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Original Article

Enhancing Therapeutic Potential: Investigating Traditional Detoxification Methods and Assessing their Influence on Anti-Microbial Efficacy, Phytochemical Composition, Heavy Metal Content and Anti-inflammatory Properties in *Trachyspermum ammi*

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ABSTRACT

Background: *Trachyspermum ammi* (*T. ammi*) holds a longstanding position in traditional medicinal practices, renowned for its diverse medicinal and pharmacological attributes. Medicinal plants not only offer significant therapeutic benefits but also hold economic importance. A noteworthy trait of phytomedicine lies in its low toxicity, which positively impacts the pharmaceutical market. Hence, traditional practices often incorporate detoxification/purification methods to mitigate the toxicity of herbs. **Objectives:** Aimed at scientifically validating these practices, our study explores the traditional detoxification method known as Sodhana and assessing its Influence on *T. ammi*. **Materials and Methods:** We evaluate the antimicrobial, phytochemical, heavy metal content and anti-inflammatory efficacy of detoxified *T. ammi* seeds. **Results:** The seeds undergo lime treatment, followed by grinding into powder and extraction with 90% ethanol. Our antimicrobial study reveals that lime-treated ethanol extract exhibits robust inhibitory activity against various microbial strains, surpassing the unprocessed extract in most cases. Notably, significant reductions in heavy metal content are observed post-lime treatment, particularly in titanium, indium, bismuth, strontium, lead, aluminum, boron, mercury, and cadmium. Phytochemical analyses via ICP/OES, GC-MS and LC-MS demonstrate alterations in compound compositions between unprocessed and lime-treated extracts, with the latter exhibiting elevated levels of thymol and fatty acids. Furthermore, our investigation highlights the considerable anti-inflammatory potential of lime-treated *T. ammi* seed extracts. **Conclusion:** In conclusion, our findings emphasize the efficacy of lime treatment in reducing toxic elements while enhancing antimicrobial and anti-inflammatory properties, thus advocating for its utilization in traditional herbal practices.

Keywords: Detoxification, Phytochemical Composition, Anti-inflammatory, *Trachyspermum ammi*.

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INTRODUCTION

Trachyspermum ammi has been extensively utilized by medieval practitioners and has demonstrated various pharmacological effects attributed to its diverse chemical composition.[1] *T. ammi*, a grayish-brown seed belonging to the *Apiaceae* family, serves as a popular aromatic herb and spice, abundantly found in the northern regions of India.[2,3] *T. ammi* is also known as ajwain or carom seeds. Ayurveda boasts a well-classified material medical primarily comprising plant-derived drugs, a practice deemed exceptionally safe due to minimal or no side effects. The utilization of herbs as complementary and alternative medicine has witnessed a remarkable surge in the past two to three decades.[4] *T. ammi* is notably rich in vitamins and minerals such as niacin, thiamine, sodium, phosphorus, potassium,

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calcium, alongside carbohydrates, fatty acids, fibers, proteins and antioxidants. Its seeds harbor an essential oil known as thymol, which imparts aromatic fragrances. In Ayurveda, *T. ammi* water finds commendable recommendation for weight management, aiding in digestive system cleansing and metabolism promotion, consequently facilitating weight loss. Moreover, it is believed to facilitate uterine and stomach cleansing for regulating menstrual cycles in females.[5]

The remarkable cholesterol-modulating properties of *T. ammi* encompass reduction of LDL (bad) cholesterol levels and regulation of HDL (good) cholesterol levels. The seeds, abundant in dietary fiber and fatty acids, contribute to maintaining healthy cholesterol levels. Thymol within the seeds assists in blood pressure management, with animal studies suggesting its calcium channel-blocking effect, effectively lowering blood pressure.[6] *T. ammi* finds widespread use in traditional medicine, particularly in rural areas, owing to its affordability and accessibility compared to modern medicine. *T. ammi* seeds have long been employed as a remedy for various ailments such as indigestion, colic, asthma, and arthritis, and are often used in poultices to relieve these conditions.[5] Additionally, when steeped in liquid form, the seeds are administered to combat diarrhea and flatulence. Beyond these traditional uses, the therapeutic spectrum of *T. ammi* extends to exhibiting antimicrobial, hypolipidemic, antispasmodic, bronchodilating, hepatoprotective, diuretic, anti-filarial, aflatoxin-detoxifying, and anti-inflammatory properties.[7]

The ancient practice of detoxification, termed Shodhana, not only purifies herbal drugs but also enhances their potency and therapeutic efficacy.[8] Various detoxification techniques, including washing, crushing, boiling, frying, heating, or immersion in specified liquids, mitigate heavy metal toxicity and deleterious compounds, thereby augmenting the suitability of herbal drugs for intended actions.[9,10] In the context of a burgeoning rate of infections, antibiotic resistance, and synthetic antibiotic side effects, herbs are emerging as preferred alternatives. *T. ammi's* antibacterial activities have been substantiated by researchers, owing to secondary metabolites such as alkaloids, flavonoids, tannins, and terpenoids.[11] The active components of *T. ammi*, predominantly thymol and carvacrol, exhibit local anesthetic, antibacterial, antifungal, antiviral, antihypertensive, antispasmodic, bronchodilator, and hepatoprotective properties.[12,13] Additionally, *T. ammi* contains trace amounts of phytochemicals such as pinene, cymene, limonene, and terpinene, alongside terpenes, glycosides, and sterols, bestowing anti-inflammatory and asthma-relieving properties.[14,15] The primary objectives of this study are to assess the detoxification method, bioassay, phytochemical composition, metal content, and anti-inflammatory properties of *T. ammi*. The findings from this research will serve to establish optimal procedures for managing the detoxification process, ensuring both the safety and efficacy of the seeds. Additionally, detailed investigations into the pharmacological potential and phytochemical diversity of detoxified *T. ammi* seeds could not only deepen our understanding but also enrich the traditional knowledge surrounding the Sodhana process.

MATERIALS AND METHODS

Processing of raw *T. ammi* seed

Seeds of *T. ammi* were obtained from the Tamil Nadu Agricultural University, Coimbatore, India and authenticated by Post Graduate and Research Department of Botany, Kalaignar Karunanidhi Government Arts College, Tiruvannamalai, Tamil Nadu, India. The obtained seeds were cleaned in distilled water to remove any dust and then dried in the shade then grinded to fine powder with homogenizer.

Detoxification Process of *T. ammi* seeds

Lime treatment

150 mL of distilled water was used to dissolve 30 g of the calcium carbonate (limestone). 25 g of *T. ammi* seeds were steeped for 3.5 days in 100 ml of Lime supernatant solution in a separate beaker. The seed is separated from the solution using Whatman filter paper rinsed with distilled water and then allowed to dry at room temperature.

Extraction method

Unprocessed and processed (lime treated) *T. ammi* (25 g of pulverized powder) for extraction with 150 mL of solvent took place over the course of 7 hr. We use water, hexane and 90% ethanol as solvent for extraction process. However, 90% ethanol extract is effective in inhibiting the growth of bacteria while aqueous and hexane extracts were found to be less effective as antimicrobial assay. The solvent was vaporized and the extract was concentrated in a rotary evaporator (40°C, 110 RPM). Finally, the extract (about 12.5 g) was collected. The yield of both the lime-treated and the unprocessed*T. ammi* ethanol extract is very similar i.e., from 2 g of seeds, 1 g extract is obtained.

Microbial Cultures

All the microbial cultures used in our study were purchased from the Microbial Type Culture Collection (MTCC), Chandigarh, INDIA. The purchased bacterial strains include

Klebsiella pneumoniae (MTCC618), *Proteus vulgaris* (MTCC426) and *Streptococcus pyogenes* (MTCC442). The fungal cultures purchased include *Candida albicans* (MTCC183), *Malassezia furfur* (MTCC1765) and *Trichophyton mentagrophytes* (MTCC7687).

Anti-Microbial Efficacy

By using the well diffusion technique, the antimicrobial activity of *T. ammi* powder and the obtained unprocessed *T. ammi*

powder extracted with 90% ethanol, n-hexane, hydro extract.[16-19] Based on the results, 90% ethanol is selected for extraction of *T. ammi* seeds processed with plain (unprocessed) and lime treated were analyzed for their antimicrobial efficacy by well diffusion method.[7]

Inoculum preparation

Bacterial colonies were inoculated in Mueller-Hinton broth and incubated for 24 hr at 37°C to prepare the inoculums for antibacterial susceptibility testing. Five or six loops full of fungal spores transferred to sterile Mueller-Hinton broth and incubate at 25°C for 24 hr.[20]

Disc Used for Agar Diffusion

HIMEDIA Antibacterial antibiotic disc (narrow-spectrum) Clindamycin (CD) 10 mcg, Erythromycin (E) 10 mcg and broad-spectrum antibiotics Tetracycline (TE) 10 mcg, Chloramphenicol (C) 10 mcg were used for the study. We used diverse Antifungal compounds belongs to the category of cutaneous antifungal drugs Nystatin (NS) 50 mcg, and Ketoconazole (KT) 10 mcg, and Subcutaneous antifungal drugs Fluconazole (FLC) 10 mcg, and Amphotericin-B (AP) 100 mcg were used.[21]

Agar Diffusion Method

The well diffusion and disc diffusion methods are the most well-known and fundamental techniques for antimicrobial study. It is common practice to utilize the Agar well diffusion technique to assess the antimicrobial property of herbal extracts.[22-24] The agar plate surface is inoculated using the disk-diffusion protocol, which involves distributing a volume of the microbial inoculum over the entire agar surface. After that, the well has been aseptically punched with a diameter of 6 mm, then a volume 20-100 µL of the unprocessed and processed *T. ammi* extracts is added to the well.[25] Then, depending on the test microorganism, agar plates are incubated under the proper conditions. The diameters of the inhibitory growth zones are measured. The inhibition zone of bacterial cultures was compared with narrow and broad spectrum antibiotic readymade discs and fungal cultures compared with cutaneous and subcutaneous readymade antibiotic discs.[26]

ICP/OES-Analytical Procedure

0.5 g of dried, unprocessed and processed *T. ammi* samples was digested with HNO_3 acid in a confined atmosphere. The volume was filled by adding the filtered digested samples, 100 mL of deionized water, and 5% HNO_3. We used Perkin-Elmer 3100 Inductively Coupled Plasma-Optical Emission Spectrometer (ICP/OES) (Norwalk, USA) equipped with an auto-sampler AS 91 and a Gem Cone-nebulizer on a cyclonic spray chamber. The digested samples, blanks, and reference materials were examined using ICP-OES.[27]

The measurements of element concentrations were confirmed using the certified values of the pertinent minerals in the reference sample. Analysis of Certified Reference Material (CRM) of ICP multi-element standard solution (10 to 50 mg/L Merck) containing the elements such as (As, Hg, Se, Zn, P, Pb, Cd, Fe, Mn, Cr, Mg, Cu, Ca, Na and K) and Multi-standard IV - standard solution (Merck), which contained Ag, Al, B, Ba, Bi, Ca, Cd, Co, Cr, Cu, Fe, In, K, Li, Mg, Mn, Na, Ni, Pb, Sr, Tl and Zn at the concentration of 1000 mg/kg was used for the preparation of calibration solutions. The preparation of standard solutions was performed by diluting the standard. So, the concentrations of standards for the calibration curves (5 points) were selected to match the expected concentrations for all the elements of the sample studied. The correlation coefficient r^2 obtained for all cases was 0.9999.

Gas Chromatography Coupled to Mass Spectrometry (GC-MS) Compound Analysis

GC-MS analysis involved the examination of detoxification processed and unprocessed *T. ammi* seeds ethanol extracts. GC–MS in combination with derivatisation prefers small polar metabolites covering primary metabolism. The analysis was performed utilizing an Agilent 7890-An instrument under specified conditions, and compound identification was achieved by comparing spectra to NIST and EPA/NIH mass spectral libraries.

Liquid Chromatography Coupled to Mass Spectrometry (LC-MS) Analysis

LC–MS/MS analysis of detoxification processed and unprocessed *T. ammi* seeds ethanol extracts. LC-MS covers large hydrophobic metabolites predominant in secondary metabolism. The analysis was carried out using an Agilent 1100 LC system consisting of degasser, binary pump, auto sampler, and column heater. The column outlet was coupled to an Agilent MSD Ion Trap XCT mass spectrometer equipped with an ESI ion source. Data acquisition and mass spectrometric evaluation were carried out on a personal computer with Data Analysis software (Chemstations). For the chromatographic separation, a Zorbax 300 Å Extend-C-18 Column (2.1 × 150 mm) was used. The column was held at 95% solvent A (0.1% formic acid in water) and 5% solvent B (0.1% formic acid in acetonitrile) for 1 min, followed by an 11 min step gradient from 5% B to 100% B, then it was kept for 4 min with 100% B, finally, the elution was achieved with a linear gradient from 100% B to 5% B for 2 min. The flow rate was 200 l/min and the injection volume was 5 l. The following parameters were used throughout all MS experiments: for electrospray ionisation with positive ion polarity the capillary voltage was set to 3.5 kV, the drying temperature to 350°C, the nebulizer pressure to 40 psi, and the drying gas flow to 10 l/min. The maximum accumulation time was 50 ms, the scan speed was 26,000 m/z/s (ultra scan mode) and the fragmentation time was 30 ms.

In vitro Anti-inflammatory Assay

Evaluation of anti-inflammatory properties involved the preparation of a reaction mixture comprising egg albumin, phosphate buffered saline, and both processed and unprocessed *T. ammi* seeds ethanol extracts. Turbidity measurements is 660 nm at specific intervals enabled the determination of the percentage inhibition of protein denaturation.

The percentage inhibition of protein denaturation was calculated through the formula:

$$\% \text{ inhibition of denaturation} = 100 \times (A1 - A2/A1)$$

where A1 represents the absorption of the control sample and A2 signifies the absorption of the test sample.

RESULTS

Our results reveal that lime-treated ethanol extract significantly enhances antimicrobial efficacy against bacterial and fungal cultures, except for *M. furfur* and *T. mentagrophytes*. The lime-treated extract exhibits exceptional antibacterial activity, surpassing the unprocessed *T. ammi* ethanol extract (Figure 1, Table 1). The maximum antibacterial effect was observed in lime-treated extract which showed the inhibition zone recorded as 20≥17≥19 mm against *Klebsiella pneumonia, Proteus vulgaris* and *Streptococcus pyogenes* followed by unprocessed *T. ammi* ethanol extract. A significant antifungal effect was identified on lime-treated extract which showed the inhibition zone was recorded as 20≥20≥20 mm against *Candida albicans, Malassezia furfur, Trichophyton mentagrophytes* followed by unprocessed *T. ammi* ethanol extract (Figure 2, Table 2).

The analysis of 26 elements by ICP-OES reveals that comparison between unprocessed and detoxification processed *T. ammi* seeds ethanol extracts show considerable changes in concentration of major elements between the treatments (Figure 3, Table 3). Trace minerals such as Aluminum, Strontium, Barium, Lead, Thallium, Indium, Bismuth, Boron, Cadmium, Mercury, and Lithium exhibited substantial decreases following lime treatment. Conversely, Zinc, Nickel, Cobalt, and Silver showed no decline due to their extremely low concentrations. Non-essential heavy metals, including Barium, Strontium, Aluminum, Lead, Cadmium, Silver, Arsenic, Thallium, Mercury, and Lithium, were significantly reduced in samples treated with lime. This reduction highlights the effectiveness of lime treatment in minimizing heavy metal content in *T. ammi* seeds.

GC-MS Exploration of *T. ammi* Seed Extracts: Results unveiled sixteen secondary metabolites in the extracts. Specifically,

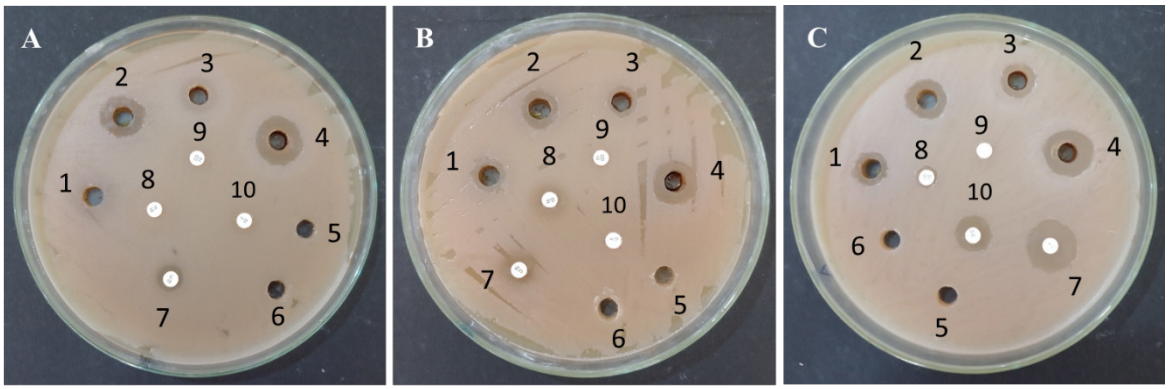

Figure 1: Antimicrobial efficacy of Unprocessed and Detoxification processed *T. ammi* seeds ethanol extracts against bacterial Pathogens. A-*Klebsiella pneumoniae,* B-*Proteus vulgaris* and C-*Streptococcus pyogenes.*

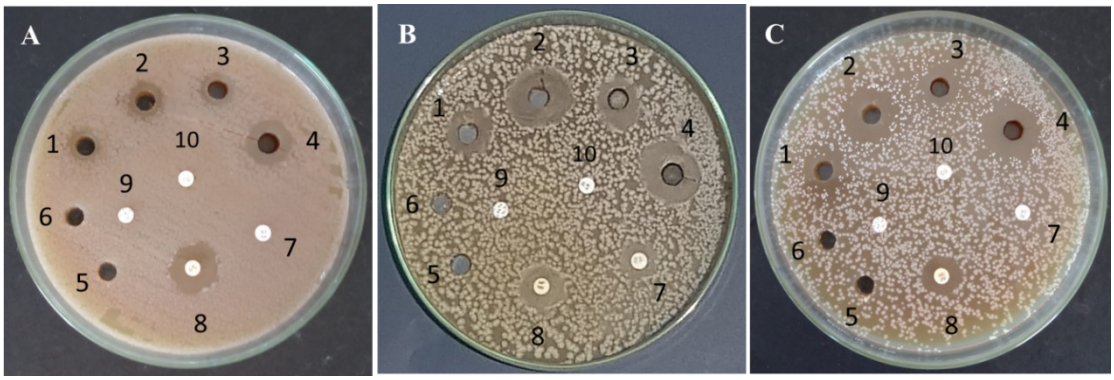

Figure 2: Antimicrobial efficacy of Unprocessed and Detoxification processed *T. ammi* seeds ethanol extracts against fungal Pathogens. A-*Candida albicans*, B-*Malassezia furfur,* C-*Trichophyton mentagrophytes.*

Table 1: Antimicrobial Efficacy of Unprocessed and Detoxified *T. ammi* Seed Ethanol Extract Against Bacterial Pathogens

Sl. No.		Product Names	*Klebsiella pneumoniae*	*Proteus vulgaris*	*Streptococcus pyogenes*
1.	*T. ammi* ethanol extract	Unprocessed *T. ammi* ethanol extract 100 µL	11±1.5	12±1	14±1
2.		Unprocessed *T. ammi* ethanol extract 200 µL	18±1.5	16±1	18±1.5
3.		Lime treated 100 µL	13±1	11±1.5	14±1
4.		Lime treated 200 µL	20±1	17±1	19±1
5.	Negative Control	Ethanol	0	0	0
6.		Lime supernatant	0	0	0
7.	Antibiotics	Chloramphenicol 10 µg (Positive control)	12±1.5	12±1	20±1
8.		Tetracycline 10 µg (Positive control)	0	16±1	11±1.5
9.		Clindamycin 10 µg (Positive control)	0	0	0
10.		Erythromycin 10 µg (Positive control)	0	0	14±1

Measured by Inhibition zone in mm, each zone value is the mean of three observations.

Table 2: Antimicrobial Efficacy of Unprocessed and Detoxified *T. ammi* Seed Ethanol Extract Against Fungal Pathogens.

Sl. No.		Product Names	*Candida albicans*	*Malassezia furfur*	*Trichophyton mentagrophytes*
1.	*T. ammi* ethanol extract	Unprocessed *T. ammi* ethanol extract 100 µL	14±1.0	13±1.5	14±1.0
2.		Unprocessed *T. ammi* ethanol extract 200 µL	16±1.5	20±1.0	20±1.5
3.		Lime treated 100 µL	14±1.5	13±1.5	14±1
4.		Lime treated 200 µL	20±1.5	20±1	20±1.5
5.	Negative control	Ethanol	0	0	0
6.		Lime supernatant	0	0	0
7.	Antibiotics 1	Ketoconazole 10 mcg (Positive control)	0	14±1.0	0
8.		Ampotericine-B (Positive control)	20±1.5	16±1.5	16±1.5
9.		Fluconazole 10 mcg (Positive control)	0	0	0
10.		Nystatin 50 mcg (Positive control)	0	0	0

Measured by Inhibition zone in mm, each zone value is the mean of three observations.

the unprocessed extract contained 11 compounds, including Thymol, 9,12-octadecadienoic acid, 3-methoxy methoxy butyric acid, Thiamine, and Oleic Acid (Figure 4). On the other hand, lime-treated ethanol extract revealed 12 compounds, with 9,12-octadecadienoic acid, Thymol, Oleic Acid, and 3-methoxy methoxy butyric acid as primary constituents (Figure 5). Notably, four major compounds -9,12-Octadecadienoic acid, Thymol, 3-Methoxy-methoxybutyric acid and Oleic acid-were recurrently present in both extracts (Tables 4 and 5).

Our LC-MS analysis of the *T. ammi* seed extracts revealed seven compounds in unprocessed *T. ammi* seed ethanol extract, with Thymol, 8,11-octadecadienoic acid, Palmitic acid, and Linoleic acid as abundant constituents (Figure 6). In lime-treated ethanol extract, seven compounds were identified, with Thymol, Palmitic acid, and Linoleic acid as major components (Figure 7). Notably, both samples contained Thymol, Oleic acid, 8,11-octadecadienoic acid, Thiamine, Palmitic acid, Linoleic acid, and Stigmasterol (Tables 6 and 7).

In our study, anti-inflammatory properties of both detoxified and non-detoxified *T. ammi* revealed that lime-treated extracts exhibited the highest inhibition of protein denaturation at a concentration of 1000 µg/mL compared to the unprocessed extract (Table 8).

LC-MS Result of Lime Treated *T. ammi seeds* ethanol extract.

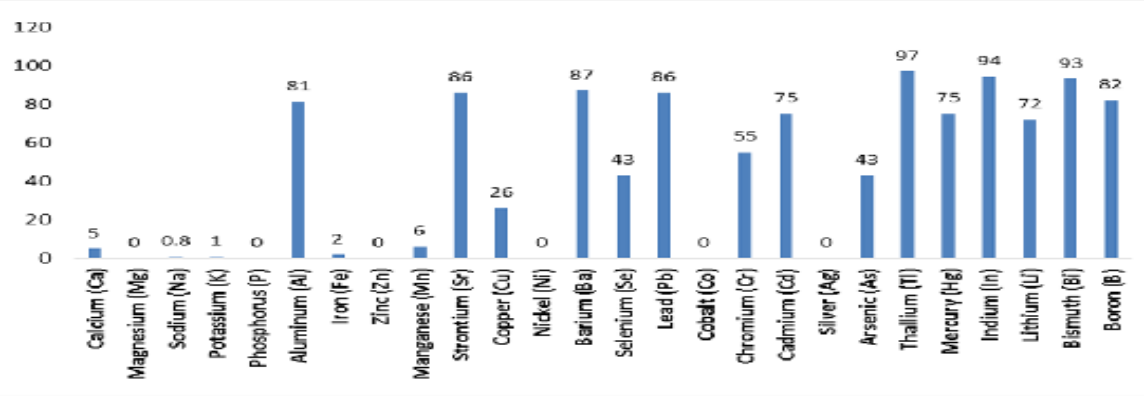

Figure 3: Processed *T.ammi* seeds ethanol extract ICP-OES study. Percentage of metal reduction in detoxification processed *T. ammi* seeds ethanol extracts.

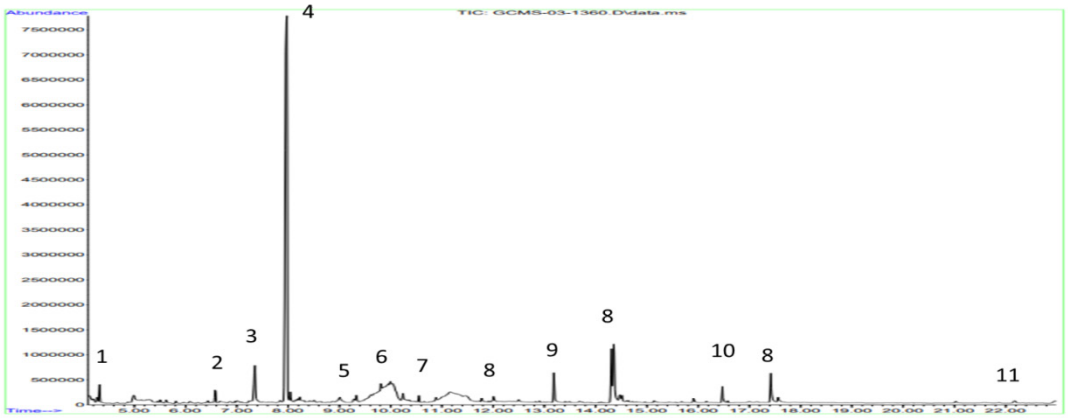

Figure 4: GC-MS analysis of the unprocessed *T.ammi*.

1. p-Cymene, 2. cis-beta. -Terpineol, 3. Thiamine, 4. Thymol, 5. n-Decanoic acid, 6. 3-methoxy methoxy butyric acid, 7. Phenol, 4-methoxy-2,3,6-trimethyl, 8. 9,12-Octa decadienoic acid (Z,Z), 9. Oleic Acid, 10. Hexadecanoic acid, 11. Stigmasterol.

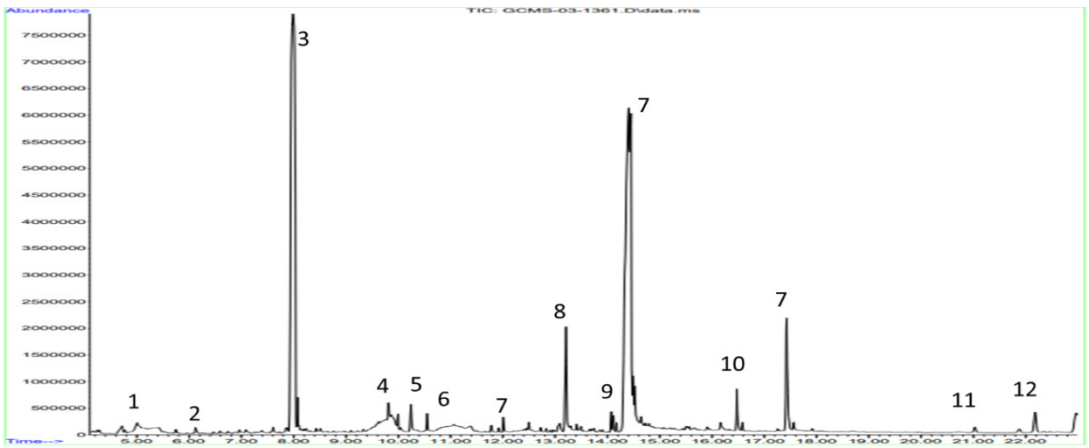

Figure 5: GC-MS analysis of the Lime treated *T.ammi.*

1. Hexanoic acid, 2. cis-.beta.-Terpineol, 3. Thymol, 4. 3-Methoxymethoxybutyric acid, 5. Phenol,2,4 bis(1,1-dimethyl ethyl), 6. Phenol, 4-methoxy-2,3,6-trimethyl, 7. 9,12-Octadecadienoic acid (Z,Z), 8. Oleic Acid, 9. Carvacrol, 10. Hexadecanoic acid, 11. 5-Cholestene-3-ol, 2-4-methyl/Campesterol, 12. Stigmasterol.

Table 3: Efficacy of detoxification process in reduction of macro and micro minerals in *T. ammi* seeds evaluated by ICP-OES.

Sl. No.	Parameters PPM	Detection wavelength(nm)	Correlation Coefficient	STD Limit	Unprocessed *T. ammi* ethanol extract	Lime *T. ammi* ethanol extract	% of Reduction Lime vs. unprocessed
1	Calcium (Ca)	393.366	0.99995	Not Fixed	1853	1757	5.18
2	Magnesium (Mg)	280.271	0.99998	Not Fixed	112.01	112	0.0
3	Sodium (Na)	589.59	0.99996	Not Fixed	228	226	0.87
4	Potassium (K)	766.49	0.99999	Not Fixed	98.5	97.51	1.01
5	Phosphorus (P)	213.617	0.99996	Not Fixed	1764	1764	0.0
6	Aluminum (Al)	394.4	0.99998	<10	25.6	4.8	81.25
7	Iron (Fe)	238.94	0.99997	<150	17.89	17.52	2.0
8	Zinc (Zn)	206.20	0.99997	<27.4	2.42	2.42	0.0
9	Manganese (Mn)	257.65	0.99997	<200	41.94	39.4	6.0
10	Strontium (Sr)	407.77	0.99998	7	20.4	2.8	86.27
11	Copper (Cu)	224.7	0.99999	<15	16.8	12.4	26.19
12	Nickel (Ni)	231.67	0.99994	Not Fixed	0.01	0.01	0.0
13	Barium (Ba)	233.52	0.99995	2	10.21	1.36	86.7
14	Selenium (Se)	196.026	0.99997	<0.4	0.35	0.20	42.85
15	Lead (Pb)	220.535	0.99998	<2.0	0.14	0.02	85.7
16	Cobalt (Co)	228.616	0.99996	<0.48	0.08	0.08	0.0
17	Chromium (Cr)	283.563	0.99998	<2.0	0.2	0.09	55.0
18	Cadmium (Cd)	214.438	0.99994	0.3	0.4	0.1	75.0
19	Silver (Ag)	328.068	0.99995	Not Fixed	0.03	0.03	0.0
20	Arsenic (As)	188.97	0.99998	3	0.07	0.04	42.85
21	Thallium (Tl)	190.864	0.99998	Not Fixed	1.02	0.03	97.05
22	Mercury (Hg)	194.168	0.99994	0.1	0.04	0.01	75.0
23	Indium (In)	325.609	1.00000	Not Fixed	0.64	0.04	93.75
24	Lithium (Li)	323.262	1.00000	Not Fixed	0.43	0.12	72.0
25	Bismuth (Bi)	223.061	0.99996	Not Fixed	0.71	0.05	92.95
26	Boron (B)	182.641	0.99999	<13	5.52	1.02	81.52

Table 4: GC-MS analysis of the unprocessed *T. ammi*.

Sl. No.	Name of the Phytochemical compound	RT (min)	Molecular weight g/mol	Area % Untreated *T. ammi* ethanol extract
1	p-Cymene	4.3	134.22	0.88
2	cis-.beta.-Terpineol	6.1	154.25	0.73
3	Thiamine	7.3	265.35	3.02
4	Thymol/3-Methyl-4-isopropylphenol	8.0	150.22	41.52
5	n-Decanoic acid	9.0	172.26	0.87
6	3- Methoxy methoxy butyric acid	9.8	208.25	4.79
7	Phenol, 4-methoxy-2,3,6-trimethyl	10.5	166.22	1.08
8	9,12-Octa decadienoic acid (Z,Z)	12.1	280.44	5.79
9	Oleic Acid	13.2	282.46	1.65
10	Hexadecanoic acid,	16.4	256.42	0.92
11	Stigmasterol	22.17	412.7	0.32

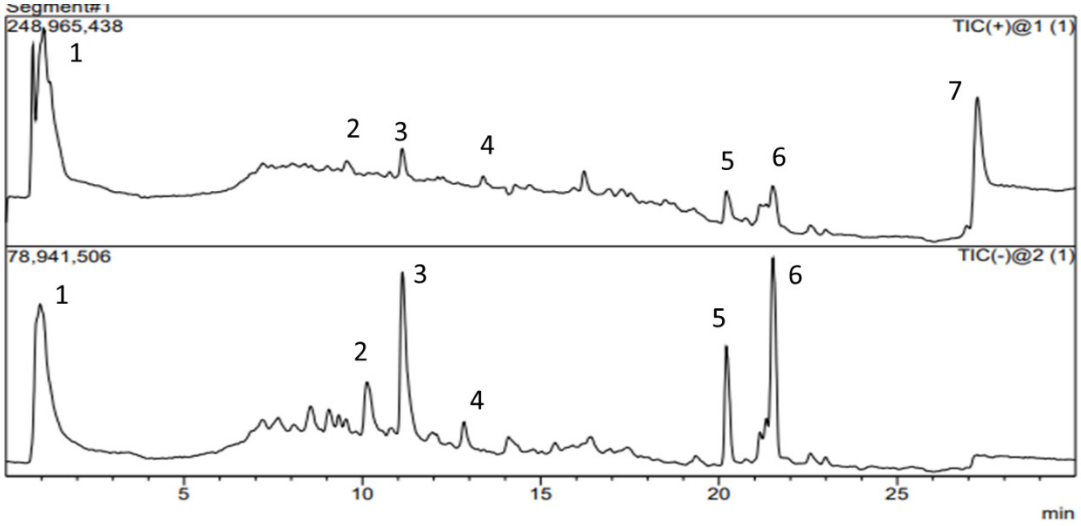

Figure 6: LC-MS Result of Unprocessed *T. ammi seeds* ethanol extract.

1.Thymol, 2. Oleic acid, 3. 8,11-Octa decadienoic acid, 4. Thiamine, 5. Palmitic acid, 6. Linoleic acid, 7. Stigmasterol.

1. Thymol, 2. Oleic acid, 3. 8,11-Octa decadienoic acid, 4. Thiamine, 5. Palmitic acid, 6. Linoleic acid, 7. Stigmasterol.

DISCUSSION

Exploring the Antimicrobial Potential

Our research focus into the antimicrobial capabilities of *T. ammi* seed ethanol extracts following detoxification. Plant secondary metabolites, widely studied, boast a broad spectrum of effects. Previous investigations, employing various solvent extraction methods, have unveiled the antibacterial properties of *T. ammi* seeds. Our findings align with prior studies,[28] demonstrating the efficacy of *T. ammi* ethanol extract in inhibiting bacterial growth, surpassing water and hexane extracts. Notably, *T. ammi* oil, recognized in pharmaceutical circles for its antibacterial properties, has been affirmed for its broad-spectrum antibacterial and antifungal activities. Interestingly, our results reveal that *T. ammi* powder exhibits greater potency than its oil counterpart against both bacterial and fungal cultures tested. This potency extends even to bacterial strains resistant to commonly used synthetic antibiotics, resembling previous discoveries highlighting *T. ammi's* impact on MRSA and vancomycin-resistant *S. aureus* strains.[29] Similarly, our investigations reinforce the superiority of ethanol extract over other solvents and oil.

Our findings parallel studies showcasing *T. ammi's* antifungal efficacy against *Candida albicans*, comparable to the antifungal medication Amphotericin-B. Moreover, our research underscores the superior suppression of *Candida albicans* by the ethanol

Table 5: GC-MS analysis of Detoxification processed *T. ammi* seeds ethanol extracts.

Sl. No.	Name of the Phytochemical compound	RT (min)	Molecular weight g/mol	Area % Lime *T. ammi* ethanol extract
1	Hexanoic acid	5.0	116.16	1.18
2	cis-.beta.-Terpineol	6.5	154.25	0.37
3	Thymol/ 3-Methyl-4-isopropylphenol	8.0	150.22	34.31
4	3-Methoxymethoxybutyric acid	9.8	208.25	2.80
5	Phenol,2,4 bis (1,1-dimethyl ethyl)	10.2	278.5	0.81
6	Phenol, 4-methoxy-2,3,6-trimethy	10.5	166.22	0.44
7	9,12-Octadecadienoic acid (Z, Z)-	12.1/14.4/17.4	280.44	42.08
8	Oleic Acid	13.2	282.46	3.24
9	Carvacrol	14.1	150.21	0.4
10	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	16.47	256.42	0.92
11	5-Cholestene-3-ol, 24-methyl/ Campesterol	21.021	400.6	0.47
12	Stigmasterol	22.17	412.7	0.97

Table 6: Detection of unprocessed *T. ammi* seeds Bioactive Compounds by LC–MS.

RT (min)	$(M+H)^+$/ $M+H)^-$	Phyto-chemical compound Names	PhytochemicalNature	Unprocessed ethanol extract
1.08	$150.1(M+H)^+$/ $(M+H)^-$	Thymol	Terpenes	+++
10.11	$282.5(M+H)^+$/ $(M+H)^{--}$	Oleic acid	Fatty acids	+
11.13	$295.3(M+H)^+$/ $(M+H)^-$	8,11-Octadecadienoicacid	Fatty acids	+++
13.9	$265.3(M+H)^+$/ $(M+H)^-$	Thiamine	vitamin B_1	+
20.24	$156.6(M+H)^+$/ $(M+H)^-$	Palmitic acid	Fatty acid	++
21.16	$293(M+H)^+$/ $(M+H)^-$	Linoleic acid	Fatty acid	+++
27.2	$413.6(M+H)^+$	Stigmasterol	Sterols	++

% of abundance is represented by ++++:75-100, +++: 50-75, ++: 25-50, +: 20- 25, -: lessthan20-nill.

extract of *T. ammi* seeds compared to the standard antibiotic Amphotericin-B.

ICP-OES Evaluation

Comparison between unprocessed and detoxification-processed *T. ammi* seeds ethanol extracts reveal considerable changes in the concentration of major elements. Twelve non-essential and fourteen essential heavy metals were identified in *T. ammi* seeds, with trace elements such as Bismuth, Indium, Mercury, Lithium, Selenium, Lead, and Silver also present. Our study provides scientific evidence supporting ancient literature on the detoxification process. While previous studies have analyzed unprocessed *T. ammi*, our research delves into the element analysis of detoxified samples,[28] revealing quantitative variations among treatments. The lime treatment proved to be the optimum method for processing *T. ammi* seeds, minimizing metal and mineral content. Notably, lime treatment resulted in a significant reduction in hazardous metals, while macro minerals remained unaffected.

GC-MS analysis of the *T. ammi* seed extracts

In our study, we employed GC-MS analysis to explore the secondary metabolites in both lime-processed and unprocessed *T. ammi* seed ethanol extracts. The results revealed the presence of sixteen distinct metabolites in the extracts. While both the processed and unprocessed extracts exhibited similar types of

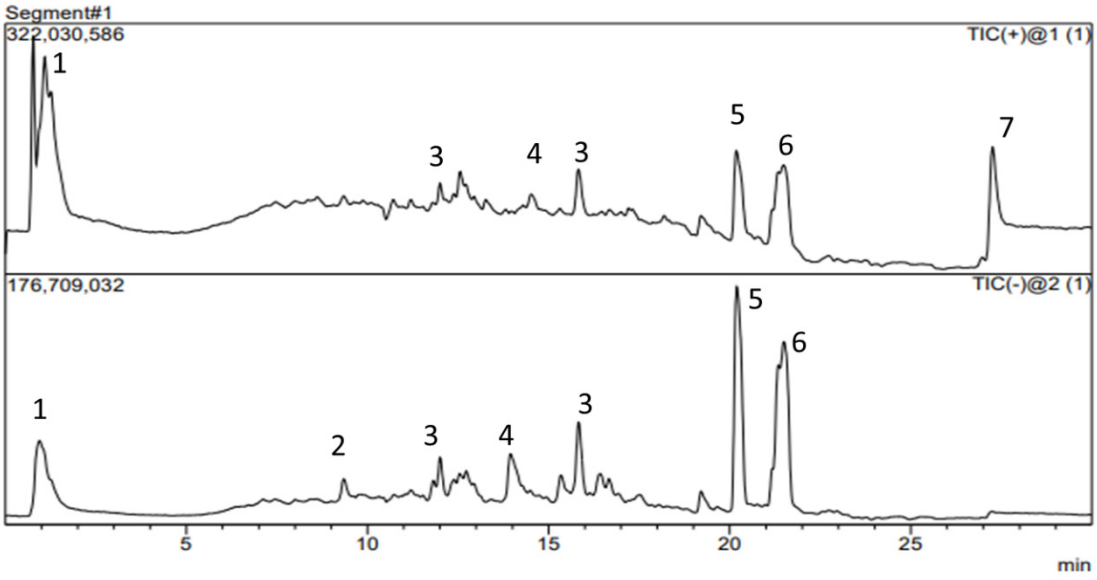

Figure 7: LC-MS Result of Lime Treated *T. ammi* seeds ethanol extract.

1. Thymol, 2. Oleic acid, 3. 8,11-Octa decadienoic acid, 4. Thiamine, 5. Palmitic acid, 6. Linoleic acid, 7. Stigmasterol.

Table 7: Detection of Lime treated *T. ammi* seeds Bioactive Compounds by LC–MS.

RT (min)	(M+H)+/(M+H)-	Phytochemical compound Names	Phytochemical Nature	Lime treated ethanol extract
0.98	150.1(M+H)+/ (M+H)-	Thymol	Terpenes	+++
9.85	282.3(M+H)-	Oleic acid	Fatty acids	+
11.3/15.2	295.3(M+H)+/ (M+H)-	8,11-Octadecadienoicacid	Fatty acids	++
12.8	265.5(M+H)+/ (M+H)-	Thiamine	vitamin B_1	+
20.24	156.6(M+H)+/ (M+H)-	Palmitic acid	Fatty acid	+++
21.16	293(M+H)+/ (M+H)-	Linoleic acid	Fattya cid	+++
27.2	413.6(M+H)+	Stigmasterol	Sterols	++

% of abundance is represented by ++++:75-100, +++: 50-75, ++: 25-50, +: 20- 25, -: lessthan20-nill.

metabolites, the lime-treated seeds showed a higher abundance of these compounds. Among the key bioactive compounds identified in both extracts were 9,12-Octadecadienoic acid, Thymol, 3-Methoxy-methoxybutyric acid, and Oleic acid, all of which are recognized for their antimicrobial, antidiabetic, and wound-healing properties.[30] Previous studies have already established the significance of Thymol and Oleic acid as primary bioactive components in *T. ammi* seeds. The detection of these compounds in our analysis aligns with earlier reports, reinforcing the notion that they are characteristic of *T. ammi*.[30,31]

Additionally, the presence of 9,12-Octadecadienoic acid and 3-Methoxy-methoxybutyric acid in both processed and unprocessed extracts is consistent with findings from similar studies, where these compounds were identified as major contributors to the medicinal properties of *T. ammi* seeds.[32] Our GC-MS analysis provides valuable insight into the biochemical changes resulting from seed detoxification. Further detailed investigations into the pharmacological potential and phytochemical diversity of detoxified *T. ammi* seeds could deepen our understanding and enrich the traditional knowledge of this medicinal plant.

Table 8: The percentage of albumin denaturation inhibited.

Sample	Con	Percentage
Unprocessed	1000 µg/mL	74
Lime treated		78
Dichlofenac		80

LC-MS analysis of the *T. ammi* seed extracts

Previous studies reported the presence of Linoleic acid, Palmitic acid, Oleic acid, petroselinic acid, Resin acids, and Decadienoic acid in *T. ammi*. Our results confirmed the presence of Oleic acid, 8,11-octadecadienoic acid, Palmitic acid, and Linoleic acid in both unprocessed and detoxified *T. ammi* ethanol extracts.

Thymol, recognized for its various medicinal properties, is a major component of *T. ammi*. Oleic acid, a monounsaturated fatty acid, possesses flavor and anti-carcinogenic properties.[33] 8,11-Octadecadienoic acid, an unsaturated fatty acid, exhibits antimicrobial, anti-inflammatory, and skincare activities.[34] Thiamine, also known as Vitamin B1, is used to treat beriberi and Wernicke-Korsakoff syndrome.[35] Linoleic acid and Palmitic acid, categorized as polyunsaturated omega-6 fatty acids, possess antimicrobial, antidiabetic, and wound-healing properties. Stigmasterol, a plant sterol, may impact intestinal cells and affect transporter proteins, exhibiting properties such as ear eczema treatment, anti-inflammatory effects, and hormonal imbalance treatment. Moreover, lime-treated ethanol extract contained high levels of fatty acids, particularly Linoleic acid and Oleic acid, suggesting its potential in dermatitis treatment.

Anti-inflammatory Activity

We evaluated the anti-inflammatory properties of both detoxified and non-detoxified *T. ammi* seed extracts using the egg albumin denaturation method, following the protocol.[28,36] Our findings revealed that lime-treated extracts exhibited the highest inhibition. Remarkably, these inhibitory effects closely mirrored those of diclofenac, a standard anti-inflammatory agent.

Previous studies have extensively documented the analytical and anti-inflammatory characteristics of *T. ammi* extracts, validating its traditional use in alleviating various painful and inflammatory conditions. Protein denaturation plays a crucial role in generating autoantigens associated with inflammatory ailments such as rheumatic arthritis, cancer, and diabetes, as well as during wound healing processes. Therefore, inhibiting protein denaturation holds significant promise in alleviating inflammatory responses.[37] Our study underscores the notable anti-inflammatory potential of lime-treated *T. ammi* seed extracts.

CONCLUSION

In conclusion, our research reveals that lime-treated *T. ammi* seeds exhibit strong inhibitory effects against a wide range of tested microorganisms, with the exception of *M. furfur* and *T. mentagrophytes*. Moreover, the lime treatment significantly reduces the metal and mineral content in the seeds. Importantly, this detoxification process effectively retains the key bioactive components, including major phenols, fatty acids, and flavonoids. These findings suggest that lime treatment not only reduces metal content but also preserves the seeds' pharmaceutical potential, showcasing promising antimicrobial and anti-inflammatory properties.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

T. ammi: *Trachyspermum ammi*; **LDL:** Low-density lipoproteins; **HDL:** High-density lipoprotein; **MTCC:** Microbial Type Culture Collection; **HNO_3:** Nitric acid; **ICP:** Inductively Coupled Plasma; **OES:** Optical Emission Spectrometer; **CRM:** Certified reference material; **GC:** Gas Chromatography; **MS:** Mass Spectrometry; **LC:** Liquid Chromatography; **MS:** Mass Spectrometry.

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SUMMARY

To validate the traditional detoxification method, Sodhana for *Trachyspermum ammi* (*T. ammi*) seeds, and assess its impact on antimicrobial activity, phytochemical composition, heavy metal content, and anti-inflammatory efficacy. Seeds treated with lime, ground into powder, and extracted with 90% ethanol. Evaluated for antimicrobial activity, heavy metal content, phytochemical composition, and anti-inflammatory effects. Antimicrobial activity reveals Lime-treated ethanol extract showed enhanced inhibitory activity against various microbial strains compared to the unprocessed extract. Heavy metal content of our study detected significant reductions in toxic metals, including titanium, indium, bismuth, strontium, lead, aluminum, boron, mercury, and cadmium, were observed after lime treatment. Phytochemical Composition analysis revealed Lime-treated extracts exhibited higher levels of thymol and fatty acids, with notable changes in compound composition. The lime-treated extracts demonstrated significant anti-inflammatory potential. It concluded that Lime treatment effectively reduces toxic elements and enhances the antimicrobial and anti-inflammatory properties of *T. ammi* seeds, supporting its use in traditional herbal practices.

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