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Exploring antimicrobial properties and oral health benefits of *salix subserrata* willd: unveiling the potential beyond its use as traditional chewing stick

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Abstract

Background *Salix subserrata* is one of the traditional chewing sticks used commonly in Ethiopia. It is also used traditionally against rabies and other infections. There has been no comprehensive research on the biological activity of this plant to demonstrate its role in maintaining oral health. Given the compounds previously isolated from this species, this study aims to predict which extracts among the four solvents are responsible for promoting oral health.

Method The phytochemical content and antioxidant activity of the leaves, stem, and stem bark extracted with four different solvents were evaluated using standard methods. The EC_{50} of the extracts was tested with the DPPH assay, and the silicon content of the stem was determined by a gravimetric method. The antibacterial activity was tested using an agar well diffusion method at different test concentrations of (mg/ml) crude extracts and MIC/MBC were determined using the microdilution method. Physicochemical and ADME properties were determined using the SwissADME online server. PASS online was used to predict biological activities related to the oral health of the compounds previously isolated from the plant.

Results The antioxidant activity, in terms of EC_{50}, of the leaves of *S. subserrata* was comparable to that of the standard reference, ascorbic acid. The silicon content in the stem extract of the plant was found to be 0.24%. The leaves of *S. subserrata* exhibited higher levels of total phenolic and flavonoid contents compared to other parts of the plant. Hexane, chloroform, and ethyl acetate extracts of *S. subserrata* were found to be active against the Gram-positive bacteria (*Listeria monocytogenes* and *Enterococcus faecalis)* which are known to be responsible for foodborne infections. Among the previously reported compounds from *S. subserrata*, catechin, quercetin, saligenin, catechol, gallocatechin, salicin, and triandrin were found to be predicted for the strong antioxidant, anti-infection, and anti-mucositis activities.

Conclusion All phytochemical analysis and antimicrobial activity results underscore the significance of plants traditionally used as chewing sticks. The roles of previously isolated compounds in maintaining oral health were also predicted, though toxicity tests and comprehensive profiling of the extracts necessitate further investigation.

Keywords Anti-infective, Antioxidant, Extracts, Oral health, *Salix subserrata*

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Background

Dental, periodontal, and the overall health of the oral-facial systems that enable us to chew, speak, and smile are all considered to be aspects of oral health. Tooth decay cavity disease, oral cancer, and gums (periodontal) are some of the most prevalent conditions that affect our dental health [1]. Because their strong association with overall health, oral bacteria can contribute to liver illness, inflammatory bowel disease, esophageal and pancreatic disorders, colorectal cancer, cardiovascular diseases, diabetes, rheumatoid arthritis, Alzheimer's disease, and premature birth [2].

Despite being primarily preventable, oral diseases are a significant health burden in many nations, affecting individuals at all stages of life and leading to pain, discomfort, disfigurement, and even death. It is estimated that 3.5 billion individuals worldwide suffer from oral disorders. The Global Burden of Disease 2019 report states that the most prevalent medical disease is untreated dental caries, or tooth decay, in permanent teeth. The cost of treating oral health issues is high, and most insurance plans do not cover them in Universal Health coverage (UHC). There are insufficient services available in the majority of low- and middle-income nations to prevent and treat diseases related to oral health [3]. For these all reasons traditional chewing sticks play an important role to maintain oral health at lower cost.

Oral pathogens, such as viruses, bacteria, fungus, archaea, and protozoa, are intimately linked to the mechanisms that cause oral diseases. The mouth cavity contains approximately 1,000 types of bacteria and 100 species of fungi [4]. *Porphyromonas gingivalis, Lactobacillus, Streptococcus mutans*, and other common oral bacteria are included [4, 5]. Oral bacteria that are not residents, such as MRSA strains of *Staphylococci, Klebsiella* spp., *Haemophilus influenzae*, and *Pseudomonas* spp., have been isolated [6]. Oral microbiomes are actively dynamic with the host and can exhibit significant and quick changes in composition and activity both geographically and temporally [7].

Several plant species are utilized in Ethiopia to make traditional chewing sticks (TCS), which are widely used for oral health treatments. Most of these species are chewed specifically for this purpose, with the primary parts used being stems, roots, and twigs. In several African countries, including Ethiopia, plant species like *S. persica, Salix subserrata, Vernonia amygdalina, Acacia nilotica,* and *Clausea anisata* are commonly used for making chewing sticks. TCS are produced from plant species belonging to over 35 families [8].

With more than 330–500 species and 200 hybrids, the willow (genus *Salix*) is a rapidly expanding genus of trees, shrubs, and prostrate plants that are found throughout Africa, North America, Europe, and Asia. *Salix*, is represented by indigenous species in Ethiopia. The genus is widely utilized in traditional medicine and is a rich source of biologically active substances, such as salicin, which is a prodrug of salicylic acid [9]. The genus comprises more than 300 secondary metabolites that have been described, including volatile and fatty acids as well as flavonoids, phenolic glycosides, organic acids, non-phenolic glycosides, sterols and terpenes, simple phenolics, and lignans [10, 11]. Moreover, willows have cytotoxic, antidiabetic, antibacterial, anti-inflammatory, antioxidant, anticancer, anti-inflammatory, anti-obesity, neuroprotective, and hepatoprotective properties [11].

Salix subserrata Willd. (Synonyms: *Salix axillaris, Salix cyathipoda,* and *Salix octandra*), a member of the Salicaceae family, is known as *Alelti, Alanca, Aleltu* (Or), Ahaya, Haya, or Riga (Am). It is a tree or shrub that can reach up to ten meters in height. This species thrives at elevations between 1250 and 2850 m above sea level, typically found along rivers and streams. It is widespread across various regions of Ethiopia and is also common throughout tropical Africa, reaching as far north as Egypt [12].

Figure 1A illustrates the distinctive features of *S. subserrata*, showing the stem and leaves of this noteworthy plant. Figure 1B shows how the stem is marketed for traditional chewing sticks with other alternatives. Notably, the presence of flavonoids in the aqueous methanol extract of *Salix* species leaves may be responsible for their anti-inflammatory properties. Because of its structural resemblance to aspirin, salicin, the main phenolic glycoside found in the bioactive extracts of *Salix* species, is thought to represent the pharmacologically active component [13]. It is considered a natural aspirin that reduces the effects of radiation and shown radioprotective action against alterations in electrophoretic and ultrastructural changes brought on by radiation in the spleen tissue of male rats [14].

Flavonoids from *S. subserrata* have been identified, including rutin, luteolin-7-glucoside, quercitrin, and quercetin [15]. For the first time, six compounds were isolated from *S. subserrata*: (+) catechin, 1,2-benzenedicarboxylic acid, bis (2-ethylhexyl) ester, saligenin, methyl 1-hydroxy-6- oxocyclohex-2-enecarboxylate, catechol, propyl acetate, β-sitosterol, and β-sitosterol glucopyranoside [16]. Using bioassay- and TLC-guided column chromatography, salicin, catechin, (epi)catechin-(epi) catechin, gallocatechin, triandrin, myricetin-3-O-β-d-glucoside, tryptophan, chrysoeriol-7-O-glucuronide, and phenyl alanine were also isolated [17]. Reports on *S. subserrata's* ability to alleviate inflammation, particularly when reactive oxygen species (ROS) are present, support its traditional usage. The phytochemicals that

Fig. 1 (**A**) Image for the stem and leaves of *S. subserrata* and (**B**) traditional chewing stick

were extracted included flavonoids such as quercetin-3-O-β-D-glucoside and luteolin-7-O-ß-D-glucopyranoside (cynaroside) as well as triterpenes such campesterol and stigmasterol [18]. 2D structures of the phytochemicals isolated from different parts of *S. subserrata* are shown on Fig. 2.

Salix has long been used in traditional medicine to treat a variety of illnesses affecting both humans and animals. It is used to treat headaches, stomachaches, constipation, and fever. When combined with milk, the leaves have been shown to be useful in treating rabies [10]. Bark and leaves of *S. subserrata* were used as an anti-infective and remedy for body aches [19]. Based on traditional applications, Ethiopians have been using a variety of medicinal herbs, including *S. substrata*, to cure rabies [15, 20–22]. Bark infusion or decoction for topical use as an antibiotic, antifungal, and algaecide; also used as a general painkiller, tonic, and febrifuge for rheumatic pain [23, 24]. Different parts of *S. subserrata* were used to treat different human ailments as shown in Table 1.

Apart from being used alone, the plant parts were often combined with other kinds of medicinal plants. Patient body wash (BIDUU/RISAA) using leaf infusion from *R. prinoides*, *S. subserrata*, *Acacia* species, and *Cucumis dipsaceus* Anthrax is treated by combining a mixture of mashed *Stephania abyssinica* root, *B. antidysentrica* leaf, and *S. subserrata* leaf in a drink [37]

The pH of leaf litters of *S. subserrata* was found to be less than seven [38], and its ethanol extract percentage of inhibition (%) against the K562 cell line at a conc. of 10 mg/mL was found to be 31.2 [39]. At 150 mg/kg, the ethanolic extract from its flowers was found to have hepatoprotective benefits. These effects may be attributed to the extract's anti-inflammatory and antioxidant properties [10]. Ethnopharmacological studies also shows the use of the crude extract to cure rabies together with evaluating its efficacy [22]. Tawfeek et al. also reviewed the very attempted ethnopharmacological activities for the crude extracts of different parts of *S. subserrata* [11].

The species *S. subserrata* is known for its rapid growth, ability to regenerate itself, and deeply penetrating tap root system with thin lateral roots [24]. *S. subserrata's* superior mechanical qualities and dense root system made the plant with the most potential for strengthening slopes [10]. Among the five plants tested, it yielded the best results for biological stability because of its high root density and beneficial mechanical characteristics [40, 41]. *S. subserrata* is one of the more promising plant species than grass species for stabilizing slope in adverse circumstances (wet conditions) [24]. The administration of chitosan nanoparticles loaded with *S. subserrata* bark extract (SBE.CNPs) reverses the behavioral abnormalities caused by As-induced neurotoxicity, including impairments in memory, locomotion, and exploration [42].

As demonstrated by protein modeling and docking analysis, (+)-catechin showed a higher binding affinity than kaempferol toward the target glycoprotein G in Auto Dock Vina. Additionally, it produced better full fitness and estimated ΔG values using Swiss Dock, suggesting that it may be a viable natural inhibitor of the rabies virus [43]. This is in line with the traditional uses reported in many ethnobotanical studies [28, 32].

In addition to the traditional uses mentioned above, the stem of *S. subserrata* is among the traditional chewing sticks (TCS) commonly used in Ethiopia [8]. It is popularly used next to the stem and root of *Salvadora persica*. A lot has been done on phytochemical investigations and their role in maintaining oral health [44].

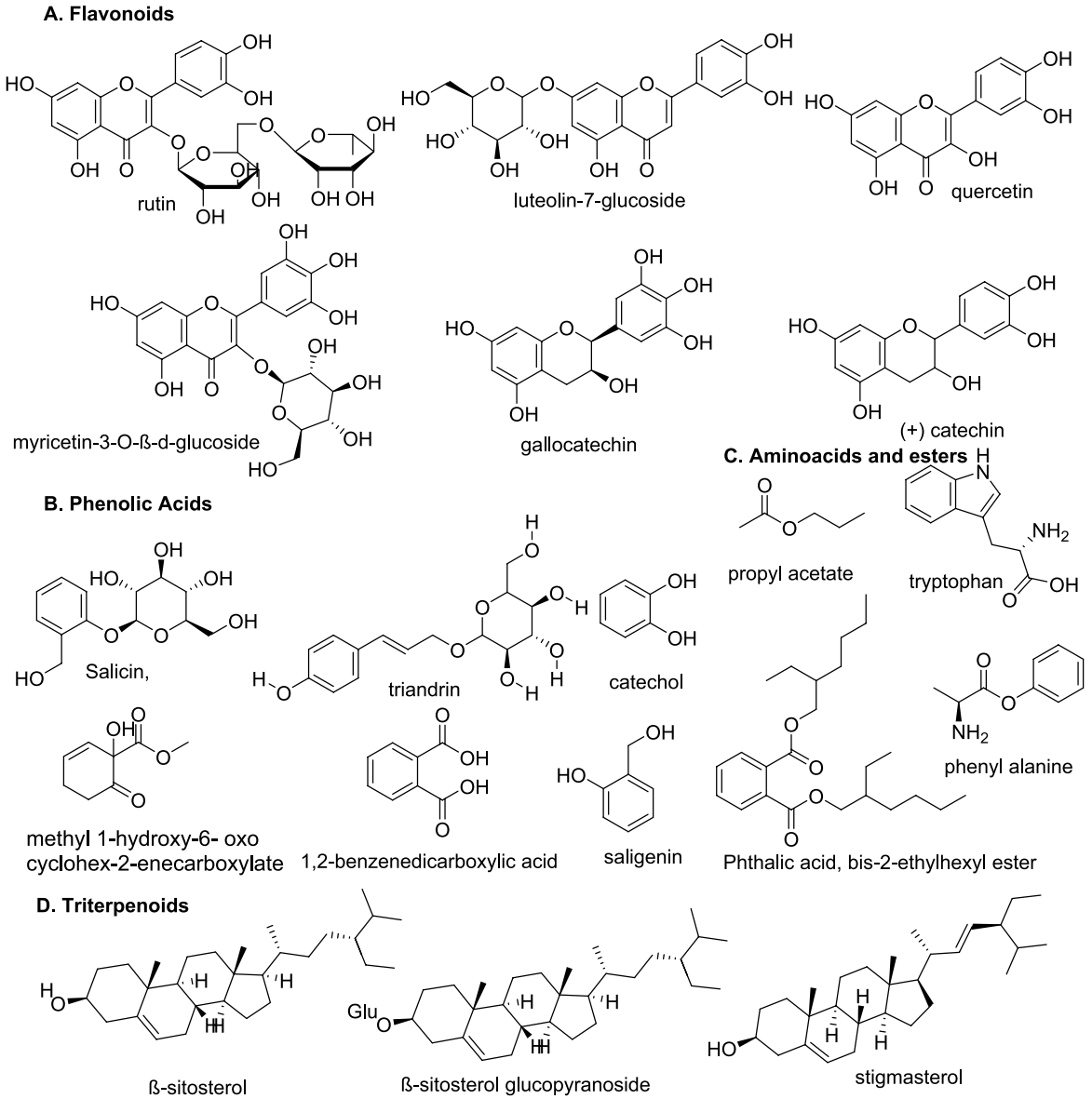

Fig. 2 2D- structures of compounds isolated form different parts of *S. subserrata*

Such studies lack for *S. subserrata* which is used as a frequent alternative for maintaining oral health. Lack of scientific justification for it its use as a chewing stick were also demanding, this current investigation aimed at phytochemical analysis, and antibacterial activities of different parts of *S. subserrata*. In addition to its role in oral and general health, the antimicrobial activities of the different parts of the plant will be compared using different solvents for extraction. For this purpose, the plant parts (stem, leaves, and stem bark) were extracted using four solvents of varied polarities.

Methods

Chemicals and apparatus

Ultrapure water was produced using a Purelab (Flex 4 Elga) 18.2 MQ water purification device at 20.6 ℃, which is used for washing plant materials and preparing all necessary reagents and standards. Analytical-grade reagents were used for the extraction, phytochemical analysis, determining silicon content, and antibacterial activity. 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ascorbic acid were obtained from Sigma (> 99.9%,

Table 1 Medicinal use of *S. subserrata* Willd.to treat human ailments, parts used, mode of preparation, and application

S. No	Human Disease	Part Used	Methods of preparation	Route of administration	Methods of application	Ref
1	Tooth abstraction	Stem	Chewing stick preparation	Oral	Additive—Vegetable drug	[25]
2	Rabies	Root				[26]
3	Rabies	Leaf	Dried and pounded and mixed with milk	Oral	Drinking	[27]
4	Retained placenta (Hobart)	Leaf	Dried leaves, Powdered, mixed with coffee	Oral	Drinking	[28]
5	Rabies (*Dhibe Sere*),	Leaves	Dried leaves, concocted, powdered, mixed with milk	Oral	Drinking	[28]
6	Joint dislocation physical worsening *	stem and leaves	The leaf ground along with the immature stem, mixed with bread and given to the cattle in problem	Oral	Feeding	[29, 30]
7	MIKEGNA-SHEREGNA	Leaves	Chewing the Fresh leaf and swallowing chewed juice(sipping) in	Oral		[31]
8	Rabies	Leaves /roots	leaves/roots were pounded and dried and then mixed with milk	Oral	Drinking	[32]
9	Skin diseases	Leaves /roots	prepared as an ointment	Topical		[32]
10	Rabies (*Dhukubaseree)*	Leaves	Crushed, mixed with water &milk	Oral	Drinking Fresh	[33]
11	Madness (*Maraatuu*)	Leaves	Concoction, crushed, squeezed with water	Oral	Drinking Fresh	[33]
12	Fever	Bark **	drink an infusion of the bark	Oral	Drinking	[34]
13	Rabies (*Vebdweshabesheta)*	Leaves	Leaves are pounded and dried, and then mixed with milk	Oral	Once a day for three days1 teaspoon powder	[27]
14	*Buginge*	Leaf	The leaf of *Salix mucronate* is pounded and place on wounded part	Dermal	Topical	[35]
15	Jaundices	stem bark	Dried stem bark powder with water and boiled is given orally	Oral	Drinking	[36]

* for Cattle

** *Salix mucronata* Thunb (*Synonym* for *Salix subserrata*)

Sigma-China). A spectrophotometer (V-770, Jasco, USA) was employed in the analyses.

A vortex mixer, an autoclave, a Leve II biosafety cabinet (BIOBASE, Chia)., culture tubes, a drying oven, Falcon tubes, an incubator, micropipettes, micropipette tips, a microscope, microscope slides, a Petri dish, barium chloride (≥ 99%, LABKEMICAL, India), Gram stain reagents, MacConkey agar(Accumix, India), mannitol salt agar (SRL, India), motility agar, Müller-Hinton agar (MHA) (HIMEDIA, India), nutrient agar (NA), DMSO (Sigma-Aldrich), nutrient broth, Simon citrate agar, triple sugar-iron agar, sulfuric acid (98% ≥, Blulux Laboratories, India), L-ascorbic acid (> 98%, Acros Organics, Belgium), and violet-red glucose-bile agar (SRL, India) were among the materials used.

Plant materials

The stem and leaves of *S. subserrata* were collected from Dessie (11°8′N 39°38′E, with an elevation between 2,470 and 2,550 m above sea level) from a nearby garden. The scientific names of plant species were identified by Mr. Melaku Wondafrash at the National Herbarium, College of Natural and Computational Sciences, Addis Ababa University, Addis Ababa, Ethiopia, and the vouchered specimen has been deposited (Voucher number—FB-006/12). The plant materials were rinsed with distilled water to remove the dust, and then the parts (leaves stem and stem bark) were separated and allowed to dry in the shed at room temperature (23 ± 3 ℃), for about a week. The samples were divided into smaller pieces and spread out on clean polyethylene plastic sheets to dry in the air. Air-dried sample was powdered before extraction with a coffee grinder, weighed and kept in a polyethylene plastic bag in a cool area, and made ready for extraction.

Extraction

20 g of each sample were taken from the dried and powdered plant parts and placed in different conical flasks.

The samples were soaked in 1.5 L of hexane for 72 h., with frequent shaking. This soaking period allowed for the effective extraction of the plant compounds into the solvent. After 72 h., the residue was allowed to settle down and filtered using Whatman no. 1 filter paper. The extracts concentrated to dryness using rotary evaporator under gentle heating and controlled evaporation of the solvent. The residue from hexane extract were further extracted by sequential soaking in 1.5 L similarly in chloroform, ethyl acetate and methanol for the same period of time. Each of the extracts were filtered and concentrated as described for hexane extract. This step allowed for a diverse range of plant compounds to be extracted into different solvents, increasing the chances of capturing a broad spectrum of bioactive components present in the plant materials. All the concentrated extracts weighed for yield calculation and kept in cool and dry place for further tests.

Phytochemical analysis: Antioxidant activity evaluation and Total Phenolic, and Flavonoid content determination

Evaluation of antioxidant activity

A modified version of the method described by Banothu et al. [45] was successfully adapted to evaluate the free-radical scavenging potential of plant extracts using the DPPH assay method. To initiate the reaction, 0.25 mL of the sample solution was mixed with 0.75 mL of DPPH. The mixture was then allowed to react in the dark for a duration of 30 min. For the preparation of the DPPH solution, 1.9716 mg of DPPH was dissolved in methanol and added to a 50 mL brown volumetric flask. The solution was adjusted to achieve an absorbance of approximately 1.000. As a reference standard, ascorbic acid was utilized. To calibrate, a standard solution with a concentration of 1000 ppm was prepared by dissolving 0.10 g of ascorbic acid in 100 ml of methanol. From this stock solution, various concentrations ranging from 25 to 500 mg/L were generated, forming a calibration curve.

Regarding the sample extracts, dilution factors of 5, 10, 25, 50, 100, 150, and 200 were recommended for the methanol extracts, with a concentration of 0.1 g/mL. To measure the absorbance at 517 nm, a spectrophotometer was utilized. A rectangular cell holder was used, with a path length of 1 mm and a cuvette volume of 500 mL. The percentage of inhibition was calculated as a measure of the radical scavenging activity, utilizing Eq. 1 to determine the DPPH radical scavenging capacity.

$$\text{Scavenging activity (\%)} = \left(\frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}}\right) x100 \quad (1)$$

Total phenol and total flavonoid content determination

The Folin-Ciocalteu colorimetric method, as reported by McDonald et al. [46] was used to estimate the total phenol content (TPC) of the sample extract with some modifications. More briefly, after mixing 0.4 mL of the extract with 0.4 mL of the 10 × diluted Folin-Ciocalteu reagent, 0.2 mL of 2% Na_2CO_3 was added. Since the sample extract concentration with dilution factor 5 consistently showed color changes and absorbances between 0.1 and 1 (within the Beers rule), it was selected for analysis. The control was methanol and reagent without extract. The mixture was incubated at room temperature for 35 min and measured using UV–Vis spectrophotometer in triplicate at 765 nm. The 1000 ppm standard gallic acid was serially diluted for calibration to prepare standard solutions of 6.25, 12.5, 25, 50, 100, 150, 200, 250, and 500 mg/L. Equation 2 was used to calculate TPC, which is expressed as mg of gallic acid equivalent (GAE) per g of extract (dry weight).

$$Concentration\left(mgGAE/100g\right) = \frac{CxVxDF}{m} x100 \quad (2)$$

where m is the mass of the sample powder used for extraction, C is the concentration determined from the calibration curve in mg/L, V is the final volume of the sample in L, and DF is the dilution factor.

Total flavonoid content (TFC) was determined using the aluminum chloride colorimetric method with slight modifications. With a few minor adjustments, the method described by Chang et al. [47] was used to assess total flavonoid content (TFC). In short, 0.3 mL of extract, 0.3 ml of 2% $AlCl_3$, 0.3 ml of 1% $NaNO_2$, and 0.3 ml of 5% NaOH were mixed and left to stand at room temperature for half an hour. The same procedure used for the TPC analysis was used to do TFC analysis in the sample extracts. Methanol served as the control.

The absorbance was measured in triplicate at 314 nm using a UV–Vis spectrophotometer. A standard stock solution of quercetin (1000 ppm; mg/L) was prepared. For the calibrating standards, comparable arrangements were made. The quercetin equivalent (QE) in milligrams per gram of dried sample extract was used to determine the TFC. The same equation as TPC was used to calculate the amount of TFC in mg QE/100 g.

Determination of Silicon content in the stem of plant extracts

For the determination of total Silicon in a wide range of solid materials, such as soils, waste products, sewage sludge, plants, organic manures, fertilizers, etc., gravimetric methods are traditional, precise techniques that work well [48].

The method of Morikawa and Saigusa [49] was followed to conduct silica content determination by gravimetrically. The plant stem was dried and then pulverized in a grinder. The platinum crucible was cleaned with dilute acids, dried, and measured with great care. Using an analytical balance, about 3 g of the dried stem powder was accurately measured out and transferred into the platinum crucible. To ensure accuracy, the analysis was performed in duplicate.

The sample was placed into a muffle furnace that was set to 575℃, and it was left there for 24 h. to undergo full ashing. The burnt sample was placed in a desiccator to cool to room temperature. Once the ashing procedure was finished, 30 mL of a solution containing 1.5 M HNO_3 and 3.7 M HCL was used to digest the ashed material. During the digestion process, the sample was washed five times with 5 mL of the mixture of HNO_3 and HCl to get rid of any remaining chloride. Distilled water was used for the final wash. The digested sample was then filtered using ash less Whatman no.1 filter paper. After the filtering process was finished, the residue was gathered again and put back into the platinum crucible. After a further 24 h. cycle at a temperature of 575℃, the crucible and residue were put back in the muffle furnace and weighed to determine the silica content based on the difference with the empty crucible. A blank determination was performed using the same procedure and the same quantity of each reagent but without the sample. The Silicon content was finally calculated using Eq. 3.

$Sicontent(\%Yield) = \frac{A-B}{C} \times 100\%$ (3).

Where A is the weight of the ash for plant samples (in g), B is the weight of the ash for blank samples in g, and C is the weight of the plant samples taken for ash in g.

Antibacterial analysis of plant extracts

Antibacterial efficacy test

The antibacterial efficacy of the plant extract was investigated in the microbiology laboratory of the Bio and Emerging Technology Institute (BETin) in Addis Ababa, Ethiopia.

Bacterial strains

Gram-negative bacteria (*Escherichia coli* (ATCC25972), *Klebsiella pneumonia* (ATTC70063), *Proteus mirabilis* (ATCC35659) and *Pseudomonas aeruginosa* (ATCC27853)) and Gram-positive bacteria (*Listeria monocytogenes* (ATCC19115), *Staphylococcus aureus* (ATTC25923) and *Enterococcus faecalis* (ATCC79112)) were used to test the antibacterial properties of the plant extracts. The selection of the bacterial strains was based on availability of authenticated strains and partly for their role to oral health and food borne diseases. One of the limitations of this study was the exclusion of highly pathogenic strains. The source of these bacterial strains was the Ethiopian Institute of Public Health in Addis Ababa, Ethiopia. To prepare inoculums, the bacterial strains were maintained on nutrient agar and sub-cultured on to Mueller–Hinton agar weekly.

Confirmation of test organism

To verify the test organism, gram staining, and biochemical identification were performed. The test organisms were cultured on nutritional agar, Mannitol Salt agar (SRL, India), Violet Red Glucose agar (SRL, India), and MacConkey agar for 18 to 24 h. at 37 ℃. Following an overnight incubation period (35 ℃ to 37 ℃), a gram reaction was conducted the next day, and biochemical tests were carried out conducted based on their characteristics. When necessary, the isolated test organisms were preserved on storage media at 2 ℃ to 8 ℃. Every test organism was standardized according to the 0.5 McFarland standard [50].

McFarland preparation

The 0.5 McFarland turbidity standard was prepared from a mixture of 1% barium chloride dihydrate ($BaCl_2{\cdot}2H_2O$) and 1% sulfuric acid [50].

Standard plant extract concentration preparation

Plant extracts were prepared using dimethyl sulfoxide (DMSO) to create standard concentrations of the various plant parts. The solvent used was 10% v/v DMSO in sterile distilled water. DMSO is a proven general-purpose solvent with no antibacterial action, making it a suitable choice as a diluent [51]. Tween 20 was also utilized as a solvent for hexane extract. Various test concentrations of crude extracts (in mg/mL), depending on the available plant mass (e.g., 200, 100, and 50) were prepared.

Antibacterial agar well diffusion assay

The antibacterial activity of the crude extract was primarily evaluated qualitatively using an agar well diffusion method at various test doses (mg/mL) based on available plant mass, The Clinical and Laboratory Standards Institute guideline (CLSI M100) [52] was followed with minor adjustments to fit the current experimental setup. The Muller-Hinton agar medium was autoclaved for 15 min at 121 ℃ after being prepared according to the manufacturer's instructions. Twenty milliliters of the sterile, melted agar medium, cooled to 45 ℃ were aseptically

transferred onto a sterile 90-mm Petri dishes and allowed to harden at room temperature in a sterile environment within the biological safety cabinet.

After 16–18 h. of incubation at 37 ℃, bacterial strains were sub-cultured in nutrient agar, suspended in sterile nutrient broth, and adjusted to 0.5 MacFarland standard as previously described. 100 all of standardized test microorganism suspension were swabbed on to sterile Mueller–Hinton agar plates using sterile cotton swabs. The Mueller–Hinton agar allowed to dry after swabbing. Using blue tips, 8-mm-diameter wells were punched into the inoculated agar medium. After 15 min, 100 µL of 10% v/v extract solution was applied to the wells. Similarly, samples of 10% v/v DMSO in distilled water and chloramphenicol (a broad-spectrum antibiotic used as a reference) were used as negative and positive controls, respectively. To allow the extract solution to diffuse evenly throughout the agar medium, each dish was pre-incubated for 2 h. at room temperature. The dishes were then incubated for 24 h at 37 ℃. The diameter of the bacterial growth inhibition zone was measured in millimeters to assess antibacterial activity. Triplicates of the experiments were carried out, with growth and sterility quality controls conducted simultaneously. The average of the results was reported [51, 52].

Minimum inhibition concentration

Microdilution method

For each crude extract tested against the chosen microorganisms, the minimum inhibitory concentration (MIC) was determined using nutrient/brain heart infusion broth. To obtain the range of concentrations, the crude extract was serially diluted with each concentration being twice as concentrated as the previous one. A stock solution of the crude extract was prepared, and then sterilized nutrient or brain heart infusion broth was used to dilute each crude extract from the stock solutions to the desired concentrations.

Preparation of inoculum

Inoculum standardization is essential for precise and repeatable susceptibility testing. Emulsifying overnight colonies from an agar medium in saltwater was the method used to prepare the inoculum. For broth dilution, a final inoculum size of 5×10^5 CFU/mL is recommended [53].

Colony suspension method

Our assay employed cultures that were 18–24 h old. By contacting the colonies with a loop, the cultures were removed and either transferred from broth suspension to saline or modified to match a 0.5 McFarland standard. The inoculum and McFarland standard suspensions were visually compared for the appearance of black lines under adequate lighting conditions (the inoculum and McFarland standard must be in the same-sized tubes). For the majority of isolated organisms, a properly adjusted suspension is thought to contain about 1.5×10^8 CFU/mL. To get 1×10^6 CFU/mL, a 10:990 dilution of the bacterial suspension (1.5×10^8 CFU/mL) was prepared with broth. Finally, by preparing the crude extract, when we add an equivalent amount of the bacterial suspension, we can get $1.5\times10.^5$ CFU/mL with a 1:2 ratio as the final inoculum [52, 53]

Viable counts

The viable count is important to ensure that the inoculum used for the test contains approximately 5×10^5 CFU/mL. The test was performed by diluting 10 µL from 1.5×10^8 and diluting to 10 mL broth to get 5×10^5 CFU/ml by calculation. Then, exactly 100 µL solution of viable counts was spread over nutrient agar, and incubated for 24 h. Approximately 50 colonies are expected from an original inoculum of 5×10^5 CFU/Ml [53].

Inoculation

A 1 mL of the adjusted inoculum to each tube containing 1 mL of crude extract in the dilution series and mixed within 15 min of the inoculum preparation as described above. This was resulted in a 1:2 dilution of each antibacterial concentration and a 1:2 dilution of the inoculums as shown on the result table (Table 2). Finally, the mixture was incubated at 35 ± 2 ℃ for 16 to 20 h. in an ambient air incubator.

Table 2 Yield of extraction of the leaves, stem, and steam bark of *S. subserrata* in different solvents

Sample Code	Solvent (Extraction)	mass of powder (g)	mass of extract (g)	% yield
SSL	Hexane	20	0.25	1.25
SSS	Hexane	20	0.17	0.85
SSB	Hexane	20	0.17	0.85
SSL	Chloroform	20	0.75	3.75
SSS	Chloroform	20	0.26	1.30
SSB	Chloroform	20	0.2	1.00
SSL	Ethyl acetate	20	0.4	2.00
SSS	Ethyl acetate	20	0.11	0.55
SSB	Ethyl acetate	20	0.19	0.95
SSL	Methanol	20	1.79	8.95
SSS	Methanol	20	1.67	8.35
SSB	Methanol	20	2.71	13.55

Sample codes for *S. subserrata* parts: *SSL* Leaves, *SSS* Stem, *SSB* Stem bark

Minimum bactericidal concentration determination

The lowest concentration of a specific crude extract that can kill 99.9% of a given bacterial strain was determined from the MIC tests that showed no visible growth by taking a loop of inoculum living test organisms from the MIC tubes by streaking on fresh Mueller Hinton agar. The streaked Mueller Hinton agar plates were incubated at 37 ℃ for 24 h. and were observed for growth. Streaked Mueller Hinton agar plates that cannot show any growth indicate a 99.9% bactericidal effect of the crude extract at that minimum bactericidal concentration (MBC) [50]. The tests were done in triplicates.

ADMET properties determination and PASS prediction

The SwissADME web page was accessed, files were imported from the external file option, and molecules' chemical structures were downloaded in SDF (structure data format) from the PubChem data bank (http://pubchem.ncbi.nlm.nih.gov/). These files were then converted into molecular sketchers using ChemAxon's Marvin JS, and ADME was calculated using the default parameters [54]. Each molecule's data are displayed in tables, graphs, and an Excel spreadsheet. For clear output and export, the SwissADME output file consists of one panel per molecule that contains all the molecules' information [55].

PASS (Prediction of Activity Spectra for Substances) is a computer software that predicts more than 8000 pharmacological effects and can be used to estimate the general biological potential of drug-like substances based on their structural formulae. For predicting in silico investigations and biologically evaluating biological activity, all the compound structures from PubChem were loaded into PASS. The foundation of this program was a thorough examination of the links between structure and activity in a diverse training set. The list of biological activity categories for which the probability of revelation (Pa) and non-revelation (Pi) values are independent and vary from 0 to 1 is known as the biological spectrum for a given drug. The study focused on oral health-related activities and found that the higher the Pa value, the lower the chance of false positives in the set of compounds chosen for investigation. Prediction confidence is determined by calculating the difference between Pa and Pi values [56].

Statistical analysis

As recommended by Chen et al. [57] for a better estimation of half maximal effective concentrations (EC_{50}), values were calculated using the relationship log [sample] vs absorbance (normalized) using GraphPad Prism version 8.0.2(263). SD for triplicate measurements computed using Microsoft Excel. The percentage inhibition of enzyme activity and EC_{50} values are presented as the mean values ± SD.

Results

Extraction yield

The stem is found to yield a lesser mass of extract as compared to the leaves and stem bark. Methanol extract yield is much more than the other extracts for all parts of the plant (Table 2).

Phytochemical analysis: Antioxidant activity evaluation and TPC, and TFC estimation

The antioxidant activity in terms of EC_{50} of the leaves of *S. subserrata* is closer to the standard reference (Ascorbic acid) as shown in Table 3. The log EC_{50} values for these are negative, indicating smaller EC_{50} values. The hillslope values are also closer to −1 assuring fair steepness of the graph.

The equation of the standard curve for the determination of TPC was y = 0.0023x—0.0693, where $R^2 = 0.9981$, and for TFC: y = 0.002x + 0.0399, where $R^2 = 0.9971$. Similarly, to the antioxidant activity, the leaves of *S. subserrata* were recorded with higher total phenolic and total flavonoids content (Table 4). The bark TPC values is also reasonably higher.

Silicon content determination

The determination of silica content involves assessing the portion utilized as a traditional chewing stick. The results

Table 3 Result summary of EC_{50} and R^2 values

log(agonist) vs. normalized response – Variable slope				
Sample	**LogEC_{50}**	**HillSlope**	**EC_{50}(µg/mL)**	**R squared**
SSL	−0.1459 ± 0.0264	−4.062 ± 0.2539	0.7155 ± 0.0436	0.9875 ± 0.0002
SSS	0.6627 ± 0.0086	−1.147 ± 0.0418	4.6 ± 0.0909	0.9296 ± 0.0120
SSB	0.6536 ± 0.0142	−1.3853 ± 0.0537	4.5053 ± 0.1489	0.9725 ± 0.0010
Ascorbic acid	−0.9075 ± 0.01	−3.439 ± 0.02	0.1237 ± 0.01	0.9910 ± 0.0200

Sample codes for *S. subserrata* parts: *SSL* Leaves, *SSS* Stem *SSB* Stem bark

Table 4 Total phenol and total flavonoid content for the sample extracts

Sample	TPC—Total phenolic Content (mg/100 g)	TFC—Total Flavonoid Content (mg/100 g)
SSL	1366.27 ± 12.34	967.67 ± 19.06
SSS	705.91 ± 2.19	331 ± 21.33
SSB	1126.2 ± 44.61	770.95 ± 33.56

Sample codes for *S. subserrata* parts: *SSL* Leaves, *SSS* Stem, *SSB* Stem bark

were obtained using the gravimetric method outlined in Eq. 3 and are presented in Table 5.

Antimicrobial and MIC studies

Hexane extract is the most active towards the selected pathogens, indicating that the less polar components of the extracts for the different parts of the plant is found to be active. Hexane, chloroform and ethyl acetate extracts were found to be active against two of the Gram-positive bacteria. Only hexane extract affects Gram-negative bacteria (Table 6). Mostly, the leaves and stem of the plant showed to inhibit the growth of the selected strains. Hexane, chloroform and ethyl acetate extracts of *S. subserrata* were found to be active against these Gram-positive bacteria. Especially both the leaves and stem of this plant show MIC of 75 mg/mL for hexane extract and 100 mg/mL for chloroform extract (Table 7).

The extracts of *S. subserrata* are not as active for Gram-negative bacteria. The leaves and stems hexane extract were only found to be active against *Escherichia coli, Klebsiella pneumonia,* and *Pseudomonas aeruginosa* (Table 6). Lower MIC (25mg/mL) was recorded for the polar fractions of stem extract against *Enterococcus faecalis* (Table 7).

Table 5 Result summary for Silica content determination of the stem of *S. subserrata*

	Sample (Trail 1)	Sample (Trail 2)	(B) Blank
(C) Initial mass (g)	3.0125	3.0080	0.0000
(A) Ash mass (g)	0.0064	0.0081	0.0000
Silica content (%)	0.2124	0.2693	-
Average Silica content (%)	0.24085		-

0.24% silica present on the stem extract of plant extract

ADMET properties and PASS prediction

Physicochemical properties like molecular weight, number of rotatable bonds, number of hydrogen bond acceptors, number of hydrogen bond donors, molar refractivity, and total polar surface area (TPSA) were computed using the SwissADME server. Lipophilicity and pharmacokinetic properties like gastrointestinal (GI) absorption, the blood–brain barrier (BBB) permeant, opioid excretion (presence of P-gp substrate), and CYP3A4 enzyme inhibition. Most of the compounds were found to be bioavailable for the respective predicted activities. From the physicochemical properties Lipinski rule of 5 is violated more than once for some compounds like chrysoeriol 7-glucuronide, myricetin-3-o-β-d-glucoside, luteolin-7-glucoside, quercitrin and rutin (Table 8).

The PASS prediction for biological activities related to oral health were managed for the components isolated from the plant to see its role to maintain oral health. The activities considered were antibacterial, antifungal, anti-infective, anti-inflammatory, antioxidant, free radical scavenging activity, antiviral (herpes), mucomembranous protector, mucositis treatment and antiseptic.

Prediction confidence was computed as the difference between Pa and Pi, and denoted as the confidence in the result of prediction. Confidence values less than 0.500 are shaded as shown in Table 9.

Triandrin, gallocatechin, Catechin, β-sitosterol glucopyranoside, methyl 1-hydroxy-6- oxocyclohex-2-enecarboxylate

Table 6 Mean of inhibition zone of different crude extracts of assay of the antibacterial test result

No	Plant extract code	Hexane Extract			Chloroform Extract			Ethyl acetate Extract			Methanol Extract		
	Test microbial organisms	SSL	SSS	SSB	SSL	SSS	SSB	SSL	SSS	SSB	SSL	SSS	SSB
1	*Enterococcus faecalis* (ATCC79112)	20	20	23.5	23	22	0	24	13	24	0	18.5	24
2	*Escherichia coli* (ATCC25972)	21	15	0	13	0	0	0	0	0	0	0	0
3	*Klebsiella pneumonia* (ATTC70063)	0	11.5	0	0	0	0	0	0	0	0	0	0
4	*Listeria monocytogenes* (ATCC19115)	16.75	21	22	0	15	0	23	15	20	0	0	0
5	*Proteus mirabilis* (ATCC35659)	0	0	0	0	0	0	0	0	0	0	0	0
6	*Pseudomonas aeruginosa* (ATCC27853)	12	13	0	0	0	0	0	0	0	0	0	0
7	*Staphylococcus aureus* (ATTC25923)	17	0	0	0	0	0	0	0	0	0	0	0

Sample codes for *S. subserrata* parts: *SSL* Leaves, *SSS* Stem, *SSB* Stem bark

Table 7 Results of minimum inhibition concentration (MIC) of microdilution

Extract code	Solvent	D1	D2	D3	D4	D5	Test bacterial strain	MIC (mg/ml)	MBC (mg/ml)
SSL	Hexane	100	50	25	12.5	6.25	*Listeria monocytogenes*	100	-
							Enterococcus faecalis	100	-
SSS	Hexane	75	37.5	18.75	9.38	4.69	*Listeria monocytogenes*	75	150
							Enterococcus faecalis	75	100
SSB	Hexane	75	37.5	18.75	9.38	4.69	*Staphylococcus aureus*	75	150
							Enterococcus faecalis	75	150
SSL	$CHCl_3$	300	150	75	37.5	18.75	*Enterococcus faecalis*	75	150
SSS	$CHCl_3$	100	50	25	12.5	6.25	*Listeria monocytogenes*	100	200
							Enterococcus faecalis	100	200
SSL	EtOAc	200	100	50	25	12.5	*Enterococcus faecalis*	100	200
							Listeria monocytogenes	50	100
SSS	EtOAc	100	50	25	12.5	6.25	*Enterococcus faecalis*	100	200
SSB	EtOAc	200	100	50	25	12.5	*Enterococcus faecalis*	50	100
SSL	MeOH	200	100	50	25	12.5	*Enterococcus faecalis*	50	100
SSS	MeOH	200	100	50	25	12.5	*Enterococcus faecalis*	25	50
SSB	MeOH	200	100	50	25	12.5	*Enterococcus faecalis*	50	100

Note that MIC; minimum inhibition concentration, MBC; Mnium bactericidal concentration, and NA; not applicable

Sample codes for *S. subserrata* parts: *SSL* Leaves, *SSS* Stem, *SSB* Stem bark

and salicin are among the responsible components for this activity with a confidence greater than 0.500 (Table 9). Most of the components showed anti-inflammatory activity with a confidence of 0.500 and above. Antioxidant activity and one of its mechanisms (free radical scavenging activity) was also predicted with a confidence of 0.500 and above for most of the compounds. Quercetin, catechol, propyl acetate, β- sitosterol, catechin, and gallocatechin predicted a biological activity of mucomembranous protector nature with a Pa -Pi value of more than 0.700.

Discussion

Successive extractions were used aiming to identify phytochemical groups responsible for biological activities. As the stem is our point of attention among the parts of the plant for oral health consideration, other parts are used for comparison and enriching the study with possible data. A comparable yield was reported from the bark of other *Salix* spp. using a mixture of methanol, water, and acetic acid (49.5:49.5:1)[58].

The existence of phenolic groups in the constituent compounds is indicated by the other part's noteworthy antioxidant activity. The stem (shoot) and stem bark were found to have comparable composition [42] and the antioxidant activity result also showed nearly similar. The antioxidant activity of the leaves is closer to the reference ascorbic acid. Strong antioxidant activity, promising antihypertensive potential, and efficient antibacterial action against *S. aureus* were demonstrated by *Salix* spp. bark polar extracts as compared with the reported in vitro antioxidant activities [58]. As flavonoids and phenolic compounds have been isolated from the different parts of the plant as shown in the introduction part the total contents presented on Table 4 is expected. Both the leaves and bark were found to be rich in phenolic compounds. This agreed to the antioxidant activity as well as to the predicted biological activities.

Silicates and silicate-based compounds play a prevalent role in dentistry, serving as fillers in various dental materials like glass ionomer cement, compomers, composites, and adhesive systems. During the setting process, these fillers react with acids, thereby enhancing mechanical properties such as physical resistance, thermal expansion coefficient, and radiopacity in acrylic filling materials. Moreover, they contribute to minimizing polymerization shrinkage, improving aesthetics, and enhancing handling properties. Furthermore, silicates establish a micro mechanical bond between the material surface and the composite matrix. Dental ceramics, widely employed in dentistry for veneers, in lays, on lays, denture teeth, full-ceramic crowns, and crown veneering materials, also contain silicates [59]. Plant materials with elevated silicon content have been employed in the production of silicon-containing toothpaste and other consumer care products [60]. Silica and porous silica, due to their superior mechanical and optical properties, are extensively used by the toothpaste and cosmetic industries. Hence, the investigated plant material, rich in both

Table 8 Physicochemical and pharmacokinetic properties of the isolated compounds of *S. subserrata*

Molecule PubChem ID	Name of Compound	MW	#Rotatable bonds	#H-bond acceptors	#H-bond donors	MR	TPSA	XLOGP3	Ali Class Solubility	GI absorption	BBB permeant	Pgp substrate	CYP3A4 inhibitor	Lipinski #violations	Bioavailability Score	PAINS #alerts
CID_14048613	Triandrin	312.32	5	7	5	76.91	119.61	−0.41	Very soluble	High	No	No	No	0	0.55	0
CID_14630700	Chrysoeriol 7-glucuronide	476.39	5	12	6	113.21	196.35	2.03	Moderately soluble	Low	No	Yes	No	2	0.11	0
CID_134601	Phenyl alanine	294.3	9	6	3	73.82	118.72	−2.74	Highly soluble	High	No	No	No	0	0.55	0
CID_6305	Tryptophan	204.23	3	3	3	57.36	79.11	−1.06	Very soluble	High	No	No	No	0	0.55	0
SID_135262673	Myricetin-3-o-β-d-glucoside	480.38	4	13	9	112.18	230.74	0.01	Moderately soluble	Low	No	No	No	2	0.17	1
CID_65084	Gallocatechin	306.27	1	7	6	76.36	130.61	0	Soluble	High	No	No	No	1	0.55	1
222,284	β- Sitosterol	414.719	6	1	1	133.23	20.23	9.34	Poorly soluble	Low	No	No	No	1	0.55	0
7997	Propyl acetate	102.13	3	2	0	27.43	26.3	1.24	Very soluble	High	Yes	No	No	0	0.55	0
289	Catechol	110.11	0	2	2	30.49	40.46	0.88	Very soluble	High	Yes	No	Yes	0	0.55	1
23,649,674	Methyl 1-hydroxy-6-oxocyclohex-2-enecarboxylate	170.16	2	4	1	40.67	63.6	0.12	Very soluble	High	No	No	No	0	0.55	0
5146	Saligenin	124.14	1	2	2	34.59	40.46	0.73	Very soluble	High	Yes	No	Yes	0	0.55	0
8343	1,2-Benzene dicarboxylic acid, bis (2-ethylhexyl) ester	390.56	16	4	0	116.3	52.6	7.45	Poorly soluble	High	No	Yes	Yes	1	0.55	0
439,503	Salicin	286.28	4	7	5	66.72	119.61	−1.22	Very soluble	Low	No	No	No	0	0.55	0
5,280,637	Luteolin-7-glucoside	448.38	4	11	7	108.13	190.28	1.46	Moderately soluble	Low	No	Yes	No	2	0.17	1
5,280,459	Quercitrin	448.38	3	11	7	109	190.28	0.86	Moderately soluble	Low	No	No	No	2	0.17	1
5,280,343	Quercetin	302.24	1	7	5	78.04	131.36	1.54	Soluble	High	No	No	Yes	0	0.55	1
9064	Catechin	290.27	1	6	5	74.33	110.38	0.36	Soluble	High	No	Yes	No	0	0.55	1
5,280,805	Rutin	610.52	6	16	10	141.38	269.43	−0.33	Moderately soluble	Low	No	Yes	No	3	0.17	1

MW molecular weight, *MR* molar refractivity, *TPSA* total polar surface area, *GI* gastrointestinal, *BBB* blood–brain barrier

silicon and phenolic components, holds potential for use in toothpaste and cosmetics formulation, given the close relationship of these components to toothpaste formulation [61, 62].

In addition to the moderately bitter test, the presence of silica with a considerable amount (Table 5) will give the stem of *S. subserrata* good quality of cleaning our teeth. The bitter test helps to produce saliva to facilitate cleaning and the presence of silica improves the abrasive property of the chewing stick [63]. Though it was not found to be determined numerically, the presence of silica and its whitening effect was reported in the stem of the popular *S. persica* chewing stick [64].

In one way or another, the selected strains are associated with food-borne infections. *Listeria monocytogenes* and *Enterococcus faecalis* are pathogens for food-borne infections [65]. Lower MIC for the hexane and chloroform extracts bring the additional role of the commonly used chewing stick as contributing to oral health as well as the well-being of the alimentary system. *Staphylococcus aureus* is one of the oral pathogens for human beings [63] Hexane extract from the leaves of *S. subserrata* shows considerable inhibition for the growth of these bacteria indicating the potential for oral health care products.

The activity of stem extracts against *E. faecalis* shows the potential of the stem (chewing stick) against food-borne infections. *Pseudomonas aeruginosa* is one of the pathogens expected in the oral cavity in association with other bacterial groups [6]. Inhibiting the growth of these bacterial colonies will contribute to the prevention of oral infection.

The bioavailability score is also less than 0.5 indicating the bio unavailability of these components [66] and it is expected that these compounds will be less effective for the intended activities. The oral bioavailability of most compounds agreed to the oral administration in most ethnobotanical preparations shown on Table 1. For the rest of the components, solubility issues, pharmacokinetic properties, and toxicity effects (PAINS alert values less than 1) were not found to be a problem. This indicates the plant is not toxic as far as the compounds considered in the study. A comprehensive profile and in vitro toxicity test might be demanded to conclude the toxicity of the plant extracts. Oral health is determinant for the overall health of our body [67, 68].

A positive Pa − Pi value indicates that compounds should be treated as potentially inhibiting the growth of bacteria, and thus, it may be selected for experimental validation. The magnitude of the confidence shows how trustworthy the results of the prediction are [69].

Most of the activities predicted for the target compounds isolated from the plant were also reported by a previous investigation [11]. The oral cavity being at the beginning of the alimentary system is more likely susceptible to a varied infection. The predicted anti-infective activities for the compounds considered showed that most of the compounds with positive confidence especially the antifungal activity was significant. This indicates the potential of the plant for antifungal activity. The antibacterial activities towards oral foodborne infections were confirmed via the antimicrobial activity. The antiviral activity for oral herpes infection was also observed as shown in Table 9. Anti-inflammatory activity with a confidence of half and above will play a role in the treatment of inflammations in the oral cavity and contribute to oral health. Triandrin, gallocatechin, catechin, quercetin, and salicin are among the potential components for the antioxidant activity excluding the less bioavailable components. Components predicted with larger confidence for mucomemebranous protector nature can be considered as potential protectors for oral health as the confidence is greater [56]. Mucositis is inflammation of the mucosa, the mucous membranes that line your mouth and the entire gastrointestinal tract. It's a common side effect of cancer treatments involving radiation or chemotherapy. Mucositis is temporary and heals on its own, but it can be painful and carries certain risks. It requires self-care and medical care to manage [70]. Phenylalanine, tryptophan, propyl acetate catechol, and saligenin were predicted for anti-mucositis activity with a confidence greater than 0.500. Quercetin, saligenin, and catechol were predicted as responsible for antiseptic activity with a confidence greater than 0.500. This will contribute significantly to maintaining oral health. Catechin which was reported to be active against the rabies virus [43] is active also for most biological activities considered like quercetin, saligenin, catechol, gallocatechin, salicin, and triandrin. This is in line with the ethnobotanical use of the plant for treating rabies as shown on Table 1. The efficacy of the crude extract of *S. subserrata* were also evaluated in support of the traditional uses against rabies [22].

The Silicon content, antioxidant activity, TFC, TPC and the antimicrobial activities test justify the significant benefits of the TCS made from *S. subserrata* stem. As oral health is the major determinant for the overall health of our body and more than 70% of the world population depends on the traditional ways of keeping oral health *S. subserrata* can be one of the alternative TCS with antimicrobial and other benefits justified in this study. In addition to the stem the other parts like the leaves and stem bark were also active towards oral pathogens indicating the potential to be developed as oral healthcare products, this will be a future concern which will also increase the oral health benefits of the plant.

Table 9 Biological activities predicted for compounds from *S. subserrata*

Pub Chem ID	Isolated Compounds	Prediction confidence of compounds isolated from *S. subserrata* (= Pa- Pi)									
		Antibacterial	Antifungal	Antiviral (herpes)	Anti-infective	Anti-inflammatory	Antioxidant	Free radical scavenger	Mucomembranous protector	Mucositis treatment	Antiseptic
14,048,613	Triandrin	0.571	0.672	0.511	0.881	0.631	0.667	0.786	0.072	0	0
14,630,700	Chrysoeriol 7-glucuronide	0.607	0.710	0.418	0.643	0.697	0.790	0.977	0	0	0
134,601	Phenyl alanine	0	0	0	0.308	0.293	0	0	0.494	0.926	0
6305	Tryptophan	0	0	0	0.327	0	0	0	0.649	0.913	0
1.35E + 08	Myricetin-3-o-β-d-glucoside	0.625	0.718	0.585	0.780	0.747	0.934	0.981	0	0	0
65,084	Gallocatechin	0.354	0.578	0.467	0	0.582	0.867	0.863	0.954	0.214	0
9064	Catechin	0.267	0.529	0.450	0	0.504	0.807	0.840	0.959	0.252	0
########	β-Sitosterol glucopyranoside	0.483	0.713	0.294	0.262	0.593	0.322	0	0	0	0
222,284	β- Sitosterol	0	0.565	0	0	0.400	0	0	0.707	0	0
7997	Propyl acetate	0	0.352	0.363	0.404	0.710	0	0	0.845	0.590	0.415
289	Catechol	0.268	0.320	0.391	0.568	0.470	0.494	0.397	0.861	0.794	0.674
23,649,674	Methyl 1-hydroxy-6- oxocy-clohex-2-enecarboxylate	0.432	0.561	0.383	0	0.366	0	0	0.224	0.445	0
5146	Saligenin	0	0.297	0.359	0.485	0.442	0.321	0.397	0.645	0.558	0.745
8343	1,2-Benzene dicarboxylic acid, bis (2-ethylhexyl) ester	0	0.361	0	0.400	0.491	0	0	0.582	0.406	0.386
5,280,343	Quercetin	0.354	0.458	0.472	0.226	0.672	0.869	0.808	0.717	0.396	0.520
5,280,459	Quercitrin	0.635	0.732	0.510	0.685	0.744	0.912	0.973	0	0	0
5,280,637	Luteolin-7-glucoside	0.588	0.712	0.544	0.858	0.704	0.838	0.950	0	0	0
5,280,805	Rutin	0.672	0.778	0.519	0.648	0.715	0.920	0.987	0	0	0
439,503	Salicin	0.509	0.593	0.439	0.894	0.601	0.644	0.676	0	0	0

Conclusion

The traditional chewing stick made from *S. subserrata* is commonly used in most parts of Ethiopia. The phytochemical analysis, antioxidant activity, and antimicrobial activity results added value to the importance of *S. subserrata* stem in maintaining oral health. The silicon content of the stem is also another beneficial value for the cleaning of our teeth. The non-polar fractions of the stem, leaves and stem bark showed better inhibition for foodborne bacteria. Physicochemical and ADME properties determination shows that most of the compounds isolated from the plant extracts are bioavailable and no significant toxicity issue was observed as far as the compounds isolated. Antioxidant and antimicrobial activities results are also supported by the predicted biological activities such as anti-infective, free radical scavenging activity, mucomembraneous protector, and mucositis of the compounds previously isolated from the different parts of the plant. A comprehensive toxicity test is demanding together with complete profiling of the extracts to come up with stronger recommendations.

Abbreviations

ADME	Absorption, Distribution, Metabolism and Excretion
DPPH	2,2-Diphenyl-1-picrylhydrazyl
EC_{50}	Effective concentration
MBC	Minimum bactericidal concentration
MHA	Müller-Hinton agar
MIC	Minimum inhibition concentration
NA	Nutrient agar
PASS	Prediction of Activity Spectra for Substances
TCS	Traditional chewing stick
TFC	Total flavonoid content
TPC	Total phenol content

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Authors' contributions

FBT and TBA conceptualized the study; RKB and MGT validated the study; MGT, YHG and TGT curated the data and helped in writing—review and editing; TGT, DSA, TGA, and MA worked on methods FBT and TBA helped in writing—original draft preparation. All authors have read and agreed upon the published version of the manuscript.

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Data availability

Data generated or analyzed during this study are included in this published article. Other datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

'Not applicable.

Competing interests

The authors declare that they have no competing interests.

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