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Antimicrobial activity and Quality Control Parameters of *Talicati Vatakam* - A Classical Siddha Drug

Research Article

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Abstract

Talicati Vatakam (TSV), a polyherbal siddha drug was chosen, it was screened for antimicrobial study and also subjected to standardization parameters. The ingredients were procured, authenticated and prepared the drug as per standard operating procedure. The ethanolic extract of TSV was screened for nine bacteria and two fungi. Then the drug was investigated for the phytochemical profile, physicochemical parameters, thin layer chromatographic photo documentation (TLC), high-performance thin-layer chromatography (HPTLC) finger print profile. Antimicrobial assay revealed inhibitory activity against all test pathogens. TLC under UV showed 8 bands at short wavelength, 13 bands at long wavelength; 8 bands showed post derivatization with vanillin Sulphuric acid reagent. The present investigation concluded that the siddha herbal preparation of TSV have great potential on antimicrobial against pathogens. This siddha formulation can be used to prevent the bacterial and fungal infections and the standards could be used for quality control of the drug.

Key Words: *Talicati Vatakam,* Staphylococcus aureus, Respiratory, Antimicrobial, Standardization.

Introduction

Plant based traditional system of medicine plays an important role in providing health care to large scale of population in the world (1). Medicinal herbs have been discovered and used in folklore and traditional medicine practices since pre historic times. There are approximately 75-100 kinds of herbs and flowers in the national library of medicine herb garden (2). Herbal medicines provide livelihood and health security to large segment of world population (3). In India 65% of rural population use traditional medicines for primary healthcare (4). The usage of these medicines has been increased in urban areas also due to intervention of pandemic situations. India is of six recognised traditional medicinal systems, Siddha is one among them. Siddha is one the oldest healing system of medicine more popular in southern part of India and has different fundamental aspects for drug formulation (5). Major herbal formulations are present in various dosage forms such as cūraṇam, māttirai, vaṭakam, iḷakam, meḻuku, decoctions, infusions, capsules, tablets and herbal powders (6). The therapeutic value of medicinal plants depends upon the presence of biologically active phyto-constituents. Antimicrobial medicines can be grouped according to the microorganism they act primarily against. TSV is one such formulation which destroys disease causing germs and also inhibits the growth of pathogenic microbes that cause communicable diseases.

Antibiotics provide the main basis for the therapy of bacterial infections (7). However, the high genetic variability of bacterial enables them to rapidly evade the action of antibiotics by developing antibiotic resistance. Thus there has been a continuing search for new and more potent antibiotics. Therefore, in the present investigation of TSV, a polyherbal formulation was evaluated for its antibacterial potential for the first time against selected human pathogenic bacteria which are known to cause many infectious diseases (8).

TSV is a classical Siddha polyherbal formulation in tablet form consisting of 14 ingredients enlisted in Table 1. It is used in the treatment of respiratory and digestive tract disorders. It is also used to treat diseases like fatigue, cold, cough, hypertension, loss of appetite, indigestion, vomiting, urinary tract disorders, skin disorders, diarrhoea and wide range of *kapha* diseases. The previous study revealed that the extract of *Tāḷicāti cūraṇam* exhibits potency against harmful bacteria (9). Major ingredient of TSV is *Zingiber officinale* rhizome which is a rich

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source of phytochemicals with antimicrobial activity against microorganisms (10-12). Major components of *Piper nigrum* and *Piper longum* are piperine and piperlongumine which act as antioxidant, antibacterial, antifungal and antimicrobial activities (13-16). Tāḷicapattiri (*Taxus baccata* Linn.) leaf has been reported to exhibit antibacterial and antifungal activities (17). In this study authors selected TSV for antimicrobial study and standardizing for its pharmacopoeia parameters.

Materials and Methods

Drug material

All the ingredients were purchased from local crude drug market (Govindaraja Mudaliyar shop, Chennai). The identity and authenticity of the drug was confirmed by the Dr.Sunil Kumar, Research Officer **(**Pharmacognosy), Siddha Central Research Institute, Chennai.

Chemicals and solvents

Analytical grade solvents toluene, ethyl acetate, ethanol and methanol were purchased from Merck. Vanillin sulphuric acid (5% sulphuric acid in methanol v/v) was used for visualisation. For microbial activity Cefepime and fluconazole were prepared in DMSO.

Instruments

For HPTLC, Auto sampler, ATS4, Visualizer, Scanner 4(Scanner_210441) linked with WINCATS software, twin trough chamber and TLC plate heater (all are from CAMAG, Switzerland) were used. For antimicrobial activity Autoclave, laminar flow, BOD incubator were used.

Sample preparation

All the 14 ingredients were taken in a vessel and stretched a cloth loosely over the mouth of the vessel securing firm binding. The drugs 1 to 12 were powdered and mixed with *karumpu vellam*. The mixture was kept in the cloth and covered with a suitable lid. The vessel was heated till the drug mixture thoroughly mixed in steam heat. The cooked drug mixture ground in a mortar, roll into one gram pills and dried. The pills were ground to a coarse powder for homogeneous extraction purpose. 5g of coarse powder was extracted with 100 ml hexane and 100 ml of ethanol by Soxhlet separately. Then extracts were filtered and evaporated. The residues were redissolved in 10 ml of each respective solvents and taken into separate sample vial for TLC/HPTLC study.

HPTLC Analysis

Analysis was performed on (6cm x 10cm) silica gel ($60F_{254}$) pre-coated aluminium plate. Ten µl of ethanolic extract of TSV was applied by ATS4 sampler with band width 8 mm and 10 mm from the bottom of the plate. Afterwards the plate was placed in a presaturated twin trough chamber (10 cm x 10 cm) containing mobile phase toluene: ethyl acetate (5:1.5 v/v) and developed till 85 mm. The developed plate was dried and photographs were taken under short UV at λ254 nm and long UV at λ366 nm followed by scanning at λ254 nm (absorption mode, D_2 lamp) and at λ366 nm (Fluorescence mode, Hg lamp) with a slit dimension 6x0.45 nm and scanning speed of 20 mm/s. The plate was dipped in vanillin sulphuric acid reagent and heated at 105°C till the appearance of coloured bands. Photograph was taken under white light followed by scanning at λ520 nm (absorption mode, W lamp).

Preparation of drug extracts for antimicrobial activity

Hundred mg of ethanolic extract of TSV was weighed accurately and dissolved in 1ml of dimethyl sulfoxide (DMSO) to make the stock solution of 100 mg/ml. Cefepime 30 µg/disc for bacteria and fluconazole 10 µg/disc for fungi were taken as a positive control and DMSO negative control for antimicrobial susceptibility test.

Culture and maintenance of microorganisms

The cleaned glassware were sterilized in hot air oven at 180^{0}C for 1 hour. All nutrients were sterilized by autoclave at 121^{0}C, 15 lbs for 20 minutes. Pure cultures of all experimental bacteria and fungi were obtained from the Microbial Type Culture Collection and gene Bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh. The pure bacterial cultures were maintained on nutrient agar medium and fungal cultures were further maintained by sub-culturing regularly on the same medium and stored at 4^{0}C before use in experiments.

For maintenance of cultures, Muller Hinton agar medium (for bacteria), Sabouraud's dextrose agar medium (for fungi) were prepared as per standard methods (18). Antimicrobial activity was determined by agar well diffusion method.

Agar well diffusion method

Agar well-diffusion method was followed to determine the antimicrobial activity. Muller-Hinton agar (MHA) and Sabouraud's Dextrose Agar (SDA) plates were swabbed (sterile cotton swabs) with 8 hour old-broth culture of respective bacteria and fungi. Wells (10 mm diameter and about 2 cm a part) were made in each of these plates using sterile cork borer. 100 µl, 75 µl, 50 µl and 25 µl of different volume of TSV extracts were added into the wells and allowed to diffuse at room temperature for 2 hours. Cefepime (5 µg/disc), fluconazole (10 µg/disc) act as positive control. The plates were incubated at 37^{0}C for 24 hours for bacterial pathogens and 48 hours at 28^{0}C for fungal pathogens. The diameter of the inhibition zone (mm) was measured and the activity index was also calculated. Triplicates were maintained and the experiment was repeated thrice, for each replicates the readings were taken in three different fixed directions and the average values were calculated.

Table 1: List of ingredients of TSV

S.No	Tamil name	Botanical name	Part used	Quantity
1	Tāḷicapattiri	*Taxus baccata* Linn	Leaf	4 parts
2	Milaku	*Piper nigrum* Linn	Fruit	4 parts
3	Cevviyam	*Piper nigrum* Linn	Root	4 parts
4	Tippili	*Piper longum* Linn.	Fruit	8 parts
5	Tippili mulam	*Piper longum* Linn.	Root	8 parts
6	Cukku	*Zingiber officinale* Rosc.	Rhizome	12 parts
7	Lavaṅkap paṭṭai	*Cinnamomum zeylanicum* Linn	Bark	1 part
8	Lavaṅkap pattiri	*Cinnamomum zeylanicum* Linn	Leaf	1 part
9	Kuṟu vēr	*Coleus vettiveroides* K.C.Jacob	Root	1 part
10	Ēlam	*Elettaria cardmomum* Linn	Fruit	1 part
11	Ciṟu nākappū	*Cinnamomum wightii* Meisn	Flower bud	1 part
12	Cittarattai	*Alpinia calcarata* Rosc.	Root	1 part
13	Karumpu vellam	*Saccharum Officinarum*	-	64 parts
14	Pacum pal	*Bos indicus*	-	Sufficient quantity

Physicochemical screening of TSV

The physicochemical parameters were determined by standard methods (19).

Preliminary phytochemical analysis of TSV

Phytochemical screening of ethanolic extract of TSV was done by standard qualitative phytochemical procedures (20).

Results

Physicochemical results

The physicochemical parameters of TSV are shown in Table 2.

Table 2: Physicochemical parameters of TSV

S.No	Parameter	Mean(n=2) SD
1	Loss on Drying at 105°C	13.37 ±0.77 %
2	Total ash	24.04 ±0.62 %
3	Water soluble ash	22.01 ±0.67 %
4	Acid insoluble ash	0.245 ±0.04 %
5	Water soluble extractives	68 ±1.39 %
6	Alcohol soluble extractives	28.8 ±1.12 %
7	pH (10% solution)	6.54±0.20 %

Phytochemical analysis

The phytochemical analysis revealed the presence of secondary metabolites which are shown in Table 3.

Table 3. Phytochemical analysis of ethanolic extract of TSV

S.No	Name of the test	Inference
1	Alkaloids (Dragendorff's test)	+
2	Flavonoids (Shinoda test)	+
3	Glycosides	-
4	Steroids (Lieberman Burchard test)	+
5	Triterpenoids (Noller's test)	+
6	Coumarins	-
7	Phenols	+
8	Tannins (Lead acetate test)	+
9	Saponins	-
10	Proteins (Biuret test)	+
11	Reducing sugar (Fehling's reagent)	+
12	Anthraquinones	-

TLC Photo documentation

TLC photo documentation of hexane extract of TSV (Fig.1) showed 8 bands with R_f 0.11, 0.15, 0.22, 0.27, 0.34, 0.49, 0.58, 0.68 (all green) under short UV ; 10 bands appeared with R_f 0.06 (blue), 0.08 (blue), 0.12 (green), 0.16 (red), 0.22 (blue), 0.31 (blue), 0.37 (blue), 0.48 (blue), 0.57 (yellowish green) and 0.67 (blue) under long UV and 11 bands appeared with R_f 0.06 (violet), 0.11 (yellow), 0.16 (violet), 0.22 (violet), 0.28 (violet), 0.39 (pink), 0.51 (violet), 0.60 (violet), 0.70 (violet), 0.81 (violet), and 0.85 (violet) under white light for post derivatized plate.

TLC photo documentation of ethanolic extract of TSV (Fig.2) revealed 8 bands with R_f 0.15, 0.22, 0.27, 0.29, 0.34, 0.40, 0.46, 0.54 (all green) under short UV; 13 bands with R_f 0.07 (pink), 0.21 (blue), 0.24 (pink), 0.26 (red), 0.29 (fluorescent blue), 0.47(fluorescent blue), 0.53(pink), 0.56(sky blue), 0.60(red), 0.62(fluorescent green), 0.64(red), 0.76(blue) and 0.92(blue) under long UV and 8 bands with R_f 0.10 (light yellow), 0.21 (dark green), 0.27 (light pink), 0.42 (red), 0.53 (green), 0.72 (pink) and 0.74 (pink) under white light for post derivatization plate.

Figure 1. TLC/HPTLC of hexane extract of TSV

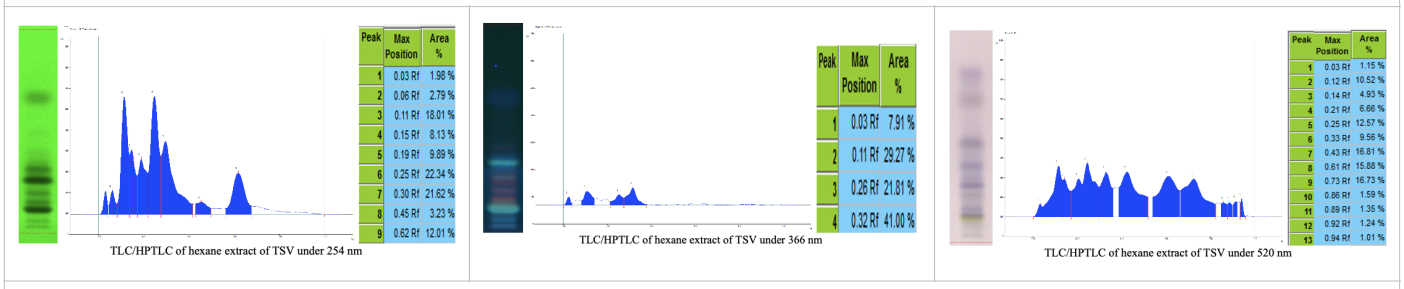
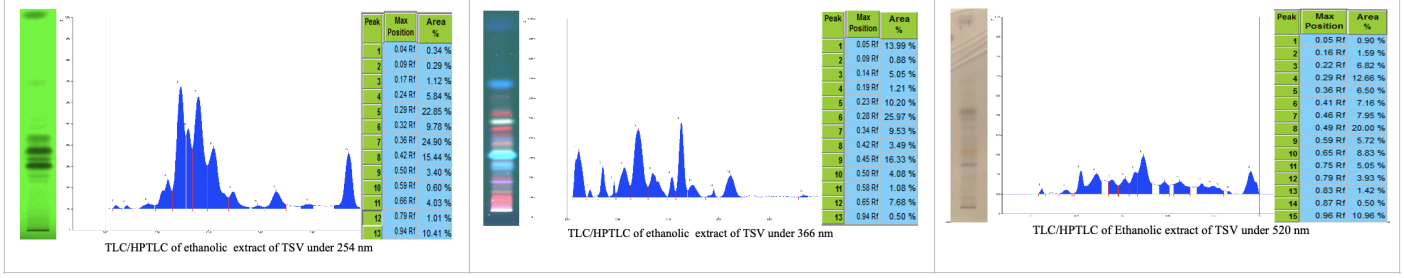


Figure 2. TLC/HPTLC of ethanol extract of TSV



HPTLC Densitometric scan

The densitometric scanning of Hexane extract of TSV at λ254 nm revealed 9 peaks at R_f 0.03 (area 1.98%), 0.06 (2.79%), 0.11 (18.01%), 0.15 (8.13%), 0.19 (9.89%), 0.25 (22.34%), 0.30 (21.62%), 0.45 (3.23%), 0.62 (12.01%) under short UV, at λ366 nm revealed 4 peaks at R_f 0.03 (area 7.91%), 0.11 (29.27%), 0.26 (21.81%), 0.32 (41%) under long UV and derivatized plate under white light at λ520 nm shown 13 peaks at R_f 0.03 (area 1.15%), 0.12 (10.52%), 0.14 (4.93%), 0.21 (6.66%), 0.25 (12.57%), 0.33 (9.56%), 0.43 (16.81%), 0.61(15.88%), 0.73(16.73%), 0.86(1.59%), 0.89(1.35%), 0.92(1.24%), 0.94(1.01%).

The densitometric scanning of the ethanolic extract of TSV at λ254 nm revealed 13 peaks at R_f 0.04 (area 0.34%), 0.09 (0.29%), 0.17 (1.12%), 0.24 (5.84%), 0.29 (22.85%), 0.32 (9.78%), 0.36 (24.90%), 0.42 (15.44%), 0.50 (3.40%), 0.59 (0.60%), 0.66 (4.03%), 0.79 (1.01%), 0.94 (10.41%). at λ366 nm revealed 13 peaks at R_f 0.05 (area 13.99%), 0.09 (0.88%), 0.14 (5.05%), 0.19 (1.21%), 0.23 (10.20%), 0.28 (25.97%), 0.34 (9.53%), 0.42 (3.49%), 0.45 (16.33%), 0.50 (4.08%), 0.58 (1.08%), 0.65 (7.68%), 0.94 (0.50%) and derivatized plate under white light at λ520 nm revealed 15 peaks at R_f0.05 (area 0.90%), 0.16 (1.59%), 0.22 (6.82%), 0.29 (12.66%), 0.36 (6.50%), 0.41 (7.16%), 0.46 (7.95%), 0.49 (20%), 0.59 (5.72%), 0.65 (8.83%), 0.75 (5.05%), 0.79 (3.93%), 0.83 (1.42%), 0.87 (0.50%), 0.96 (10.96%).

Antimicrobial activity

At 75µl and 100µl in ethanolic extract showed positive results against gram-positive *Staphylococcus aureus, Enterococcus faecalis* and Gram-negative *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Proteus vulgaris*. Also the drug showed significant results against the fungal *Candida albicans* and *Candida tropicalis.*

Table 4: Showing the zone of Inhibition

Sl. No	Organism	Zone of Inhibition (mm) Concentration (µl/ml)					
		Positive control	100	75	50	25	Negative control
1	*Staphylococcus aureus* (Gram-positive)	24	12	10	-	-	-
2	*Streptococcus pneumoniae* (Gram-positive)	28	11	-	-	-	-
3	*Bacillus subtilis* (Gram positive)	25	12	-	-	-	-
4	*Enterococcus faecalis* (Gram positive)	25	16	14	12	-	-
5	*Klebsiella pneumoniae* (Gram negative)	25	12	10	-	-	-
6	*Pseudomonas aeruginosa* (Gram negative)	24	12	10	-	-	-
7	*Escherichia coli* (Gram negative)	30	12	10	-	-	-
8	*Proteus vulgaris* (Gram negative)	30	15	12	-	-	-
9	*Candida albicans*	35	18	15	12	-	-
10	*Candida tropicalis*	27	18	15	-	-	-

Discussion

The moisture content of the drug represented by loss on drying and it was found to be 13.37±0.765%. Total ash which represents the measure of inorganic

content ie., amount of minerals present in the formulation was found to be 24.04±0.615%. Water soluble ash and acid insoluble ash were determined as 22.01±0.665 % & 0.245±0.045% respectively. The water soluble extractive and alcohol soluble extractives were calculated as 68±1.385 % & 28.8±1.12% respectively. The pH of the drug was measured as 6.54 that represents acidic nature of the drug.

Distinct separation of bands is found in all the three wave length regions (254 nm, 366 nm and 520 nm) for each hexane extract and ethanol extract of the drug TSV. Each band represents a distinct compound of which potency can be confirmed by further studies. The densitometric scanning of Hexane extract of TSV revealed major peaks at R_f 0.11 (area 18.01%), 0.15 (8.13%), 0.19 (9.89%), 0.25 (22.34%), 0.30 (21.62%), 0.62 (12.01%) Under short UV, at λ366 nm revealed major peaks at R_f 0.03 (area 7.91%), 0.11 (29.27%), 0.26 (21.81%), 0.32 (41%) and derivatized plate under white light at λ520 nm major peaks at R_f 0.12 (area 10.52%), 0.25 (12.57%), 0.33 (9.56%), 0.21 (6.66%)The densitometric scanning of the ethanolic extract of TSV under short UV at λ254 nm revealed major peaks at R_f 0.29 (area 22.85%), 0.32 (9.78%), 0.36 (24.90%), 0.42 (15.44%), 0.94 (10.41%), at λ366 nm revealed major peaks at R_f 0.05 (area 13.99%), 0.23 (10.20%), 0.28 (25.97%), 0.34 (9.53%), 0.45 (16.33%), 0.65 (7.68%) and derivatized plate under white light at λ520 nm revealed major peaks at R_f 0.22 (area 6.82%), 0.29 (12.66%), 0.36 (6.50%), 0.41 (7.16%), 0.46 (7.95), 0.49 (20%), 0.59 (5.72%), 0.65 (8.83%), 0.75 (5.05%), 0.79 (3.93%),0.96 (10.96%).

This poly-herb composition is effective in treatment of various diseases as they are rich in essential Phytochemicals (21).

Staphylococcus aureus is most dangerous staphylococcal bacteria which is in coccal shape and cause heart valve infections. It is leading cause of skin and soft tissue infection (SSTIs), present on skin and nose of a person able to spread from one another by direct contact (22). *Enterococcus faecalis* found in gastro intestinal tracts of humans and mammals. This can cause life threatening infections especially in nosocomial environment (23). *Escherichia coli* is facultative anaerobic, rod shaped coliform bacterium commonly found in lower intestine of warm blooded organisms. This can cause food poisoning (24). *Klebsiella pneumoniae* is facultative anaerobic, rod shaped bacterium. It can be found in mouth, skin and intestinal tract. It can cause severe bacterial infections leading to pneumoniae, blood stream infections, wound infections and urinary tract infections (25). *Pseudomonas aeruginosa* is a rod shaped bacterium can cause diseases in plants and animals. It is associated with serious illness such as ventilator-associated pneumoniae and various sepsis syndromes (26). *Proteus vulgaris* is a rod shaped hydrogen sulphide producing bacterium present in intestinal tracts of humans and animals. It is known to cause wound infections and urinary tract infections (27). *Candida albicans* is a pathogenic yeast detected in the gastro intestinal tract and mouth of humans. Candida causes candidiasis which results in the over growth of fungus, observed in HIV infected patients (28). *Candida tropicalis* is a species of yeast in the genus candida. It is common pathogen in neutropenic hosts, it may spread through the blood stream to peripheral organs (29).

The combination of multiple herbs has increased the efficiency of the TSV to greater extent due the presence of the secondary metabolites viz., alkaloids, flavonoids, triterpenoids, tannins and proteins. Flavonoids are known to be synthesized by plants in response to microbial infection (30). Tannins are also known as antibacterial agents and have been reported to prevent the development of microorganisms by precipitating microbial protein (31). The growth of many fungi, yeast, bacteria and virus were initiated by this compound. Alkaloids may be useful for the treating cough and has anti-tumour, analgesic properties (32) and steroids contains anti-inflammatory activity (33). Antibacterial activity shows good results against bacteria at 75 µl, 50 µl, *E. faecalis* shows good result at 50 µl and showed positive results against the fungal *C. albicans* and *C. tropicalis*.

Conclusion

In this study, a polyherbal formulation, TSV was evaluated for its antimicrobial, physicochemical & phytochemical and HPTLC studies. Presence of active secondary metabolites were recorded. It is concluded that the ethanol extract of TSV is active against gram positive and gram negative bacteria. The zone of inhibition varied suggesting the varying degree of efficacy and different phytochemical constituents of herb on the target organism. Natural medicines always have less side effects so it is best replacement for treatment of challenging diseases at present. Further studies are needed to isolate and characterize the bioactive compounds to develop new antimicrobial drugs.

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Conflict of interest

The authors declare no conflict of interest.

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