ORIGINAL ARTICLE

Studies on the therapeutic potentials of *Datura metel* L. unripen fruits

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Abstract

Introduction: *Datura metel* L. is a medicinal plant whose leaves and seeds are widely used in herbal medicine as anaesthetic, antispasmodic, antitussive, bronchodilator and as hallucinogenic. **Materials and Methods:** Qualitative phytochemical analysis was performed to detect the presence of phytochemical constituents in a formulated *Umaththankai parpam* using a common plant *Datura metel* L with *Ocimum sanctum* leaves and sulphur. Antibacterial and antifungal analysis were carried out to determine its therapeutic potentials. The phytochemical compounds present in *Umaththankai parpam* was identified by GC-MS analysis with their molecular weight (MW), retention time (RT), concentration (peak area %), Molecular formula (MF). **Results:** The qualitative phytochemical analysis revealed the presence of various secondary metabolites and found to be containing alkaloids, flavonoids, saponins, fixed oils, tannin, phenols and glycosides; while protein, amino acid, steroids and terpenoids were found to be absent. The data showed that alkaloids, glycosides and flavonoids were found as strong positive than other metabolites. The crude extract has antibacterial activity against the Gram negative bacteria such as *Pseudomonas aeruginosa* and *Escherichia coli* and the Gram positive bacteria *Staphylococcus aureus* and also the phytochemicals exhibited antifungal activity against three fungal species such as *Candida albicans, Aspergillus niger* which are causing secondary infections to COVID19 patients and *Tricophyton rubrum*. Totally 10 compounds were identified and the high peak area compounds in this sample were n- Hexadececanoic acid (54.24%) and others were 9,12- Octadecadienoyl chloride (15.63%), 9,12- Octadecadienoic acid(12.97%), Z,E-2-Methyl-3,13-octadecadien-1-ol(12.96%), Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester (2.30) and the rest of the compounds showing short peak area like 6-Tetradecanol(0.71%), 9,12-Octadecadien-1-ol, (Z,Z)- (0.54%), Benzene, 1,2-dimethoxy-4-(1-propenyl)-(0.52%), Methyl 6-methyl heptanoate (0.07%) and (S)-3,4-Dimethylpentanol (0.06%). **Discussion and Conclusions:** The peak compound n-hexadecanoic acid is a known anti-inflammatory agent and which was well documented in seaweeds but surprisingly the same compound has been found in our novel formulation. The second peak compound was 9,12-octadecadienoic acid and which has been reported as an antimicrobial compound in a different source. The study has brought out a wonderful formulation with readily available plant resources with potential therapeutic compounds and proved to be a drug source.

Key words: *Datura metel* L., phytochemicals, antibacterial, antifungal, anti-inflammatory, n-hexadecanoic acid

INTRODUCTION

Plants produce a variety of compounds to protect themselves against a variety of pathogens. *Datura metel* L. is a medicinal plant whose leaves and seeds are widely used in herbal medicine as anesthetic, antispasmodic, antitussive, bronchodilator, and as hallucinogenic. The leaves are used as a local application for rheumatic swellings of the joints, lumbago, sciatica, neuralgia, painful tumor, scabies, eczema, allergy, and glandular inflammations such as mumps, used externally for ear ache, and smoked to relieve spasmodic asthma. Seeds are also used externally for piles.[1] It is used as a good medicine to treat dandruff and lice.[2] *D. metel* are known as "baimantuoluo" and used for skin inflammation and psoriasis.[3] In Ayurvedic treatment, seeds of *D. metel* are used to treat skin rashes, ulcers, bronchitis, jaundice, and diabetes.[4] The plant is displayed as a natural source of antioxidants.[5] Hence, in this study, the scientific evaluation of phytochemicals and antimicrobial potential of "*Umaththankai parpam*" has been carried out by evaluating its physicochemical characters, preliminary phytochemical screening of the trial drug through standard

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pharmacognostical methods and gas chromatography–mass spectroscopy (GC–MS) analysis. Through FT-IR and XRF analyses, the percentage of elements and functional groups of the trial drug are estimated and the antimicrobial potential is estimated through *in vitro* antimicrobial assay.

MATERIALS AND METHODS

Collection and Authentication of the Drug

Umaththankai (unripen fruit of *D. metel*), *Thulasi* (*Osmium sanctum*) leaves, and *Kanthagam* (sulfur) were the major ingredients of *Umaththankai Parpam*. Unripen fruit of *D. metel* and leaves of *O. sanctum* have been used in the present study which were collected in and around Tamil University campus and the agriculture waste lands. The mineral drug sulfur was collected from Siddha medical shop at Thanjavur. The specimen sample of each ingredient was labeled separately and kept in the laboratory for future reference [Figure 1].

Preparation of *Umaththankai Parpam* and Extract

The collected unripen fruit was opened and filled with sulfur and then closed with the same fruit particles. Then, the processed unripen fruits were covered with cow dung and it was taken into "*Pudam"* process (calcination). After Pudam, process the drug was removed from the cow dung. *O. sanctum* (Thulasi) leaves were added with the processed *Umaththankai* and made into powdered form [Figure 2a-f]. Ten grams of *Umaththankai Parpam* were weighed accurately and placed in a clean beaker and a few drops of concentrated hydrochloric acid were added. This was allowed to cool and dry at room temperature. One hundred milliliters of distilled water were added, stirred well, and filtered. The filtrate was subjected to further evaluations such as preliminary phytochemical screening, compounds identifications, and determination of antimicrobial potentials.

Qualitative Phytochemical Studies

Qualitative phytochemical analyses were carried out using the procedures of Kokate *et al*. (1995).[6] Alkaloids, carbohydrates, tannins and phenols, flavonoids, gums and mucilage, proteins, fixed oils and fat, volatile oil, and saponins were qualitatively analyzed.

Figure 1: *Datura metal* L. source plant and parts collected

Carbohydrates

Three hundred milligrams of ethanolic extract were dissolved in distilled water and filtered. The filtrate was boiled with Fehling's and with Benedict's solution. Formation of brick red precipitate in Fehling's and Benedict's solution was the positive result for reducing sugars and non-reducing sugars, respectively.

Alkaloids

The extract was dissolved in diluted hydrochloric acid and filtered. The filtrate was tested with alkaline reagents such as Mayer's, Dragendorff's, Hager's, and Wagner's reagent separately. The cream, orange, brown, yellow, and reddish-brown precipitate appearances in response to the above reagents, respectively, indicated the presence of alkaloids.

Steroids

Minimum amount of chloroform was taken and in which three drops of acetic anhydride and two drops of concentrated sulfuric acid were added along with test solution. The presence of steroids was denoted by the appearance of purple color changing to blue color.

Tannins and Phenols

Small quantity of ethanol extract was dissolved in water and to that ferric chloride solution (5%) was added. Appearance of blue color with ferric chloride indicated the presence of tannins and phenols.

Saponins

In 20 ml of water, 1 ml of the extract was added and shaken in graduated cylinder for 15 min. The presence of saponins was indicated by the formation of 1 cm layer of foam.

Figure 2: Processing steps of prepare parpam from *Datura metal* L. (a) Unripen fruits before filling sulfur. (b) Unripen fruits after filling sulfur. (c) Processed unripen fruits. (d) Initial stage of "*Pudam.*" (e) Final stage of "*Pudam.*" (f) End product "*Umaththankai parpam*"

Fixed Oils and Fats

A drop of concentrated extract was pressed and kept undisturbed in between two filter papers: Oil stains on the paper indicate the presence of oils and fats.

Gums and Mucilage

To 25 ml of absolute alcohol, about 10 ml of extract was slowly added under constant stirring and formation of precipitation indicated the presence of gums and mucilage.

Proteins and Free Amino Acids

In a few ml of water, a small quantity of extract was added and subjected to Millon's or Biuret tests. The presence of proteins was indicated by the appearance of pink-purple or red color.

Flavonoids

A few ml of alcohol was added to the extract and it was heated with magnesium and then conc. HCl was added under cooling. The pink color indicated the presence of flavonoids. When the extract was treated with few ml of aqueous NaOH, the appearance of yellow color and changes to colorless with HCl indicated the presence of flavonoids.

Volatile oils

Hydrodistillation was carried out by placing 50g of powdered sample material in a volatile oil estimation apparatus for the detection of volatile oil. The distillate was collected within the graduated tube of the assembly during which the aqueous portion was automatically separated from the essential oil. If it is present in the drug, it returns back to the distillation flask.

Gas Chromatography–Mass Spectroscopy (GC-MS) Analysis (Kumaravel *et al.*, 2017)

The powdered sample (20 g) was soaked and dissolved in 75 ml of methanol for 24 h. By evaporating under liquid nitrogen, the filtrates were collected. The GC–MS analysis was carried out using a Clarus 500 PerkinElmer (Auto system 37 XL), gas chromatograph equipped and paired to a mass detector TurboMass gold – PerkinElmer TurboMass 5.2 spectrometer with an Elite-1(100% dimethylpolysiloxane), 300 mm × 0.25 mm × 1 × m df capillary column. The instrument was set to an initial temperature of 110°C and maintained at this temperature for 2 min. After 2 min, the oven temperature was raised up to 280°C, at the speed of a rise of 5°C/min and maintained for 9 min. Injection port temperature was ensured as 250°C and helium flow as 1 ml/min. The ionization voltage was 70 eV. The sample was injected in split mode as 10:1. Mass spectral scan range was set at 450 MHz. The chemical constituents were identified by GC–MS. The fragmentation patterns of mass spectra were compared to those stored within the spectrometer using National Institute of Standards and Technology Mass Spectral database. The percentage of every component was calculated from the relative peak area of every component within the chromatogram.

Physicochemical Parameters

Organoleptic characters (color, appearance, odor, and touch), qualitative analysis, and different quantitative parameters such as pH, ash (%w/w), acid-insoluble ash (%w/w), loss on drying @105°C, water-soluble extractive, alcohol-soluble extractive, calcium content, and foreign matter were tested according to the prescribed standard methods of Indian Pharmacopoeia[7] and the instrumental analyses, namely, FTIR, XRF standard methods adopted in CARISM, SASTRA University, Thanjavur.

Color and Odor

In a clean glass beaker, about 5 g of *Umaththankai parpam* was taken and tested for its color by viewing against a white opaque background under direct sunlight. In a 100 ml beaker, about 5 g of the *Umaththankai parpam* was placed and tested for its odor by wafting the air above the beaker.

pH

The pH of the *Umaththankai parpam* was detected as per the method prescribed in the Indian standard (IS) – 6940 (1982). 1.0 g of the *Umaththankai parpam* was taken into a 100 ml graduated cylinder containing about 50 ml of water and filled up to the mark with water. The cylinder was shaken vigorously for 2 min and allowed the suspension to settle for an hour at 25 to 27°C. Twenty-five milliliters of the clear aqueous solution were transferred into a 50 ml beaker and tested for pH using digital pH meter.

Loss on Drying

The powdered sample was dried in an electrical oven at 105°C until it reaches a constant weight.

Total Ash Value and Acid-Insoluble Ash Value

Around 5 g of test sample was ignited in an electric furnace at 500–550°C in silica crucible until the sample reaches a constant weight. Total ash obtained was heated with addition of 25 ml of dilute HCl for 10 min. It was filtered in an ashless filter paper (Whatman No. 41) and the residue was ignited in the furnace to get a constant weight.

FTIR Analysis

FTIR was used to identify the functional group.[8] The spectrum appeared denoted the molecular transmission and absorption. It forms the molecular fingerprint of the sample and was recorded as the wavelength and the peaks seen in the spectrum indicate the amount of material present. The IR Tracker-100 – The new Fourier-transform infrared spectrophotometer for high sensitivity, high-resolution, and high-speed applications was used to derive FTIR spectra of *Umaththankai parpam.*

XRF Analysis

Analysis XRF (X-ray fluorescence spectroscopy) analysis of *Umaththankai parpam* was done at SASTRA University, Thanjavur, Tamil Nadu, using Bruker S8 Tiger XRF Spectrometer. X-ray fluorescence was used to determine the chemical elements both qualitatively and quantitatively by measuring their characteristic radiation of the sample. The sample holder was filled with 2 g of boric acid and 1 g of the *Umaththankai parpam* was topped over it for achieving better accuracy and precision. This was pelletized by a 25-tonne hydraulic press to achieve 34 mm diameter pellets.

In Vitro Antimicrobial Susceptibility Test

The *in vitro* method proposed by National Committee for Clinical Laboratory Standards for testing molds[9] was followed for the present study.

Medium Preparation

In distilled water, the Mueller-Hinton Agar and Sabouraud dextrose agar – HiMedia were dissolved with the aid of heat and pH was adjusted to 7.0 and 5.6, respectively, using dilute alkali or dilutes acid. Mueller-Hinton agar and Sabouraud dextrose agar were prepared and autoclaved at a pressure of 15 psi (121°C) for not less than 15 min. The sterilized medium was transferred into a sterile Petri dish.

Inoculum Preparation

The inoculum for the experiment was prepared fresh in nutrient broth and Sabouraud broth from preserved frozen slant culture. It was incubated at 37°C and 28°C for 24 h and 48 h, respectively, for bacteria and fungi and used after standardization.

Microorganisms

Microorganisms were obtained from the Doctors Diagnostic Laboratory, Tiruchirappalli, Tamil Nadu, India. Among six microorganisms investigated, three bacterial species were *Pseudomonas aeruginosa*, *Escherichia coli,* and *Staphylococcus aureus* while three fungal species were *Candida albicans*, *Aspergillus niger,* and *Trichophyton rubrum* [Figure 3]. All the microorganisms were maintained at 37°C and 28°C on nutrient and Sabouraud dextrose agar slants, respectively, for bacteria and fungi.

Working Conditions

The entire work was done using horizontal laminar air flow hood so as to provide aseptic conditions. Before commencement of the work, air sampling was carried out using a sterile nutrient agar plate and exposing it to the environment inside the hood. After incubation, it was checked for the growth of the microorganism and absence of growth confirmed aseptic working conditions.

Disc Diffusion Method

In vitro disc diffusion assay[10] will be used to evaluate the antimicrobial potential of the plant drugs. To perform the antimicrobial assay for bacterial and fungal strains, Mueller-Hinton agar and Sabouraud dextrose agar (purchased from HiMedia, Mumbai) will be used, respectively. Discs were made from Whatman No.1 filter paper. Fifteen milliliters of sterilized molten agar media were poured aseptically into sterilized Petri plates. The plates were allowed to solidify for 5 min and inoculum suspension of both the group of microorganisms was swabbed uniformly and the inoculum was allowed to dry for 5 min. The paper discs impregnated with the plant extract (100 µg/disc) were placed on the test organism seeded medium. The plates were pre-incubated for l h at room temperature and then incubated at 37 ± 2°C for 24 h and 28 ± 2°C at 48 h for fungal and bacterial growth for their antimicrobial response. Fluconazole (10 µg/disc) and ciprofloxacin (5 µg/disc) were involved as reference standard for fungi and bacteria, respectively. All the test microorganisms were tested in triplicates. Antimicrobial growth was qualitatively evaluated by measuring inhibition zone. After the incubation period, the no microbial growth area in the culture plate was recorded by measuring the diameter in mm.

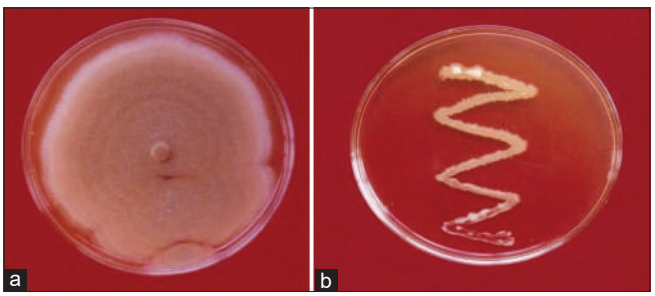

Figure 3: Microbial pure cultures. (a) Pure culture of *Trichophyton rubrum*. (b) Pure culture of *Staphylococcus aureus*

Well-diffusion Method

By the well-diffusion method, the *in vitro* antimicrobial activity of the plant extract was assessed. To perform the antimicrobial assay for bacterial and fungal strains, Mueller-Hinton agar and Sabouraud dextrose agar (purchased from HiMedia, Mumbai) will be used, respectively. The test organisms taken are the bacterial organisms (*P. aeruginosa*, *E. coli,* and *S. aureus)* and the fungal organisms (*A. niger, C. albicans,* and *T. rubrum*). Fifteen milliliters of sterilized molten agar media were poured aseptically into sterilized Petri plates. The plates were allowed to solidify for 5 min and inoculum suspension of both the group of microorganisms was uniformly swabbed and the inoculum was allowed to dry for 5 min. After 5 min, 5 mm sized wells of three numbers in each Petri plate were made with the help of cork borer. The extract of the test drug *Umaththankai parpam* at 100 µg/ml was aseptically loaded in each well in the help of micropipette. The plates were pre incubated for 1 h at room temperature and then incubated at 37± 2° C for 24 h and 28± 2° C at 48 h for antibacterial and antifungal growth for their antimicrobial response. Ciprofloxacin (5 µg/disc) and fluconazole (10 µg/disc) were involved as reference standard for bacteria and fungi, respectively. All the test microorganisms were tested in triplicates. Antimicrobial growth was qualitatively evaluated by measuring inhibition zone. After the incubation period, the non-microbial growth area in the culture plate was recorded by measuring the diameter in mm. The zones of diameter of three replicates were measured in mm and were tabulated.

RESULTS

Qualitative phytochemical analysis was performed to detect the presence and absence of phytochemical constituents in the *Umaththankai parpam*. The qualitative phytochemical analysis showed the presence of the secondary metabolites listed in Table 1. The extract of the *Umaththankai parpam* contains alkaloids, flavonoids, saponins, fixed oils, tannin, phenols, and glycosides; while protein, amino acid, steroids, and terpenoids were found absent. The data showed that alkaloids, glycosides, and flavonoids were found as strong positive than other metabolites.

The phytochemical compounds present in *Umaththankai parpam* were identified by GC–MS analysis with their molecular weight, retention time, concentration (peak area %), and molecular formula which are presented in Table 2. A total of 10 compounds were identified. The high peak area compounds in this sample were n- Hexadecanoic acid (54.24%), 9,12-Octadecadienoyl chloride (15.63%), 9,12-Octadecadienoic acid (12.97%), Z,E-2-Methyl-3,13-octadecadien-1-ol(12.96%), Hexadecanoic acid, and 1-(hydroxymethyl)-1,2-ethanediyl ester (2.30), and the rest of the compounds showing short peak area such as 6-Tetradecanol(0.71%), 9,12-Octadecadien-1-ol, (Z,Z)- (0.54%), Benzene, 1,2-dimethoxy-4-(1-propenyl)-(0.52%), Methyl 6-methyl heptanoate (0.07%), and (S)-3,4-Dimethylpentanol (0.06%). GC–MS chromatogram of *Umaththankai parpam* is shown in Figures 4 and 5.

Table 1: Preliminary phytochemical screening of *Umaththankai parpam*

S. No.	Phytochemicals	Aqueous extract
1.	Alkaloids	++
2.	Saponins	+
3.	Tannins	+
4.	Flavonoids	++
5.	Glycosides	++
6.	Phenol	+
7.	Protein and amino acids	–
8.	Steroids	–
9.	Terpenoids	–
10.	Fixed oil and fats	+

The organoleptic characteristic features of *Umaththankai parpam* were solid in nature, light gray color in day light and the smell and taste were not to be found characteristic. The values of the physicochemical parameters depicted in Table 3.

The total ash value of *Umaththankai parpam* was found to be 9.16% w/w. The method of preparation of this drug was based on calcinations procedure. The inorganic contents of the formulation value of total ash indicate that they were below the limits. Water-soluble extractive value of this formulation was 0.72%; while acid-insoluble ash was found to be 36.50%w/w.

The FTIR spectra were shown in with ranges from 400 to 4000 cm^{-1}. From this spectrum, it was observed that hydroxide stretching peaks present in 3300–3500 cm^{-1}. The sharp peak 2925 cm^{-1} denotes the presence of aliphatic compounds (or fragment), where we observed the asymmetric C–H stretch of methyl and methylene groups. The peak 1650 cm^{-1} was alkenyl group. 1458 cm^{-1} peak point indicates the presence of sulfate groups. 1241 cm^{-1} implies the presents of sulfonate groups and 1031 cm^{-1} peak signifies the iron oxide and also silicone peak was found at 721 cm^{-1a} [Table 4 and Figure 6].

XRF results of *Umaththankai parpam are* shown in Table 5. The elements in oxide as well as elemental form are analyzed by the XRF analysis. Chemical properties of the formulated Umaththankai parpam was found to be having 6.16% of total ash and mong the total percentage, water soluble ash was found to be higher than other types [Figure 7]. Elements present in of Umaththankai parpam in oxide form was determined using XRF and among the thirteen elements studied, So3 was found to be higher [Figure 8]. Presence of essential & non-essential metal elements in Umaththankai parpam was analysed using XRF and Cu (91ppm) has been found to be higher than other

Table 2: GC–MS analyses of *Umaththankai parpam*

S. No.	Name of the compound	Retention time (min)	Peak area	% peak area
1.	Name: (S)-3,4-Dimethylpentanol Formula: C7H16O MW: 116	15.22	93,100	0.06
2.	Name: Benzene, 1,2-dimethoxy-4-(1-propenyl)- Formula: C11H14O2 MW: 178	16.16	852,798	0.52
3.	Name: Methyl 6-methyl heptanoate Formula: C9H18O2 MW: 158	31.99	110,834	0.07
4.	Name: 6-Tetradecanol Formula: C14H30O MW: 214	33.03	1,162,044	0.71
5.	Name: n-Hexadecanoic acid Formula: C16H32O2 MW: 256 (Palmitic acid)	33.37	88,373,384	54.24
6.	Name: 9,12-Octadecadien-1-ol, (Z,Z)- Formula: C18H34O MW: 266	35.48	878,638	0.54
7.	Name: 9,12-Octadecadienoic acid (Z,Z)- Formula: C18H32O2 MW: 280	36.35	21,132,392	12.97
8.	Name: Z,E-2-Methyl-3,13-octadecadien-1-ol Formula: C19H36O MW: 280	36.53	21,114,178	12.96
9.	Name: Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester Formula: C35H68O5 MW: 568	37.55	3,740,474	2.30
10.	Name: 9,12-Octadecadienoyl chloride, (Z,Z)- Formula: C18H31ClO MW: 298	40.38	25,471,556	15.63

MW: Molecular weight

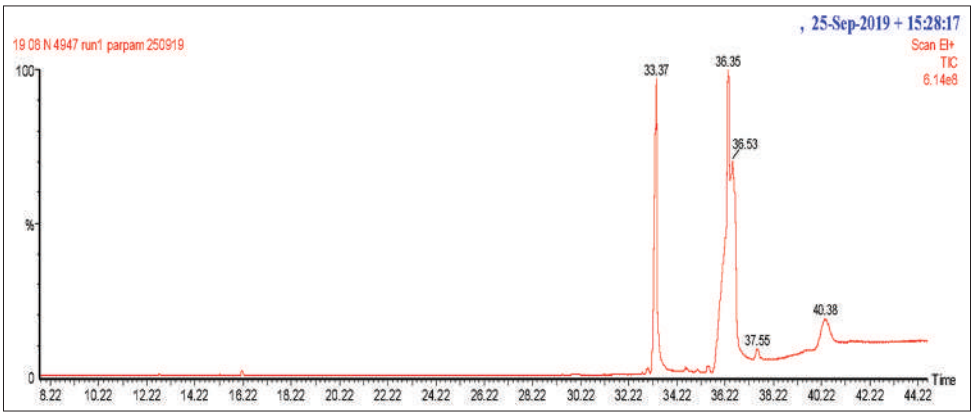

Figure 4: Chromatogram of gas chromatography–mass spectroscopy of *Umaththankai parpam*

elements [Figure 9]. It was observed that so many elements were present in *Umaththankai parpam.* Elements such as sulfur, potassium, and calcium were the key compounds playing a major therapeutic role in *Umaththankai parpam*. Level of oxide form of sulfur (SO_3) was found to be higher (55.21%) when compared to elemental form (30.52%). However, the level of elemental form of potassium, chloride, calcium, and silicon was found to be higher when compared to oxide form.

Table 3: Physicochemical analyses of *Umaththankai parpam*

S. No.	Parameters	Results	Reference of test method
1.	Description	Green color powder	IP Vol-I, 1996, p 7
2.	pH (1% w/v solution)	6.86	IP Vol-I, 2014, p 169
3.	Total ash	9.16 %w/v	IP Vol-I, 2014, p 98
4.	Acid-insoluble ash	1.57 %w/v	IP Vol-I, 2014, p 98
5.	Water-soluble ash	4.15 %w/v	IP Vol-I, 2014, p 98
6.	Loss on drying	4.02 %w/v	IP Vol-I, 2014, p 162

Table 4: XRF analyses of *Umaththankai parpam*

a. Element in oxide form

S. No.	Formula	Concentration (%)
1	SO_3	55.21
2	K_2O	17.39
3	Cl	6.03
4	PbO	5.06
5	SiO_2	4.52
6	CaO	4.11
7	P_2O_5	3.86
8	MgO	1.09
9	AL_2O_3	0.82
10	MOO_3	0.68
11	FeO_3	0.68
12	Na_2O	0.14
13	ZnO	0.14

Umaththankai parpam extract showed potent antimicrobial activity against all test pathogens, namely, bacteria (*E. coli*, *P. aeruginosa,* and *S. aureus*) and fungal (*A. niger*, *C. albicans,* and *T. rubrum*) pathogens. Figure 10 shows about Antibacterial activity of the Umaththankai parpam and results shows high sensitivity against the Gram positive cocci Staphylococcus aureus compared to Gram negative bacteria Escherichia coli and Pseudomonas aeruginosa. The antibacterial assay on the standard bacterial antibiotic ciprofloxacin (5µg) corresponding to test organisms is listed in Table 6a and 6b. The maximum IZD (inhibition zone of diameter) of 28 mm was observed in ciprofloxacin (5 µg) against *S. aureus*, 16 mm was observed against *E. coli,* and 15 mm was observed against *P. aeruginosa*. Compared to the standard antibiotics, the extract was found to have good activities against *Staphylococcus aureus*, *C. albicans,* and *E. coli* (Plate-V and VI).

Table 5: XRF analyses of *Umaththankai parpam*

b. Element form

S. No.	Formula	Concentration (%)
1	S	30.52
2	K	29.49
3	Cl	12.05
4	Pb	8.97
5	Ca	5.12
6	Si	4.62
7	P	3.07
8	Pd	1.79
9	Mg	1.28
10	Mo	1.02
11	Al	0.77
12	Fe	0.77
13	Zn	0.26
14	Cu	91PPM

Table 6a: Antibacterial activity of *Umaththankai parpam* by disc diffusion method

S. No.	Microorganisms	(100 µg / disc) (mm)	Ciprofloxacin (5 µg/disc) (mm)
1	*Staphylococcus aureus*	24	28
2	*Escherichia coli*	14	16
3	*Pseudomonas aeruginosa*	12	15

Table 6b: Antibacterial activity of *Umaththankai parpam* by well-diffusion method

S. No	Microorganisms	(100 µg/ disc) (mm)	Ciprofloxacin (10 µg/disc) (mm)
1.	*Staphylococcus aureus*	15	28
2.	*Escherichia coli*	11	16
3.	*Pseudomonas aeruginosa*	11	15

Table 6c: Antifungal activity of *Umaththankai parpam* by disc diffusion method

S. No.	Microorganisms	(100 µg/ well) (mm)	Fluconazole (10 µg/well) (mm)
1.	*Candida albicans*	16	18
2.	*Aspergillus flavus*	13	17
3.	*Trichophyton rubrum*	10	11

Twenty-four millimeters of inhibition zone were exhibited in the extract of *Umaththankai parpam* and the test extract at

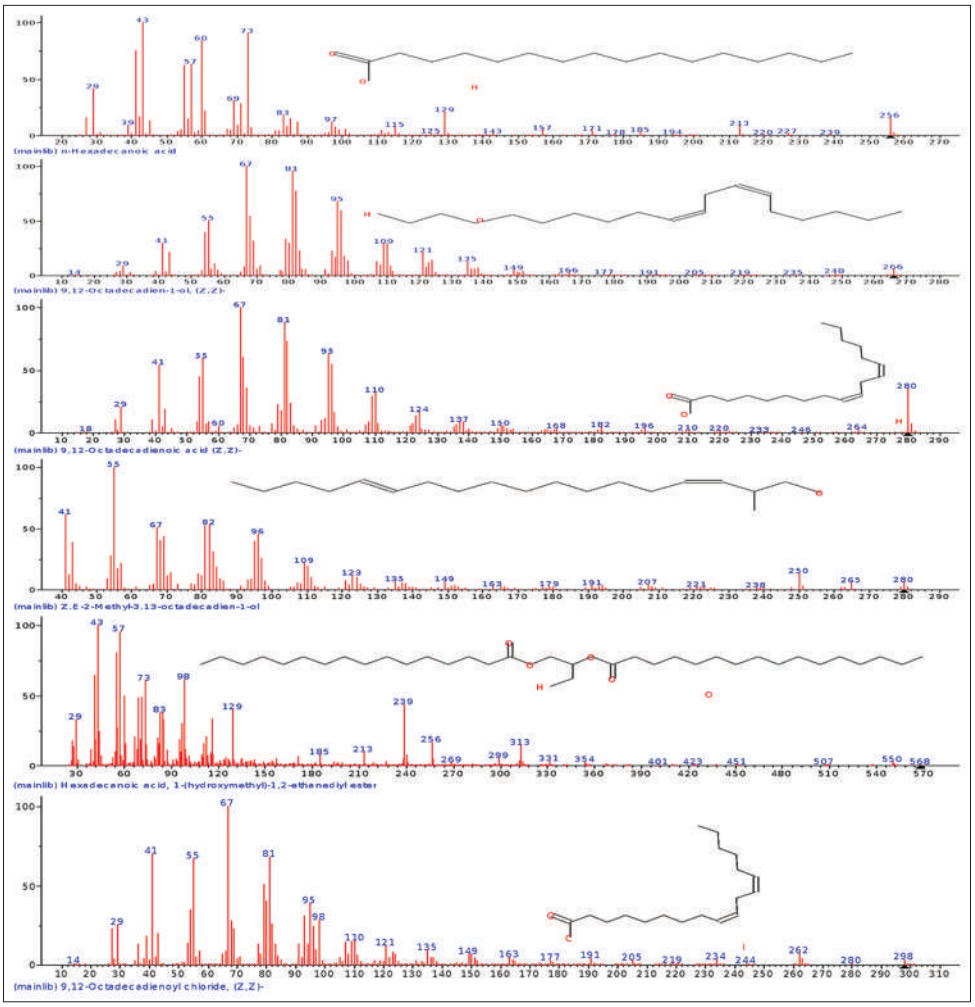

Figure 5: Isolated compounds of *Umaththankai parpam* by gas chromatography–mass spectroscopy

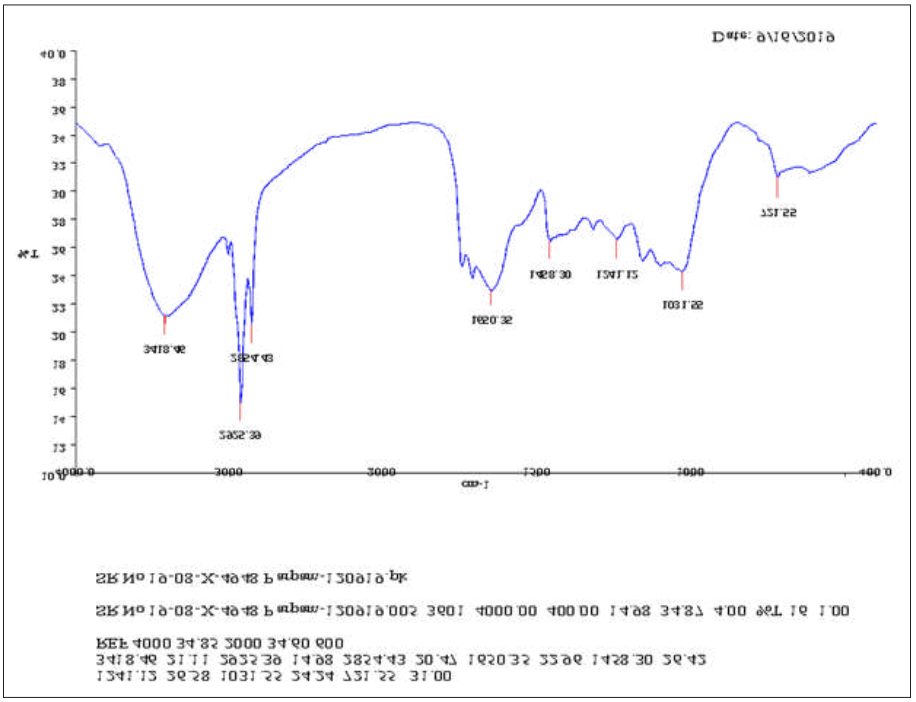

Figure 6: Chromatogram of FTIR of *Umaththankai parpam*

Table 6d: Antifungal activity of *Umaththankai parpam* by well-diffusion method

S. No.	Microorganisms	(100 µg/well) (mm)	Fluconazole (10 µg/well) (mm)
1.	*Candida albicans*	12	18
2.	*Aspergillus flavus*	10	17
3.	*Trichophyton rubrum*	10	11

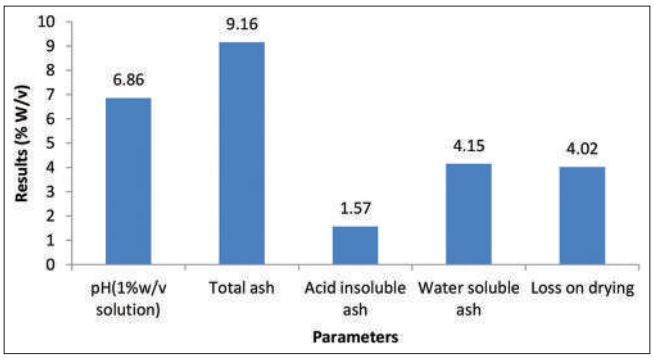

Figure 7: FTIR analyses of *Umaththankai parpam*

Wave number (cm^{-1})	Vibrational modes of Sangu Parpam in IR region	Functional group
3300–3500	Hydroxide stretch	Alcohol, phenols
2935	C-H stretch	Methylene group
1650	C = C Bend	Alkenyl group
1458	N-H Bend	Amine
1241	N-O Stretch	Nitro compound
1031	C-O Stretch	Primary alcohol
721	C-Cl Stretch	Alkyl halide

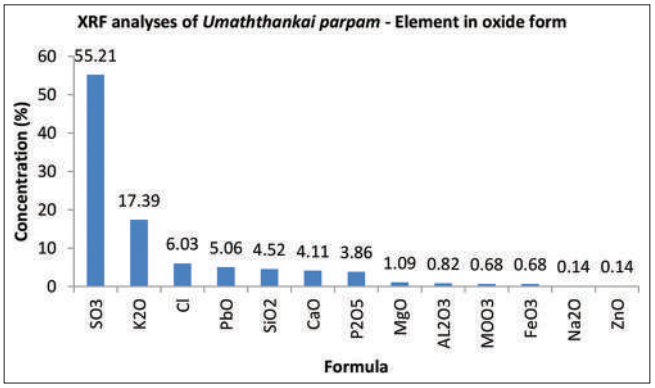

Figure 8: About the percentage availability of elements in oxide form and among the 13 elements, So3 was found to be higher

100 µg/ml against *S. aureus* in disc diffusion method while maximum bacterial inhibition of 15 mm of the extract was found in well-diffusion method.

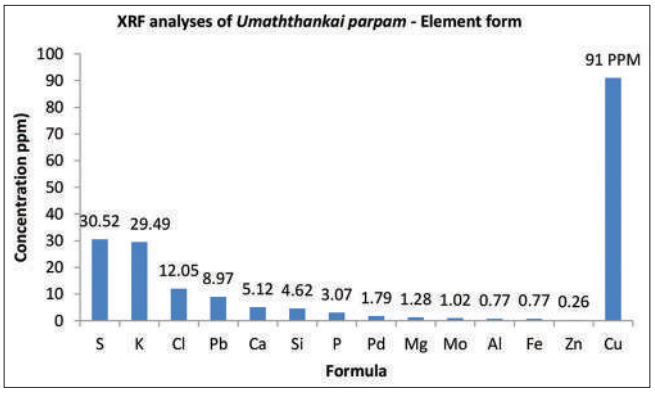

Figure 9: The result shows the presence of essential and non-essential metal elements in *Umaththankai parpam*. Some elements are found to be very high (Cu 91 ppm)

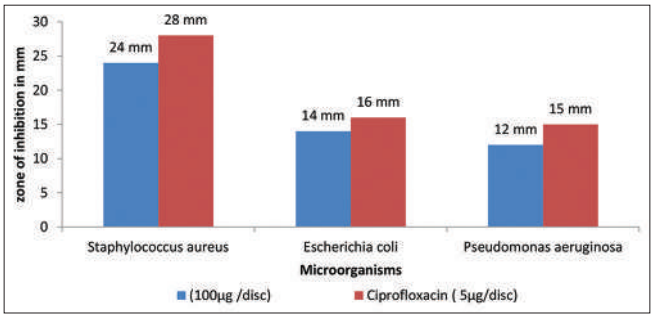

Figure 10: Antibacterial activity of the *Umaththankai parpam and* results shows high sensitivity against the Gram-positive cocci *Staphylococcus aureus* compared to Gram-negative bacteria *Escherichia coli and Pseudomonas aeruginosa*

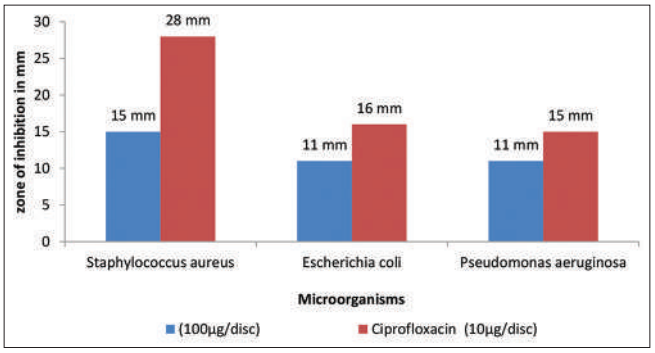

Figure 11: The well-diffusion method and it was found to be comparatively less effective than disc diffusion method. Although the *Umaththankai parpam* showed inhibitory activity against bacterial pathogens, it was less effective than the commercial antibiotics ciprofloxacin

The extract of *Umaththankai parpam* exhibited good antifungal activity against all test fungal microorganisms while compared with standard fungal antibiotic fluconazole. The maximum IZD (inhibition zone of diameter) of 16 mm was observed in fluconazole (10 µg) against *C. albicans*, 10 mm was observed against *A. niger,* and *T. rubrum* 15 mm was observed against *P. aeruginosa*. It was observed that the extract exhibited the highest antifungal activity against

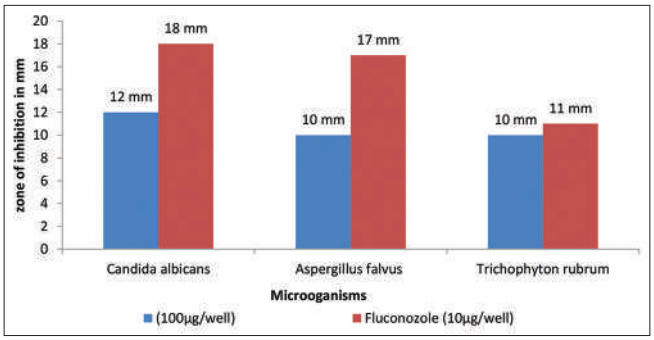

Figure 12: Antifungal activity of *Umaththankai parpam* and showed comparatively higher sensitivity against *Candida albicans* than the other two opportunistic pathogen *Aspergillus flavus* and *Trichophyton rubrum*

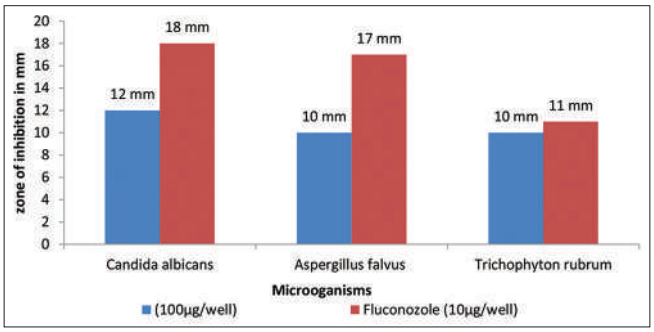

Figure 13: About antifungal activity of the drug. As for as antifungal activity tests, there is no significant difference in the sensitivity pattern but it was found to be almost similar in disc diffusion method and well-diffusion method. Among the three chosen fungal pathogens, *Candida albicans* showed little higher sensitivity than other two

C. albicans with inhibition zone 14 mm when compared with the standard drug with inhibition zone of 16 mm in disc diffusion method.

The inhibition zone of diameter was obtained in well-diffusion method, *S. aureus* and *P. aeruginosa* with the diameter 15 mm and 11 mm, respectively. Similarly, the extract showed the inhibition zone with diameter of 12 mm in *C. albicans* and 10 mm in *T. rubrum* and *A. niger.* The antifungal activity of the fluconazole and the *Umaththankai parpam* were found to be nearly similar since the fluconazole is the drug of choice for *C. albicans.* Figure 11 depicts the well diffusion method and it was found to be comparatively less effective than disc diffusion method. Though the *Umaththankai parpam* showed inhibitory activity against bacterial pathogens, it was less effective than the commercial antibiotics ciprofloxacin. Figure 12 shows Antifungal activity of *Umaththankai parpam* and showed comparatively higher sensitivity against *Candida* albicans than the other two opportunistic pathogen *Aspergillus flavus* and *Trichophyton rubrum*. Figure 13 depicts about antifungal activity of the drug. As for as antifungal activity tests, there is no significant difference in the sensitivity pattern but it was found to be almost similar in disc diffusion method & well diffusion method. Among the three chosen fungal pathogens *Candida albicans* showed little higher sensitivity than other two [Table 6a-6d].

DISCUSSION

The present work has been carried out on the scientific evaluation of phytochemicals and antimicrobial potential of "*Umaththankai parpam.*" The phytochemicals identified from the *Umaththankai parpam* through qualitative screening were alkaloids, flavonoids, saponins, tannins, phenols, glycosides, and fixed oils which are secondary metabolites of plants that serve as defense mechanisms against predation by many microorganisms, insects, and herbivores.[11]

Phytochemicals derived from plant products functions as a prototype to develop less toxic and more effective medicines in controlling the microbial growth.[12] Several plants and spices containing flavonoid derivatives have found application as disease preventive and therapeutic agents in traditional medicine in Asia for thousands of years.[13] Flavonoids are mentioned as nature's biological response modifiers due to strong experimental evidence of their inherent ability to switch the body's reaction to allergies, virus, and carcinogens.[14] The analysis shows that *Umaththankai parpam* was found rich of flavonoids. Saponins from plants sources are also liable for some pharmacological effects such as anti-inflammatory, molluscicidal, antimicrobial, antispasmodic, antidiabetic, anticancer, hypocholesterolemic, antioxidant, anticonvulsant, analgesic, anthelmintic, antitussive, and cytotoxic activities.[15] Alkaloids, flavonoids, tannins, phenols, and saponins were used to function as antioxidants, antibacterial, antifungal, and antiviral.[16] The presence of tannins inhibits the pathogenic fungi and improves the antimicrobial activity.[17] The phytochemicals alkaloids, flavonoids, saponins, tannins, phenols, glycosides, and oils were present in the study drug *Umaththankai parpam* has the effective therapeutic properties. This was evidenced and supported by the work of above-mentioned researchers.

The major compounds identified from the *Umaththankai parpam* through GCMS were n-Hexadecanoic acid ($C_{16}H_{32}O_2$), 9,12-Octadecadienoic acid (Z,Z)- ($C_{18}H_{32}O_2$), 9,12-Octadecadienoyl chloride (Z,Z) (linoleic acid chloride), $C_{18}H_{31}ClO$, and 9,12-Octadecadienoic acid (Z,Z)- ($C_{18}H_{34}O$). n-Hexadecanoic acid (54.24%) was the minor compound that had been found within the *Umaththankai parpam*. This phytochemical compound was reported to possess larvicidal activity, antibacterial, antifungal, antioxidant activity, hypocholesterolemic, nematicide, pesticide, antiandrogenic flavor, and hemolytic activity.[18] 9,12-Octadecadienoic acid (Z,Z)- (12.97%) was the carboxylic acid that can be found in the *parpam*. These carboxylic acid was reported to possess various uses such as antimicrobial,[19] anti-inflammatory, and anti-tumor activity.[20] 9,12-Octadecadienoyl chloride (Z,Z), (linoleic acid chloride) (15.63%), and 9,12-Octadecadienoic acid (Z,Z)- (0.54%) were reported to have anti-inflammatory,

hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, antieczemic, and anti-acne.[21]

FTIR instrumental analysis was done through IR Tracer 100. The tested drug was identified to possess eight peaks values. They are the functional groups present within the *Umaththankai parpam*. Table 4 and the Figure 4 show the presence of aliphatic compounds, C–H stretch of methyl and methylene groups, alkenyl group, sulfate groups, sulfonate groups, the iron oxide, silicone, and ferrous sulfate compound. Vikesh *et al*., 2018,[22] stated that these functional groups are liable for the therapeutic effect of the drug.

Medicinal plants contain rich amount of trace elements which increase the curative effect.[23] The trace elements and its oxide forms detected within the *Umaththankai parpam* are given in Table 5 which were found in descending sequence, that is, sulfur (S), potassium (K), chlorine (CL), lead (Pb), calcium (Ca), silicon (Si), phosphorus (P), magnesium (Mg), and zinc (Z). Sulfur is anticancerous, lowers blood cholesterol and skin infirmities.[24] The presence of silicon in these *parpam* is reported for the 1st time. This element is vital because it is required for skeletal and animal tissue growth, anticancerous, antidiabetic, and antioxidant.[25] Zn has antioxidant property and enhances the function of immune system, DNA replication, olfactory, and taste senses.[26] K is a vital electrolyte for sustaining fluid equilibrium in cells and has defensive role against hypertension, kidney stones, and osteoporosis.[27] Phosphorous participates in genetic material formation and energy management also for the development of strong bone and teeth and tissue repair.

Several reports are available that indicate the antibacterial capacity of extract against bacteria.[28] Numerous studies are conducted with the extracts of many plants, screening antimicrobial activity also for the invention of new antimicrobial compounds.[29]

In the present investigation, *Umaththankai parpam* was evaluated for exploration of their antimicrobial activity against certain bacteria and fungi which was considered as human pathogenic microorganism. Susceptibility of extract was tested by agar disc diffusion and agar well-diffusion method was evaluated. In the present study, the preliminary investigation showed that the extract of the *Umaththankai parpam* was active against the human pathogens such as *E. coli*, *S. aureus,* and *C. albicans*. Out of the two methods used for screening the antimicrobial study, the disc diffusion method showed the maximum activity against the test organisms than the well-diffusion method.

Satnami and Yadav, 2016,[30] also reported that the phytochemical seed analysis of *D. metel* revealed the presence of saponins, tannins and alkaloids, glycosides, and flavonoids and also tested that tannins, glycosides, and alkaloids were present in the methanolic compounds of the plant. Compounds were active against *E. coli*, *P. aeruginosa, Klebsiella pneumonia*, *E. coli, Trichoderma viride,* and *A. niger*, medically important organisms. The present study was supported by the earlier findings. The presence of the secondary metabolites with the ingredient sulfur has been effectively prevented the pathogenic bacteria and fungi.

The extract of *Umaththankai parpam* possesses significant inhibitory effect against tested pathogens. The drug possesses necessary secondary metabolites and proved the presence of active therapeutic chemical compounds and possesses effective antimicrobial activity. The test drug *Umaththankai parpam* extract also found actively suppresses the pathogenic activity of the infectious bacteria and fungi. The findings of the study lead the development of new antimicrobial drugs.

CONCLUSION

The test drug was prepared as per the standard operative procedure mentioned in Siddha literature. All the ingredients were identified and authenticated by the experts. The drug *Umaththankai parpam* formulated using unripen fruit of *D. metel* L. as key ingredients was found be containing many vital compounds which was reported to be an anti-inflammatory compounds and many compounds' therapeutic potentials are yet be explored. In addition to its anti-inflammatory compounds, the extract has showed its antibacterial and antifungal activities. Antibacterial activity of the *Umaththankai parpam* results showed high sensitivity against the Gram-positive cocci *S. aureus* compared to Gram-negative bacteria *E. coli* and *P. aeruginosa.* Among the three chosen fungal pathogens, *C. albicans* showed little higher sensitivity than other two which was an opportunistic infection causing fungi and its recent outbreak among COVID-19 patients was alarming.

The present study concluded that the drug possesses necessary secondary metabolites and proved the presence of active therapeutic chemical compounds and possesses effective antimicrobial activity. The present study has given more insights about the elements, phytochemicals, and the antimicrobial activities of the formulated siddha drug *Umaththankai parpam.*

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