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RESEARCH ARTICLE

Identification of Bioactive Molecules in Traditional *Siddha* Formulation "*Nilaavarai Choornam*"

S. Selva Sivagami, S. Abirami Swetha, V. Swetha, N. Ravichandran, R. Lavanya, P. Rajalakshmi* and P. Brindha

Centre for Advanced Research in Indian Systems of Medicine, School of Chemical and Biotechnology, SASTRA Deemed University, Thanjavur - 613401, Tamil Nadu, India; rajalakshmi@carism.sastra.edu

Abstract

The *Siddha* system of medicine is developed by ancient Tamil Siddhars, through their spiritual power. In the present work, the *Siddha* herbal formulation *Nilaavarai choornam* (NVC) is selected and chemically evaluated for its major active constituents. All standardization parameters were evaluated for the in-house and market samples of NVC. These parameters will also be useful in checking the quality of the ingredients and the end product. Its ingredients are senna, ginger, black pepper, ajowan and embelia. It is administered to treat constipation, indigestion, flatulence and intestinal worms. Pharmacognostic parameters of *Nilaavarai choornam* were determined through powder microscopy and chemical studies. Preliminary phytochemical screening was carried out using petroleum ether, chloroform, acetone, alcohol and water extracts obtained through successive extraction methods. Fluorescence analysis was also performed to detect chromophores present in this formulation. HPTLC Fingerprinting profile was determined to identify the active molecules present in this formulation. Powder microscopy of this formula suggested the presence of characteristic, calcium crystals, oleoresin, stone cells, endosperm cells, striated cuticle and parenchyma cells. The powdered material showed 2.8% loss on drying for NVC-M and 2.5% for NVC-H, 3% of ash for both samples and 0.54% of acid insoluble ash for NVC-M and 0.09% for NVC-H. Preliminary phytochemical screening of various extracts revealed the presence of proteins, sterols, terpenoids, carbohydrates, flavonoids, tannins, quinones, alkaloids and glycosides. The HPTLC fingerprinting profile of the chloroform fraction of NVC-M and NVC-H showed characteristic spots. Also, HPTLC determination was done with the standard gingerol and piperine and the results obtained confirmed the presence of active ingredients in both the samples.

Keywords: Fluorescence Analysis, HPTLC Fingerprinting, *Nilaavarai Choornam,* Pharmacognostic, Phytochemical, *Siddha* System

1. Introduction

India has a vast heritage of traditional systems of medicine like *Ayurveda* and *Siddha*. Both systems contribute significantly to the healthcare of mankind but the Greatest lacuna existing in these systems is the lack of quality control parameters. As the global market for herbal medicinal products is increasing tremendously, there is an urgent need to determine quality control parameters for the herbal medicines belonging to these systems. *Ayurveda* originated along the river bank of the Indus. *Siddha* is the mother medicine of the ancient era. It originated in South India. Persons who established *Siddha* are Siddhars[1]. *Siddha* formulations contain herbal, mineral and metal ingredients[2]. To determine quality control parameters *Nilavaarai choornam* (NVC) a poly herbal formulation is selected for the present work. Chemical standardisation and scientific validation are necessary for international recognition and global acceptance of these formulations. Hence in the present study selected formulation is subjected to quality control studies as per WHO protocols.

Choornam is are fine dry powder form of medicine, that retains its potency for three months[3]. This formulation contains senna leaf, dry ginger, black

*Author for correspondence

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pepper, ajowan and embelia which are powdered separately and mixed homogeneously[4]. These ingredients of the selected formulations help in the proper absorption, distribution, metabolism and excretion. This ADME concept of the modern approach is fulfilled in the selected *Siddha* formulations, Siddhars have selected these plant ingredients that can help in ADME contributing to the proper maintenance of health.

2. Therapeutic Efficacy of the Ingredients of NVC

Ingredients such as the whole plant of senna in Tamil is called *Nilavaarai* are botanically known as *Cassia angustifolia* L (Figure 1(a)). Sanskrit's name is *Swarna pathra* and in English, it is called Indian senna or Tinnevelly senna. It has laxative action and is used to manage constipation and piles, it is given at a dose level of two grams with hot water at bedtime. The next one is the dry ginger botanical equivalent *Zingiber officinale* Roscoe (Figure 1(b)), it is one among the 108 *kayakarpa* plants (promoting health as well as a prophylactic medicine) in the *Siddha* system. Medicinally, it is given for respiratory problems such as cough, wheezing, sinusitis, headache, fever and digestive disorders, loss of appetite, gastritis, gastric reflexes, gastric ulcer, flatulence, diarrhoea and anaemia. Another ingredient is a black pepper seed botanical named *Piper nigrum* L (Figure 1(c)). It is an important herbal drug in all traditional healthcare systems. In *Siddha,* it acts as an antidote, carminative, antiperiodic, stimulant, resolvent and antipathy drug. Therapeutically it is used to manage colds, coughs, fever, wheezing, sinusitis, indigestion, itching and insect bite[5,6].

Ajowan helps to improve indigestion, Tamil name is *Omam*. Its botanical name is *Trachyspermum ammi* (L.) (Figure 1(d)). Therapeutically it acts as a stomachic, carminative, antispasmodic and sialogogue[7]. The last ingredient is *Embelia ribes* Burm. f. (Figure 1(e)). Its Tamil name is *Vaaividangam*, it enhances the metabolism destroys intestinal worms and enhances digestive activity[2,8].

In total NVC can prevent gastrointestinal diseases (Figure 1(f)) and is unique to the *Siddha* system of medicine. Attempts were also made to identify the key molecules present in the formulation that might contribute to the therapeutic actions employing the HPTLC technique. This same standardization technique is followed in the *Ayurvedic* polyherbal formulation *Hinguvacadi* churna[9].

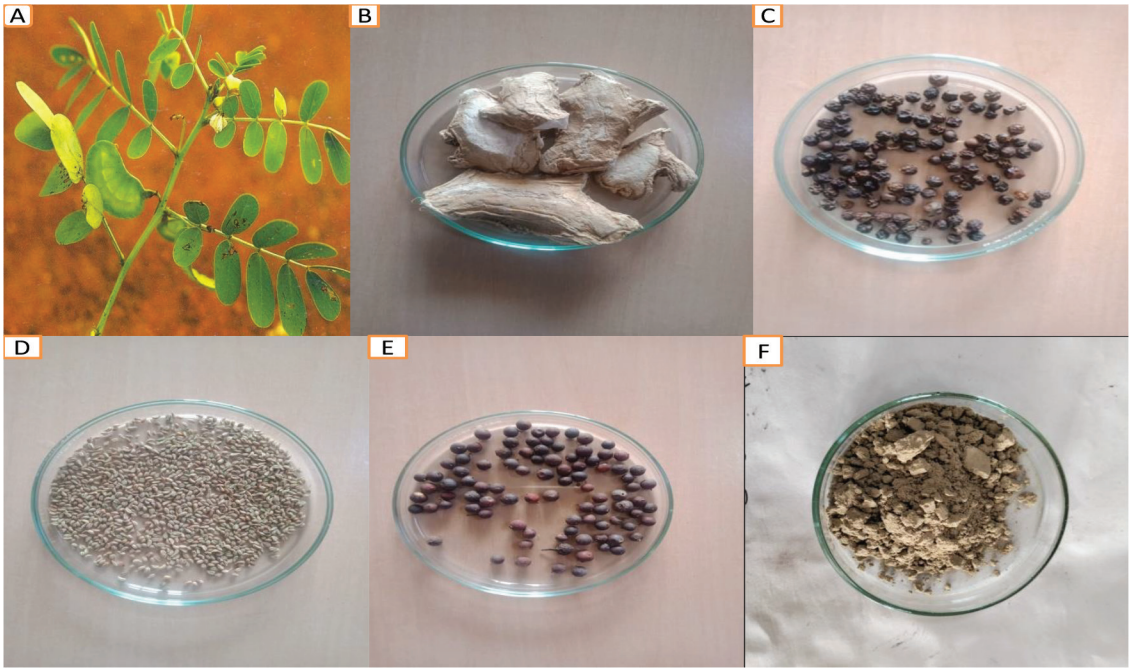

Figure 1. Herbal ingredients of NVC: (a) *Cassia angustifolia*, (b) *Zingiber officinale*, (c) *Piper nigrum*, (d) *Trachyspermum ammi*, (e) *Embelia ribes*, (f) *Nilavarai choornam.*

3. Material and Methods

3.1 Preparation of the Formulation of NVC

The raw materials were purchased from *Siddha* drug shops of Thanjavur market Tamil Nadu, India, and authenticated in the NABL accredited lab of CARISM, SASTRA University and identified using macroscopic and microscopic features. Senna leaf, dry ginger, black pepper, ajowan and embelia were dried and pulverized separately. More than 80% of the powder passed through the sieve number complying with the fineness of the powder. The fine powder thus obtained is mixed to get a homogenous blend and preserved in airtight containers so that the powder is free from light and moisture. Market samples were also procured from *Siddha* medicine dealers, in Thanjavur, Tamil Nadu. All standardization parameters were evaluated for the in-house and market samples of NVC. In house sample is named NVC-H and the market sample is named NVC-M.

3.2 Powder Microscopy

Powder microscopy is a commonly employed quality control technique to authenticate powdered plant materials as per the WHO protocol[10]. Lignin was identified using 1% phloroglucinol in ethanol. After draining the phloroglucinol, add a few drops of HCl. After draining out the extra acid, add a few drops of 30% glycerol, and cover with a cover glass[11]. Lignin-containing cells will stain red. The presence of starch grains was detected by taking a pinch of the powdered plant material on a microscopic slide and the Iodine-Potassium Iodide (IKI) stain used to colour it. Covered with a cover glass and observed through a microscope. The grains will turn dark blue. The presence of lipids was detected by taking a pinch of powdered drug and staining with 1-2 drops of Sudan black B, heated gently and the preparation was irrigated with ethanol (70%), and the slides were mounted and observed under the microscope. The lipids if present will appear black. For viewing crystals with clarity chloral hydrate treatment method was used[12].

3.3 Physicochemical Studies

Physicochemical standards were determined in triplicates following the standard protocol for both NVC-H and NVC-M samples[13,14].

3.3.1 Total Ash

Weigh accurately 2-3 g of the air-dried powdered drug NVC-H and NVC-M, in a previously ignited, tarred silica crucible. Incinerate gently at first, and gradually increase the temperature to 675°±25° until free from carbon. Cool and weigh. If a carbon-free ash cannot be obtained in this way, extract the charred mass with hot water, collect the insoluble residue on an ashless filter paper, incinerate the residue and filter paper until the ash is white or nearly so, add the filtrate, evaporate to dryness and ignite and evaporate to dryness and heat the whole to a temperature of 675°±25°. The % of total ash is calculated as follows:

$$\text{Percentage of total ash (\% w/w)} = \frac{\text{Weight of ash (g)} \times 100}{\text{Weight of the sample (g)}}$$

3.3.2 Acid Insoluble Ash

The ash obtained from Total ash was boiled with 25 ml of 2M Hydrochloric acid for 5 minutes. Collect the insoluble matter on an ashless filter paper, and wash the residue with hot water until free from acid. Ignite the crucible with filter paper at a temperature of 675°±25°, cool it in a desiccator and weigh it.

Calculation:

$$\text{Percentage of acid insoluble ash (\%w/w)} = \frac{\text{Weight of acid insoluble matter (g)} \times 100}{\text{Weight of the sample (g)}}$$

3.3.3 Loss on Drying

Weigh a glass stoppered shallow weighing bottle that has been dried under the same conditions to be employed in the determination. Weighed 1g of sample NVC-H and NVC-M in the shallow weighing bottle, covered it and weighed the bottle with the sample accurately. Distribute the sample evenly as practicable to a depth not exceeding 10mm/remove the stopper and place the loaded shallow weighing bottle in the drying chamber as directed in the monograph. Dry the sample to constant weight or till two consecutive weights remain within ± 0.5 After drying is completed open the chamber, close the bottle and cool the sample to room temperature in the desiccator before weighing. Weigh the shallow weighing bottle and the contents.

Calculation:

$$\text{Loss on drying (\%w/w)} = \frac{\text{Loss in weight (g)} \times 100}{\text{Weight of the sample (g)}}$$

3.3.4 Alcohol Soluble Extractive Values

Macerate 5g of coarsely powdered drug NVC-H and NVC-M, with 100ml of ethanol, of the specified strength (as specified in the monograph) in a closed flask for 24 hours Shake frequently during the first six hours and allow to stand for eighteen hours. Filter rapidly, taking precautions against loss of Ethanol, Evaporate 25 of the filtrate to dryness over a water bath, in a tarred flat-bottomed shallow dish dry at 105°C to constant weight and weight. Calculate the percentage of alcohol-soluble extractives.

Calculation:

$$\text{Percentage of alcohol soluble extractive (\%w/w)} = \frac{\text{Weight of residue (g)} \times 100 \times 100}{\text{Weight of the sample (g)} \times 25}$$

3.3.5 Water Soluble Extractive Values

Macerate 5g of the coarsely powdered drug NVC-H and NVC-M with 100 ml of chloroform water in a closed flask for twenty-four hours. Shake frequently during the first six hours and allow standing for eighteen hours. Filter rapidly, evaporate 25 of the filtrate to dryness in a tarred flat-bottomed shallow dish over a water bath dry at 105°C, in an oven, to constant weight and weigh the dish to calculate the percentage of water-soluble extractive concerning the air-dried drug.

Calculation:

$$\text{Percentage of water soluble extractive (\%w/w)} = \frac{\text{Weight of the residue (g)} \times 100 \times 100}{\text{Weight of the sample (g)} \times 25}$$

3.4 Phytochemical Evaluation

3.4.1 Extract Preparation

5g of NVC-H and NVC-M were macerated with 100 ml of solvents like petroleum ether (40-60°C), chloroform, acetone, alcohol and water in a closed conical flask for 24h. The extract was shaken frequently for 6h and allowed to stand for 18h. The solutions were filtered using Whatman no.1 filter paper. The filtrate was concentrated and used for preliminary phytochemical screening.

3.4.2 Phytochemical Screening

Preliminary phytochemical analysis for steroids, triterpenes, tannins, flavonoids, alkaloids, carbohydrates, proteins and glycosides were carried out as per standard procedures[15].

3.4.3 Fluorescence Analysis

The plant powder was subjected to fluorescence analysis as per Pratt and Chase[16]. The fluorescence characteristics of plant powders were observed before and after treatment with various chemical reagents under UV 254, UV 366 and visible light[17].

3.4.4 HPTLC Instrumentation Methodology

HPTLC fingerprint profiles NVC-H and NVC-M were determined using the HPTLC Linomat system (CAMAG, Muttenz, Switzerland). HPTLC analysis was performed on 10cmx10cm aluminium sheets coated on a silica gel 6F254 (E-Merck, Germany). Sample and standards solutions were applied as bands 7mm wide, 15mm apart and 15mm from the bottom of the plate using a CAMAG Linomat 5 sample applicator fitted with a 100µL Hamilton syringe. The mobile used for the development of chloroform extract was Toluene: Ethyl acetate (9.3:0.7). The mobile phase used for the development of gingerol was n-hexane: diethyl ether (4:6) and for piperine Toluene: Diethyl ether: Dioxane (62.5:21.5:16). The mobile was prepared in a CAMAG twin trough chamber previously saturated for 30min. The plates were allowed to develop in the mobile phase and after development the plates were allowed to dry in a hot air oven at 105°C. The developed plates were scanned at UV 366nm using TLC scanner 3. The images were captured at UV 254 and UV 366nm using CAMAG REPROSTAR III[18].

4. Results

4.1 Powder Microscopic Studies

The results of powder microscopic studies of NVC-H are presented in Figure 2. The powder microscopic studies revealed the presence of xylem vessels with scalariform and spiral thickening and unicellular and uniseriate long and curved warty covering trichomes, dome-shaped unicellular trichomes are seen. The parenchyma cells are filled with brown content, yellow to orange oleoresin, druses of calcium oxalate and acicular crystals. The parenchyma cells also consist of reticulate and pitted thickened. The powder microscopic studies also found that paracytic stomata, wavy and anticlinal palisade cells. elongated septate fibres with pits, beaker-shaped and rectangular stone cells are found. The epidermal cells contain a cuticle

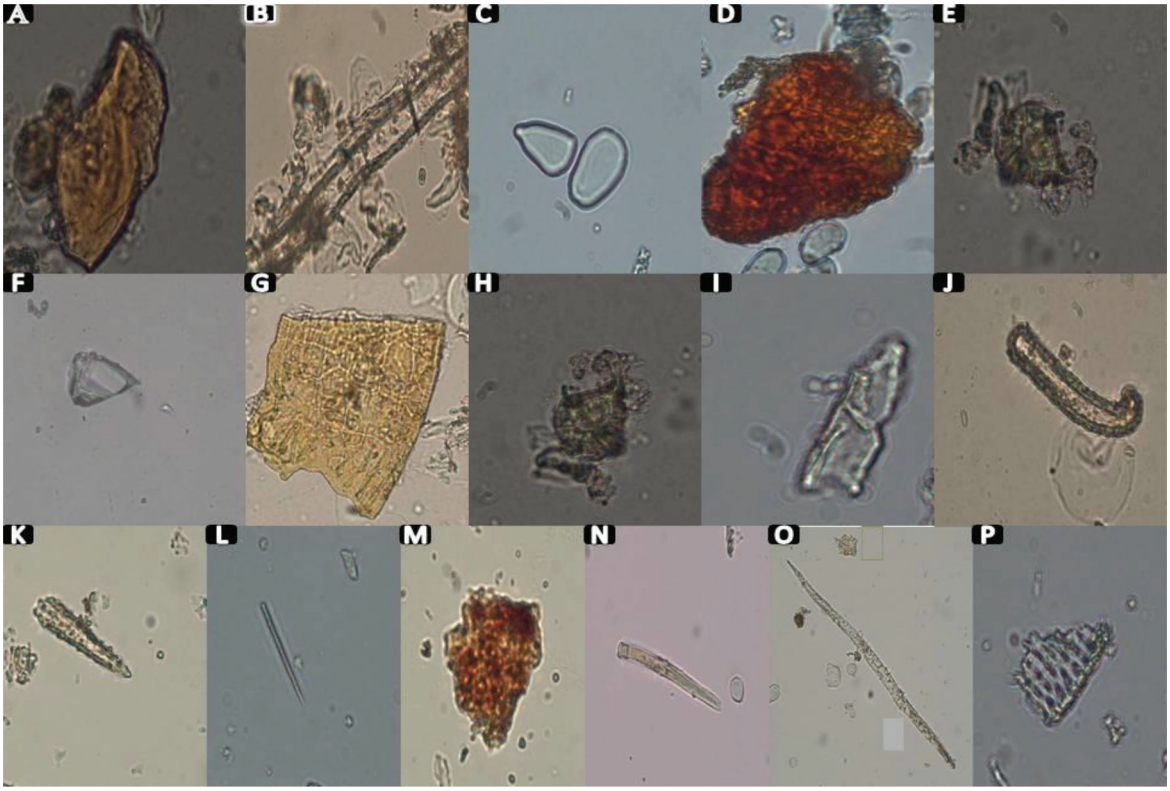

Figure 2. Powder microscopic images: (a) Oleoresin-containing cell, (b) Septate fibber, (c) Beaked starch grains, (d) Reddish brown cells, (e) Stone cells, (f) Prismatic crystals, (g) Epidermal cells with wrinkled cuticle, (h) Beaker shaped stone cells, (i) Sclereids, (j) Unicellular uniseriate trichome, (k) Warty trichome, (l) Acicular crystals, (m) Reddish brown cells, (n) Unicellular trichome with bulging, (o) Striated unicellular trichome and acicular calcium oxalate crystal, (p) Pitted parenchyma cells.

Table 1. Physicochemical parameters of NVC-H and NVC-M samples

PARAMETER	NVC-M(%w/w)	NVC-H(%w/w)
Loss on drying at 105˚C	2.878	2.537
Total ash	3.477	3.025
Acid insoluble ash	0.5474	

with striation, and the endosperm cells are filled with fixed oils.

4.2 Physicochemical Studies

Table 1 reveals the physicochemical features of NVC-H and NVC-M samples. In this formulation LOD level was below 3%, which indicates that the formulation has low moisture content and it indicated the product may have a good shelf life. The ash values obtained in the present work showed low total ash content values and lower acid-insoluble ash values which indicates this formulation contains less inorganic substances.

Table 2. Extractive values of different extracts

EXTRACT	NVC-M(%w/w)	NVC-H(%w/w)
Pertoleum ether	5.080	4.528
Chloroform	5.538	6.006
Acetone	7.408	5.263
Ethanol	7.263	8.050
Water	49.32	52.07

4.3 Preliminary Phytochemical Screening of Various Extracts

The data of the result obtained in the Preliminary phytochemical screening of various extracts were given in Tables NVC-H (Table 3) and NVC-M (Table 4) showing the presence of sterols, terpenoids, flavonoids, saponin, tannin, quinine, alkaloids and glycosides.

4.4 Fluorescence Analysis

Both samples were treated with various chemical reagents like ammonia, acetic acid, pet ether, chloroform,

Table 3. Phytochemical screening of NVC-H

Solvent	Pet ether	Chloroform	Acetone	Alcohol	Water
Protein	-	-	+	+	+
Sterols	+	+	-	-	-
Terpenoids	-	+	-	-	-
Carbohydrates	-	-	+	+	+
Flavonoids	-	-	+	+	+
Saponins	-	-	-	-	-
Tannins	-	-	-	+	+
Lignin	-	-	-	-	-
Quinones	-	-	+	+	+
Mucilage	-	-	-	-	-
Alkaloids	-	+	-	-	-
Glycosides	-	+	-	+	-

Table 4. Phytochemical Screening of NVC-M

Solvent	Pet ether	Chloroform	Acetone	Alcohol	Water
Protein	-	-	+	+	+
Sterols	+	+	+	-	-
Terpenoids	-	+	-	-	-
Carbohydrates	-	-	+	+	+
Flavonoids	-	-	+	+	+
Saponins	-	-	-	-	-
Tanins	-	+	+	+	+
Lignin	-	-	-	-	-
Quinones	-	-	-	+	+
Mucilage	-	-	-	-	-
Alkaloids	-	+	-	-	-
Glycosides	-	+	-	+	-

ethyl acetate, methanol, water, 50% hydrochloric acid, 50% sulphuric acid, 50% nitric acid, 50% ferric chloride and 1N sodium hydroxide[17]. The colour developed was examined under visible and under UV light (254 nm and 365 nm) and the results were presented in Table 5 and Table 6. The chromophores developed in the fluorescent analysis indicate the presence of various types of phytoconstituents in both NVC-H and NVC-M.

4.5 HPTLC Analysis

HPTLC fingerprint profiles of chloroform fractions of NVC-M and NVC-H samples using Toluene: Ethylacetate (9.3:0.7) as mobile phase was shown in Figure 3. Chloroform fraction of NVC-M showed seven distinct spots at R_f 0.02 (blue), 0.06 (green), 0.22 (pink), 0.26 (blue), 0.40 (pink), 0.46 (blue), 0.76 (yellow) under UV366nm and NVC-H also showed seven spots at R_f 0.02 (blue), 0.06 (green), 0.22 (pink), 0.29 (blue), 0.40 (pink), 0.47 (blue), 0.06 (green), 0.22 (pink), 0.29 (blue), 0.40 (pink), 0.47 (blue), 0.78 (yellow) under UV366nm. The peak display of NVC-M (a) and NVC-H (b) is shown in Figure 4. HPTLC analysis of chloroform extracts of NVC-H and NVC-M indicates the presence of various phytoconstituents in selected extracts. And also identified the presence of gingerol and piperine in the NVC-H and NVC-H.

Table 5. Fluorescence analysis of NVC-H

Experiment	Visual observation	UV Fluorescence	
		254 nm	365nm
Powder+Ammonia	brown	brown	green
Powder+Aceticacid	brown	green	green
Powder+Pet ether	green	brown	brown
Powder+chloroform	green	green	green
Powder+Ethylacetate	yellow	green	light green
Powder+Methanol	brown	brown	green
Powder+Water	brown	brown	green
Powder+50%Hcl	brown	green	yellow
Powder+50%H_2SO_4	brown	green	yellow
Powder+50%HNO_3	yellowish brown	brown	green
Powder+$FeCL_3$	dark brown	brown	green
Powder+1N NaOH	dark brown	brown	green

Table 6. Fluorescence result of NVC-M

Experiment	Visual observation	UV Fluorescence	
		254 nm	365nm
Powder+Ammonia	brown	brown	green
Powder+Aceticacid	brown	brown	green
Powder+Pet ether	brown	brown	green
Powder+chloroform	brown	green	green
Powder+Ethylacetate	yellow	brown	green
Powder+Methanol	brown	dark brown	green
Powder+Water	brown	green	green
Powder+50%Hcl	yellowish brown	brown	yellow
Powder+50%H_2SO_4	brown	brown	green
Powder+50%HNO_3	brown	brown	green
Powder+$FeCL_3$	brown	brown	green
Powder+1N NaOH	dark brown	brown	green

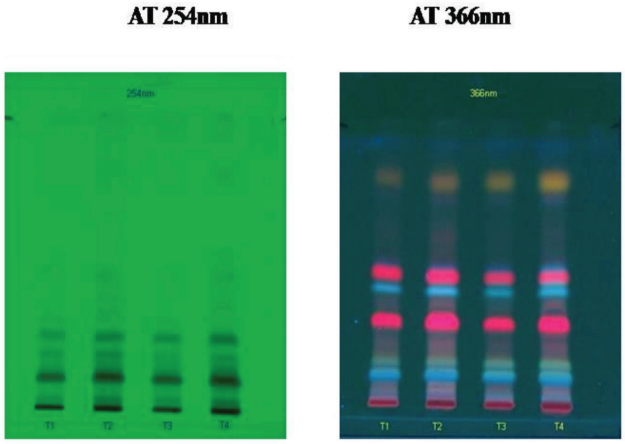

Figure 3. HPTLC photo documentation of chloroform extract of NVC-M and NVC-H. T1, T2-5µl and 10µl of *Nilavagai chooranam* (Market) and T3, T4-5µl, 10µl of *Nilavagai chooranam* (In-house).

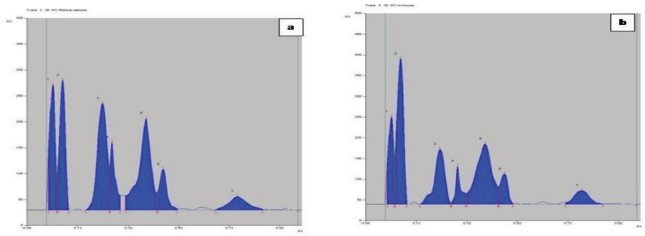

Figure 4. Peak display of NVC-M(a) and NVC-H(b).

The gingerol representative HPTLC profile of Dichloromethane extract of NVC-M and NVC-H using n-hexane: diethyl ether (4:6) as Mobile phase is shown in Figure 5. There were 11 spots observed in NVC-M at R_f 0.10 (blue), 0.16 (pink), 0.20 (blue), 0.25 (green), 0.34 (pink), 0.42 (pink), 0.53 (pink), 0.60 (blue), 0.79 (yellow), 0.88 (pink), 0.92 (blue) and also NVC-H showed 11 spots at R_f 0.09 (blue), 0.15 (pink), 0.24 (blue), 0.33 (green), 0.41 (pink), 0.53 (pink), 0.59 (pink), 0.79 (blue), 0.86 (yellow), 0.91(pink), 0.15 (pink), 0.24 (blue), 0.33 (green), 0.41 (pink), 0.53 (pink), 0.59 (pink), 0.79 (blue), 0.86 (yellow), 0.91 (pink), 0.99 (blue). The peak display of standard gingerol(a), NVC-M(b) and NVC-H(c) at UV 366nm is shown in Figure 6.

The piperine representative HPTLC fingerprinting of Dichloromethane extract of NVC-M and NVC-H using Toluene: Diethyl ether: Dioxane (62.5:21.5:16) as mobile phase is shown in Figure 7. There were 10 spots identified in NVC-M at R_f 0.09 (blue), 0.18 (yellow), 0.25 (pink), 0.39 (blue), 0.52 (blue), 0.59 (green), 0.66 (red), 0.72 (pink), 0.82 (pink) whereas NVC-H showed 9 spots with and 9 spots at R_f 0.09 (blue), 0.18 (yellow), 0.27 (pink), 0.39 (blue), 0.53 (blue), 0.62 (green), 0.72 (pink), 0.79 (pink). Peak display of piperine(a), NVC-M(b) and NVC-H(c) at UV 366nm was shown in Figure 8.

5. Discussion

5.1 Powder Microscopic Studies

The results of powder microscopic studies helped to confirm whether all the ingredients of the formulation

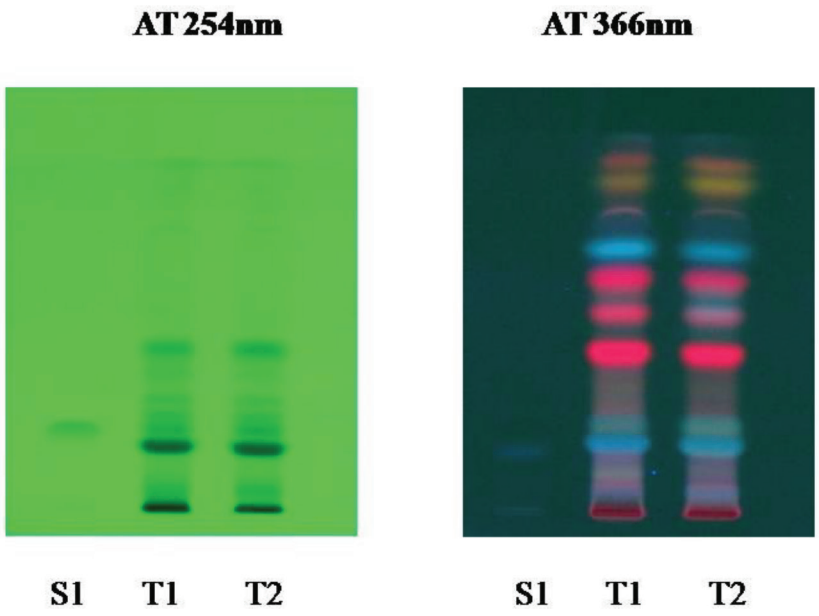

Figure 5. HPTLC finger profile of NVC-M and NVC-H based on gingerol using -hexane: diethylether (4:6) as mobile phase. S1-5µl of standard gingerol, T1 and T2-10µl of NVC-M and NVC-H.

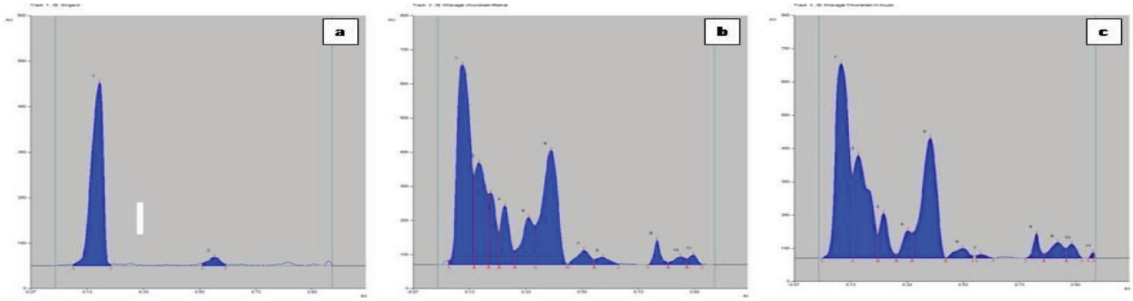

Figure 6. Peak display of standard gingerol(a), NVC-M(b) and NVC-H(c) at UV 366nm.

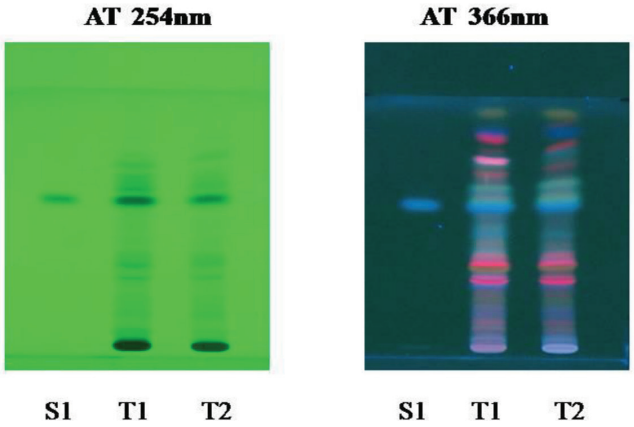

Figure 7. HPTLC finger profile of NVC-M and NVC-H based on piperine using Toluene: Diethyl ether: Dioxane (62.5:21.5:16) as mobile phase. S1-5µl of standard piperine, T1 and T2-10µl of NVC-M and NVC-H.

were included or not. These studies of these formulations confirmed the presence of all the ingredients such as *C. angustifolia, Z. officinale*, *P. nigrum*, *E. ribes* and *T. ammi* as per *Siddha* literature *"Siddha vaithiya thirattu"*[4]. Previously these powder microscopic techniques were carried out for *noccik kudineer choornam*[19].

5.2 Physicochemical Studies

The LOD studies revealed the NVC-H and NVC-M have low water content (<3%). Which indicates the increased shelf life of the *nilavaarai choornam*. Ash values are crucial pharmacognostic parameters that help to determine the quality and purity of herbal medicines. The ash content is typically thought of as a by-product of incineration that simply refers

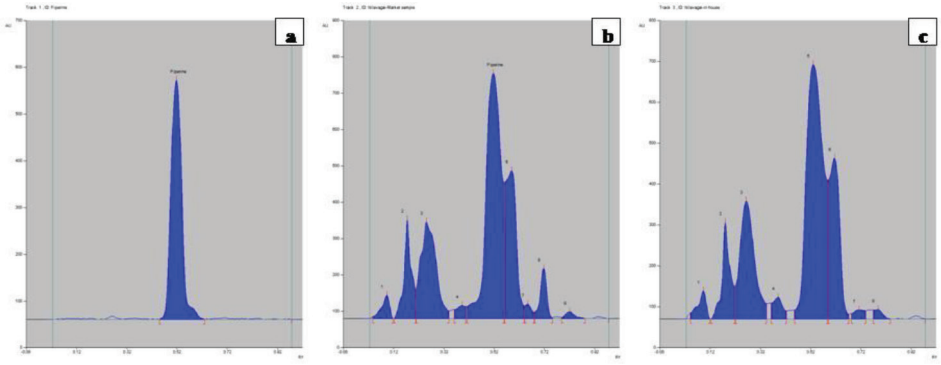

Figure 8. Peak display of piperine(a), NVC-M(b) and NVC-H(c) at UV 366nm.

to inorganic salts that are present in or adhering to crude plant material. The ash values obtained in the current study were low which indicates the selected formulations have fewer inorganic substances and it is highly pure[20].

5.3 Preliminary Phytochemical Screening of Various Extracts

The preliminary phytochemical screening tests revealed that both the samples exhibited the presence of phytochemicals such as proteins, sterols, terpenoids, carbohydrates, flavonoids, tannins, quinones, alkaloids and glycosides.

5.4 Fluorescence Analysis

Both samples were treated with various chemical reagents like ammonia, acetic acid, pet ether, chloroform, ethyl acetate, methanol, water, 50% hydrochloric acid, 50% sulphuric acid, 50% nitric acid, 50% ferric chloride and 1N sodium hydroxide[17]. Colours developed were examined under visible light and also under UV light (254 nm and 365 nm) Fluorescence analysis with different reagents revealed the presence chromo-phores such as yellow, green, brown, and greenish-yellow under ordinary light and UV light which indicates the presence of various phytochemicals such as flavones sterols and phenols[21]. This analysis is one of the pharmacognostic methods for detecting qualitatively the nature of phytoconstituents present in plant materials.

5.5 HPTLC Analysis

It is a very important analytical tool for qualitative analysis and the identification of major constituents. It is also one of the most common techniques used in phytochemical investigations[22,23]. Both NVC-M and NVC-H showed similar spots indicating the presence of volatile oil from *T. ammi, P. nigrum* and *Z. officinalis*[24]. The similar pattern of chromatogram with these R_f values indicated that all these ingredients are present in the formulation. The presence of gingerol and piperine represents the selected formulation contains the *P. longum*[26] and *Z. officinale*[25] and it can be used as a marker compound for quality standardization studies of *Nilavaarai chooranam*.

6. Conclusion

The quality standardization studies of NVC were carried out to determine its quality control parameters. Quality control studies can help in developing quality herbal medicines for the better health care of mankind. Quality standards for NVC were determined employing WHO quality control protocols and the HPTLC profile was determined as per Ayush protocols. The *choornam* was evaluated for both botanical and chemical standardization of NVC a safe poly herbal formulation containing many phytoconstituents. This *choornam* has the potential of Absorption, Digestion, Metabolism, and Excretion (ADME) which is essential for proper health maintenance. Major constituents detected in this formulation are piperine and gingerol. Piperine promotes a positive effect on nutrient bioavailability and acts as a bioenhancer. Gingerol facilitates gastrointestinal motility which improves immunity. To conclude the following standards were determined and reported for the *Siddha* polyherbal formulation *Nilavaarai choornam.*

7. References

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