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Antimicrobial activity and *Escherichia coli* biofilm destruction potency of *Siddha* formulation *Sagadevinei*

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The primary objective is to evaluate the microbial efficacy and biofilm destruction potency of Siddha polyherbal formulation "*Sagadevinei*'' and secondary objective is to correlate the ingredients with *Siddha* principles such as *Suvai* (Taste) and *Panchabootha thathuvam* (five elements theory). The *In vitro* microbial activity of *Sagadevinei* (SDN) has been carried out through Agar well diffusion method and evaluated the *Escherichia coli* biofilm destruction potency, by growing of *Escherichia coli* to form biofilm in coverslip. The coverslip insisted with biofilm were stained by Acridine Orange (AO Staining), and the potency has observed under fluorescent microscope. The result shows significant microbial activity in five common microorganisms and the drug revealed marked potency against *E. coli* strain. Further bio-film destruction potency of the test drug was also carried out and discussed. There is a huge need for a drug from herbal origin to tackle the situation of a post antibiotic era. In day to day clinical practice, almost 80% of cases are from *Escherichia coli*, Gram-negative bacteria alone. To overcome the antibiotic resistance, reinfection, and reversion, the novel herbal replacements are quite effective in nature, easily affordable and safe.

Keywords: Burning micturition, *Neersurukku, Sagadevi nei*, Siddha medicine, Urinary Tract Infection (UTI), *Vernonia cinerea*

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Sagadevinei is a Siddha polyherbal formulation mainly consisting of *Vernonia cinerea* (*Neichitti in Tamil*) referenced from *Siddhar Pothu Maruthuvam*[1]. It is widely in practice by the Traditional Practitioners of *Kanyakumari* district (Tamilnadu) for the management of *Siruneeraga Paathainoigal.* The term *Siruneeraga Paathainoigal* can be correlated with Urinary tract Infections[2] (UTI).

UTI is one of the most common medical condition creating most serious distressing and worrying symptoms like pain, lower abdominal pain, and burning micturition. The occurrence of UTI associated problems at least slaps once in the year[3]. A gram-negative bacterium, *Escherichia coli* alone is the reason in 80% of cases. The incidence of UTI is quite higher in women than in men, 40% to 50% and Lower urinary tract infection is also common among women than in men[4]. There are few shortcomings in current therapeutics causing resistance, reinfection, and reversing of infections which underlines the need for new age antibiotics[5].

On the contrary scientists and health seekers are always looking for an alternate, having its origin in nature, which is devoid of adverse effects which rules out the existing shortcomings. *Sagadevinei* is an age-old classical preparation[6] which have been used for the treatment of Urinary tract ailments[7]. We had taken this potent preparation into this research study.

The plant kingdom mentioned in *Siddha* materiamedica bears a lot of inexhaustible source of active ingredients, polyherbal combinations for the treatment of many intractable, challenging[8] and chronic diseases. Not only from the vegetable plant sections, the treatment protocol was also framed from the key ingredients taken from metal, mineral and animal products. Worldwide health status information shows that infectious diseases are the leading cause of death. Various research studies have carried out in herbal ingredients and identified various effective compounds that have similar action as antibiotics.

The key ingredient used for the preparation was the leaves of *Vernonia cineria*[9] which has a wide variety of traditional uses and well documented for its diuretic action[10], antimicrobial activity, larvicidal action[11], anti-malarial property, antilithiatic properties, antioxidant properties[12], analgesic and antipyretic

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activities[13]. Many research articles also state that the plant has gold nanoparticles which can be used to treat a variety of clinical conditions. In *Siddha*, Urinary diseases are classified into two major divisions. One is the clinical conditions which reduce urine output (*Neerarukkalnoigal* ex. Renal calculi, Acute urinary retention, Anuria, Urinary incontinence, etc) and secondly certain clinical conditions with increased urine output (*Neerinaiperikkalnoi* ex. Diabetes mellitus and Diabetes insipidus, etc.)[1]. Based on the literature review and classical text evidence, our primary objective is to evaluate the microbial efficacy and biofilm destruction capacity of *Sagadevinei*, a potent Siddha herbal formulation, and the secondary objective is to correlate the ingredients of preparation with Siddha principles such as *Suvai* (Taste) and *Panchabootha thathuvam* (Five elements theory)[18].

The chief ingredient *Vernonia cinerea* has six major triterpene compounds elucidated through GCMS[14] (Misra et al) *6-amyrin acetate, 3P-acetoxyurs- 13(18)-ene (l), P-amyrin, a-amyrin acetate, P-amyrin acetate, aamyrin,and others caryophyllene oxide, Guaiol*etc. Therapeutically helps to engulf infections and promotes immune system[15].

Materials and Methods

Collection of raw drugs

The raw drugs mentioned in Table 1 & Table 2. used for the preparations contain fresh leaf and fresh root, were collected from wet ground and others from the Traditional raw drug store, Nagercoil, Tamilnadu. The fresh leaf of *Vernonia cinerea* (*Sagadevi*) and roots of *Nymphaea pubesens* (*Allithandu*) were collected by the initial knowledge-based authentication process.

Test Materials

The cow ghee (Butter fat) was purchased from Aavin Milk Dairy farms, Tirunelveli, which is a standardized Agmark product of Government of Tamilnadu.

Authentication of raw drugs

The raw drugs were authenticated by faculties of Department of Botany and Department of *Gunapadam* (Siddha Pharmacology), Government Siddha Medical College, Palayamkottai, Tirunelveli, Tamilnadu, India. The polyherbal preparation *Sagadevi nei* was referenced from *Siddha Maruthuvam Pothu-Valakku muraigal.*

Method of preparation

The Siddha formulation *Sagadevi nei* was prepared as per SOP (Standard Operative Procedure) from

Table 1 — Ingredients list of the test drug -*Sagadevi nei*

Fresh herbs		
Neichittikeerai (Vernonia cineria)	Parts collected	Quantity
	Fresh leaves	100 *mL*
Dry raw drugs used		
Athimathuram (Glycyrrhiza glabra)	Dried root	10 *g*
Santhanam (Santalum album)	Dried bark	10 *g*
Thippili (Piper longum)	Dried fruit	10 *g*
Nannari (Hemidesmus indicus)	Dried root	10 *g*
Kadugarogini (Picro rhizakurroa)	Dried Whole part	10 *g*
Ilavangam (Syzygium aromaticum)	Dried fruit	10 *g*
Vettiver (Vettiveria zizanoides)	Dried root	10 *g*
Vilamichuver (Plectranthus vettiveroides)	Dried root	10 *g*
Allithandu (Nymphaea pubesens)	Dried stem	10 *g*

Table 2 — Classification of raw drugs based on *Suvai* (taste) and *Panchabootha thathuvam* theory (five elements theory)

Ingredients	Suvai (Taste)	Thanmai (Character)	Pirivu (Post digestive taste)
Vernonia cineria	*Kaippu*	*Veppam*	*Karppu*
Glycyrrhiza glabra	*Inippu*	*Seetham*	*Inippu*
Santalum album	*Kaippu Siruthuvarppu*	*Thapam Veppam*	*Inippu*
Piper longum	*Inippu*	*Veppam*	*Inippu*
Hemidesmus indicus	*Inippu Sirukaippu*	*Thatpam*	*Inippu*
Picorrhiza kurroa	*Kaippu Karppu*	*Veppam*	*Karppu*
Syzygium aromaticum	*Karppu Viruviruppu*	*Veppam*	*Karppu*
Vettiveria zizanoides	*Inippu*	*Thatpam*	*Inippu*
Plectranthus vettiveroides	*Kaippu*	*Seetham*	*Inippu*
Nymphaea pubesens	*Siruthuvarppu*	*Thatpam*	*Inippu*

NOTE: *Inippu* (Sweet), *Pulippu* (Sour), *Kaippu* (Bitter), *Karppu* (Pungent), *Thuvarppu* (Astringent), *Veppam* (Hot), *Thatpam* (Cold)

Department of *Gunapadam*, Government Siddha Medical College, Palayamkottai. The *Vernonia cinerea* leaf was detoxified by cleaning with fresh water and unwanted pulpy leaves were removed. It was grounded in a mortar and the fresh juice was collected without adding water. The fresh root of *Alli kilangu* was collected, dried used for the preparation process. Later all other dried ingredients were pounded finely and triturated with 50 *mL* of *Vernonia cinerea* juice and taken it as a *Karkam,* a herbal spherical mass. After that, it was poured into cow's ghee and the procedure was carried out. It is indicated for *Neer surukku* (retention of urine) and based on clinical experience used for Urinary tract infections.

Experimental method

The methods such as Siddha principle analysis, antimicrobial activity, *E. coli* biofilm destruction activity has been followed.

Siddha principle correlation with the drug

The ingredient of the drugs was correlated with the basic principles of *Siddha* pathology such as *Suvai* (taste) and *Panchabootha thathuvam* theory (five elements theory) in Table 2.

Antimicrobial activity

The antimicrobial activity has been carried out through *agar well* diffusion method and the processes were described.

Principle

The antimicrobials present in the *Sagadevinei* (SDN) extract are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of the zone of inhibition can be measured in centimeters[16,19].

Reagents used

Nutrient Agar Medium (1 L)

The medium was prepared by dissolving 28 g of the commercially available Nutrient Agar Medium (*Hi-Media*) in 1000 *mL* of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 min. The autoclaved medium was mixed well and poured onto 100 *mm* Petri plates (25-30 *mL*/plate) while still molten[17].

***Nutrient broth** (1L)*

One litre of nutrient broth was prepared by dissolving 13 *g* of commercially available nutrient medium (*Hi-Media*) in 1000 mL distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 min.

Streptomycin: a standard antibacterial agent was used as a control with the concentration of 10 *mg/mL.*

Procedure

Petri plates containing 20 *mL* Muller Hinton medium were seeded with the 24 h culture of bacterial strains such as *Escherichia coli*, *Pseudomonas auroginosa, Staphylococcus aureus, Streptococcus mutans,* and *Klebsiella pneumonia* wells of approximately 10 *mm* was bored using a well cutter and sample of 25, 50, and 100 *µL* concentration were added. The plates were then incubated at 37°C for 24 h. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993). *Streptomycin* was used as a positive control. Concentration of Sample Stock: 1 *mg/mL* DMSO

***E. coli* biofilm destruction activity (AO staining)**

E. coli biofilms were established in coverslips by incubating them for a week. The sample was added to the biofilm and was kept for incubation for 24 h at 37ºC. The untreated biofilm was taken as control. After incubation, the coverslips were thoroughly washed with PBS (Phosphate-buffered Saline) without disturbing the attached biofilm. The coverslips were then mounted on to glass slides. The biofilms were stained with a drop Acridine Orange (AO staining) for 10 min at dark. The stain was washed off with PBS and another coverslip was mounted above it. The dark condition was provided after staining to get the staining. The glass slides were observed under a fluorescent microscope for the visualization of stained biofilm.

Results and discussion

Based on *Suvai* (taste) concept, the UTI are mainly due to an elevation of *pitham* in the body. Each ingredient was analyzed in Table 1 that showed almost equal number of ingredients with *Kaippu* (50%) and *Inippu* (40%) (Fig. 1 & Fig. 2), which pacifies the dominant *Pitham* as the result the disease burden can be reduced. The symptoms of UTI are also related to *pitham* oriented and it can be pacified by *Neer* (Water) and *Prithuvi* (Earth) *Bootham*. By comparing this basic principle, which help to understand more about the treatment selection pattern based upon the combination of drugs.

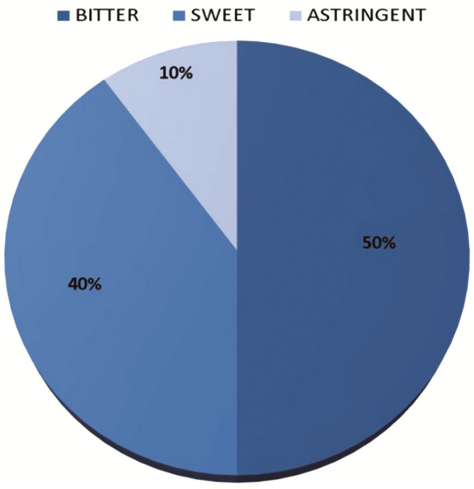

Fig. 1 — Distribution of taste contents in each of the ingredients of *Sagadevi nei*

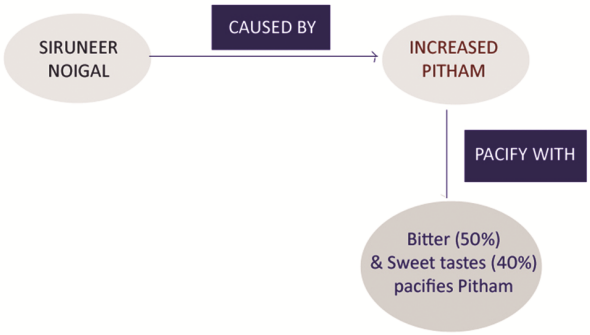

Fig. 2 — Diagrammatical representation of Pathological aspects of UTI and pacifying process based on tastes (*Suvai)*

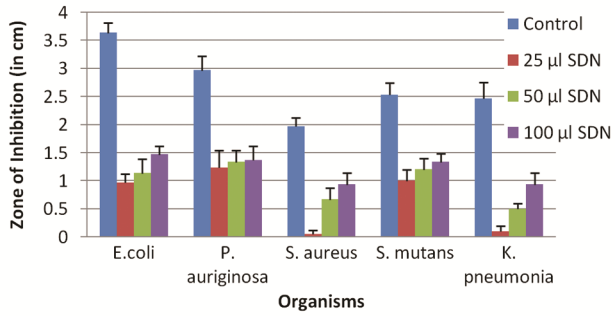

Fig. 3 — Graphical representation of the antimicrobial activity of *Sagadevi nei*

The anti-microbial activity of *Sagadevinei* mentioned in Table 3 shows significant antibacterial engulfing potency. It can help in evading the microbial infections especially *E. coli* and Pseudomonas auroginosa, which is clearly mentioned in Fig. 3. The *E. coli* biofilm has cultured over coverslip and AO Staining has made as given in the procedure. The result of this study shows in Fig. 4 and Fig. 5 clearly show that the test drug has significant *Escherichia coli* biofilm destruction potency. Fig. 5 clearly shows that live cells are stained in green where as dead cells are stained in orange-red.

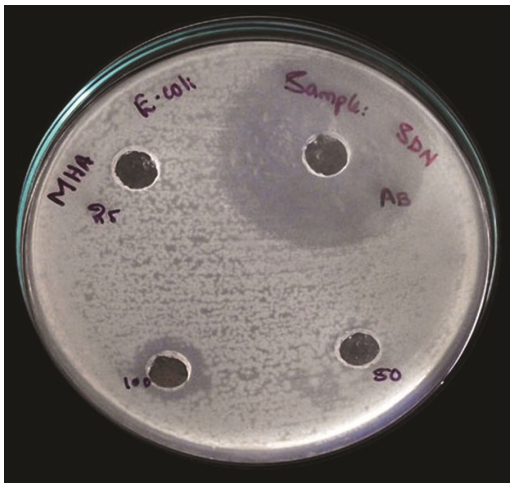

Fig. 4 — *E. Coli* microbial activity of *Sagadevi nei* (SDN) and its Zone of inhibition against *Streptomycin*

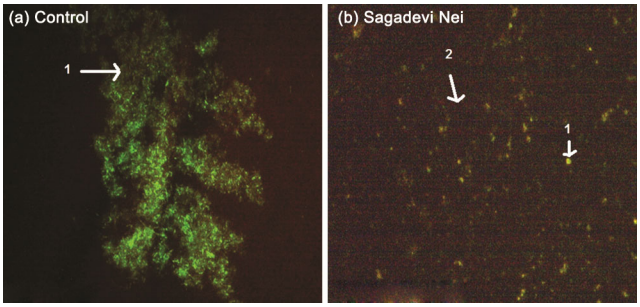

Fig. 5 — Images of *E. coli* biofilm destruction potency of *Sagadevi nei* (AO Staining). In a) Control b) *Sagadevi nei* live cells are stained with green (1) whereas dead cells are stained in Orange-red (2)

Table 3 — Antimicrobial Inhibition Zone of *Siddha* Medicine *Sagadevinei*

	Escherichia coli	*Pseudomonas auroginosa*	*Staphylococcus aureus*	*Streptococcus mutans*	*Klebsiella pneumonia*
Control (*Streptomycin*)	3.6±0.18	2.97±0.25	1.97±0.15	2.53±0.21	2.47±0.29
25 *µL* SDN	0.97±0.15	1.23±0.31	0.05±0.07	1±0.2	0.1±0.1
50 *µL* SDN	1.13±0.25	1.3±0.21	0.67±0.21	1.2±0.2	0.5±0.1
100 *µL* SDN	1.467±0.15	1.37±0.25	0.93±0.21	1.3±0.15	0.93±0.21

SDN=Sagadevinei, Values are expressed as Mean ± Standard deviation (SD), (n=3).

The Test sample (Fig. 5) has the presence of orange red-stained cells shows dead bacteria in the biofilm.

Conclusion

A novel herbal antimicrobial drug discovery is need of the hour and many infectious diseases have been treated with herbal formulations throughout the history of mankind. The herbal formulations can be used by all age groups. Some traditional remedies have already produced many compounds that are more effective against clinically significant bacterial strains. Similarly, the test drug S*agadevinei* has a significant role to combat the current situation (i.e., Post antibiotic era)[20] in combination with supportive medications mentioned in materiamedica. The drug S*agadevinei* can be used to treat the recurrent UTIs and causatives of *Escherichia coli*. Based upon the leads received, it is the appropriate time to move for experimental randomized control trials (RCTs) to prove its clinical evidence to the scientific world. We the team is yet to carry out furthermore docking studies in this drug and to uphold the drug on scientific platforms.

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Conflict of interest

The authors have declared that no conflict of interest exists.

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