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Evaluation of phytochemical, physicochemical and biochemical analysis of saaranai ver chooranam (the root of *Trianthema decandra* linn,) - a Siddha herbal medicine

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## Abstract

Abundant herbal remedies separately or in combination have been suggested in different medical treatises for the remedy of various illnesses, *Trianthema decandra* Linn, (Family: Aizoaceae) familiarly known as *Vellai Saaranai* (Tamil) and *Punarnavi* (Sanskrit) is a procumbent herb widespread in the tropical and subtropical provinces of the globe, and also found abundantly in India. It has been widely known in various systems of traditional treatments, used efficiently in *Siddha* for the treatment of diseases and ailments of human beings. The current study aims to endow up-to-date standardized data of *Saaranai ver chooranam* (SVC) indicated for *Gunmam* with distinct observations such as physicochemical, phytochemical, biochemical, HPTLC, analysis of heavy metal, microbial load, specific pathogen, pesticide residue, aflatoxin parameters were evaluated as per PLIM guidelines, gathered and orchestrated in a prompt manner on this paper, to explore and inspire further ethnobotanical and ethnopharmacological research and investigations towards drug discovery.

**Keywords:** *Trianthema decandra* root, *Vellai saaranai*, Saaranai ver chooranam, *punarnavi*

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## Introduction

*Trianthema decandra* Linn (Aizoaceae) Stem elongated, prostrate, not much diverged, angular and striate, glabrous. Leaves sub fleshy, 2-3.8 by 0.6-1.6 cm. Opposing Pairs are somewhat unequal, elliptic-oblong, spherical, and usually apiculate at the apex; petioles 6-13 mm puberulous are extremely enlarged and amplexicaul at the base, not having the flowers. Flowers in dense axillary sub umbellate clusters, peduncles, and pedicels are short, thinly membranous bracteoles. Calyx 4 mm, long, tube very fast, lobes much longer than the

tube, oblong, obtuse, with scarious margins, and different long assumptions at the back below the apex. Stamens 10. Styles 2. Capsules not held in the calyx tube, four seeded, the cap especially truncate, 3 mm. long, solid, subcylindric with a narrow critical rim around its base, bearing away two seeds. Kernels are orbicular-reniform, striate, and black [2].

## Therapeutic Significance

Root chewed up with milk and shared internally is detailed in orchitis. Juice of the leaves slipped into the nostrils, reducing-sided headache [1]. The juice of leaves is utilized to treat the black quarter illness in Cattles, and these bitter roots are utilized for healing Ulcers and bacterial infections. It is also given in mix with ginger as a cathartic. The leaves have a prodigious quantity of Vitamin C, used to manage edema. The decoction of the herb is employed as a vermifuge and is valid in rheumatism. It is also an antidote to Alcoholic toxins [4]. The plant is a sound source of Zinc (1.007± 0.188) and

Copper ( $0.416 \pm 0.057$  mg/ 100g) In Andhra Pradesh, and the roots are used in veterinary pharmaceuticals as an eye mixture for eye damage and eye illnesses in cattle [3]. Keeping because of the medicinal prominence of *T. decandra*, the current study was enterprises to provide scientific bases for to use of this plant in the standard *Siddha* system of treatment, and have been evaluated for its bio-potentiality. An extensive range of Qualitative Analytical Parameters such as Biochemical, HPTLC, Analysis of heavy metal, Microbial load, Specific pathogen, Pesticide residue, and Aflatoxins specifications following PLIM guidelines was evaluated [6].

## Materials and Methods

### Collection

The raw drug *Saaranai ver* (*Trianthema decandra* Linn,) was procured from outskirts of Dharmapuri, Tamilnadu, and South India.

### Identification Authentication

The solitary ingredient *T. decandra* roots are recognized and certified by the professionals from Botanist at Govt Siddha Medical College, Arumbakkam, and Chennai. The Voucher specimen *Trianthema decandra* Linn was labelled as 1014/PGG/321912104/GSMC-CH/2019-2022 and deposited to the laboratory of P.G *Gunapadam* department for prospective considerations.

### Preparation and Purification

The selection, preparation and purification was done as per the verses denoted in *Siddha* classical literature "*Agasthiyar Mani 4000 ennum vaithiya sinthamani venba- Muthal pagam*", Page No-180 [5].

### Process for Purification



Fig: no: 1 *Trianthema decandra* Linn, root



Fig: no: 2 boiling process

### Materials required

1. Milk from the Cow's (*Bos Taurus*)
2. Milk from the Goat's (*Capra aegagrus hircus*)

The well matured *saaranai* roots were selected cut into pieces and then boiled with cow's milk and goat's milk separately for purification. Repeat this process about 7 times.

### Preparation Process

Purified *T.* roots were taken in anhydrous form pound well and grounded in a stone mortar. The powder was sieved through a mesh (80- 100) particle size and maintained it in a clean Airtight Container. It was marked as "*Saaranai ver chooranam*" (SVC). The contents were examined repeatedly to evade wetness and microbes.

- Physicochemical Analysis was done [7]
- Finding Whole Ash was done
- Resolution of Acid Insoluble Ash was done
- Resolution for the Alcohol Soluble Extractive
- Water-Soluble Determination Extractive was done
- pH Determination is also done
- Solubility test was done
- Particle Size Determination [9] was done

### Phytochemical Analysis was done for the following tests [8, 10]

- Alkaloids analysis – was performed with Mayer's Test
- Coumarins
- Saponin
- Tannins
- Glycosides - Borntrager's Test
- Flavonoids
- phenols - Lead acetate test
- steroids
- Triterpenoids
- Cyanins
  - A. Anthocyanin
  - B. Betacyanin
- Carbohydrates - Benedict's test
- Proteins (Biuret Test)
- HPTLC was done [11]
- Chromatogram Development was done
- Scanning was done
- Heavy Metal Analysis By AAS Standard was done [12]

### Standard preparation

- As & Hg- 100 ppm sample in 1mol/L HCl
- Cd & Pb- 100 ppm sample in 1mol/L HNO<sub>3</sub>

Test for Sterility was done by Pour Plate Method [13]

Test for Specific Pathogen [15]

Table: no: 1 – complete info about Specific Medium and their abbreviation

Organism	Abbreviation	Medium
E-coli	EC	EMB Agar
Salmonella	SA	Deoxycholate agar
Staphylococcus aureus	ST	Mannitol salt agar
Pseudomonas /aeruginosa	PS	Cetrimide Agar

- Pesticide Residue was done [15, 16]
- Test for Aflatoxins was done [17]
- Standard

Table: no: 2 – Aflatoxins

1	Aflatoxin B <sub>1</sub>
2	Aflatoxin B <sub>2</sub>
3	Aflatoxin G <sub>1</sub>
4	Aflatoxin G <sub>2</sub>

- Solvent was done for analysing the combination of chloroform and acetonitrile

## Results and Discussion

### SVC Organoleptic Characters

The following organoleptic characters have been noted in *Saaranai ver chooranam*.

Table: no: 4 Results of Organoleptic Characters

Sl No	Parameter	Result
1	State	Solid
2	Nature	Fine
3	Odour	Characteristic
4	Touch	Soft
5	Flow Property	Free Flowing
6	Appearance	Pale Brownish
7	Taste	Bitter

#### Interpretation:

The organoleptic parameters revealed that SVC is solid in nature, pale brownish in colour, soft content, free flowing property and bitter in taste which stimulates the taste buds and this produces reflex secretion of gastric juice.



Fig: No: 3 Saaranai Ver Chooranam  
SVC Microscopic Observation of Particle Size

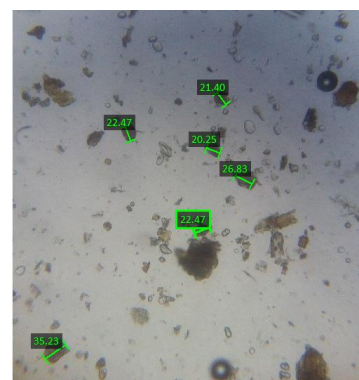


Fig: no: 4 Particle size  $24.36 \pm 5.15 \mu\text{m}$  of SVC

#### Interpretation

Microscopic observation of the particle dimensions examination indicates that the mean intermediate particle dimension of the sample was sized up to be  $24.36 \pm 5.15 \mu\text{m}$  further, the sample has particles with the size range of lowest  $20 \mu\text{m}$  to most elevated  $35 \mu\text{m}$ , which ensures the solubility, processing belongings, bioavailability, product uniformity, strength and the medicinal result of the SVC [19].

#### Physico – Chemical Analysis of SVC

Table: no: 5 SVC Solubility Profile

Sl No	Solvent Used	Solubility/ Dispensability
1	Chloroform	Insoluble
2	Ethanol	Soluble
3	Water	Soluble
4	Ethyl Acetate	Insoluble
5	DSMO	Soluble

#### Interpretation

##### 1. Solubility

- Solubility is the primary factor that exploits the bioavailability of a drug substance. It is valuable to decide the form of the drug and the processing of its dosage format [20].

- SVC is soluble in preliminary solvents and sparingly soluble in some solvents, establishing that its effectiveness of solubility in the gut indirectly raises the bioavailability.

**Table: no: 6 Results of Physicochemical Analysis**

SI No	Parameter	Result
1	pH	7.1
2	Loss On Drying 105 <sup>o</sup> c (%)	6.533±1.007
3	Total Ash (%)	5.067±2.055
4	Acid Insoluble Ash (%)	0.05±0.0167
5	Water Soluble Extractive	20.47±3.356
6	Alcohol Soluble Extractive	8.233±1.531

### 2. pH

- It is an extent of hydrogen ion engagement. It is the extent of acidity. 7.0 is unbiased and above 7.0 is alkaline, and below is acidic.
- The pH of the drug SVC is 7.1, which is Alkaline. Alkalinity is the buffering ability of the water body. It calculates the ability of water bodies to neutralize acids and bases, holding a well harsh pH as the concentration of CaCo3 gains in SVC, thereby enhancing the alkalinity and the risk of acidification lowering [21].

### 3. Loss on Drying

- It demonstrates the quantity of volatile essence and wetness current in the medication [22, 23].
- The defeat on drying ratio of SVC is 6.53%. This tells the strength and shelf life of the drug SVC are acceptable.

### 4. Decisiveness of Total Ash

- The purity of the drug is associated with ash analysis<sup>22, 23</sup>.
- Here, the total ash value of SVC is 5%. This indicates that the drug SVC has no impurities and it is safe to treat the Peptic Ulcer Diseases.

### 5. Decisiveness of acid insoluble ash

- The acid-insoluble ash worth of the drug represents the portion of siliceous value [dust, sand, etc. current in that medication [22, 23].
- The grade of the medication is more useful if the acid insoluble ash significance is low. Here, acid insoluble ash significance of SVC is 0.05%. Hence, it means the superior grade of the drug SVC.

### 6. Decisiveness of Water Soluble Extractive

- Water-soluble extractive is a portion of the entire ash value, representing the drug's

diffusion ability [22, 23]. Here, the water-soluble extractive Value of SVC is 20%, which denotes easy facilitation of diffusion and osmosis mechanism.

### 7. Decisiveness for Alcohol Soluble Extractive:

- Extraction of Alcohol soluble is useful for quality as well as purity of the drug<sup>22, 23</sup>. Here, the alcohol-soluble extractive value of SVC is 8.23%.

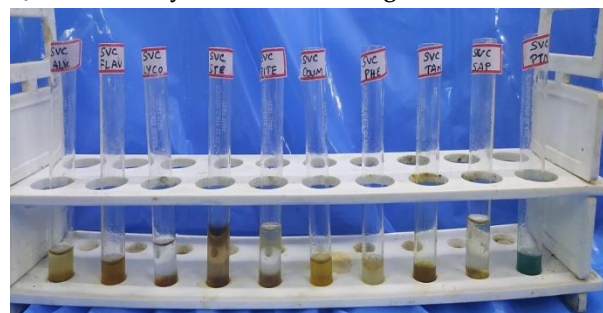
### Phytochemical Analysis of SVC

#### Phytochemical Analytical Report

SI No	Test	Observation
1.	Alkaloids	+
2.	Flavonoids	+
3.	Steroids	+
4.	Coumarins	+
5.	Phenol	+
6.	Tannin	+
7.	Saponin	+
8.	Sugar	+

+ = Indicates Positive

#### Qualitative Phytochemical Investigation of Svc



#### Interpretation

The Phytochemical screening of SVC indicates the actuality of alkaloids, flavonoids, steroids, coumarins, phenols, tannins, sugar and saponins.

#### Alkaloids

- Alkaloids, any of an inherently occurring organic nitrogen-containing bases. Generally the alkaloids are bitter in taste and have pronounced physiological activity.
- This increase the motility of the gastrointestinal muscle contraction, inhibition of gastric acid secretion. This indicates the presence of alkaloid in the drug SVC has an anti-ulcer activity.

#### Flavonoids

- Flavonoids are vital for genuine outcomes; notably, they belong to secondary plant metabolites with a polyphenolic structure,

widely found in fruits, vegetables, and certain liquids. Excluding their activity as gastroprotective, flavonoids also act in the rehab of gastric ulcers, and further, these polyphenolic blends current in the medicine SVC can be new choices for quelling or suppressing peptic ulcers associated with peptic ulcers H.pylori [25].

#### Coumarins

- Coumarins are the chemical combinations in the benzopyrone class of organic combinations seen in numerous plants.
- These compounds possessing gastro protective and inhibits the gastric acid secretion. This indicates that the drug SVC has an antiulcer and antacid activity [26].

#### Phenols

- Phenols exist as an organic combination holding a hydroxyl crowd and a benzene ring, the most uncomplicated of which is phenol.
- It shows several natural shiftings in the gastroprotective area, including anti-secretory, cytoprotective and antioxidant activities, and these combinations in the drug SVC cover the gastrointestinal mucosa from lesions built by peptic ulcers [27].

#### Steroids

- Steroids are lipids because they are hydrophobic and insoluble in wetness, but they do not compare to lipids since they have a design formed of four connected rings.
- This compound reducing the gastric pH with proton pump inhibitors in peptic ulcer and the drug SVC acts as an antiulcer property [28].

#### Tannins

- Tannins [tannic acid] are intrinsically emerging complex chemicals discovered in plants. These proanthocyanidin polyphenols dominate various vascular plants, including fruits [mostly grapes], teas, legumes, and grasses.
- In ulcers in the gastric, this tannin-protein intricate layer shields the stomach. Which present in the drug SVC promotes more excellent antagonism to chemical and automated harm or irritation [29].

#### Sugar

- Sugar is any innumerable sweet, pale, water-soluble mixtures present in the sap of seed plants and the milk of mammals and causes up

the numerous distinctive group of carbohydrates.

- These compound present in the drug SVC indicates gastric emptying was faster and inhibits the effect of the osmotic receptors are not changed in peptic ulcer [30].

#### Saponins

- Saponins are secondary metabolites in terrestrial plants and aquatic invertebrates and include one of the considerable prominent families of biological products. They are responsible for imparting a bitter taste, astringency and have strong detergent properties.
- Saponin mainly those of triterene type like glycyrrhetic acid and carbenoxolone, present in the drug SVC have been found as anti - ulcer agent, whose action is mediated by the formation of mucous on the gastric mucosa [31].

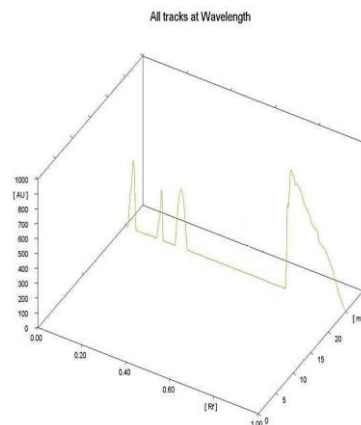
#### HPTLC Analysis

HPTLC is an analytical chemistry approach employed to isolate the elements in a variety, identify each element, and quantify each element.

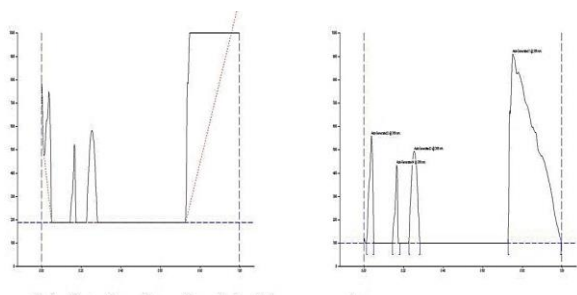
#### TLC Visualization of SVC



Fig: no: 4 TLC Plate Visualization At 366 nm of SVC



Graph: no: 1 3D View of Chromatogram



Graph No: 2 HPTLC Finger Printing Of Sample SVC

Table: no: 7 Table For HPTLC Peak

Peak	Start Rf	Stationary height	Max Rf	Max height	Max x%	End Rf	End height	Area	Area %
1	0.01	5.8	0.04	460.4	22.99	0.05	14.8	615.5	6.29
2	0.14	5.5	0.17	336.2	16.79	0.08	0.0	308.9	3.16
3	0.23	11.7	0.25	394.6	19.71	0.08	3.5	918.2	9.39
4	0.36	36.7	0.75	811.0	40.51	1.0	11.0	794.0	81.16

**Interpretation**

- HPTLC fingerprinting investigation of the specimen demonstrates the existence of three notable mounts reaching the presence of three versatile photo components.
- Rf value of the mounts varies from 0.01 to 0.73. Peak 2 populates a substantial portion of the extent of 3.61, representing the significant presence of such a blend.
- This approach was employed successfully to concoct a chemical fingerprint for authentication and trustworthy verification of the existence of bioactive compounds in the sample SVC.
- So, the presence of medicinally important phytochemicals in the sample drug SVC was strengthened by TLC and comparing the Rf of corresponding spot with that of standards.
- The drug SVC possesses anti-ulcerogenic property. Hence, the result support the ethno

medical uses of the drug SVC in the treatment of PUD [11].

**Analysis pf Heavy Metal: AAS (Atomic Absorption Spectrometry)**

Table: No: 8 Test Report For SVC

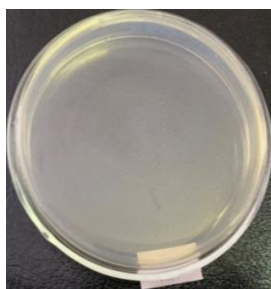
SL NO	Heavy metal Name	Absorption Max $\Delta$ max	Result Analysis	Maximum limit
1	Lead	217.0 nm	BDL	10 ppm
2	Arsenic	193.7 nm	BDL	3 ppm
3	Cadmium	228.8 nm	BDL	0.3 ppm
4	Mercury	253.7 nm	BDL	1 ppm

- **BDL- Below Detection Limit**
- **Interpretation**
- The results of absence of the heavy metal ensures the quality of the drug and safety of the drug SVC is extremely safe for the diseases PUD [12].
- **Microbial Load – Sterility Test**
- **Table: No 9 Results of Sterility Test By Pour Plate Method**

Sl No	Test	Result	Specification	As Per Ayush / Who
1	Total Bacterial count	Absent	NMT 10 <sup>5</sup> CFU/g	As per AYUSH Specification
2	Total Fungal count	Absent	NMT 10 <sup>3</sup> CFU/g	



Fig: No: 5 Sterility Test Of SVC – Total Bacterial Count



**Fig: No: 6 Sterility Test Of SVC – Total Fungal Count Observation**

No development was marked after the incubation span in any plates, indicating an apparent pathogen's lack.

**Interpretation**

This revealed that the drug SVC is free from the viable microorganisms and the absence of total bacterial and fungal count which may indicate that the drug SVC has good quality and safer drug to treat PUD [14].

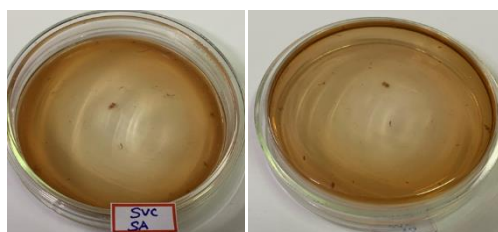
**Test for Specific Pathogen**

**Table: No: 10 Results of Specific Pathogen Test**

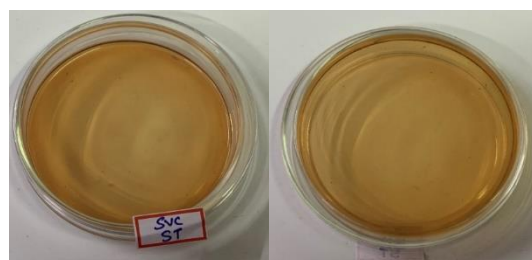
SL NO	Organism	Specificati on	Result	Method
1	E-coli	Absent	Abse nt	As per AYUSH specificati on
2	Salmonella	Absent	Abse nt	
3	Staphylococ cus Aureus	Absent	Abse nt	
4	Pseudomona s Aeruginosa	Absent	Abse nt	



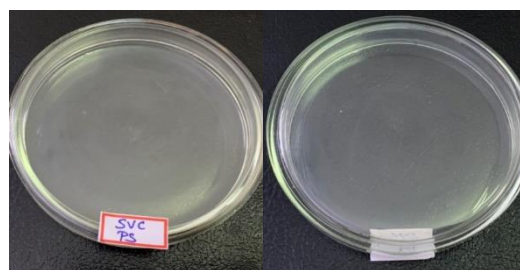
**Fig: No: 7 Culture plate with E-coli (EC) specific medium**



**Fig: No: 8 Culture plate with Salmonella (SA) specific medium**



**Fig: No: 9 Culture plate with Staphylococcus aureus (ST) specific medium**



**Fig: No: 10 Culture plate with Pseudomonas Aeruginosa (PS) specific medium**

**Observation**

No development was marked after the incubation span in any plates, indicating an apparent pathogen's lack.

**Interpretation**

The results of the specific pathogen test showed that the drug SVC were able to prevent the growth of the microorganisms such as E.Coli, Salmonella species, Staphylococcus aureus, Pseudomonas aeruginosa and it indicates that the drug SVC used to reduce the morbidity and mortality from chronic diseases [15].

**Pesticide Residue**

**Table: No: 11 Test Result Analysis Of The Sample SVC**

SI No	Pesticide Residue	Sample SVC	Ayush Limit [Mg/Kg]
<b>I.Organo Chlorine Pesticides</b>			
1.	Alpha BHC	BQL	0.1 mg/kg
2.	Beta BHC	BQL	0.1 mg/kg
3.	Gamma BHC	BQL	0.1 mg/kg
4.	Delta BHC	BQL	0.1 mg/kg
5.	DDT	BQL	1 mg/kg
6.	Endosulphan	BQL	3 mg/kg
<b>II. Organo Phosphorus Pesticides</b>			
1.	Malathion	BQL	1mg/kg
2.	Chlorpyriphos	BQL	0.2mg/kg
3.	Dichlorovos	BQL	1mg/kg
<b>III. Organo Carbamates</b>			
1.	Carbofuran	BQL	0.1mg/kg

IV. Pyrethroid			
1.	Cypermethrin	BQL	1 mg/kg

BQL- Below Quantification Limit

**Interpretation**

- Pesticide precipitate study confines the parent combinations' metabolites and degradation outcomes [32].
- The effects displayed that the drug SVC has no toxicity, slow degradation, and bioaccumulation [16].

**Aflatoxins**

**Table: no: 12 Aflatoxins Report of the Sample SVC**

Sl No	Aflatoxins	Sample SVC	AYUSH Specification Limit
1.	B <sub>1</sub>	Not detected- Absent	0.5 ppm
2.	B <sub>2</sub>	Not detected- Absent	0.1 ppm
3.	G <sub>1</sub>	Not detected- Absent	0.5 ppm
4.	G <sub>2</sub>	Not detected- Absent	0.1 ppm

**Interpretation**

- Aflatoxins produced by the Aspergilla's species are highly toxic, carcinogenic and cause severe contamination to food sources leading to serious health consequences.
- Here, the results showed that there was no spots were been identified in the test sample SVC loaded TLC plates when compare to the standard, which indicates that the sample SVC were free from Aflatoxin B<sub>1</sub>, Aflatoxin B<sub>2</sub>, Aflatoxin G<sub>1</sub>, Aflatoxin G<sub>2</sub> [33].

**Analysis for Bio-Chemical**

**Table: No: 14 Results of Acid & Basic Radicals**

Sl No	Specific Radicals	Test Report
<b>I. Acid Radicals</b>		
1.	Carbonates	Positive
2.	Chlorides	Positive
<b>II. Basic Radicals</b>		
1.	Zinc	Positive
2.	Iron	Positive
3.	Copper	Positive

**Interpretation**

The biochemical study reveals the presence of Carbonate and Chloride, Iron, Zinc & Copper in the drug SVC.

**Carbonates**

- Stanford (Calif) University Medical Center routinely specified long-term calcium carbonate antacid treatment to aid in preventing peptic ulcer conditions.
- Carbonate present in the drug SVC is used as an antacid to relieve heartburn, acid indigestion and upset stomach [34].

**Chlorides**

Chloride secretion in the medicine SVC instantly determines mucosal hydration throughout the GI tract, and chloride transportation is also pivotal in controlling fluid secretion by organs that drain into the intestine [35].

**Iron**

The iron present in the drug SVC in a colloidal form would be smallish to irritate the GI tract. A mixture of the colloidal hydroxides of iron and aluminium has been newly presented beneath the name of alumina in the colloidal gel form and is soluble in dilute acid; it also performs as an antacid like that of Aluminium hydroxide [36].

**Zinc**

- Zinc present in the medicine SVC is also required to restore broken tissue and has guarded against stomach ulceration in animal investigations.
- Daily management of 220 mg of zinc sulfate diminished peptic ulcer dimensions, and also, patients with regular groups of serum zinc had better recovery processes [37].

**Copper**

- Copper-glycinate complex Cu (II)(glycinate)<sub>2</sub> in the medicine SVC rescues by lowering the ulcer index and interfering with gastric perforation and dying. Substantial boosts in gastric juice volume and superoxide dismutase (SOD) activity in the gastric mucosa and blood Plasma were seen with both copper complexes [38].
- Copper (tamrabhasma) causes a reduction in the total acid and pepsin output and an addition in the carbohydrate/protein ratio, implying increased mucus secretion in the gastric secretion of rats [39].

## Conclusion

Standardized work is one of the most powerful but least used lean tools. By documenting the above results and observations of Saaranai ver chooranam reveals that physicochemical results LOD  $6.533 \pm 1.007$ , total ash(%)  $5.067 \pm 2.055$ , acid insoluble ash (%)  $0.05 \pm 0.0167$ , water soluble extractive(%)  $20.47 \pm 3.356$ , alcohol soluble extractive (%)  $8.233 \pm 1.531$ . solubility nature shows soluble in ethanol, water & DMSO, Phyto chemicals reported are Alkaloids, flavonoids, steroids, Coumarins, phenol, Tannin, Saponin & sugar, Heavy metal analysis falls out within BDL, There were free from viable microorganisms in Sterility test, seldom microbial growth were seen in Specific pathogen test, Pesticide residue of Organochlorine, Organophosphorous, Organocarbamates, Pyrethroids on below quantification limit, Aflatoxins are not detected, In Biochemical test Acid radicals found are Carbonates & Chlorides and Basic radicals found are Zinc & Iron. The current research article confirms that the Saaranai ver chooranam is potent medicinal drug indicated for Acid peptic diseases. It reduces the variations of the process and improves the quality of *Trianthema decandra* Linn, and its processes which proved that the plant has a leading capacity for the evolution of new drugs in future, which might supply a consequential way for promotion of the Traditional Siddha wisdom of the herbal medicinal plants.

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## Footnotes

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## Author Contribution

All authors contributed equally

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