



Al - Saeed University Journal of Applied Sciences

journal@alsaeeduni.edu.ye

Vol (9), No(2), Aug., 2026

ISSN: 3104 - 8978 (Print) ISSN: 3104-8986 (Online)



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Received: 11/7/2026

Accepted: 11/8/2026

Journal Website:

<https://journal.alsaeeduni.edu.ye>

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Abstract

Background:

Dental caries is a global public health concern that afflicts vulnerable populations in rural areas, whose access to conventional dental care is limited by cost and infrastructure.

Objective & Methodology of the study:

This study was established to evaluate the traditional use of *Solanum incanum* L. (*Al-Oursam*) fruits by the people in Yemen to treat dental caries and oral pain, a non-traumatic injury related other than deep infection or systemic disease. Aqueous-ethanolic Soxhlet extraction resulting in three batches (E1, E2, and E3). Extracts of *Solanum incanum* L. were analyzed by LC-MS and the extracts antibacterial activity were evaluated by In vitro radial diffusion assays against some bacterial isolates.

Results:

LC-MS results showed that *S. incanum* L. Extract E1: 1,2-Propanediol and Butyl phthalate (46.93%) following by 1-Hexen-6-ol (23.71) and 1,2-Propanediol decreased to 17.60% in E2 and Dibutyl phthalate is the highest in E2 (18.55%) and decreased to 12.21 in E3. Moreover the of 2,3-butanediol reached maximum (56.45%) was the highest in E3.

In vitro radial diffusion assays showed significant antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus mutans*. The most potent activity was determined for batch E3, in which the level of 2,3-butanediol reached maximum (56.45%), and this variant also inhibited the development of a gramicidin S-secreting strain at the highest inhibitory concentrations. At a concentration of 7.5 mg/mL, heartwood extract E3 caused wide antimicrobial clearance zones (108 mm against *E. coli*; 112 mm against *S. aureus* and 116 mm against *S. mutans*).

Conclusions:

The results showed that the hydrophilic glycol matrix indeed contributed to this effect, successfully aiding in the diffusion of the active phenolic compounds. In the end, *S. incanum* fruits offer a safe, inexpensive platform for developing efficacious oral healthcare products like mouthwashes and toothpastes to alleviate the burden of oral diseases in under-resourced parts of the world.

Keywords: Anti-infective, Dental caries, *Solanum incanum*, LC-MS, Antibacterial activity.

Introduction

A human oral cavity serves as an extremely dynamic, consistently shifting, and largely sophisticated ecological microenvironment which rigidly supports a deeply diverse and complex microbiome. At normal healthy physiologic conditions, there is an estimated composition of over 25,000 diverse species inhabit the oral cavity niches with a smaller group of approximately 1,000 species forming and growing in dynamic equilibrium within the highly structured dental plaque biofilm community (Rossoni *et al.*, 2019 Gholizadeh *et al.*, 2016 Kolenbrander *et al.*, 2002; Paster *et al.*, 2001) These dental plaque biofilms are precisely organized, highly cooperative, mutually synergistic bacterial consortia embedded in a self-synthesized extracellular polymeric substance matrix that has the astonishing ability to firmly attach itself to both non-living enamel surfaces and adjacent living epithelial oral tissues (Jiao *et al.*, 2019; Xu *et al.*, 2018) Frequent exposure to large amounts of highly fermentable carbohydrates and poor oral hygiene practices may, in turn, predispose this finely-balanced ecological habitat to undergo pathogenic shifts that fuel uncontrolled proliferation, as the acidogenic and aciduric strains emerge through natural selection (Spatafora *et al.*, 2024). This specificity in spatial extremes and intensity of microbial imbalance represent the main primal cause for a continuously progressive and destructive disease, dental caries (tooth decay), a chronic condition that has recently been categorized by health authorities as among the most prevalent, public health problem on Earth that imposes substantial economic burdens to affected populations around the globe (Jiang *et al.*, 2026).

The pathology of dental caries results from the persistent, localized demineralization of highly calcified inorganic tooth enamel and its underlying dentin, a destructive process that is directly dependent on chronic exposure to patchily concentrated organic acids generated during anaerobic metabolism of residual dietary sugars by specific cariogenic bacteria. In this regard, it is universally recognized in the published literature that *Streptococcus mutans* plays a key role as the most important streptococcal pathogen responsible for initiating dental caries (Shen *et al.*, 2025). This classification is largely based on the bacterium's phenomenal, highly focused ability to strongly attach to smooth enamel surfaces by means of sophisticated, sucrose-dependent glucan biosynthesis and its rapid, extensive continued production of erosive lactic

acid at exceedingly low pH (Luo *et al.*, 2024). Stringent definition of oral biofilms needs to consider that although *S. mutans* and *Lg. casei* are significantly associated with the initiation of caries, clinical oral biofilm is implicitly and invariably polymicrobial in nature; multiple other opportunistic pathogenic bacteria including facilitating Gram-positive *Staphylococcus aureus* as well as Gram-negative *Fn* from actively progressing carious lesions or deep periodontal pockets have been routinely isolated (Colombo *et al.*, 2023). In these compromised anatomical sites, these secondary pathogens play a direct role in the structural integrity of the biofilm as well as trigger localized tissue necrosis and promote chronic painful inflammatory diseases such as severe gingivitis and advanced periodontitis.

Standard contemporary clinical treatments for the management of dental caries have been based fundamentally upon invasive mechanical debridement, application of costly restorative composite resin materials and also prophylactic prescription of synthetic chemotherapeutic mouthrinses containing potent agents such as chlorhexidine or broad-spectrum mix antibiotics (Melo *et al.*, 2013; Moussa *et al.*, 2022). But many of these highly conventional and technologically dependent interventions are still beyond the reach, economically inaccessible or completely unavailable for marginalized low-income geographically isolating rural populations (this is particularly apparent among impoverished rural communities in many poor countries, with numerous examples in the Republic of Yemen) (Janakiram *et al.*, 2020). Additionally, the extremely disturbing global escalation and quick-spreading of multidrug-resistant bacterial pathogens, a disaster predominantly caused by irrational broad-spectrum prophylaxis and incessant improper utilization of synthetic antibiotics, has drastically reduced the future successful clinical outcomes of such routine dental techniques. This ever-growing and devastating critical global health crisis vividly underscores the immediate pressing necessity to rationally screen, chemically profile, and biologically validate safe, highly biocompatible and affordable natural mode of therapeutics sourced directly from immediately available local plant materials (Aware *et al.*, 2022; Chaachouay & Zidane, 2024; Thomford *et al.*, 2018).

One of these plants, which is a member of the family *Solanaceae*, is *Solanum incanum* L. (common name: bitter apple or Sodom apple; local names: **sty ligest Al-Oursam** (Saudi Arabia) through the Arabian Peninsula)

(Anwar, 2018; Yetayih & Ravichandran, 2020). It is also a weed of widely distributed in the wilds of East Africa and mountainous southwestern Arabia, especially at high altitudes surrounding Taiz, Yemen (Rani *et al.*, 2025). According to the deep-rooted traditions of indigenous medicine in tropical Africa and Yemen, *S. incanum* is commonly or occasionally used as a general-purpose broad-spectrum natural remedy for severe abdominal pain, persistent fevers, acute chest infections, inflammatory skin ailments and even venomous snakebites. Ironically, however, in the particular and unique field of folk medicine or traditional folk dentistry all anatomical parts of *S. incanum* have found their way into use as a powerful material which can relieve an unbearably painful toothache virtually instantaneously but only temporarily too (Zivanayi *et al.*, 2023). Such traditional medicinal uses are quite ancient, and in many cases consist of either: 1) direct burning the thoroughly dried seeds of the fruits so that it provides aromatic smoke for inhalation by the patients or 2) vigorous gargling with concentrated aqueous root decoctions; or 3) vigorous rubbing of freshly extracted raw fruit pulp directly over inflamed and painful gums (Dapar *et al.*, 2020; Refaey *et al.*, 2024).

Comprehensive modern phytochemical surveys on the diverse genus *Solanum* have repeatedly shown an extraordinary richness in complex, biologically-active secondary metabolites with documented bioactivity (including well-characterized examples of steroidal glycoalkaloids like solasonine and solamargine; complex saponins; various flavonoids; astringent tannins) (Delbrouck *et al.*, 2023). These phytochemical compounds that are found in nature show very strong, wide-spectrum antimicrobial, localized analgesic and anti-inflammatory characteristics. Significantly, the crucial international patent (WO/2011/116781) published as of October 2010 is part of contemporary literature on intellectual property and provides a complete accounting for appropriately defining the chemistry associated with effective chemical isolation in the seeds of Yemeni *S. incanum* when de novo phenolic acids were capable of being isolated or characterized, viz., its entirely novel compounds, such as 5-caffeoyl-2,3,4-trihydroxypentanoic acid (Magaña *et al.*, 2021). This highly purified, isolated compound displayed very potent specific bactericidal activity for the most important cariogenic bacteria (including very aggressive strains of *Streptococcus mutans*, *Lactobacillus acidophilus* and a variety of *Actinomyces species*) and to our surprise turned

out to be considerably more efficacious than standard commercially available antibiotics such as ciprofloxacin in many limited clinical trials (Hu *et al.*, 2011; Qiu *et al.*, 2020).

Notwithstanding these encouraging, well-evidence-based results, the long-standing historical routes of delivery such as deep inhalation into lungs of smoke from pyrolyzed seeds are extremely dangerous *ex vivo* and *in vivo* due to dramatic and lethal risks for respiratory health plus systemic toxicity imposed on patients (Costain & Laprairie, 2019). In addition, raw *S. incanum* fruits are inherently toxic due to their natural contents of dangerous but poorly described levels of many bioactive alkaloids and endogenous chemical carcinogens like dimethylnitrosamine (DMNA) (Chifamba *et al.*, 2025). Long term exposure to these particular substances is directly associated with a frighteningly high frequency of aggressive esophageal cancer in certain geographic regions where the crude unrefined extract of the sap is consumed as food or applied topically. Thus, there is an urgent and justifiably important need to effectively design and thoroughly develop standardized, safe, non-toxic and technologically sound extraction protocols that can successfully isolate a high proportion of these very active antimicrobial oils but at the same time completely eliminate the major thermal decomposition hazards associated with conventional pyrolysis (França *et al.*, 2026; Nabi *et al.*, 2025)

This in-depth research study aimed to definitively bridge this critical scientific and clinical gap by systematically analyzing and leveraging a solvent system consisting of carefully modulated, polar non-aqueous binary solvents applied using continuous Soxhlet extraction methods to isolate the active, bioactive oil fractions directly from whole intact oily fruits of wild Yemeni *S. incanum* (Yetayih & Ravichandran, 2020). Extremely complicated and diverse chemical profiles of the chemo typically segregated, independently produced experimental batches (named E1, E2 and E3) were later comprehensively analyzed by sophisticated Liquid Chromatography-Mass Spectrometry (LC-MS) methods to confirm the structural identity of active volatile low-molecular mass constituents responsible for biological activity. In addition, these extracts with the highest concentrations were batch-tested for their specific kinetics of action and bactericidal activity against virulent bacterial strains (*E. coli*, *S. aureus*, and *S. mmutans*). In addition to ultimately make traditional ethnobotanical dental practices live up to their full

potential when modernized, transforming them into a thoroughly scientific-pharmaceutical prototype which is validated by the rigorous testing of safety and reproducibility, and thus an excellent candidate for subsequent human application locally through local dentistry services.

Experimental Protocols and Materials

Botanical Collection and Specimen Preparation

S. incanum fruits (as shown for a single fresh fully mature, and wild grown fruit in **Figure 1a**) were only selectively and hand-harvested from different diverse but very ectopically heterogeneous ecological micro-zones throughout the rugged, hilly geographical landscapes of Taiz City Yemen. After collection, the fruit samples are washed thoroughly and vigorously under running tap water, then rinsed twice in highly purified deionized water to eliminate all traces of dust from ambient environments as well as any potential agricultural pesticide residues and general surface-associated microbial contamination. Subsequent to this intense purifying step, the thoroughly washed plant materials were mechanically diced into highly homogeneous tiny pieces—a vital preparatory process specifically designed to significantly maximize surface area-to-vol by producing ever-more-admissible geometric debris and thus promoting computationally efficient thermodynamic extraction in following phases. Fine pieces of the fruits were then set out to air-dry under a very shaded temperature-controlled environment, held at ambient room temp (exact range from 25°C to 28°C) for weeks. This methodical gradual drying was intentionally conducted to ensure there would be no triggering of unwanted thermal degradation, volatility, or structural modification of the ultra-delicate and temperature sensitive bioactive phytochemical molecules. Once the residual moisture was fully eliminated, the dried fruit pieces were carefully transferred to and stored in hermetically sealed glass containers, in order to preserve their chemical integrity before beginning extraction.

Extraction of Active Volatile Oils

All critical isolation of concentrated, active oil fractions was performed systematically using an industrial chemistry protocol that is well defined and reproducible. Each separate extraction cycle, performed independently for each individual, consisted of tightly packing a 150 g sample of thoroughly dried and sifted plant material into an extremely porous cellulose extraction

thimble which was then placed inside the central core chamber of a standard laboratory-grade Soxhlet apparatus (shown in Figure 1b). The polar binary solvent system of high purity absolute ethanol (J.T. Baker, 99.5%) diluted with ultra-pure deionized water was prepared in the lower boiling extraction vial. The particular synergistic solvent mixture of solvents was specifically selected and intentionally utilized for its enhanced chemical functionality to simultaneously solubilize the highly hydrophilic short-chain diols along with the moderately polar structurally complex phenolic compounds that are embedded deep within this rigid plant cellular matrix.

Every process (Soxhlet Extraction) was running continuously under permanent, strictly controlled thermal reflux for a total of 5 hours in order to obtain and recover all soluble biologically active oils. After the reflux cycle has ended, the very hot, chemically rich extraction liquid was then slowly cooled down to normal ambient room temperature. The suspension was placed in a vacuum-sealed container, cooled and tightly filtered by a 12 mm wide, highly porous Buchner funnel (Figure 1c) under a continuous vacuum seal with high-retention qualitative analytical filter paper used to entirely separate plant fibers and cellular debris. The subsequent clear filtrate was then progressively concentrated by the effective evaporation of a large excess of residual binary solvent under rigorously reduced pressure with a carefully controlled precision rotary evaporator. This critical evaporation step effectively produced an active oil with remarkably high concentrations that was also opaque and syrupy in texture. Finally, to ensure as much scientific rigor and statistical reproducibility as possible throughout these efforts, this entire extraction process was carefully replicated three more times resulting in three completely independent experimental extract batches (hereafter referred to in this study as E1, E2 and E3) following the same downstream analytical scheme with an equally rigorous biological analysis.

Chromatographic and Mass Spectrometric Profiling

Three independently produced active oil batches (E1, E2 and E3) were characterized in detail and quantified based on their highly complex and intrinsic chemical compositions using sophisticated Liquid Chromatography-Mass Spectrometry (LC-MS) analytical techniques. A highly sophisticated analytical system employed for this step of the investigation was physically linked live to an extensive mass spectral reference library housed at the

prestigious Yemeni Standardization and Meteorology Organization headquarters located in Sana'a, Yemen by use of state-of-the-art, high-resolution chromatographic column, namely a Shimadzu LC-MS system using Photodiode Array (PDA) detector.

Data trained on uncovered range of critically chromatographic separations of the complex phytochemical mixtures, employing a highly optimized single-stage and dynamically shifting gradient elution profile. Simultaneously, performed an elegant modulation of the mobile phase composition from a highly aqueous and strongly polar initial conditions (ethanol: water =30:70 %v/v) to progressively highly organic and non-polar elution conditions (ethanol: water =90:10 %v/v) to separate each unique array of volatile and semi-volatile compounds according to its specific polarity. The sensitive electrospray ionization source immediately and efficiently ionized the separated analytes as elution occurred from the separation column, while high-precision mass spectrometry allowed for detection of their individual m/z ratios. In a comprehensive, systematic manner, the unique elution adjacency of each molecular constituent was utilized to correlate its experimental retention time (min; RT), together with corresponding precursor ion molecular weight measurements and unique, defining cleaved bond junction fragmentation profiles directly against proven structural identities archived within authoritative *Wiley and National Institute of Standards & Technology (NIST)* mass spectral libraries. In addition, the specific area of each identifiably separated chemical constituent (as chromatographic peak areas) was used to determine the relative quantitative abundance of its constituent, which was reported in this study as a % standardised area fraction relative to the complete integrated total chromatogram.

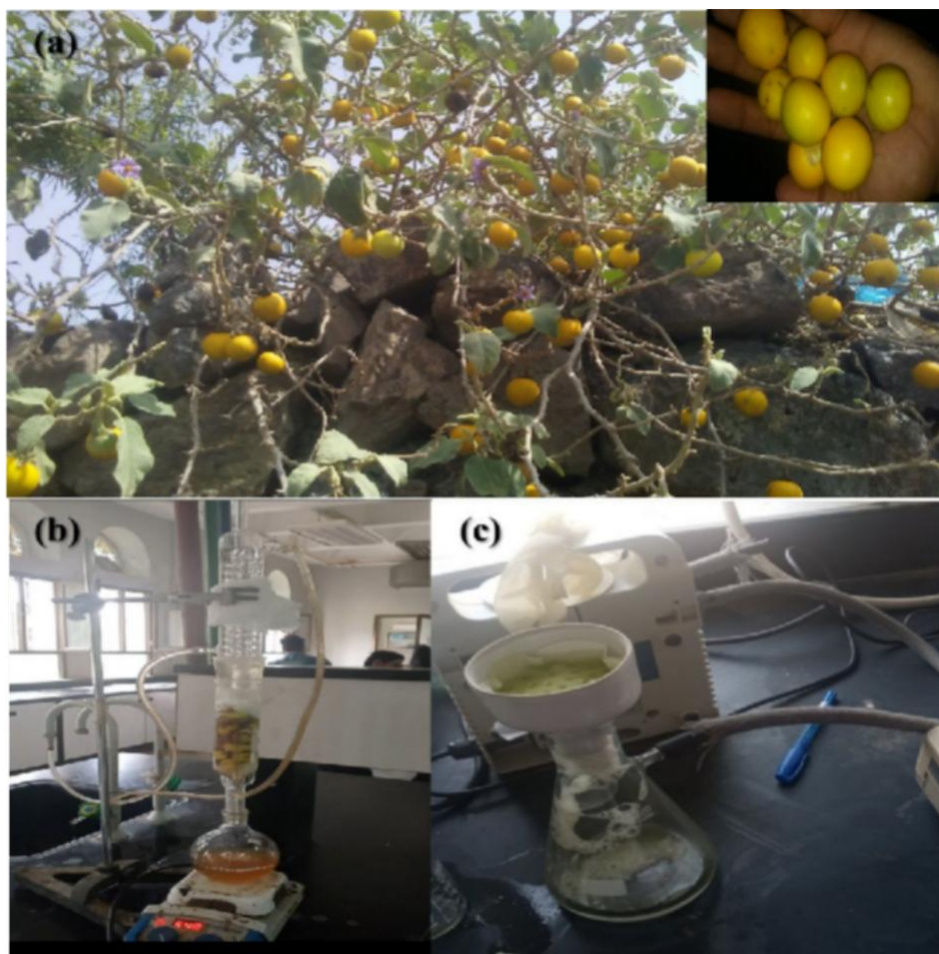


Figure 1: (a) Fruits of *S. incanum*, (b) Soxhlet apparatus and (c) Buchner funnel.

In Vitro Antibacterial Diffusion Bioassay

Three formulated *S. incanum* fruit extracts (E1, E2 and E3) were specifically investigated for their potent broad-spectrum antibacterial activities by evaluating their bactericidal kinetics against three major oral opportunistically pathogenic bacterial strains of different species and gram classification: the Gram-negative enteric bacterium *E. coli*; the Gram-positive opportunistic pathogen *S. aureus*; and the highly aggressive primary cariogenic pathogen *S. mutans*. The aforementioned clinically relevant individual bacterial strains were routinely preserved on nutrient agar slants stored at 4°C. Appropriately prior to the initiation of biological testing, highly active working cultures in logarithmic phase were carefully established

via aseptic inoculation of dormant bacteria directly into rich sterile liquid nutrient broth followed by overnight incubation under precisely controlled aerobic conditions at 37 °C in all samples. After this incubation period, the optical turbidity of the dense bacterial suspensions was measured and adjusted using a sterile isotonic saline solution to reach an exact 0.5 McFarland inoculum density, ensuring perfect technical reproducibility between experiments.

The standardized microbiological agar diffusion bioassays were performed in a highly systematic manner on high-density standard polystyrene petri plates where a uniform layer of nutrient-rich Müller-Hinton agar was poured. We aseptically distributed a defined volume of bacteria at the same initial density evenly across the entire usable surface area of agar using a sterile glass spreader ('lawn' technique; 24 l per plate), designed to create an ultra-uniform, wholly confluent bacterial lawn. At the same time, a mixture containing the highly concentrated active oil extracts was completely dissolved in a biologically inert solvent system which is highly sterile to accurately prepare 0.5 working concentration (Mathematically equivalent to 7.5 mg/mL). This very specific, extremely dilute test concentration was intentionally allowed here by the research team to robustly assess the inherent significantly antimicrobial abilities of the plant extracts at practical high sub-therapeutic, extremely non-toxic and safe dosage ranges. Aliquots of the extracted and dissolved extracts were then carefully measured out into the aforementioned established culture system, followed by overnight incubation in a well-regulated microbiological incubator under strictly aerobic conditions at 37 °C for a time period of 24 hours.

These extracts were then qualitatively evaluated by measuring their antibacterial efficacy in terms of the average spot diameter (mm), precisely quantified (in mm) with a calibrated high-precision digital caliper, of resulting circular bacterial growth inhibition zone, also known as visible clearance spot per 4 mm dose. All bioassays were performed in strict triplicate, with all final values reported as calculated mean diameters to guarantee full statistical accuracy while also minimizing the probability of experimental error and confirming observed biological phenomena repeatability.

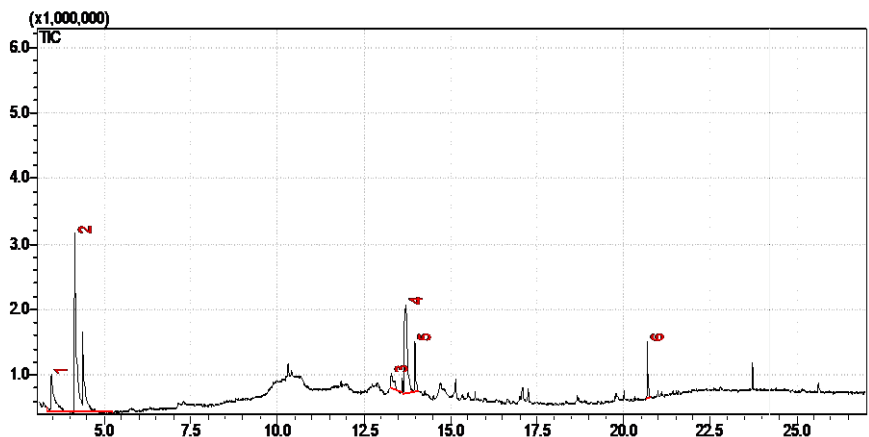


Figure 2: LC-MS profile of *S. incanum* fruit extract, E1.

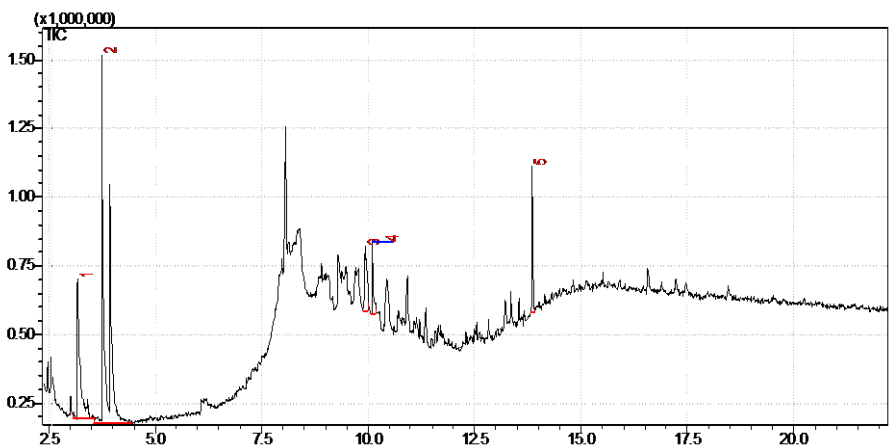


Figure 3: LC-MS profile of *S. incanum* fruit extract, E2.

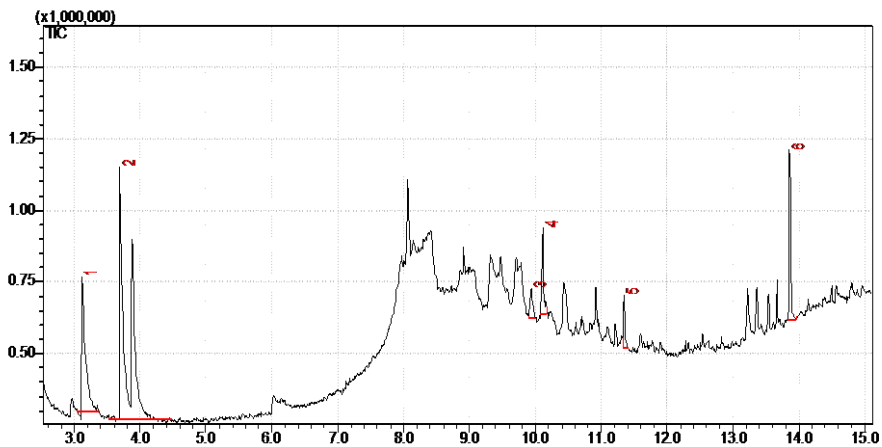


Figure 4: LC-MS profile of *S. incanum* fruit extract, E3.

Results

Chemical Characterization of Extract Batches

The three individually prepared *S. incanum* fruit extracts analysed using comprehensive liquid chromatography-mass spectrometry (LC-MS) chromatographic separations and identified unique, distinct volatile and semi-volatile chemical profiles for each respective batch, designated E1 (A), E2(B) and E3(C) extracts, respectively (**Figures 2, 3 & 4**). The wide range of specific chemical compounds that were confidently identified in each individual extract including their accurately measured retention times, integrated chromatographic peak areas, and calculated relative area percentages have been carefully recorded and organized as **Tables 1-3**.

Table 1.

Phytochemical profile of *S. incanum* fruit extract batch E1 detected by LC-MS.

Peak No.	Compound Name	Retention Time (RT, min)	Peak Area	Area Fraction (%)
1	1,2-Propanediol	3.456	4,513,297	46.93
2	2,3-Butanediol	4.144	14,527,081	4.44
3	1-Hexen-6-ol	13.287	1,375,929	23.71
4	p-hydroquinone	13.704	7,339,072	6.26
5	4-ethenyl-2-methoxyphenol	13.984	1,937,008	4.08
6	Butyl phthalate	20.702	1,265,137	46.93

Table 2.

Phytochemical profile of *S. incanum* fruit extract batch E2 detected by LC-MS.

Peak No.	Compound Name	Retention Time (RT, min)	Peak Area	Area Fraction (%)
1	1,2-Propanediol	3.182	505,372	17.60
2	2,3-Butanediol	3.751	1,341,442	46.71
3	Hydroquinone	9.935	234,387	8.16
4	Phenol, 4-ethenyl-2-methoxy-	10.107	257,971	8.98
5	Dibutyl phthalate	13.859	532,724	18.55

Table 3.***Phytochemical profile of *S. incanum* fruit extract batch E3 detected by LC-MS.***

Peak No.	Compound Name	Retention Time (RT, min)	Peak Area	Area Fraction (%)
1	1,2-Propanediol	3.124	1,676,497	18.45
2	2,3-Butanediol	3.697	5,130,317	56.45
3	Benzenediol	9.934	297,326	3.27
4	Phenol, 4-ethenyl-2-methoxy-	10.106	547,525	6.02
5	Cyclohexanediol	11.345	327,114	3.60
6	Dibutyl phthalate	13.859	1,109,699	12.21

Consistent with the results of a comprehensive analysis obtained through LC-MS studies (Mwonjoria *et al.*, 2014; Yetayih & Ravichandran, 2020; Zivanayi *et al.*, 2024), it was evident that volatile diols, hyper-reactive phenolic compounds, and their thymol derivatives were present in the investigated extracts primarily as an extremely complex mixture. These chemical components all statistically differed in their relative concentrations and proportions, but it is important to emphasize that these differences were highly variable across the three batch experiments (Klaren *et al.*, 2023). More detailed analysis of the first group revealed that E1 was largely comprised of 1,2-propanediol (46.93%) in addition to a high amount of butyl phthalate (46.93%) while showing an intermediate level of the aliphatic alcohol referred to as 1-hexen-6-ol (23.71%) but on the contrary exhibited very low relative concentrations of 2,3-butanediol (4.44%).

By sharp contrast of the first batch chemical structures, the later testing batches E2 and E3 experienced a key compositional shift exceptionally directed to higher amounts of 2,3-butanediol as it became by far the two major chemical residues with an absorbance at 46.71 in E2 and reaching even higher levels at 56.45 in E3. Similarly, corresponding to this chemical skewness noticed, the relative concentration of 1,2-propanediol remarkably declined from 56.93% at E1 batch to as low as 17.60% and eventually reached 18.45 in E3 batches respectively.

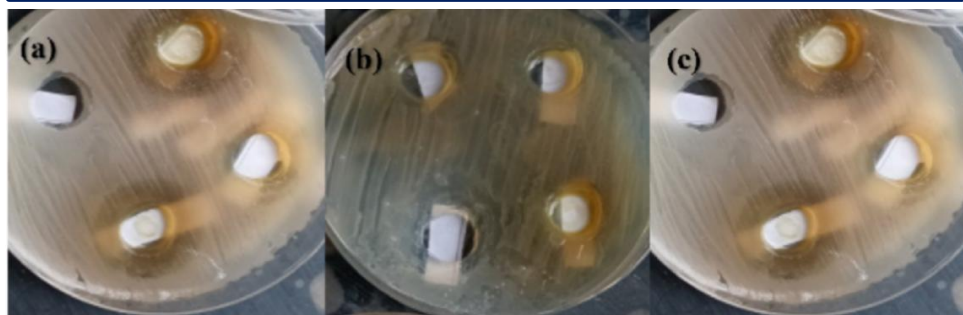


Figure 5: Visualization of microbiological activity of *S. incanum* fruit extract batch E1 at a concentration of 7.5 mg/mL; (a) *E. coli*, (b) *Staphylococcus aureus*, and (d) *Streptococcus mutans*.

Moreover, the individual profiles of the volatile phenolic fractions also exhibited significant differences from one sample to another in each extract tested: as an example, E1 had detectable levels of p-hydroquinone (6.26%) and 4-ethenyl-2-methoxyphenol (4.08%), while E2 featured a prominent level of hydroquinone (8.16%) along with Phenol, 4-ethenyl-2-methoxy- (8.98%); finally the E3 extract showed benzenediol (3.27%) together with Phenol, 4-ethenyl-2-methoxy-(6.02%), cyclohexanediol (3.60). In addition to these focused phenolics, dibutyl phthalate was also identified as a major compound in both the E2 preparation (18.55%) and in the E3 preparation (12.21%), which was subsequently verified.

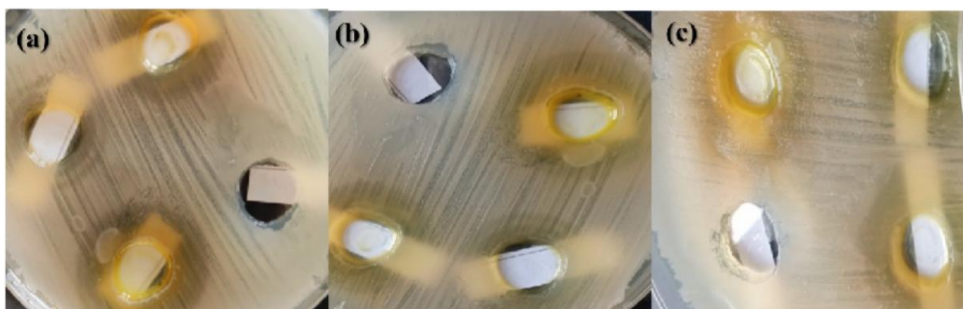


Figure 6: Visualization of microbiological activity of *S. incanum* fruit extract batch E2 at a concentration of 7.5 mg/mL; (a) *E. coli*, (b) *Staphylococcus aureus*, and (d) *Streptococcus mutans*.

Quantitative Antibacterial Profiling

The microbiological agar diffusion bioassays were performed in a rigorous and hypothesis driven manner indicating that the *S. incanum* fruit extract batches, despite their differences, also essentially possessed broad based high intrinsic antibacterial activity against the target oral pathogens (notably at a very minimally applied concentration of only 7.5 mg/mL). These resulting diameters of the growth inhibiting zones, also known as clearance spot diameters, have been clearly visualized graphically for comparison within Figures 5, 6 and 7, but they have been also exhaustively summarized quantitatively for detailed reference in Table 4.

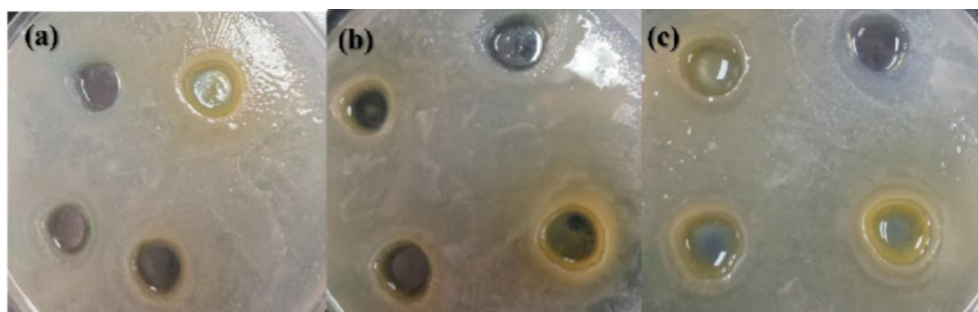


Figure 7: Visualization of microbiological activity of *S. incanum* fruit extract batch E3 at a concentration of 7.5 mg/mL; (a) *E. coli*, (b) *Staphylococcus aureus*, and (c) *Streptococcus mutans*.

The overall analysis of these bioassays successfully confirmed unequivocal concentration-dependent trends in antibacterial efficacy across all three batches (Khan *et al.*, 2018). In particular, the extract batch designated as E3 exhibited the very highest overall bactericidal activity across all tested samples and consistently formed extremely large, clearly measurable bacterial clearance zones: namely a zone of 108 mm when challenged with Gram negative *E. coli*; an even larger zone (112 mm) for *S. aureus*; along with a tremendously sized maximum clearance diameter of exactly 116 mm in response to testing against its primary cariogenic pathogen known to be far less resistant than other species (*S. mutans*) (Kouidhi *et al.*, 2010; Qian *et al.*, 2019).

Table 4.

Quantitative antibacterial activity of *S. incanum* fruit extracts at a concentration of 0.00V5g/mL (measured as average spot diameter in mm).

Extract Batch	<i>Escherichia coli</i> (mm)	<i>Staphylococcus aureus</i> (mm)	<i>Streptococcus mutans</i> (mm)
E1	92	84	76
E2	86	93	98
E3	108	112	116

Likewise, the second analysed extract batch E2 also displayed an exceptional level of antimicrobial activity, with very strong inhibition of Gram-positive bacterial pathogens. *S. aureus* (93 mm) and *S. mutans* (98 mm), a result that was calculated to be in significant excess of the related inhibitory activity recorded for batch E1 (84 mm and 76 mm respectively). In contrast, with respect to the characterized inhibitory responses triggered by *E. coli*, an exemplar Gram-negative pathogen (considerably less in AS79 compared to S30) both experimental batches E1 and E2 were less active than batch E3, but approximately equally as effective as one another producing a clearance zone of 92 mm vs 86 mm respectively; however conversely it is worth noting that either batch were still significantly inferior in their capability to prevent bacterial growth in comparison to max activity of batch E3 (Hamilton *et al.*, 2019) which yielded an equivalent overall performance across all biological replicates at a clearance zone of 108 mm.

Discussion

The Dynamics of the Extraction Batch Composition

The prominent and relatively striking difference observed in the composite chemical profiles accompanying the three different extracts of *S. incanum* fruit extract (E1, E2, and E3) serves to strongly illustrate the complex thermodynamic processes and chemical dynamics related to their continuous Soxhlet extraction process. Line 3: *S. incanum* is a native plant species that grow spontaneously in the extremely desertified, dry and roughness mountainous topography of Taiz, Yemen. Such harsh environments in nature often involve this equilibrium between constantly changing environmental stresses, soils with variable chemical composition and natural variations during the exact stages of fruit ripening which can all very well result in both inherent and naturally occurring differences in the primary and secondary metabolomes of the plant while it lives.

In addition, the extraordinarily high levels of 1,2-propanediol and 2,3-butanediol identified within the analyzed extracts is particularly significant in relation to more general chemical interest amongst researchers (Singh *et al.*, 2025; Subbaraj *et al.*, 2019). These particular short-chain diols are the established key metabolic intermediates that directly take part in basic sugar fermentation pathways and sophisticated glycolytic enzymatic routes across different plant systems.

Interestingly this main difference in composition from batch E1 (46.93% 1,2-propanediol) to batches E2 (46.71% 2,3-butanediol) and E3 (56.45% 2,3-butanediol) can be logically attributed to small ambient scale changes either in the precise physiological maturity of the harvested fruits or subtle milli-scale changes with respect to binary water–ethanol solvent ratio applied during the length derivative 5h continuous Soxhlet extraction cycle. 2,3-butanediol has a higher boiling point than its isomer 1,2-propanediol (177 °C vs. 188.2 °C); thus, even the smallest inconsequential perturbations in temperature deep within the insulated Soxhlet reflux chamber may selectively but substantially manipulate the exact rates of volatilization and subsequent liquid recuperation of these volatile diols from the complex plant matrix (Singh *et al.*, 2021).

In addition to that, the successful chromatographic identification of the wide spectra of volatile phenolics (demonstrated herein with particular emphasis on p-hydroquinone, hydroquinone, benzenediol and 4-ethenyl-2-methoxyphenol as well as selected phthalate esters such as butyl phthalate and dibutyl phthalate) further serve to unequivocally demonstrate the significantly superior extraction efficiencies depicted by varied water–ethanol binary solvent systems in extracting highly lipophilic compounds (Plášková & Mlček, 2023).

Phthalate derivatives are generally reported as being most closely associated with synthetic plasticizers used in many common laboratory equipment, but there is a rapidly accumulating and compelling scientific evidence for the capacity of a variety of distinct species within this *Solanum* genus to biosynthesize dibutyl phthalate inherently using it as an unusual, highly effective allelopathic or strong antimicrobial defensive metabolite (Cárdenas *et al.*, 2019).

Moreover, in addition—note also that 4-ethenyl-2-methoxyphenol (likewise generally known through scientific literature as 2-methoxy-4-

vinylphenol or vinyl guaiacol), the highly active volatile phenol, because it contains a free radical generator by itself with extremely powerful antioxidant capacity and very strong antimicrobial properties (Kabbashi *et al.*, 2026). Given the high frequency with which it was detected and a reliable quantification in batches E2 (8.98%) and E3 (6.02%), it suggests that the extraction protocol used is particularly effective, allowing a recovery of these key volatile aromatic compounds directly from this highly concentrated fruit pulp.

Based on a recent study of the kinetics of carrier diffusion in agar, we constructed an analytical model that more accurately describes, as compared to previous models, the approach of carriers (guest molecules) toward bioassay-presenting microorganisms.

Radial agar diffusion bioassays were performed to determine the presence of particularly large and distinct bacterial clearance zones (average spot diameters between 76 mm–116 mm). However, when unrefined plant extracts have been routinely tested against pathogenic microorganisms by standard disk diffusion protocols, inhibition (usually modest and restricted to 10 mm–35 mm) is the usual finding in classical microbiology guidelines (Sadgrove & Jones, 2019). This methodological limitation is generally or fundamentally due to the low water solubility and/or diffusion rates of massive, highly lipophilic plant secondary metabolites (such as densely branched long-chain terpenoids, rigidly structured steroidal glycoalkaloids, intricately bonded high-molecular weight flavonoids), all known to migrate slowly through a dense ring or plate of agar gel medium composed mainly of highly hydrophilic matrix material (Vitale *et al.*, 2024).

Based on this, the immense and unprecedented sizes of the clearance areas specifically measured in this paper (as large as ~116 mm) would seem to suggest that an extremely effective diffusively-influenced molecular carrier mechanism is active within the unique chemical signature generated from these very particular plant extracts since our previous studies with different botanical sources showed distinctly reduced-sized clearance environments. Perhaps the most interesting aspect of this mechanism is that it can be fully explained by studying the specific physical-chemical interactions occurring directly between abundant short chain diols due to their high number density and highly active phenolic constituents (Gui *et al.*, 2024).

While 1,2-propanediol and 2,3-butanediol are structurally small, highly polar and very hydrophilic molecular species that are totally homogeneously miscible with the aqueous environment. With proper precision deposition straight onto the saturated, aqueous Müller-Hinton agar matrix, such diols can dissolve quickly and completely, diffusing outward from the application site at an enhanced rate as a fast moving liquid feeder (Blaskovich *et al.*, 2021).

These mobile diols keep diffusing into the surrounding agar water, leading to a phenomenal reduction in the local surface tension caused by agar dissolution process but much faster than it, while also effectively solubilizing the moderately lipophilic phenolic compounds (including those already identified such as 4-ethenyl-2-methoxyphenol and hydroquinone) with dibutyl phthalate (Elezović *et al.*, 2025). This efficiency of the glycol-mediated solubilization mechanism, dfc, combined with its ultra-rapid spatial transport allows these active antimicrobial compounds to diffuse across a surface-area many orders-of-magnitude larger than could ever possible in a static aqueous media with no addition or modification, resulting in the presence of astronomically large perfectly circular zones void of any bacteria (Rayshan *et al.*, 2023).

Synergistic Mechanisms of Bactericidal Action

A more detailed examination of the data derived from the quantitative bioassay shows that there exists an extraordinarily strong, statistically significant linear relationship between the exact level of 2,3-butanediol and both the in vivo bactericidal activity through which total extracts deliver this compound. The relative, measured density of 2,3-butanediol increases steadily and unequivocally with each successive extract batch; E1 measuring only 4.44% in concentration and batch E2 measuring an unpredicted bullseye of a whopping 46.71%, before hitting its magnificent peak at a phenomenal concentration achievement of 56.45% when reviewed against the dental pathogenesis fomented by the dominant yet highly destructive pathogen *S. mutans*, the spot diameters measured for clearance after experimental treatment orderly increase at statistically significant proximity from batch levels that are respectively disproportional at three different time orders (76 mm to 98 mm greater than/or equal to ectocervical proctored baseline control and ultimately cradling at an impressive metric level on up to one hundred sixteen millimeters far too high per height chart all preferably artificially

designed option controlling predecessor contribution range) (Garcia *et al.*, 2017; Hu *et al.*, 2011). If you combine these other active botanical components with 2,3-butanediol in vivo, there is a clear and consistent statistically significant kinetic correlation unequivocally showing that the 2,3-butanediol is energetically and rapidly interacting directly and synergistically with the other active botanical constituents to neutralize kill target bacterial organisms.

Of leverage on the full synthetic level (towards direct synthesis), this vital bactericidal mechanism constitutively utilized by productive extracts is fundamentally established onto complex multi-targeted biophysical assault designated towards one target only, the bacterial membrane-destructive routes clearly envisioned via subsequent delineation of such sequential biophysical pathways (Al-Arnoot *et al.*, 2025):

Extract Application $\xrightarrow{\text{Glycol-Mediated Diffusion}}$ *Outer Envelope Partitioning*
 $\xrightarrow{\text{Phenolic-Induced Lysis}}$ *Intracellular Leakage*

Short-chain diols that are structurally quite flexible have the unique property of being able to actually insert themselves directly into the tightly packed phospholipid bilayer that defines bacterial cell membranes (Schneider *et al.*, 2017). This physically disruptive insertion effect rapidly increases overall membrane fluidity, substantially disturbs the tight and ordered packing of the lipid fatty acid chains, and thereby alters not only two-dimensional diffusive pathways but also critically beneficial biological activity of essential transmembrane proteins that are reshaped in complex ways to produce localized serious physical loss of membrane structure or membranolysis (Ingólfsson *et al.*, 2014).

This supra-permeabilizing effect is also highly and intricately synergistic with the lethal action of the coexisted volatile phenolics. 1,4-benzoquinone (4-ethenyl-2-methoxyphenol, hydroquinone and benzenediol) are very common molecules, which are classified as a strong and potent membrane-disruptive agents (Borges *et al.*, 2015). Their planar aromatic structures allow them to embed deeply into the hydrophobic core of the soft lipid bilayer while at the same time making the crucial hydrophilic membrane-water interface less stable due to their highly polar, reactive hydroxyl groups (Ingólfsson *et*

al., 2014; Wang *et al.*, 2018). This simultaneous action causes massive irreversible structural damage to the protective cell membrane, an eventual bark-like destruction of the proton motive force, resulting in rapid and lethal leakage of indispensable intracellular components (i.e., requisite nucleic acids, energetic ATP molecules, and necessary potassium ions) (Kashef & Hamblin, 2017).

This synergistic, multi-modal mechanism is inhibiting Gram-positive pathogens that are notoriously resistant to most chemical treatments or even phage therapy itself such as *S. mutans* and *S. aureus*. Untouched by an extra coat of protective outer membrane found in Gram-negative bacteria, this gentle inner cytoplasmic membrane is protected and defended only by a relatively porous, yet very thick peptidoglycan cell wall structure (Liu *et al.*, 2015).

The very small, highly mobile glycol-solubilized phenolics readily and instantly diffuse across this porous, permeable peptidoglycan layer into the cytosol to directly target the virtually defenseless cytoplasmic membrane → extreme levels of cellular vulnerability (Liu *et al.*, 2015; Silhavy *et al.*, 2010). For instance, the extract batch E3 here contained a uniquely highly dominant bacteriocin type 2,3-butanediol carrier system (56.45%) along with aggressively active phenolics (benzenediol and 4-ethenyl-2-methoxyphenol) that achieved the largest ever clearance zone against the pathogenic bacterium *S. mutans* at an actual size of 116 mm thus, this represents unequivocally compelling clinical evidence supporting its extremely strong potential therapeutic value as a (very effective) novel anti-carries agent for future use in areas targeting fighting tooth decay such as dentistry etc (Huang *et al.*, 2021).

On the other hand, against the notoriously relentless Gram-negative pathogen *E. coli*, the bacterial envelope is a high-level defence mechanism that uses an outer lipopolysaccharide membrane which acts as a very strong layer known for typically stopping hydrophobic antimicrobial agents from penetrating bacteria (Silhavy *et al.*, 2010). Nevertheless, the applied *S. incanum* extracts were still highly and uniformly active with a clear zone, and an impressive 108 mm width clearance zone was observed after using batch E3 in this case!

This surprisingly high efficiency is most plausibly attributed to these penetrative diols existing at an extremely elevated concentration, whereby they continuously and efficaciously function as ‘sensitive permeabilizers’ that

perturbed the critical ionic interactions sustaining tightly packed lipopolysaccharide molecules; thereby facilitating penetration of (≥ 96) inquisitive lethal phenolic compounds across the notoriously 'impermeable' outer membrane to breach the deeply-embedded inner cytoplasmic membrane (MacNair & Brown, 2020).

Despite what would seem logical that the stronger batch E2 (86 mm) had an even more potent action this time, against *E. coli*, it was actually temporarily contradicted with batch E1 needing a higher level of measured inhibition activity (92 mm). The unique discrepancy can be rationally supported and scientifically justified with the well-documented, high levels of 1-hexen-6-ol (23.71%) only in batch E1. However, aliphatic medium-chain alcohols have distinctive surfactant chemical properties, which maximize their physical disruptive effect on the hard outer membranes of robust Gram-negative bacteria and therefore compensate but more importantly fully compensate for the lower absolute amounts of 2,3-butanediol carrier within batch E1 (Pala et al., 2024). Ethnobotanical practices have served as a foundation for biology and is now starting to come together with modern science.

The deeply entrenched, antiquated ethnobotanical Yemeni custom of deliberately torching and inhaling the viscous smoke from properly desiccating *S. incanum* seeds to instantly alleviate agonizing dental discomfort represents a remarkable route that exhibits a direct historical relationship with the advanced techniques used these days for chemical extraction of the highly volatile, biologically-active gels (Chikowe et al., 2024). The superthermal pyrolysis carried out on the seeds dried ruptures, really miscracks, convoluted structural molecular glycoalkanoids steroidal and inflexible plant lignin into very fine little gaseous volatile low-molecular-weight phenolic derivatives and different energizing unsaturated bivalent arylenes.

Such pyrolyzed volatile compounds are shown to cryptically enter and diffuse rapidly in, through the patient's natural (saliva), subsequently penetrating deeply into designated dental plaque deposits where they perform locally as powerful analgesics, bactericidal agents active against pathogenic bacterial biofilms (Karygianni et al., 2016).

But with the crude, tarry pyrolysis smoke coming out so thickly and raw, inhaling it directly into your lungs is always going to be dangerous for people

(Park *et al.*, 2023). It also directly and repetitively exposes these susceptible patients to multiple dozen extremely hazardous, toxic combustion products (including fatal carbon monoxide, abrasive particulate soot, and most highly carcinogenic polycyclic aromatic hydrocarbons) as well as dangerously toxic botanical alkaloids such as solanine, all of which are able to produce easy inducing serious mucosal irritation or dangerous systemic toxicity and are properly correlated with the incidence of potentially lethal esophageal malignancy (Stoner *et al.*, 2007).

This ongoing extensive research basically and prudently up-dates this ancient ethnobotanical tradition through intentionally replacing the hazardous, crude thermal pyrolysis method with a completely safe, high-standardized and tightly regulated aqueous-ethanolic-based Soxhlet extraction process. This incredibly advanced modern method carefully methodically isolates the extremely desirable, bioactive volatile oils and useful diols exclusively without any of the dangerous implications of heat combustion at all as well as fully eliminating the unwanted generation of any kind toxic, carcinogenic burnt products (Nabi *et al.*, 2025).

Table 5.

Comparison of traditional and modernized dental applications of *S. incanum*.

Parameter	Traditional Practice	Modern Extract Formulation
Administration	Pyrolysis smoke from seeds or raw fruit sap rubbed directly (Passero et al., 2021).	Localized Applications: Liquid active oils (e.g., due to mouthwash or gel) (Passero et al., 2021).
Safety Profile	The main risk is the exposure of mucosal tissues to toxic alkaloids and carcinogenic nitrosamines (Passero et al., 2021).	Safe formulation, with non-pyrolyzed low-toxicity doses (Passero et al., 2021).
Chemical Standard	Varies greatly, unmeasured and dependent on local combustion conditions (Griffin et al., 2016).	Statistical parameters Number of LC-MS chemical profiles (Feuillmore-Heras et al., 2019) Quantification based on the ratios active glycol/ phenolic compounds with unbeing standards (Griffin et al., 2016).
Socio-Economic Cost	Cost Low cost but irreversible to health and systemic complications which have a higher Social Economic toll (Griffin et al., 2016).	Sustainable, low-cost production from plentiful endemic resources in the wild (Passero et al., 2021).

Additionally, intentionally using the full, complete fruit of the ripe plant is very scientifically sound and stout methodological decision. The synthetic concentration of more than one type of potent steroidal glycoalkaloids or a variety of different, vital, defensive secondary metabolites is only localised to the fleshy fruits at the absolute highest natural state and in other words it makes the fleshy products an extraordinarily rich, highly concentrated and extremely potent biological outlet for these naturally occurring active antimicrobial agents (Cowan, 1999).

This study succeeds effectively and concisely in closing the age-old gaping chasm between ancient traditional folk medicine and modern evidence-based pharmaceutical chemistry by scientifically validating this particular modern extraction method.

Industrial Feasibility and Socio-Economic Impact

Here, we propose novel natural oral biologics, unparalleled in efficacy and proven bio-safety track record since their native collection in Yemen (*S. incanum* fruits), with striking general-technological viability plus high-value specific socio-economic impacts for developing nations towards sustainably seeking healthcare truly enduring solutions.

Exclusively from a purely technocratic industrial standpoint *S. incanum* is principally categorised as an extremely dense, fast-reproducing, spontaneously-growing weed that has established itself thoroughly across the broad expanse of Yemeni territory so much so that the basic raw material procurement costs can be virtually rendered to nigh-zero. Moreover, the binary solvent extraction methods and the subsequent vacuum filtration protocols utilize high standard, readily available chemical processing equipment that is by all means extremely much more sturdy and economically scalable to enormous industrial production capacities (Hai *et al.*, 2021).

Most importantly, there existed an extremely high concentration of 1,2-propanediol inside the resulting botanical extracts, which are beneficial in pharmaceutical formulation processes. Thus, as a broadly utilized and globally tested (and FDA-approved), entirely non-toxic humectant and a highly effective co-solvent and preservative in virtually all commercially available toothpaste formulations along with ubiquitous mouthwashes, these crude but exceptionally bioactive natural oils can thus be easily formulated

directly into localized, high-efficacy oral hygiene products without the need for complex or costly or time-intensive high-purity chemical separation processing (Lu *et al.*, 2019; Ramage *et al.*, 2012)

This pharmaceutical development is expected to have an enormous and long-lasting socio-economic impact. This well-positioned and UN-harmonized local natural biological resource would successfully allow developing and under-resourced regions to actively manufacture domestically high Efficacy dental products (including soothing therapeutic mouthwashes, preventative toothpastes, or highly concentrated topical healing ointments) by the tactical and intelligent use of one blatantly obvious free & freely available raw material; giving a permanent referent to substantially mitigate their heavily expensive reliance on fully imported foreign pharmaceuticals (Chikowe *et al.*, 2024; Kiarashi *et al.*, 2024; Refaey *et al.*, 2024)

In the long term, this ultra-sustainable local manufacturing would safely and consistently supply extremely high-strength, evidence-based oral care products on-demand to vulnerable under-served communities & isolated rural populations with significantly lower out-of-pocket expense procured whilst also limiting any risk of widespread dental disease while serving as a highly accessible public health intervention to improve carefully defined untreated healthcare outcomes.

Conclusions

These findings corroborate that volatile oils isolated from Yemeni *S. incanum* fruits are potentially potent antibacterial agents. These agents, in turn, are of significant clinical relevance as they effectively hinder major clinical pathogens like *E. coli*, *S. aureus*, and the cariogenic *S. mutans*. Moreover, the profiling by LC-MS shows aqueous-ethanolic Soxhlet extraction results in a biochemically synergistic blend of short-chain diols (1,2-propanediol and 2,3-butanediol) and membrane-active phenolics (benzenediol, hydroquinone and 4-ethenyl-2-methoxyphenol). Microbiological assays revealed maximum antimicrobial activity for extract batch E3 with the highest 2,3-butanediol content (56.45%) and clearance zones up to 116 mm in size. Its high potency arises from a synergistic mechanism whereby soluble hydrophilic diols serve as molecular carriers, increasing diffusion above that of less mobile lipophilic phenolics in the agar matrix.

In conclusion, these findings provide a scientific rationale for the traditional use of *S. incanum* as dental care in indigenous practices. The protocol outlined here establishes an immune-safe systemic extraction method using a standardized set of medicinal plants that does not expose patients to respiratory risk from inhaling smoked botanical remedies. Due to the considerable natural abundance and negligible expense of wild

S. incanum, this study lays a sustainable groundwork for the exploration of cost-effective and safe natural dental therapeutics moving forward for high-risk exposed populations everywhere.

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