JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2024/35671

SHORT COMMUNICATION

Analysis of Antimicrobial and Antihistaminic Activity of *Siddha* Medicine *Sarva Sangaara Uppu Parpam*

Barathi Kannamma Govindarasu[1*], Kavitha Kannan[2], Bharathkumar Annamalai[3], Ramalingam Shanmugam[4] and D. Mantela[5]

[1]Department of Noi Naadal Government Siddha Medical College, Chennai – 600106, Tamil Nadu, India; barathi097@gmail.com
[2]National Institute of Siddha, Chennai – 600047, Tamil Nadu, India
[3]Almaa Clinic, Vellore – 632001, Tamil Nadu, India
[4]ArutJothi Siddha Clinic, Thanjavur – 613005, Tamil Nadu, India
[5]Tamil University, Thanjavur – 613010, Tamil Nadu, India

Abstract

Background: Respiratory disorders lead to mental stress and reduce the quality of life. Hence, there is a need to intervene with traditional healthcare systems. One such healthcare system being practised in the Southern Peninsula is *Siddha*. *Siddhars* used herbs, inorganic substances, and animal products to formulate a medicine for maintaining the balance of trihumors (*Vali, Azhal* and *Aiyam*). *Sarva Sangaara Uppu Parpam* is one of the formulations made using herbs indicated for respiratory disorders. **Aim:** The current study was carried out to analyse the antihistamine and antimicrobial of *Siddha* medicine *Sarva Sangaara Uppu Parpam* (SSUP). **Methods:** Antihistaminic activity was done by isolated chick ileum method and anti-microbial activity for respiratory pathogens like *Streptococcus pneumoniae, Staphylococcus aureus, Klebsiella pneumonia, Candida albicans, Escherichia coli, Pseudomonas aeruginosa* was determined by disc diffusion method. **Result:** The results show a statistically significant difference in the response curve of histamine with mean and standard deviation values of 6.5 and 2.258 at $p < 0.001$. Thus, the drug was effective against pathogens such as *K. pneumonia*, *P. aeruginosa* and fungal pathogen *C. albicans* with a maximum inhibitory zone of 12 mm. This medicine has antihistaminic and antimicrobial activity against *K. pneumoniae*, *P. aeruginosa* and *C. albicans.* The results of this study should have extended analysis through further preclinical analysis and clinical trials are necessary to establish the safety and efficacy of SSUP.

Keywords: Antihistaminic Activity, Bronchial Asthma, *Candida albicans, Klebsiella pneumoniae, Pseudomonas aeruginosa, Sarva Sangaara Uppu Parpam*

1. Introduction

Among respiratory disorders, bronchial asthma is looming as one of the most significant and sensible medical conditions worldwide. These disorders lead to mental stress and reduce the quality of life. Bronchial asthma is a chronic inflammatory, non-communicable airway disease resulting from the air passage's narrowing[1]. The underlying pathophysiology of bronchial asthma involves airway inflammation, smooth muscle contraction, epithelial sloughing, mucous hypersecretion, bronchial hyper-responsiveness and mucosal edema[2]. This leads to breathlessness, increased heart rate (tachycardia), wheezing, chest tightness, cough that can vary in intensity and over time, and other allergic symptoms[2-4]. The risk factors of asthma include family history, viral respiratory infections, allergies, occupational exposures, smoking, air pollution, and obesity. According to WHO, over 235 million people are affected by asthma. Among them, 15 to 20 million people are from India[5]. Individuals between the age group of 5 and 34 have a mortality rate

*Author for correspondence

Article Received on: 21.11.2023 Revised on: 20.04.2024 Accepted on: 22.05.2024

of 0.1-0.8 per 100,000 persons in developed countries[6]. As per the recent report, more than 100 million people by 2035 will be affected by asthma[7]. Severe asthmatic exacerbation requires urgent action to prevent mortality and morbidity, which in turn increases the economic burden.

Hence, it is the need of an hour to intervene in this ailment using traditional medicines and to validate them scientifically. Herbal medicine is an important part of the traditional healthcare system in India. *Siddhars* use herbs, inorganic substances, and animal products to formulate a medicine for maintaining the balance of trihumors (*Vali, Azhal* and *Aiyam*) Most of the plants are rurally available and traditionally accepted. Its effectiveness in treating diseases has to be studied widely. In the *Siddha* system of medicine, the medicines are classified as Internal and external.

Parpam, which is a high-end medicine in *Siddha*, is classified as one of the 32 types of internal medicine. As with every medicine, *parpam* also has a shelf life. The medicine, even though it seems to be fresh, it is not effective after a shelf lifetime. So, the medicines should not be used after a certain period. As per the *Siddha* literature *Agamarunthupadal* in *Gunapadam (Materiamedica Part 2, Thathu- jeevam)* text, the shelf life of *Parpam* is 100 years[8]. SSUP is one such medicine indicated for *Ilaippu* (Tuberculosis), *Iraippu* (Bronchial asthma) and *Irumal* (Cough) in the *Siddha* classical literature *Noigalukku Siddha Parigaaram-Part-I* which was published by the Department of Indian Medicine and Homeopathy (DIMH)[9]. 96% of *Siddha* physicians are using *parpam* such as *Muthuchippi, Thalagam, Pavalam, Palagarai* and *Kanthaparpam* in the treatment of *Iraippu*. These *parpam* are herbo-mineral preparations[6], SSUP differs from the mentioned *parpam* as it is an herbal formulation. Metals, minerals and bones of some animals are incinerated to form *parpam* – a calcified powder form of medicine. SSUP is a unique preparation of *parpam* made from easily available potent herbs. All the ingredients of this formulation have been found to have bronchodilator, antibacterial, antifungal and antihistaminic activity in previous research studies. Hence, this research work was carried out as a preliminary work to screen SSUP's antihistaminic action and antimicrobial potential against respiratory pathogens (*S. pneumoniae, S. aureus, K. pneumoniae, C. albicans, E. coli, P. aeruginosa)* which can cause the secondary respiratory infections in immune suppressed individuals.

2. Materials and Methods

2.1 Medicine Preparation

The SSUP is a compound drug used for respiratory disorders such as asthma and tuberculosis[9]. The raw drugs *Kuppaimeni* (*Acalyphaindica*) and *Pirandai* (*Cissusquadrangularis*) were collected from Velumailu *Siddha* Medical College's garden. All the raw drugs were identified and authenticated by the experts of the Department of *Gunapadam* (Materiamedica) and by the botanist at Velumailu *Siddha* Medical College, Sriperumbudur, Tamil Nadu, India. The specimen samples of the identified raw drugs were preserved at the *Gunapadam* department laboratory for future reference.

20 kg of fresh *Kuppaimeni* whole plant was collected and allowed to dry well for ten days. Then, it was placed on large cast ironware (Figure 1a), and the ash was collected after being burnt (Figure 1b). 1800 gm of ash obtained from the above process was diluted in cow's urine in an earthen pot. It was allowed to remain stable for three days. On the fourth day, it was flamed and kept on flame until the cow's urine fully evaporated (Figures 1c and d). The deposited particles on the earthen pot are called as *Uppu* (Figures 1e and f). 260 gm of *Uppu* collected from the process was ground with 150 ml of *Pirandaichaaru* (juice of *Cissusquandrangularis)* and allowed to dry (Figures 1g and i). Then it was placed in an earthen plate; the same sized earthen plate was chosen, and their mouths opposed each other. A three-layered muddy wet cloth covered the gap between their mouths, allowing it to dry. Then, it was subjected to incineration with 15 cow dung cakes (Figure 1k). After finishing the incineration process, it was left undisturbed to give away heat. The seal was opened, and the *Parpam* was collected (Figure 1l). The *Parpam* was preserved in a well-stopper air-tight glass container and labelled as SSUP.

2.2 Screening of Antimicrobial Activity by Disc Diffusion Methods

The medicine, SSUP, was tested against the microbes *S. pneumonia, S. aureus, P. aeruginosa, E. coli, K. pneumonia* and *C. albicans.* The disc diffusion method

Figure 1. Preparation of *Sarva Sangara Uppu Parpam*. **(a).** Burning process of *Kuppaimeni* in a large iron cast; **(b).** Ash of Kuppaimen; **(c).** Burning process of ash of *Kuppaimeni* with cow's urine; **(d).** Cow's urine is dried; **(e).** *Uppu* deposited; **(f).** *Uppu* collected after burning **(g).** Juice of Pirandai; **(h).** Juice of Pirandai; **(i).** Drying process; **(j).** After drying process; **(k).** Incineration; **(l).** SSUP.

which is the standard method for testing antimicrobial susceptibility was used in this study[10]. Mueller-Hinton Broth (MHB) was the culture medium used for the target microorganisms. MHB is a loose agar, it allows antibiotics to diffuse in a better way when compared with the other media. So, this cultural media was selected. The better diffusion leads to a truer zone of inhibition[11]. The suspensions were changed to a normal subculture dilution after 24 hrs.

The bacterial strain was diluted and grown in Petri dishes with Muller Hinton Agar (MHA) medium. Fungal strains were grown using Sabouraud dextrose. A 6 mm diameter Whatman No. 1disc was kept in an aseptic chamber after being pre-sterilized. The sterile disc papers were injected with 100, 500, 1000, 2000, and 4000 µg doses concentration of test samples. After that, the culture media was added to the produced discs. To find the sensitivity of each examined microbial species, the standard medication streptomycin (10 µg) was employed as a positive reference standard, and 20 µl of Dimethyl Sulfoxide (DMSO) was utilized as the vehicle control. The inoculation plates were incubated for 24 hrs for bacteria and 72 hrs for fungus at 37°C. The antimicrobial property of the disc was determined by the inhibition zone measured by callipers and recorded in units of millimetres.

2.3 Determining the Antihistaminic Activity by Chick Ileum Method

As there are limitations in performing experiments using animals, bioassays are preferred for screening studies. Anti-histaminic activity was screened by using the chick ileum method, a suitable method for performing bioassay histamine[12].

A section of the chick ileum, about 2 to 3 cm long, was obtained from chicken sacrificed for food which is a waste product easily available from slaughterhouses. It was put right into the watch glass with the physiological salt solution inside. Adequate measures were implemented to prevent harm to the stomach muscle. Before introducing the test medication, the tissue was given 30 min to acclimate and a bath volume of roughly 25 ml. The ileal smooth muscle which is recommended to show anti-histaminic properties and its contraction reaction captured on a kymograph using a frontal writing lever. A 30 s contact period and a 5 min time interval were maintained to ensure accurate response recording. Following the measurement of the normal response, the test drug was briefly incubated at a concentration of 500 µg (0.5 ml) in the ileal preparation. The concentration-response curve of histamine was then observed, and the height of the response before and after the test drug incubation was measured to determine the test drug's antagonist effect.

3. Results

3.1 Effect of SSUP on the Response of Isolated Chick Ileum Preparation

The data from this experiment revealed that before incubation with SSUP, the height of response of the histamine concentration-response curve ranges from 10 mm to 32 mm. The height of the response curve showed a positive decline following Incubation with test agent SSUP, which varies from 10 mm to 32 mm before incubation and 3 mm to 22 mm after incubation (Table 1, figure 2).

It was concluded that the Sample SSUP possess significant antihistamine properties by a decrease in the concentration-response curve between before and after the inoculation of SSUP (Table 2).

Table 1. Effect of SSUP on the response of isolated chick ileum preparation

Dose in mcg	Initial response in mm (Before Incubation)	Final response in mm (After Incubation with Test drug SSUP)
10	10	3
20	12	6
40	16	10
80	18	15
160	27	20
320	32	22

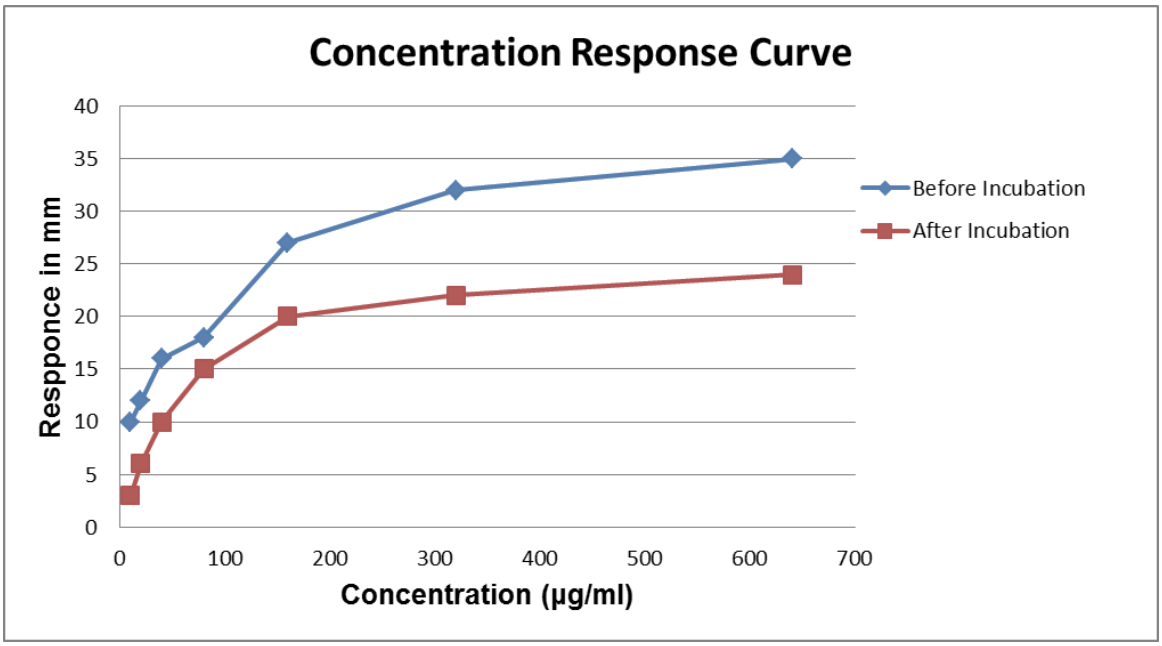

Figure 2. Concentration-response curve before and after incubation.

Results show a statistically significant difference in mean and standard deviation values of 6.5 and 2.258 at $p < 0.001$. There was a promising decrease in the height of the response curve after incubation with the test drug SSUP (Table 2). Thus, the result of this study shows SSUP has significant antihistaminic activity.

3.2 Effect of SSUP on Response to Microbes

Authors used *E. coli, P. aeruginosa, S. aureus, K. pneumoniae, Staphylococcus epidermidis* and *C. albicans* microbes for anti-bacterial and antifungal testing. The diameter of the clear zone around the disc was measured and expressed in millimetres as its antifungal property Table 3 and Figure 3.

The result reveals that the Sample SSUP was effective against pathogens such as *K. pneumoniae* and *P. aeruginosa* with inhibitory zones of 12 mm and 8 mm at 4000 µg and it was further observed that the sample SSUP is not effective against *S. aureus, S. epidermidis* and *E. coli.* The sample SSUP was highly effective against the fungal pathogen *C. albicans* with a maximum inhibitory zone of 12 mm. Hence, it was observed that SSUP has shown inhibitory zones of 12 mm, 8 mm, and 12 mm, respectively to *K. pneumoniae*, *P. aeruginosa* and *C. albicans* at the dose of 4000 µg and 10 mm, 6 mm, 10 mm at the dose of 2000 µg. Thus, SSUP has exhibited both antibacterial and antifungal properties.

4. Discussion

SSUP has antihistaminic activity and antimicrobial activity against gram-negative bacteria (*K. pneumoniae* and *P. aeruginosa)* and, fungi (*C. albicans).* This formulation possesses significant antihistaminic activity and can be attributed to having broncho-dilating

Table 2. Difference in response curve of histamine in the absence and presence of sample SSUP on Isolated chick ileum in optimized condition

	Before SSUP		After SSUP			95% CI for MeanDifference				
Outcome	**M**	**SD**	**M**	**SD**	**N**	**M**	**SD**	**r**	**T**	**Df**
	19.17	8.635	12.67	7.633	6	6.50	2.258	0.92*	7.050*	5

* $p < 0.001$.

Table 3. Zone of Inhibition data of Antibacterial activity and antifungal activity

Microorganisms /Sample	Zone of Inhibition in mm						
	4000 µg	**2000 µg**	**1000 µg**	**500 µg**	**250 µg**	**DMSO**	**Streptomycin 10 µg**
Sample Code: SSUP							
Escherichia coli	-	-	-	-	-	-	11
Klebsiella pneumoniae	12	10	-	-	-	-	19
Staphylococcus aureus	-	-	-	-	-	-	20
Staphylococcus epidermidis	-	-	-	-	-	-	20
Pseudomonas aeruginosa	8	6	6	6	6	-	19
Microorganisms/ Sample	4000µg	2000µg	1000µg	500µg	250µg	DMSO	Flucanazole10 µg
Candida albicans							
SSUP	12	10	10	10	8	-	11

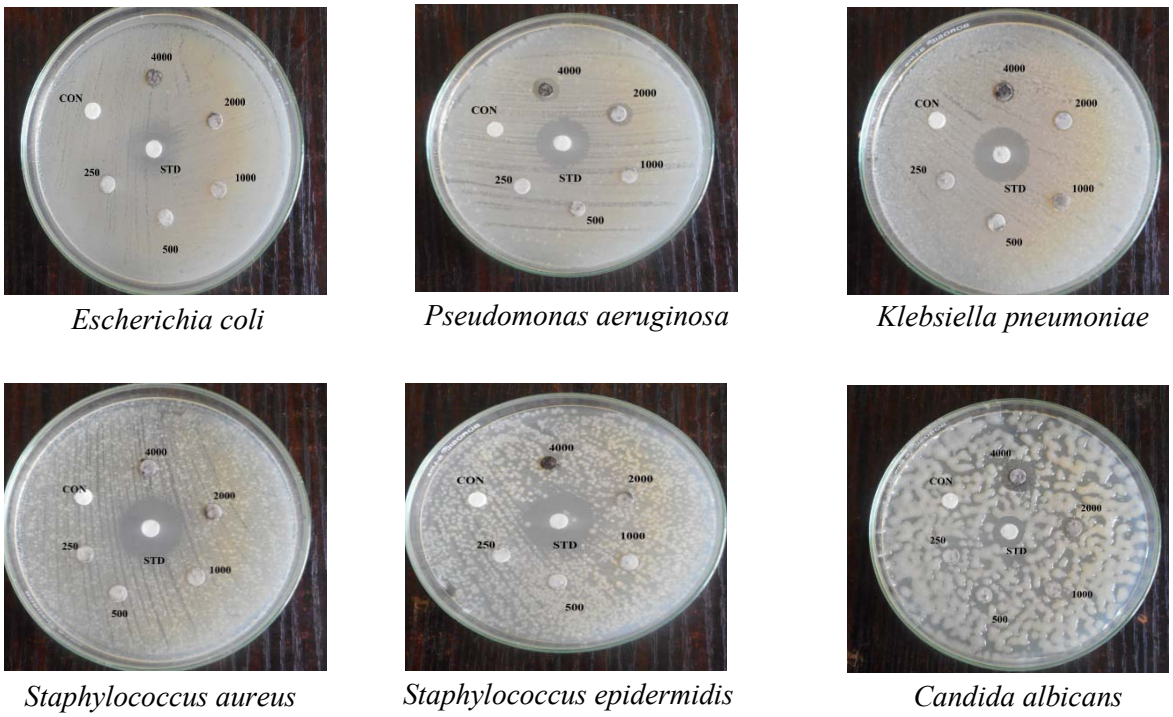

Figure 3. Anti-microbial effect of SSUP on bacterial and fungal.

activity. Since this is a preliminary study to screen the above-mentioned activities, further detailed studies are needed to evaluate the activities by both *in vitro* and clinical studies.

This formulation is indicated for the diseases *Iraippu* (Bronchial asthma*), Ilaippu* (Tuberculosis), and *Irumal* (Cough). Respiratory disorders such as asthma and tuberculosis are explained as *Iraippu* and *Ilaippu* in our *Siddha* literature, respectively. To pacify this deranged *Kabam* tastes like *Kaippu* (Bitter) and *Kaarppu* (Pungent) should be used[13]. Here, the ingredients of SSUP, such as *Acalyphaindica* and *Cissusquadrangularis,* have *Kaippu* and *Kaarppu* taste[14]. According to the concept of humour, *Iraippu* and *Ilaippu* are due to the vitiation of *Kabam*. *Sangaaram* means suppression[15]. SSUP can suppress the vitiated *Kabam.* The ingredients of SSUP have activities like bronchodilators, antibacterial, and so on, which supports this study. According to modern concepts, respiratory disorders are mainly caused by bronchoconstriction and pathogens. To rectify this, the medicines which are having bronchodilator, antihistaminic and antimicrobial activity are selected.

Kuppaimeni is widely used in the *Siddha* medical system for treating *Thanthamoolapini* (disorders of tooth and gums), *Thee thanthidum pun* (burns and scalds), *Kunmam* (gastric and duodenal ulcer), *Uthiramoolam* (bleeding haemorrhoids), *Thinavu* (itching), *Swaasakaasam* (bronchial asthma), *Peenisam* (headache), *Soothagavaayu* (polycystic ovarian syndrome), *Azhal Keel Vaayu* (osteoarthritis)[16-19]. This plant has antimicrobial, anti-mutagenic, bronchodilation, anti-diabetic, anti-tumourous, cytotoxic, antibiotic, chemotherapeutic, anti-teratogenic properties which make it a unique medicinal plant and is referred to as, "A Boon to Mankind"[20].

*Pirandai*has the ability to cure *Seriyamai* (indigestion)and derangements of *Kabam*[14]. It has bone healing[21] and antibacterial activity[22]. This herb is used for treating osteoarthritis, rheumatoid arthritis, osteoporosis, asthma, haemorrhoids, bowel infections, anaemia, scurvy,ottorrhoea and eye disorders[23]. Cow's urine has immune modulatoractivity[24] antimicrobial activity against multidrug resistance *E. coli* and *K. pneumoniae*. Further, it has powerful antioxidant properties, and anthelminthic activity and is useful in hypersensitivity reactions[25].

So, the medicine *Sarva Sangaara Uppu Parpam* will be effective against bronchial asthma and co-infections

caused by the pathogens *K. pneumoniae, P. aeruginosa* and *C. albicans*. In the future, clinical trials should be done to predict the efficacy of this medicine. Nowadays, asthma and co-infections caused by respiratory pathogens are treated by corticosteroids, which are high-cost and may lead to many side effects such as hypertension, diabetes, osteoporosis, etc., so it is vital to provide a cost-effective treatment for those who are living in low socio-economic status.

There are some limitations in this study which include both the disc diffusion and chick ileum methods being invitro in nature further *in vivo* studies are needed to substantiate the findings of this study. There is a lack of standard control for antihistaminic activity. Clinical trials are necessary to establish the efficacy and safety of SSUP.

5. Conclusion

The SSUP formulation has:

- Antihistaminic activity with a significant decrease in the response curve of histamine from 10 mm to 3 mm at the lowest (10 mcg) dose of SSUP and from 32 mm to 22 mm at the highest (320 mcg) dose of SSUP.
- Antimicrobial activity against respiratory pathogens- *K. pneumoniae, P. aeruginosa* and *C. albicans* (with a maximum zone of inhibition of 12 mm, 8 mm and 12 mm at 4000 µg dose of SSUP).

Herbs used in this formulation are easily available. The present study is, therefore, essential for the scientific validation of traditional knowledge. Further preclinical and clinical studies should be conducted to enhance the findings of this study.

6. References

1. Hashmi MF, Tariq M, Cataletto ME. Asthma. StatPearls. Treasure Island (FL): StatPearls Publishing; 2021.
2. Padem N, Saltoun C. Classification of asthma. Allergy Asthma Proc. 2019; 40(6):385-8. https://doi.org/10.2500/aap.2019.40.4253 PMid:31690376
3. Rehman A, Amin F, Sadeeqa S. Prevalence of asthma and its management: A review. J Pak Med Assoc. 2018; 68(12):1823-7.
4. Quirt J, Hildebrand KJ, Mazza J, Noya F, Kim H. Asthma. Allergy Asthma Clin Immunol. 2018; 14(Suppl 2):50. https://doi.org/10.1186/s13223-018-0279-0 PMid:30275843 PMCid:PMC6157154
5. Singh SK, Gupta J, Sharma H, Pedgaonkar SP, Gupta N. Socio-economic correlates and spatial heterogeneity in the prevalence of asthma among young women in India. BMC Pulm Med. 2020; 20:190. https://doi.org/10.1186/s12890-020-1124-z PMid:32664897 PMCid:PMC7362630
6. Anbarasi MG, Pavithra K, Chitra U, Anbu N. Assessment of various prescription practice among *Siddha* physicians in the treatment of Swasakasam (Bronchial Asthma)-A cross sectional study. Int J Trans RES Ind Med. 2022; 4(1):25-32.
7. Kudo M, Ishigatsubo Y, Aoki I. Pathology of asthma. Front Microbiol. 2013; 4:263. https://doi.org/10.3389/fmicb.2013.00263 PMid:24032029 PMCid:PMC3768124
8. Thiyagarajan R, *Gunapadam Thathu Jeevavaguppu,* Eighth edition, Vols 2 and 3; 2013.
9. Shanmugavelu M. *Noigalukku Siddha parigaram,* Second edition, Part 1; 2012.
10. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Ameri J Clin Pathol. 1966; 45(4):493-6. https://doi.org/10.1093/ajcp/45.4_ts.493 PMid:5325707
11. Nassar MSM, Hazzah WA, Bakr WMK. Evaluation of antibiotic susceptibility test results: how guilty a laboratory could be? J Egypt Public Health Assoc. 2019; 94(1):4. https://doi.org/10.1186/s42506-018-0006-1 PMid:30686832 PMCid:PMC6329728
12. Daude RB, Mishra SV, Ghegade RY, Amrutkar SV. Bioassay of histamine by using isolated chicken ileum. World J Pharm Pharmaceut Sci. 2015; 5(1):1221-5. https://doi.org/10.4103/0250-474X.25727
13. Shanmugavelu M. Noi naadal noi muthal naadal thirattu, Vol 1; 2016.
14. Muthaliar KSM. *Siddha Materiamedica* (Medicinal plants division), Ninth edition; 2013.
15. Sambasivampillai TV. Tamil- English dictionary of Medicine, Chemistry, Botany and Allied Sciences. The Research Institute of Siddhar's Science, Madras, India, Vol 3; 1992.
16. Kanakaavalli K, Parthibhan P, Geetha V, Chithradevi R, Latharani M, Thillaivanan S. A review on anti-allergic herbs in *Siddha* system of medicine. Int J Pharm Sci. 2014; 3(6):312-20.
17. Kaaseem RM. Pathaartha guna chinthamani, Seventh edition; 2007.
18. Sathya R, Kanimozhi K, Manimekalai V, Matheshvaran S, Saravanadevi MD. An approach to the therapeutic management of polycystic ovarian syndrome (Soothagavayu) in *Siddha* system of medicine–A literature review. World J Pharm Res. 2023; 12(4):323-41.
19. NasiyaBanu M. A study on Azhal Keel Vayu (Osteoarthritis). [dissertation]. Government *Siddha* Medical College, Palayamkottai; 2018.

20. Mondal R, Negi A. *Acalypha indica* – A boon to mankind. World J Pharm Res. 2021; 10(3):765.
21. Buddhadev S, Buddhadev S. A review update on plant *Cissus quadrangularis* L. Punarnav. 2014; 2(4):01-10.
22. Kashikar ND, George I, Antibacterial activity of *Cissus quadrangularis* Linn. Indian J Pharm Sci. 2006; 68(2):245-7.
23. Sundaran J, Begum R, Vasanthi M, Kamalapathy M, Bupesh G, Sahoo U. A short review on pharmacological activity of *Cissus quadrangularis*. Bioinformation. 2020; 16(8):579-85.
24. Kekuda PT, Nishanth BC, Kumar PS, Kamal D, Sandeep M, Megharaj HK, Cow urine concentration: A potent agent with antimicrobial and anthelminthic activity. J Pharm Res. 2010; 3:1025-7.
25. Randhawa GK, Sharma R. Chemotherapeutic potential of cow urine: A review. J Intercult Ethnopharmacol. 2015; 4(2):180-6. https://doi.org/10.5455/jice.20150222100320 PMid:26401404 PMCid:PMC4566776