2CO_3 solution was mixed and kept in the dark (90 min, 20 °C) and mixed randomly until a homogenous solution was achieved. Lastly, using a spectrophotometer (Thermo Fisher EVO300PC, USA) at 765 nm, the absorbance was estimated and compared with the calibration curve, which was formulated applying known quantities of gallic acid. The findings are measured as milligrams of gallic acid equivalent (GAE)/100 g [25].

Antioxidant assay

DPPH dissolvent

The 0.01 mM DPPH ($\text{C}_{18}\text{H}_{12}\text{N}_5\text{O}_6$, M.W. 394.32 g/mol) dissolvent was formulated by dissolving 1.9716 mg of DPPH (2,2-diphenyl-1-picrylhydrazyl) in 500 ml methanol.

DPPH radical scavenging assay

Antioxidant activity was measured by DPPH radical-scavenging assay given by Brand-Williams [26] with several alterations. The fresh stock solution 0.01 mM of DPPH (Sigma-Aldrich Co., Germany) was prepared by dissolving in methanol (SRL, Mumbai, India). 2.7 ml of prepared DPPH was added to various concentrations of samples (20–200 $\mu\text{g}/\text{ml}$), which was then incubated in pitch-dark for 15 min at ambient temperature (20 °C). A gradual decline in absorbance was determined at 517 nm in a spectrophotometer (Thermo Fisher EVO 300PC, USA). Ascorbic acid (SRL, Mumbai, India) solution of the same concentration can be used as a positive control for the free radical scavenging assay. The test was conducted in three replicates. The findings were expressed in IC_{50} value, ascertained by plotting the percentage of scavenging activity against concentration.

The potential ability to scavenge DPPH free radical was estimated by the below equation:

$$\% \text{inhibition ratio} = \frac{\text{Absorbance of the control} - \text{Absorbance of the tested compound}}{\text{Absorbance of the control}} \times 100$$

- Absorbance of control = absorbance of solution that contain all reagents except sample
- Absorbance of compound = absorbance of sample or standard

Antimicrobial test

Antibacterial test

The antibacterial activity of crude extract of *S. urens* roots was checked against bacterial strains *Escherichia coli*, *Bacillus subtilis*, and *Klebsiella pneumoniae* by employing agar well diffusion assay [27]. Obtained bacterial cultures were maintained at 37 °C in Mueller Hinton nutrient broth (Himedia, Mumbai, India). The bacterial cell suspension culture was prepared in sterile saline water, and it was optimized to 0.5 McFarland turbidity standards with the help of a spectrophotometer to achieve the desired cell density (1.5×10^8 cfu/ml). Each bacterial culture uniformly streaked on Mueller Hinton agar plates, and 100 μl hydro-methanolic root extract was filled in the wells with 200 and 300 $\mu\text{g}/\text{ml}$ concentration. Bacterial plates were kept at temperature 37 °C and incubated for about 24 h. Later, antimicrobial activity was reckoned by measuring the inhibition zone.

Antifungal test

The experiment of diffusion in agar well was performed in compliance with the Clinical Laboratory Standard Institute document M51-A2 [25], with several adaptations. Initially, species were cultured in Sabouraud's agar medium (Himedia, Mumbai, India), which was kept at 30 °C temperature for 7 days of incubation. The fungal

spore suspension culture of *Penicillium glaucum* was prepared using sterile PBS (phosphate-buffered saline). Spore concentrations were standardized using haemocytometer to 10⁵ spores/ml limit. The root extract (50 µl) was added with a concentration of 150 and 250 µg/ml, after punching the agar plates with a sterilized cork borer (2 cm in diameter). The plates were then kept at 30 °C temperature for 48 h immediately followed by the measurement of inhibition halos.

Statistical analysis

Each value obtained is an aggregate of three replicas, and it was denoted as mean ± standard deviation. All the recorded data were analysed with the help of the statistical tool of the Window's statistical software package SPSS (release 8.0). The test of significance was conducted by one-way analysis of variance (ANOVA; $P < 0.05$).

Results

Yield value and extract properties

The present study highlights yield value to quantify the number of active constituents relative to the amount of the crude drug material which was found co-related. Hydro-methanolic root extract recorded significantly high yield by 5.68%, along with the physical properties like the color (dark red), stickiness (non-sticky), and appearance (dry solid).

The formula used for calculating the yield value of crude methanol extract follows:

$$\% \text{yield} = \frac{\text{The final weight of extract obtained}}{\text{The initial weight of extract}} \times 100$$

Preliminary phytochemical screening

Hydro-methanolic root extract exhibited the presence of tannins, flavonoids, alkaloids, phenols, saponins, glycosides, steroids, and proteins and the absence of carbohydrate or sugar. Besides, methanol efficiently extracted phytochemical constituents indicating maximum polar molecules like phenolics (Table 1).

Thin-layer chromatography

The number of compounds in the extracts was calculated using the TLC method of analysis. The chromatographic spots which were representative of compounds in the various extracts were observed, and their R_f values determined (Table 2). The extract exhibited the separation of phenolics with a yellowish-brown spot having the highest R_f value of 0.92, whereas separation of alkaloids revealed a yellowish spot was having R_f value 0.72 in visible light, red and blue at UV365. Further, flavonoids resulted in a brown spot having an R_f value of

0.84 in visible light, orange, red, and green at UV365. TLC assay separated steroids with purplish spot having R_f value 0.74 in visible light, red and blue at UV365. The analysis showed the presence of tannins with a greenish-brown spot (visible), red and blue at UV365, having the lowest R_f value of 0.52. R_f values denote relative migration, while absolute values vary by position based on different parameters, including humidity and temperature.

Fourier-transformed infrared (FT-IR) spectra analysis

Hydro-methanolic dried root extract was measured sourcing ATR-FTIR (Fig. 3) to analyze functional groups and to identify the peaks characteristic of each species. The possible functional groups and peak value interpretation (obtained by FTIR analysis) are shown in Table 3. Eighteen significant bands were spotted between 500 and 4000 cm^{-1} ; the transmission was converted into absorbance, and evaluation was generated. Band (A) at 529 cm^{-1} , (B) at 546 cm^{-1} , and (C) at 576 cm^{-1} appeared as three small peaks, which could indicate the C-halogen (Out-of-plane-ring bending). Fourth band (D) was distinguished at 631 cm^{-1} , representing skeletal vibration of C-Cl, an alkyl aldehyde stretches, and at 763 cm^{-1} , the band (E) detects C-H vibration indicating alkene structure. A sharp infrared band (F) at 1063 cm^{-1} was visualized, representing a C-O stretch of primary alcohol. Similarly, a peak at 1111 cm^{-1} (G) revealed C-H wag bending vibration of alkyl halide. A peak of medium intensity was spotted at 1156 cm^{-1} (H), exhibiting C-O band verifying a tertiary alcohol group. A C-O-C stretch of aromatic amine is detected as band (I) at 1280 cm^{-1} . Between the frequency range of 1550–1300, three bands appeared at 1373 cm^{-1} (J), 1446 cm^{-1} (K), and 1517 cm^{-1} (L), assigning bands of C-H, C-H, and NH indicating possible groups like alkanes, alkanes, and secondary amines respectively, while stretching of C=C indicates vibrations of alkenes on band (M) at a strong peak of 1610 cm^{-1} . Likewise, bands of medium intensity were recognized at 2085 cm^{-1} (N) and 2357 cm^{-1} (O); the vibrations correspond to amide and carboxylic acid stretch respectively.

Band (P) 2852 cm^{-1} and (Q) 2921 cm^{-1} indicate a strong stretch of C-H corresponding to an alkane group. At 3356 cm^{-1} (R), a broad intensity peak was detected, which is specifying OH vibration of alcohol, aldehyde, or carboxylic acid.

Total phenolic content analysis

The 90% root extract consisted of a significant amount of total phenolic content with 705 ± 0.40 mg GAE/g DE. The calibrated curve equation is described below.

Table 1 Phytochemical analysis of hydro-methanolic extract of *Sterculia urens* Roxb. root

Phytochemical test					
Alkaloid	Wagner's test	+	Tannins	Potassium dichromate test	+
	Dragendorff test	+		Lead acetate test	+
	Mayer's test	+		Ferric chloride test	+
Flavonoids	Pew test	+	Steroids	Liebermann sterol test	+
	Shinoda test	+		Acetic anhydride test	+
	Alkaline reagent test	+		Liebermann Buchard test	+
	Zinc hydrochloride reduction test	+		Sugar/carbohydrates	Iodine Test
Phenols	Alkaline reagent test	-	Proteins/amino acids	Fehling's test	-
	Lead acetate test	+		Molisch's test	-
	Ferric chloride test	+		Benedict's test	-
	Potassium dichromate test	+		Xanthoproteic	+
Saponins	Frothing test	+		Ninhydrin test	+
Glycosides	Killer-Killiani test	+		Million's test	+

Note: (+) = present; (-) = absent

$$y = 0.0345x + 0.9659; R^2 = 0.9683$$

The current study investigated and confirmed that the hydro-methanolic extract of *S. urens* root represents high phenolic content as well as antioxidant activity.

Antioxidant activity

The DPPH assay is often used to test the antioxidant potential of the natural compounds. It is known as one of the standards and an effective and rapid method for colorimetric evaluation of antioxidant properties of biological samples. The basic principle of the assay is that the DPPH is a stable molecule that tends to absorb at 515 nm and gives purple colour in methanol. The reduction of DPPH by the antioxidant agent has resulted in a change from purple to yellow colour with a consequent decline in absorbance at 515 nm, which is monitored by spectrophotometer [28]. In the present investigation, the DPPH radicals were used for the determination of the prospective antioxidant properties of *S. urens* root. Ascorbic acid was chosen for positive control. Findings were expressed as 50% inhibition concentration (IC_{50}) computed from the linear regression equation obtained

through a plot of concentration against the percentage of inhibition (Fig. 4). The lower IC_{50} value of the compound indicates its stronger antioxidant properties [29]. In this study, the IC_{50} value of a hydro-methanolic plant extract and ascorbic acid was estimated to be 27.055 $\mu\text{g/ml}$ and 37.244 $\mu\text{g/ml}$, respectively, which suggests that root extract possesses strong antioxidant properties.

Antimicrobial activity

The present investigation of antimicrobial potency of hydro-methanolic extract of *S. urens* roots was tested against two strains of gram-negative bacteria *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*), one gram-positive bacteria strain *Bacillus subtilis* (*B. subtilis*), and one fungal species *Penicillium glaucum* (*P. glaucum*) by measuring the zone of inhibition (Fig. 5). For *E. coli*, the result obtained from the current investigation shows a higher zone of inhibition, 30 mm and 50 mm for 200 $\mu\text{g/ml}$ and 300 $\mu\text{g/ml}$ concentration of root extract, respectively, which indicates good antimicrobial activity against *E. coli* bacteria.

The results of the current study revealed that root extract with a concentration of 300 $\mu\text{g/ml}$ shows significant

Table 2 Thin layer chromatography of hydro-methanolic extract of *Sterculia urens* Roxb. root

Compound name	Solvent system	Confirmatory test	Spot band colour		R_f value
			Visible light	Ultraviolet 365 nm	
Phenolics	Toluene to acetone (9:1)	Alcoholic ferric Chloride	Brownish-yellow	NII	0.92
Alkaloid	Diethylamine to methanol (8:1:1)	Dragendorff reagent	Yellow	Blue, red	0.72
Flavonoid	Glacial acetic acid to ethyl acetate to formic acid to water (0.5:10:0.5:1.3)	10% methanolic sulfuric acid	Brown	Orange, red, green	0.84
Steroid	Butanol to methanol to water (16:4:0.2)	10% methanolic sulfuric acid	Purple	Blue, red	0.74
Tannins	Methanol to chloroform: to water (35:65: 10)	Vanillin solution	Brownish green	Blue, red	0.52

(NII negligible)

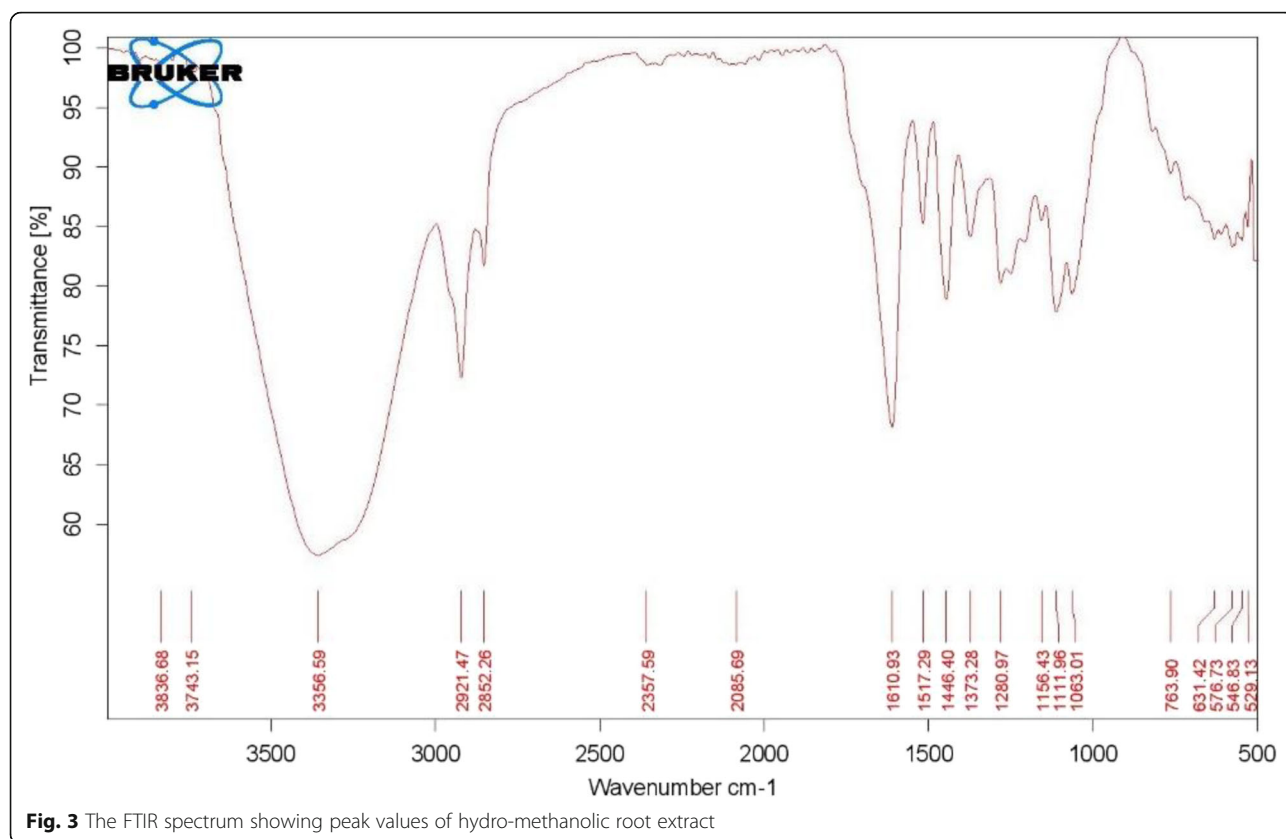
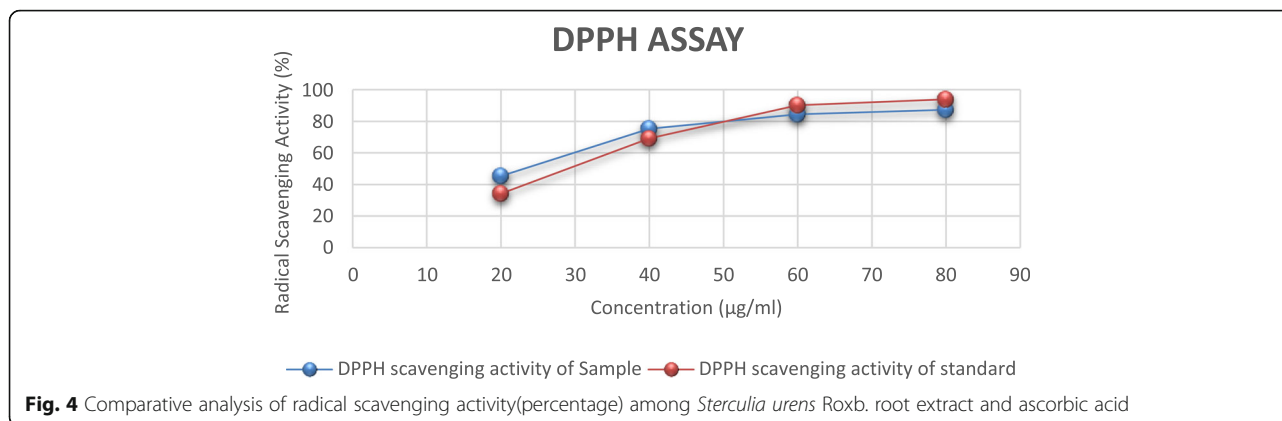


Table 3 FT-IR analysis revealed the presence of functional groups in the *Sterculia urens* Roxb. root

Frequency range	Band	Band location cm^{-1}	Band interaction	Band assignments	Possible compounds
800–400	A, B, C	529, 546, 576	Out-of-plane ring bending	C-halogen	Halogen compounds
	D	631	Stretch	C–Cl	Alkyl halides
	E	763	Stretch	C–H	Alkenes
1100–800	F	1063	Stretch	C–O	Primary alcohol
1300–1000	G	1111	Stretch	C–H wag	Alkyl halides
	H	1156	Stretch	C–O	Tertiary alcohol
	I	1280	Stretch	C–O–C	Aromatic amine
1550–1300	J, K	1373, 1446	Bend	C–H	Alkanes
	L	1517	Bend	NH	Secondary amines
1650–1550	M	1610	Stretch	C=C	Alkenes
2800–2000	N	2085	Bend	C=N	Amide group
	O	2357	Stretch	C–O	Carboxylic acid
3000–2800	P	2852	Stretch	C–H	Alkane
	Q	2921	Stretch	C–H	Alkane
3700–3100	R	3356	Stretch	–OH	Alcohol, aldehyde, carboxylic acid



antimicrobial activity (zone of inhibition 35 mm) against *K. pneumoniae*. In contrast, root extract with a concentration of 200 µg/ml shows limited antimicrobial activity (zone of inhibition 18 mm).

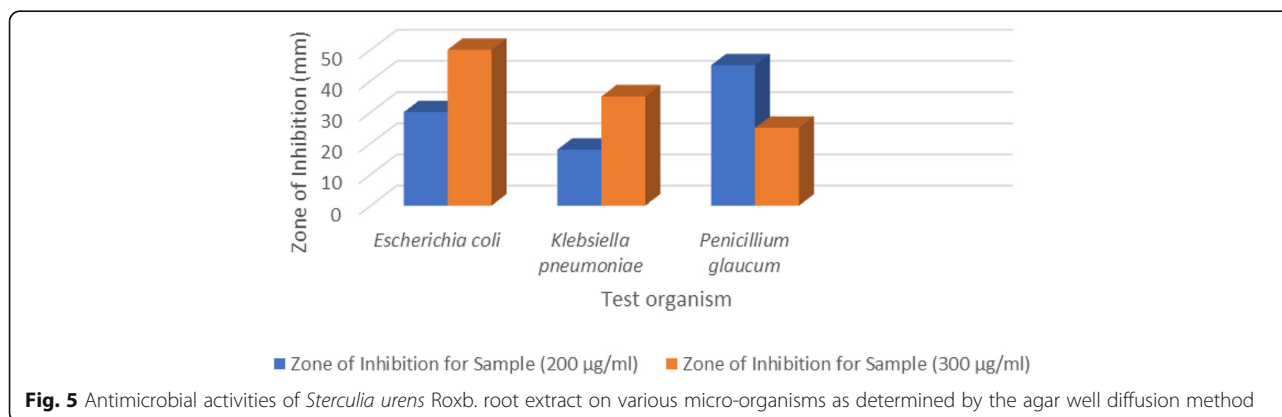
In this study, we found that root extract was ineffective for *B. subtilis* strain. Penicillium is the most familiar and widespread fungus; several members of the genus produce the antibiotic penicillin while others are used to make cheese [30]. Hydro-methanolic extract of *S. urens* root with concentrations of 200 µg/ml and 300 µg/ml showed 45 mm and 25 mm zone of inhibition, respectively, which indicates unusual antifungal activity against *P. glaucum*.

Discussion

At present, research in herbal medicines and their natural substances is substantially focused. *S. urens* has its traditional significance in treating different human diseases. Moreover, ethnomedicinally, its roots are used for facilitating childbirth, relieving constipation, and reducing body swelling [31]. For utilizing this plant in modern therapeutic systems, rigorous experimental evidence, toxicity analyses, and human clinical trials are needed.

Henceforth, the main purpose of this research was to investigate and elucidate the medicinal values of *S. urens*. This is a first study that we are reporting since there is less prior literature on the phytochemical characterization of the root. Consequently, *Sterculia urens* Roxb. was selected and standardized on their physicochemical parameters such as extractive values, phytochemical study, qualitative estimation, TPC, TLC along with FTIR analysis, antioxidant activity, and antimicrobial activity for the examination.

Yield value of *S. urens* root extract is noteworthy which suggests that the plant contains a variable number of active constituents or relative amounts of soluble compounds in methanol extract. There will be common secondary metabolites found in two distinct species of a similar genus. It has been claimed that the genus *Sterculia* includes phenolics, steroids, terpenes, alkaloids, and flavonoids, but literature on *S. urens* root chemistry is not yet documented. It poses a challenge when it comes to discovering new constituents from *S. urens* root. Moreover, several biological activities in genus *Sterculia* like *Sterculia tavia*—anti-proliferative activity, *Sterculia villosa*—antidiabetic activity, *Sterculia guttata*—larvicidal activity, *Sterculia foetida*—anti-fertility and anti-inflammatory activity,



and *Sterculia quadrifida*—antioxidant activity are studied [32]. Despite a successful investigation into the genus *Sterculia*, *S. urens* was underexplored for its chemical constituents and biological activities. Further, experiments are underway to isolate and classify bioactive substances obtained from *S. urens*. Later, bioactive phytoconstituents were confirmed using TLC.

S. urens displayed notable concentration of TPC which is supported by various studies in the same genera. *S. quadrifida* root is traditionally known to cure diabetes and cancer-like ailments. The findings obtained in our analysis for TPC are higher than the results published by Lulan et al. [32], who reported a maximum of 116.84 ± 1.22 mg of GAE/100 g *S. quadrifida* extracted with methanol. Few researchers stated total phenolic content values of *S. oblongata* bark ranges from 100 to 600 mg GAE/g with chloroform, ethyl acetate, and methanol extracts which supports the phenolic content of the current study [33]. Phenolic compounds act as antioxidant because of their redox properties. According to Annapandian and Rajagopal [34], TPC substantially impacts its antioxidant activity, which is crucial for radical scavenging. Several studies claimed to have phenolics as crucial substances showing different biological activity and medicinal properties.

The synergistic role of the phytochemicals can serve as a natural cure for oxidative damage resulting in chronic inflammation. Corresponding outcomes were reported by Haque et al. [35], in crude methanolic extract of *S. villosa* bark (IC_{50} value 27.28 μ g/ml), and Dillak et al. [36], in the ethanolic extract of *S. quadrifida* root (IC_{50} value 20.55 ± 0.42). The result derived from this analysis was in good agreement towards earlier reports suggesting the use of methanol extract for the radical scavenging activity analysis [37]. Different metabolic processes of the living system produce reactive species called free radicals; they spread throughout the body and damage important biomolecules of the body by disrupting their structure and interfering with their function, leading to cellular dysfunction and cell death. This can result in serious health problems such as cancer and ageing, which can be prevented by radical scavenging potential of flavonoid and phenolics [38].

Since the last few decades, multiple antibiotic drug resistance has been acquired by pathogenic bacteria. For that, alternative strategies are required to combat antibiotic resistance. *Escherichia coli* is a widely occurring bacterium in human's intestine and homeothermic organisms. The vast majority of *E. coli* strains are innocuous. Nonetheless, a few other strains, including enterohemorrhagic *E. coli*, may cause severe foodborne diseases. In general, *K. pneumoniae* is an encapsulated, non-motile opportunistic pathogen that is found in the environment and targets principally people with compromised immune

systems and causes nosocomial infection. *K. pneumoniae* is also responsible for various diseases like urinary tract, bloodstream, and septic infection [39, 40]. The presence of considerable phytoconstituents like phenolics, alkaloids, flavonoids, saponins, terpenoids, glycosides, tannins, and steroids in root extract might be the reason behind its significant in vitro antioxidant and antimicrobial activity. Such results offer a comparable bioactivity study and serve as an example of their future application in the pharmaceutical and food industries.

Conclusion

The principal objective of the study is to identify the presence of different secondary metabolites, their functional groups, antioxidant and antimicrobial activity of *Sterculia urens* root. The presence of these bioactive metabolites implies that the hydro-methanolic root extract of *S. urens* has quite a few pharmaceutical products that would have quite several medicinal applications. It showed high phenolic content, which is a valuable reference for antioxidant properties. The extract of root exhibited high DPPH radical scavenging activity, indicating *S. urens* as a potential candidate for antioxidant study for further studies. Additionally, isolation, purification, and characterization of the phytochemicals will make remarkable studies. This primary information will simplify in leading further studies on the discovery of bioactive ingredients, resolve their efficacy by in vivo studies, and demonstrate their safety and effectiveness in clinical trials. The study suggests that crude extract possesses promising antimicrobial and antioxidant activity; thus, organic antioxidant and antimicrobial medicines can be generated for possible therapeutic applications. This is the fundamental collective report on phytochemical screening, TLC, TPC, FTIR, antioxidant, and antimicrobial activity on the roots of *Sterculia urens* Roxb.

Abbreviations

DE: Dried extract; DPPH: 1,1-Diphenyl-2-picrylhydrazyl; FC: Folin-Ciocalteu; FT-IR: Fourier transform infrared; GAE: Gallic acid equivalent; Na_2CO_3 : Sodium carbonate; TLC: Thin layer chromatography; TPC: Total phenolic content; PBS: Phosphate buffer saline

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Study involving plants

1. The plant material was authenticated by Dr. Bharat Maitreya. The herbarium specimen was deposited in Plant Systematics Division, Department of Botany, Bioinformatics and Climate Change Impacts Management, Gujarat University, India, with reference ID (GUSU-01).
2. As per the local and national guidelines and legislation and the required or appropriate permissions and/or licenses for the study.

Authors' contributions

AS analysed and interpreted the data regarding the phytochemical characterization and bioassay and inscribed the major part of the manuscript. KD performed significant contribution in writing the manuscript.

NM was associated in supervising, advising, positioning, and structuring the manuscript. All authors read and approved the final manuscript.

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