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Comparative evaluation of phytoconstituents in *Nayopayam Kwatha* (An Indian polyherbal formulation) and its component Herbs

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Abstract
Ayurveda is largely based upon usage of herbs either singly or in combination (polyherbal). Polyherbal formulation has been used all around the world due to its curative and beneficial application. In present research work, an attempt was made to compare the phytoconstituents in Nayopayam Kwatha (An Indian polyherbal formulation) and its component Herbs. The component herbs in the polyherbal formulation were collected, authenticated and processed. Ayurvedic fomulation was prepared as per standard methodology. The prescribed ratio of Nayopayam kwatha is 10:2:2, Sida: Cumin: Ginger. Accordingly 90 g of S. cordifolia roots, 18 g of C. cyminum fruits and 18 g of Z. officinale rhizome were weighed and taken for kwatha preparation. The individual plant extracts of the three raw drugs of Nayopayam kwatha *viz*. Sida, Cumin and Ginger were prepared in the same standard methodology followed for the preparation of Nayopayam kwatha. The differential separation of prepared of kwatha and individual extracts was done using different solvents such as hexane, chloroform, methanol and distilled water. Preliminary phytochemical analysis, Total phenolic content assay, The antioxidant activity analysis and High Performance Thin Layer Chromatography (HPTLC) Studies were done to compare phytoconstituents in Nayopayam Kwatha (An Indian polyherbal formulation) and its component Herbs. The results of preliminary phytochemical evaluations indicate that, the highest number of different phytoconstituents was observed in the Nayopayam kwatha compared to component herbs. The comparative HPTLC fingerprinting of hexane, chloroform, methanol and water fractions of Nayopayam kwatha and individual decoctions provided valuable information on the quality of the samples and showed great variation in the number of bands and their intensities. The present study was an attempt to find out the chemical changes during the processing of polyherbal formulations by comparing it with the individual source plants. The present study also shows that HPTLC fingerprinting is a reliable method to assess these chemical changes during Ayurvedic processing. Quantitative and qualitative differences were also observed with the individual source plants and the polyherbal formulations.

Keywords: phytoconstituents, *nayopayam kwatha*, polyherbal formulation, HPTLC

1. Introduction
India has a rich heritage of traditional medicine constituting different components like Ayurveda, Siddha, Unani etc. has been flourishing in this country for many centuries [1]. The earliest documents of Indian medicine are found in the Vedas in between 3000 and 1000 B.C and they were compiled in Sanskrit [2].
Ayurvedic medicine is available in a variety of dosages form such as Avaleha (electuary), Asava-Arishta (alcoholic preparations), Ghrita (fat-based medicine), Taila (oil based medicine), Churna (powder), Swarasa (juice), Vati (tablet), Kwath (decoction), and much more [3]. The development of this traditional system of medicine with perspectives of safety, efficacy and quality will help not only to preserve the traditional heritage but also to rationalize the use of natural products in health care. Majority of the remedies are based on plants and plant products along with animal origin as well as minerals. The subject of herbal drug standardization is massively wide and deep [4].
Drug formulation in Ayurveda is based on two principles: Use as a single drug and use of more than one drug, in which the latter is known as Polyherbal formulation (PHF). This key traditional therapeutic herbal strategy exploits the combining of several medicinal herbs to achieve extra therapeutic effectiveness, usually known as polypharmacy or poly herbalism. It is evident that better therapeutic effects can be reached with a single multi-constituent formulation. For this, a lower dose of the herbal preparation would be needed to achieve the effect and herbals may act on multiple targets at the same time to provide a thorough relief [5].
The concept of poly herbalism is peculiar to Ayurveda although it is difficult to explain in terms of modern parameters. Sarandghar Samhita highlights the concept of synergism behind polyherbal formulations. Ayurveda has fundamental aspects for drug formulation. The herbs are selected according to disease and other herbs are used to prevent side effects arising from chief herb [6]. Synergy is defined as the interaction of two or more agents to produce a combined effect greater than the sum of their individual effects.
Use of herbal products requires great care towards the quality standard of natural ingredients. The standardization of herbal formulation using various analytical techniques helps to establish quality parameters and composition of formulation may also be ensured. The quality standardization by analytical technique also helps to confirm marker compounds of formulation in qualitative as well as quantitative manner. These modern analytical techniques such as HPLC, HPTLC and UV visible spectroscopy offer great advantages towards the high quality standardization of herbal products [7].
Nayopayam kwatha is a herbal decoction that contains Sida cordifolia L. (Bala), Cuminum cyminum L. (Jiraka) and Zingiber officinale Rosc. (Viswam) as described in

sahasrayoga. This kwatha is a very good bronchodilator and carminative in actions. It is indicated for respiratory diseases like hiccup (hikka), bronchial asthma (thamakaswasa), gas trouble, cardiac diseases etc.In Ayurveda, single or multiple herbs (polyherbal) are used for the treatment. The active phytochemical constituents of individual plants are insufficient to achieve the desirable therapeutic effects. When combining the multiple herbs in a particular ratio, it will give a better therapeutic effect and reduce the toxicity [5].Due to synergism, polyherbalism confers some benefits not available in single herbal formulation. It is evident that better therapeutic effect can be reached with a single multi-constituent formulation. For this, a lower dose of the herbal preparation would be needed to achieve desirable pharmacological action, thus reducing the risk of deleterious side-effects. Besides, polyherbal formulation bring to improved convenience for patients by eliminating the need of taking more than one different single herbal formulation at a time, which indirectly leads to better compliance and therapeutic effect. All these benefits have resulted in the popularity of polyherbal formulation in the market when compared to single herbal formulation. There is no scientific explanation for the synergistic action of most of the drugs resulting in less global recognition. This is an attempt to find out an explanation for the appearance and desertion of biomolecules in polyherbal formulations.

Material and Methods

Collection and processing of plant material

Roots of Sida cordifolia L., fruits of Cuminum cyminum L. and rhizome of Zingiber officinale Rosc. were used for the present study. The raw drugs used for the preparation of Nayopayam kwatha were collected from raw drug department of Arya Vaidya Sala, Kottakkal. Sida was obtained in fresh and the plant was washed with water, dried in shade; root portion was separated and cut into small pieces. After drying, the plant materials were ground separately using mechanical blender into fine powder and transferred into airtight containers at ambient temperature with proper labelling for future use (Plate: 1).

Preparation of Nayopayam kwatha

Ayurvedic fomulation was prepared as per standard methodology (API Part-I, 2003). The prescribed ratio of Nayopayam kwatha is 10:2:2, Sida: Cumin: Ginger. Accordingly 90 g of S. cordifolia roots, 18 g of C. cyminum fruits and 18 g of Z. officinale rhizome were weighed and taken for kwatha preparation. Generally the weighed drug is powdered and 16 times water is added to it, boil it till it is reduced to 1/4th quantity. Kwatha indicated for drinking is directed to be reduced up to 1/8th quantity. 504 ml (1/4th) of water was added to the pot and the level was marked before adding the rawdrugs. The weighed raw drugs were taken into the pot and 2016 ml (16 times) of water was added to the mixture. It was boiled on a low flame till reduced to 1/4th of the total volume (504 ml). The extract was allowed to cool and filtered through muslin cloth. It is further centrifuged at 12,000 rpm for 5 minutes and the supernatant was taken and stored in air tight glass containers in low temperature.

Preparation of individual plant extracts

The individual plant extracts of the three raw drugs of Nayopayam kwatha *viz*. Sida, Cumin and Ginger were prepared in the same standard methodology followed for the preparation of Nayopayam kwatha. Sida decoction was prepared by 90 g of S. Cordifolia roots, weighed and taken in the pot. 2016 ml (16 times) of water was added to the mixture. It was boiled on a low flame till reduced to 1/4th of the total volume (504 ml). 18 g each of cumin and ginger were weighed and taken for the preparation of Ginger decoction and Cumin decoction respectively. Sixteen times of water (2016 ml) was added to the mixture and was boiled till reduced to its one fourth of the total volume (504 ml). The Sida decoction, Cumin decoction and Ginger decoction was allowed to cool and filtered through muslin cloth. The extracts were centrifuged at 12,000 rpm for 5 minutes and the supernatant was taken and stored in low temperature.

Preparation of individual plant extracts

The individual plant extracts of the three raw drugs of Nayopayam kwatha *viz*. Sida, Cumin and Ginger were prepared in the same standard methodology followed for the preparation of Nayopayam kwatha.

Differential separation of kwatha and individual plant extracts

The differential separation of prepared of kwatha and individual extracts was done using different solvents such as hexane, chloroform, methanol and distilled water. 25 ml each of centrifuged Nayopayam kwatha, Sida decoction, Cumin decoction and Ginger decoction were taken in separating funnel and 10 ml of hexane was added three times subsequently. The fractions were shaken well. Allowed to settle for 15 minutes and then hexane fraction was collected each time. It was followed by chloroform fractionation in the same manner. The chloroform fractions were centrifuged at 5000 rpm using high speed refrigerated centrifuge and the residue was taken for methanol separation. For obtaining methanol fraction the remaining kwatha and individual extracts were evaporated in Petri plate in a water bath, made up with methanol and centrifuged at 5000 rpm. The sediment was dissolved in 5 ml water for obtaining water fraction. All the fractions were reduced to 5ml and separately kept in labelled glass vials. Preliminary phytochemical analysis to identify the presence of various metabolites different preliminary analysis were conducted.

Total phenolic test

Total phenolic content was measured by the Folin-Ciocalteu phenol colorimetric assay [8].

Antioxidant assay

The antioxidant activity of samples was evaluated by the phosphomolybdenum method according to the procedure of Umamaheswari and Chatterjee [9].

High Performance Thin Layer Chromatography (HPTLC) Studies

Stationary phase Aluminium backed pre-coated Merk silica gel plate 60 F 254 plate (10×8 cm). Procedure Hexane, Chloroform, Methanol and Water fractions of the Nayopayam Kwatha and individual plant extracts were applied on the plate respectively, for a total of four plates using Camag automatic TLC sampler 4 attached to Camag HPTLC system. Applied 10µl of test solutions on a pre-coated silica gel 60 F254 TLC plate (E. Merk) of uniform

thickness of 0.2 mm plate in the form of bands with width 8mm using Hamilton syringe (100µl). Develop the plate in their respective solvent systems as of TLC analysis in a twin trough chamber to a distance of 9 cm. The plate was then derivatized using Anisaldehyde sulphuric acid (ANS) reagent. Visualization Observed the plate under UV light at 254nm and 366nm. Plate was visualized at 550nm after ANS derivatization. The water fractions were derivatized using AlCl3 and visualized under 366nm. Scanning Densitometric scanning of the plates was done by using Camag TLC scanner at 254, 366 and 550 nm.

Results and Discussion

Preliminary phytochemical analysis The preliminary phytochemical analysis of Nayopayam kwatha and individual decoction was done using different phytochemical tests. The potential phytoconstituents present in the samples were analysed and characteristic differences were observed regarding the chemical composition of different extracts.

In *Sida* decoction, phytochemical constituents such as flavanoids, phenols, quinones and saponins were present. Whereas alkaloids, coumarins, glycosides, steroids, tannins and terpenoids were absent.

In Cumin decoction, phytochemical constituents such as coumarins, flavanoids, phenols, quinones and tannins were present. Whereas alkaloids, glycosides, saponins, steroids and terpenoids were found to be absent in the phytochemical screening.

In Ginger decoction, phytochemical constituents such as coumarins, flavanoids, phenols and quinones were present. Whereas alkaloids, glycosides, saponins, steroids, tannins and terpenoids were absent.

In Nayopayam kwatha, phytochemical constituents such as alkaloids, coumarins, flavanoids, phenols, quinones and saponins were present. Whereas glycosides, steroids, tannins and terpenoids were absent.

The comparative analysis of the phytochemical components of Nayopayam kwatha and individual decoction confirmed the presence of various phytochemicals. The highest number of different phytoconstituents was observed in the Nayopayam kwatha. The coumarins were found to be absent only in Sida decoction. Cumin decoction revealed the only potential presence of tannins. Flavanoids, phenols and quinones are present in all the samples and glycosides, steroids and terpenoids were observed to be absent. Alkaloids were found to present only in Nayopayam kwatha.

Quantitative analysis

Total Phenolic Estimation

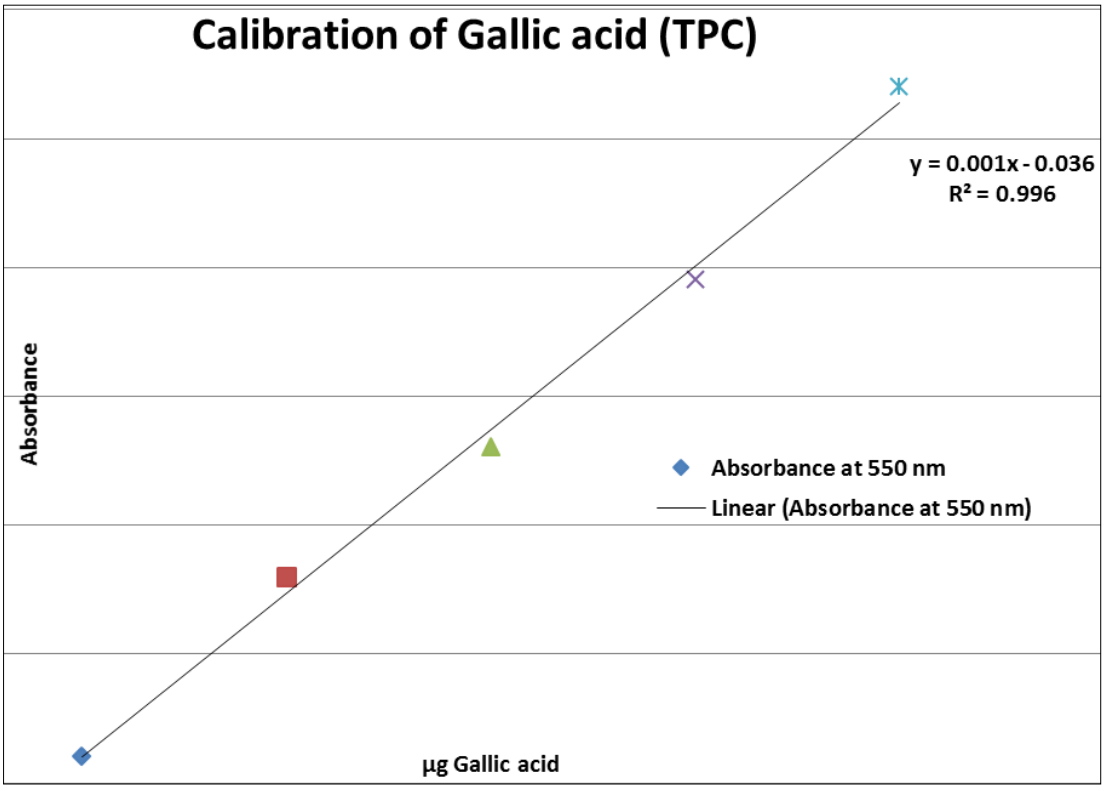

Fig 1: Callibration of Gallic acid

Table 1

Name of the extract	TPC (mg GAE/g)
Sida	20.34 ± 0.976
Cumin	36.067 ± 2.359
Ginger	15.59 ± 0.721
Nayopayam kwatha	13.10 ± 0.564

Folin-Ciocalteau colorimetry is based on a chemical reduction of the reagent, a mixture of tungsten and molybdenum oxides. Singleton adapted this method to wine analysis [8]. The products of the metal oxide reduction have a blue colour that exhibits a broad light absorption with a maximum at 765 nm. The blue complex was formed by the reduction of reagent by phenolic compounds in extract. The

intensity of light absorption at that wavelength is proportional to the concentration of phenols [10]. The total phenol content was estimated in the *Nayopayam kwatha* and individual decoction using Folin-Ciocalteu reagent (FCR) using standard Gallic acid curve standardized in the lab for the calculation of Gallic acid equivalent (GAE) per gram of extracts **(Fig. 1).**

The total phenolics content of the samples were studied using the Gallic acid calibration curve. Cumin decoction had the maximum total phenolic content (TPC) of about 36.067 ± 2.359 and the least was observed in Nayopayam kwatha (13.10 ± 0.564).

Antioxidant assay

Phosphomolybdate assay

The phosphomolybdate method has been routinely used to evaluate the antioxidant capacity of extracts. In the presence of extracts, Mo (VI) is reduced to Mo (V) and forms a green coloured phosphomolybdenum V complex, which shows a maximum absorbance at 700 nm [11]. The antioxidant activity of the extracts was estimated with that of the standard ascorbic acid **(Fig. 2).**

The total antioxidant capacity is expressed as ascorbic acid equivalents. Among the samples tested, the *Sida* decoction contains 22.13 mg ascorbic acid equivalent per gram. The least was observed in the *Nayopayam kwatha* (12.22mg). The antioxidant activity increased in the order of *Sida* decoction > cumin decoction > ginger decoction > Nayopayam kwatha.

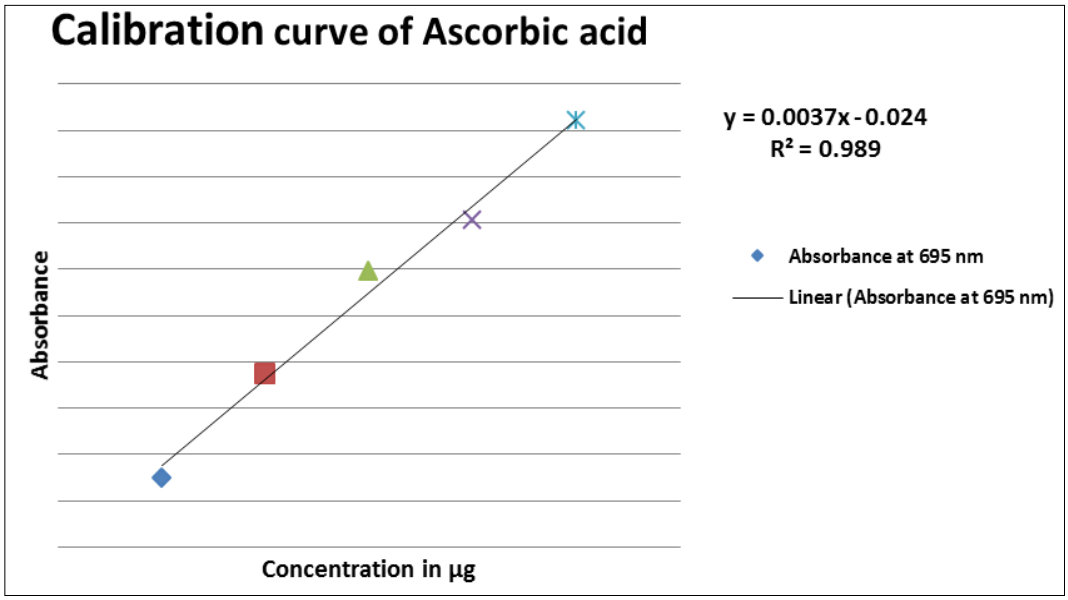

Fig 2: Callibration of Ascorbic acid

Table 2

Name of the extract	Antioxidant capacity (mg AAE/g)
Sida	22.13 ± 1.792
Cumin	20.94 ± 1.10
Ginger	17.44 ± 0.912
Nayopayam kwatha	12.22 ± 0.102

HPTLC profiling

Nayopayam kwatha and individual decoctions were differentially fractionated individually using hexane, chloroform, methanol and water to obtain four different fractions each. The HPTLC profile was developed for the hexane fraction, chloroform fraction, methanol fraction and water fraction. The solvent systems for the different fractions were standardized. Disparities were observed in terms of number of bands and band intensity of the HPTLC profile developed for different fractions of *Nayopayam kwatha* and individual decoctions. These disparities in turn show the qualitative and quantitative deviation in chemical constituents.

Toluene: Ethyl acetate mobile phase combination in the ratio 9: 1 was the best solvent system for the separation of hexane fraction**.** The developed chromatographic plate was visualized under 254 nm, 366nm and they were derivatized using Anisaldehyde sulphuric acid (ANS) reagent and visualized at 550 nm. The hexane fractions at 254nm showed a total of only three bands. The band at Rf 0.04 was specific to cumin decoction and a common band at Rf 0.15 was absent in *Sida* decoction. There was a common band for ginger decoction and *Nayopayam kwatha* at Rf 0.51 (Table-1 and Plate-2).

Under 366 nm, a total of 12 compounds was found. Among the 12 bands, four of the bands were common to ginger and *Nayopayam kwatha*. A single band was found to be common for *Sida* and *Nayopayam* at Rf 0.19. The *Sida* decoction had a unique band at Rf 0.49. The bands at Rf 0.54 and 0.60 was specific to cumin decoction. Four bands were observed to be unique for the ginger decoction (Table-1 and Plate-2).

A total of 9 bands were found to be present in the ANS derivatized hexane HPTLC plates visualized at 550 nm. No bands were observed for *Sida* decoction in 550 nm. The ginger hexane fraction showed a single unique band at Rf 0.30. A specific band for cumin and *Nayopayam* was seen at Rf 0.11. A total of three bands were common to cumin, ginger and *Nayopayam* except the *Sida* decoction. Ginger and *Nayopayam kwatha* together possessed a total of four unique compounds (Table-1 and Plate-2).

The comparative HPTLC profiling of hexane fraction of *Nayopayam* and individual decoction under different wavelengths had a total of 24 different Rf values and no bands in common among all samples. A total of four bands were present in all samples except the *Sida* decoction. Five specific compounds were present in ginger decoction and

three unique bands were observed in cumin decoction. A single band was found to be specific to *Sida* decoction. Nine bands were found to be common for ginger and *Nayopayam*. *Sida* and *Nayopayam* had one common Rf value. Cumin and *Nayopayam kwatha* also had a common band. Among the individual decoctions most number of phytoconstituents was found to be in ginger (18) and the least was in *Sida* (2). *Nayopayam kwatha* had a total of 15 compounds.

HPTLC profile was developed for chloroform fractions of *Nayopayam kwatha* and individual decoctions. Toluene: Ethyl acetate mobile phase combination in the ratio 8: 2 was the best solvent system for the separation of chloroform fraction. The developed plate was visualized at different wavelengths. A total of six compounds were found to be present in the chloroform decoction at 254 nm. Two common bands at Rf 0.10 and 0.32 was only absent in *Sida* and the Rf 0.20 was found to absent only in ginger decoction. The compounds at Rf 0.04 and 0.06 was seen in both *Sida* and *Nayopayam kwatha*. The cumin decoction had a specific band at Rf 0.40 (Table-2 and Plate-3).

The chloroform decoction visualized at 366 nm had a total of nine compounds. The compound at Rf 0.19 was a common band to all of the four samples. Ginger decoction had two bands in common with the *Nayopayam kwatha* at Rf 0.72 and Rf 0.96. The *Sida* decoction and cumin decoction shared a band with *Nayopayam kwatha* at Rf 0.34 and Rf 0. 87 respectively. Two compounds at Rf 0.04 and 0.26 was unique to the ginger decoction and a specific band at Rf 0.06 was unique to *Sida* decoction. The *Nayopayam kwatha* showed a specific compound at Rf 0.80 (Table-2 and Plate-3).

The ANS derivatized HPTLC plate of chloroform extract at 550 nm had a total of 14 compounds. Among 14 plates five bands were present in both ginger decoction and *Nayopayam kwatha*. Two compounds at Rf 0.23 and 0.27 were common in Cumin decoction and *Nayopayam kwatha*. A band at Rf 0.05 was present among the *Sida* decoction and cumin decoction. *Sida* and ginger decoction had two unique bands and cumin had a unique band at Rf 0.12. The *Nayopayam kwatha* had a specific band at Rf 0.56 80 (Table-2 and Plate-3).

The comparative HPTLC profiling of chloroform fractions of the *Nayopayam kwatha* and individual extracts at different wavelengths had a total of 29 different compounds. There was a single common band present in all of the four samples studied under 366 nm at Rf 0.19. The ginger decoction had the most number of unique bands (4) and the cumin decoction had the least (2) among the three individual decoctions. *Nayopayam kwatha* had two bands that are specific to the formulation alone. Two common bands were absent in *Sida* decoction and one common band was seen absent in ginger decoction. Among the 29 bands seven bands were found to be present in both the ginger and *Nayopayam kwatha*. Three bands were observed in both *Sida* decoction and *Nayopayam kwatha*. Cumin decoction and *Nayopayam kwatha* also possess three common bands. A single compound was found to be present in both *Sida* and cumin decoction. Among the individual decoctions most number of phytoconstituents was found to be in ginger (14) and the least was in *Sida* (9). *Nayopayam kwatha* had a total of 19 compounds.

The HPTLC studies were used to develop the chromatogram of the methanolic fractions of *Nayopayam kwatha* and individual decoctions at different wavelengths. Toluene: Ethyl acetate: Methanol: Acetic acid mobile phase combination in the ratio 7: 3: 2: 0.3 was found to be the best solvent system for the separation of methanol fractions. The HPTLC plate visualized at 254 nm had a total of about 12 compounds. A common band at Rf 0.72 was present in the individual decoctions and *Nayopayam kwatha*. Three common bands were absent in the ginger decoction. The *Sida* and ginger decoction had a common band at Rf 0.03. Two common bands at Rf 0.06 and 0.41 was observed in cumin and *Nayopayam kwatha*. Two bands were specific to each of the *Sida* decoction and cumin decoction respectively. *Nayopayam kwatha* had one unique band at Rf 0.12 (Table-3 and Plate-4).

A total of 16 compounds were visualized in the methanolic fractions at 366 nm. The maximum number of bands in methanolic fraction was observed under 366 nm. No common bands were observed among the four samples. A common band was found to be absent in the cumin decoction at Rf 0.72. Four bands at Rf 0.06, 0.09, 0.23 and 0.28 was present in cumin decoction and *Nayopayam kwatha*. *Sida* decoction and ginger decoction had a unique band at Rf 0.02. A total of five bands were unique to cumin decoction and four compounds were specific to *Sida* decoction. A unique band was observed for *Nayopayam kwatha* at Rf 0.12 (Table-3 and Plate-4).

The HPTLC chromatogram after ANS derivatization was visulaized at 550 nm and had a total of 10 compounds. Two bands at Rf 0.57 and 0.72 was found to be common to all of the four different samples. A common band was absent in ginger decoction alone at Rf 0.07. The compound at Rf 0.03 was specific to cumin and ginger decoction. Cumin and *Nayopayam kwatha* had a common band at Rf 0.23. Two bands were specific to each of the *Sida* decoction (Rf 0.18 and 0.28) and ginger decoction (0.08 and 0.24) respectively. *Nayopayam kwatha* had a unique band at Rf 0.51 (Table-3 and Plate-4).

The comparative HPTLC analysis of methanol fractions of the individual extracts and *Nayopayam kwatha* at various wavelengths had a total of about 38 phytoconstituents. The most number of bands among the individual decoction was seen in the cumin decoction (22) and the least was observed in the ginger decoction (9). Three common bands were present in all of the four samples. Four common bands were absent in ginger decoction and one common band was seen absent in cumin decoction The *Sida* decoction had the most number of unique bands (8) and the ginger decoction had the least (2) among the three individual decoctions. *Nayopayam kwatha* had three bands that are specific to the formulation alone. Seven bands were found to be present in both the cumin and *Nayopayam kwatha*. Two bands were observed in both *Sida* decoction and ginger decoction. A single compound was found to be present in both ginger and cumin decoction.

The best solvent system for the separation of water fraction was found to be Toluene: Ethyl acetate: Methanol: Acetic acid mobile phase combination in the ratio 7: 3: 2: 0.3. HPTLC chromatogram was developed for the water fractions of *Nayopayam kwatha* and individual decoctions at different wavelengths. A total of 6 compounds were observed under 254 nm wavelength of UV light in water fractions. Four common bands (Rf 0.07, 0.11, 0.29 and 0.76) were found to be absent in ginger decoction. No bands were observed for ginger decoction. Two bands at Rf 0.20

and 0.76 was specific to cumin decoction and *Nayopayam kwatha* (Table-4 and Plate-5).

Under 366 nm, a total of 11 compounds were observed in the HPTLC chromatogram of water extracts. No common bands were seen among the four samples. A common band was present in all samples except the ginger decoction at Rf 0.05. Two bands at Rf 0.09 and 0.73 was specific to cumin decoction and *Nayopayam kwatha*. *Sida* decoction and *Nayopayam kwatha* had two specific bands at Rf 0.12 and 0.46. Four bands (0.21, 0.25, 0.29 with red colour and 0.55) were specific to cumin decoction alone and two bands (0.16 and 0.29 with ash colour) were unique to the *Sida* decoction (Table-4 and Plate-5).

The developed HPTLC plate of water fraction was derivatized using Aluminium chloride ($AlCl_3$) and visualized at 366 nm. A total of 15 bands were observed in the water extract. Five common bands were found to be present in *Sida* decoction and *Nayopayam kwatha*. Cumin decoction and *Nayopayam kwatha* had one specific band at Rf 0.74. Six specific compounds were seen in the cumin decoction and a unique band was observed for the *Sida* decoction at Rf 0.05. Cumin decoction had two characteristic unique yellow coloured bands at Rf 0.09 and 0.19. *Nayopayam kwatha* had two unique bands at Rf 0.04 and 0.09 (Table-4 and Plate-5).

The comparative HPTLC profiling of water fractions of *Nayopayam* and individual decoctions under different wavelengths had a total of 32 different compounds. No band was found to be in common among all samples. No band was found to be present in the ginger decoction even after derivatization. A total of five bands were present in all samples except the cumin decoction. Ten specific compounds were present in cumin decoction and three unique bands were observed in *Sida* decoction. Nine bands were found to be common for ginger and *Nayopayam*. *Sida* and *Nayopayam* had seven common bands. Cumin and *Nayopayam kwatha* had a total of five common bands. Among the individual decoctions most number of phytoconstituents was found to be in cumin (22) and the least was in ginger (9). *Nayopayam kwatha* had a total of 18 compounds.

The comparative analysis of the HPTLC profiling of hexane, chloroform, methanol and water fractions of *Nayopayam kwatha* and individual decoctions showed that the maximum number of phytoconstituents was found to be present in the methanol fraction. A total of 38 different phytoconstituents were present in the methanol fraction of the extract. The least number of compounds (24) were observed in the hexane fraction of the samples. The *Nayopayam kwatha* and individual extracts were prepared using water as the solvent. The phytoconstituents with slightly high polarity close to that of the water will be mainly present in the extracts and formulation.

Among the *Nayopayam kwatha* and individual extracts, *Nayopayam kwatha* had the maximum number of phytoconstituents in total (71). Cumin decoction had the maximum compounds (60) and least was for the ginger decoction (41). *Sida* decoction had 44 bands in total. Cumin decoction had the maximum phytochemical compounds found to be common with that of the *Nayopayam kwatha* (36). Ginger decoction had the least number of similar bands with that of the Nayopaym *kwatha* (23). *Sida* decoction showed a total of 26 compounds present in both *Sida* and *Nayopayam kwatha*. However, there were a number of compounds/bands that are unique to the individual decoctions and *Nayopayam kwatha*. The cumin decoction had the maximum number of unique bands (22). Ginger decoction had the least number of specific bands (11). *Sida* decoction had a total of 15 unique bands that are absent in all other samples. It had been observed that a total of seven unique bands were observed in *Nayopayam kwatha* which was not present in individual extracts of the source plants. In addition a total of 10 common bands were found to be absent only in the ginger decoction. The *Sida* decoction had lacked 6 common bands and the cumin decoction had showed the absence of only a single common band in total.

Plate 1: Raw drugs of Nayopayam kwatha. A and B: sida cordifolia L. root and its powdered from respectively. C and D cuminum Cyminum L. fruit and its powdered from respectively. E and F: Zingiber officinale Rosc. rhizome and its powdered from respectively.

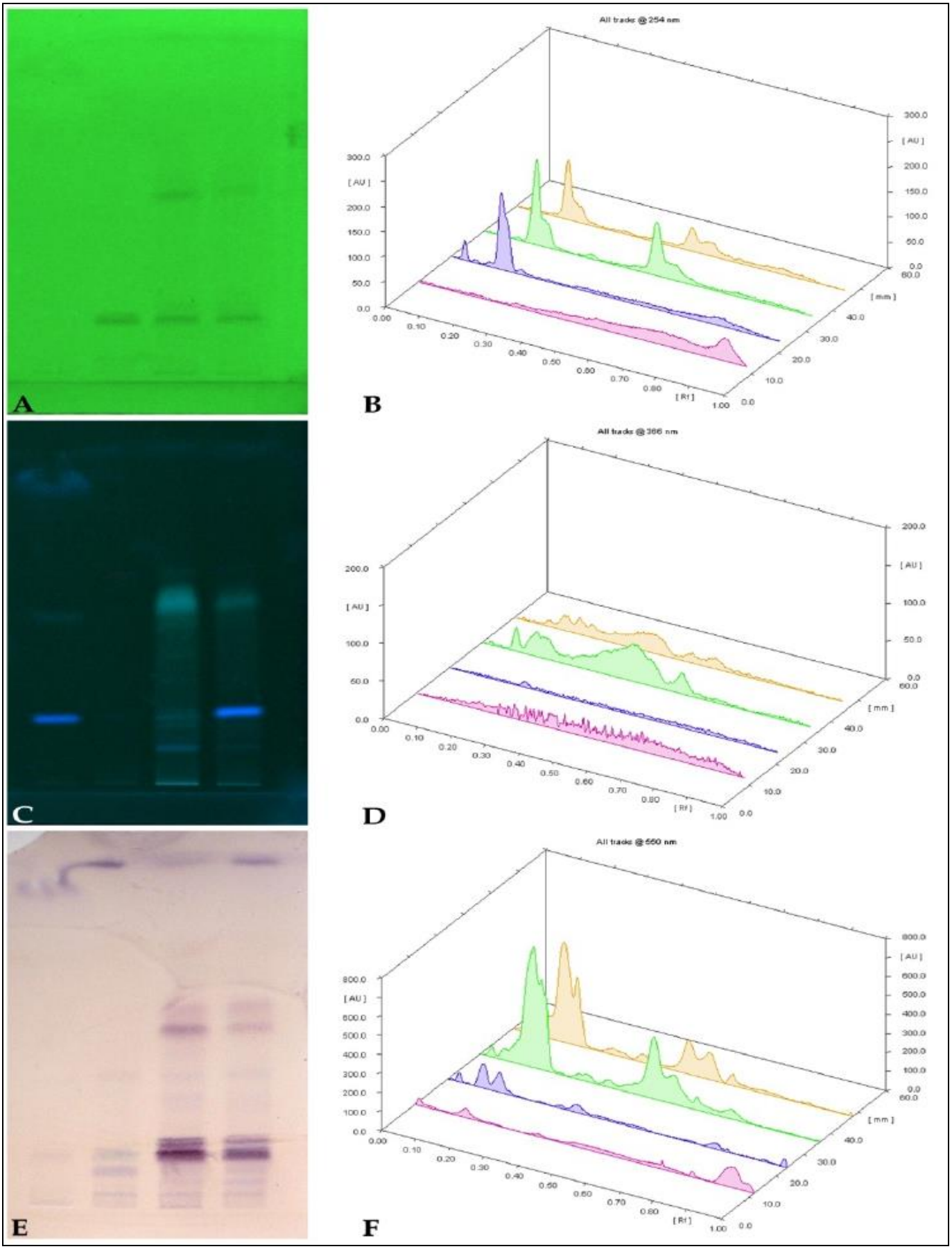

Plate 2: HPTLC Profile of hexane extracts of nayopayam kwatha and individual decotion. A and B: HPTLC chromatogram and densitometric scanning image under 254 nm respectively. C and D: HPTLC chromatogram and densitometric scanning image under 366 nm respectively. E and f: HPTLC chromatogram and densitometric scanning image under 550 nm respectively. Track-1: Sida decoction, Track-2: cumin decoction, Tracke-3: Ginger decoction, Track-4: Nayopayam Kwatha

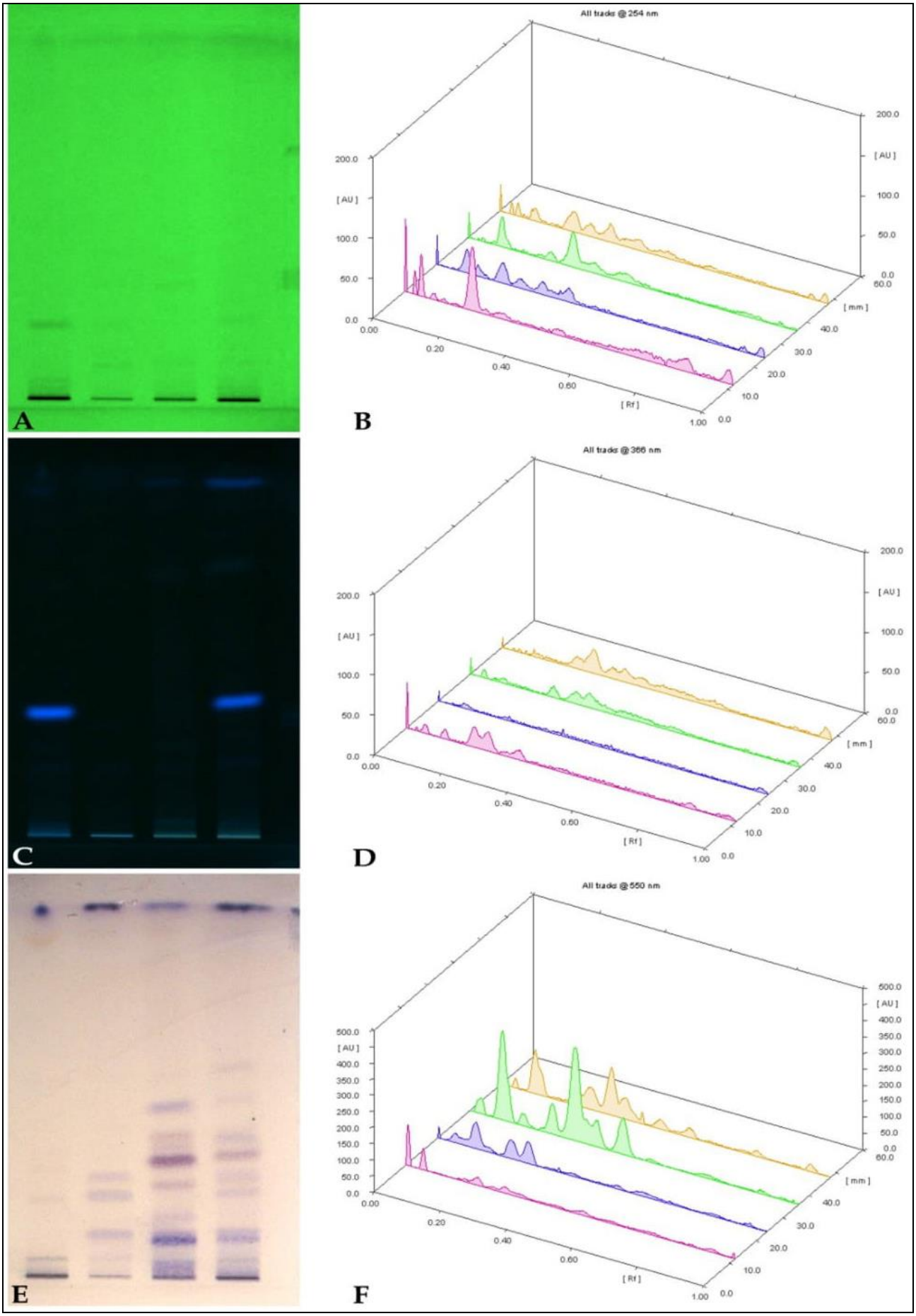

Plate 3: HPTLC Profile of hexane extracts of nayopayam kwatha and individual decotion. A and B: HPTLC chromatogram and densitometric scanning image under 254 nm respectively. C and D: HPTLC chromatogram and densitometric scanning image under 366 nm respectively. E and f: HPTLC chromatogram and densitometric scanning image under 550 nm respectively. Track-1: Sida decoction, Track-2: cumin decoction, Tracke-3: Ginger decoction, Track-4: Nayopayam Kwatha

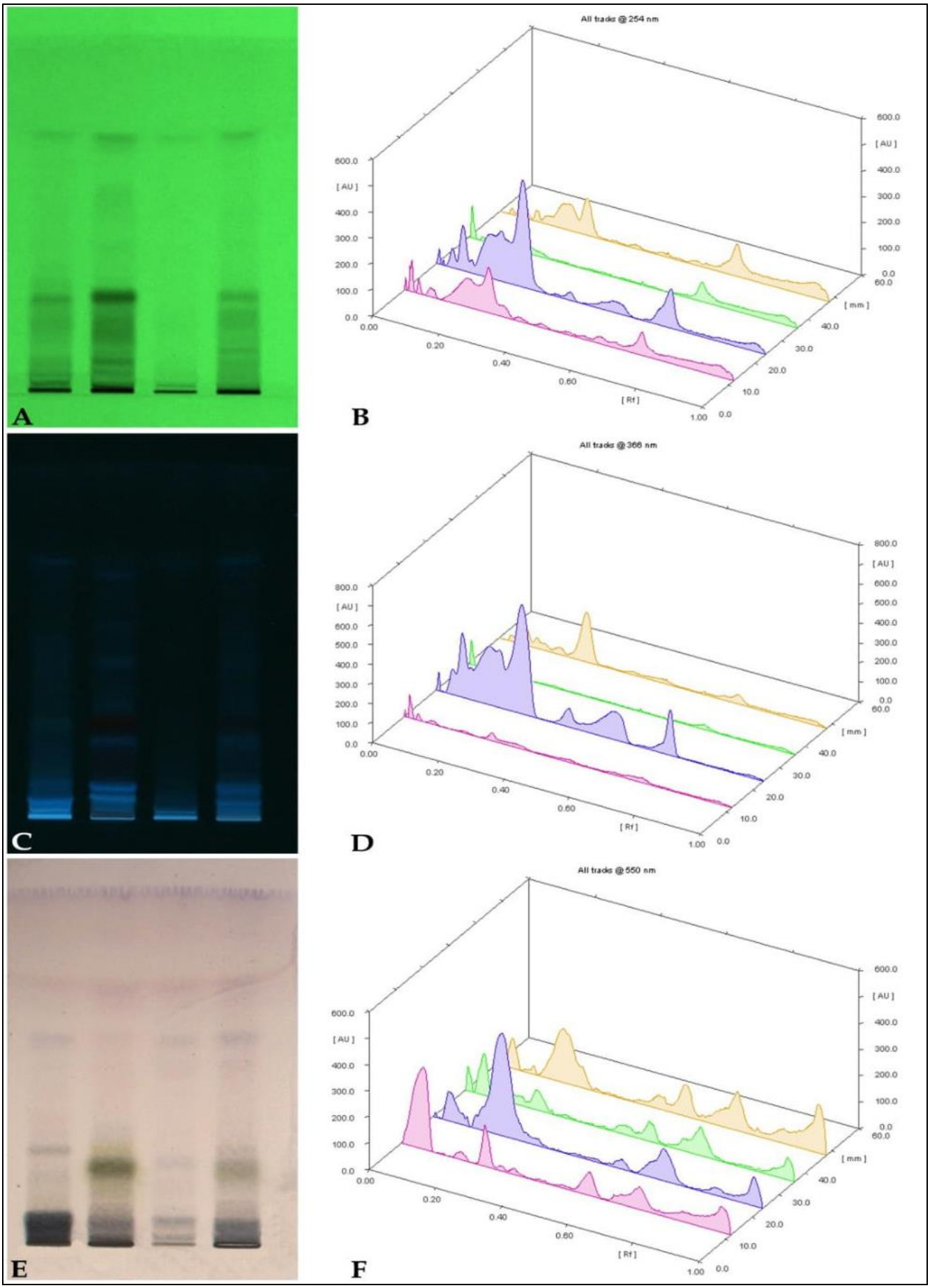

Plate 4: HPTLC Profile of hexane extracts of nayopayam kwatha and individual decotion. A and B: HPTLC chromatogram and densitometric scanning image under 254 nm respectively. C and D: HPTLC chromatogram and densitometric scanning image under 366 nm respectively. E and f: HPTLC chromatogram and densitometric scanning image under 550 nm respectively. Track-1: Sida decoction, Track-2: cumin decoction, Tracke-3: Ginger decoction, Track-4: Nayopayam Kwatha

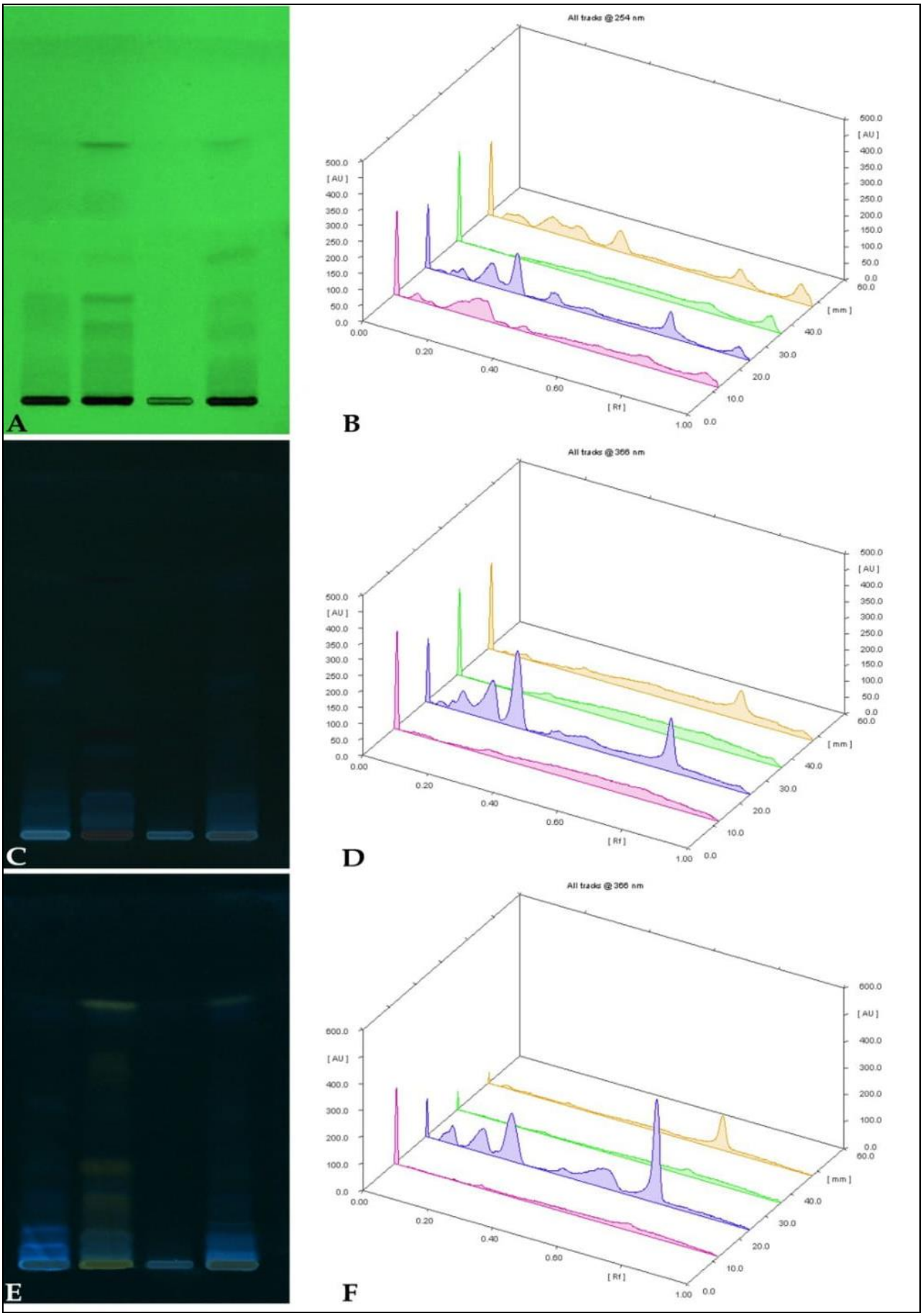

Plate 5: HPTLC Profile of hexane extracts of nayopayam kwatha and individual decotion. A and B: HPTLC chromatogram and densitometric scanning image under 254 nm respectively. C and D: HPTLC chromatogram and densitometric scanning image under 366 nm respectively. E and f: HPTLC chromatogram and densitometric scanning image After $AlCl_3$ Derivatization Under 366 nm respectively. Track-1: Sida decoction, Track-2: cumin decoction, Tracke-3: Ginger decoction, Track-4: Nayopayam Kwatha

Table 3: Comparative HPTLC analysis of hexane extract of *Nayopayam kwatha* and individual decoction at 254 nm, 366 nm and 550 nm

S. No.	Rf	Name of the extract				Band colour
		Sida	**Cumin**	**Ginger**	***Nayopayam***	
254 nm						
1.	0.04	0	1	0	0	Black
2.	0.15	0	1	1	1	Black
3.	0.51	0	0	1	1	Black
366 nm						
4.	0.1	0	0	1	1	Grey
5.	0.16	0	0	1	0	Grey
6.	0.18	0	0	1	0	Grey
7.	0.19	1	0	0	1	Blue
8.	0.3	0	0	1	0	Grey
9.	0.38	0	0	1	0	Grey
10.	0.49	1	0	0	0	Grey
11.	0.52	0	0	1	1	Light red
12.	0.54	0	1	0	0	Light red
13.	0.56	0	0	1	1	Grey
14.	0.6	0	1	0	0	Red
15.	0.63	0	0	1	1	Grey
550 nm						
16.	0.04	0	1	1	1	Violet
17.	0.07	0	0	1	1	Violet
18.	0.11	0	1	0	1	Violet
19.	0.15	0	1	1	1	Dark violet
20.	0.18	0	0	1	1	Violet
21.	0.3	0	0	1	0	Violet
22.	0.38	0	1	1	1	Light violet
23.	0.52	0	0	1	1	Dark violet
24.	0.56	0	0	1	1	Dark violet

Table 4: Comparative HPTLC analysis of chloroform extract of *Nayopayam kwatha* and individual decoction at 254 nm, 366 nm and 550 nm

S. No.	Rf	Name of the extract				Band colour
		Sida	**Cumin**	**Ginger**	***Nayopayam***	
254 nm						
1.	0.04	1	0	0	1	Black
2.	0.06	1	0	0	1	Black
3.	0.1	0	1	1	1	Black
4.	0.2	1	1	0	1	Black
5.	0.32	0	1	1	1	Black
6.	0.4	0	1	0	0	Black
366 nm						
7.	0.04	0	0	1	0	Grey
8.	0.06	1	0	0	0	Grey
9.	0.19	1	1	1	1	Light blue
10.	0.26	0	0	1	0	Grey
11.	0.34	1	0	0	1	Blue
12.	0.72	0	0	1	1	Grey
13.	0.8	0	0	0	1	Feeble grey
14.	0.87	0	1	0	1	Red
15.	0.96	0	0	1	1	Blue
550 nm						
16.	0.04	0	0	1	1	Blue
17.	0.05	1	1	0	0	Blue
18.	0.1	0	0	1	1	Blue
19.	0.12	0	1	0	0	Dark blue
20.	0.16	0	0	1	0	Blue
21.	0.22	1	0	0	0	Blue
22.	0.23	0	1	0	1	Blue
23.	0.25	0	0	1	0	Blue
24.	0.27	0	1	0	1	Blue
25.	0.3	1	0	0	0	Blue
26.	0.32	0	0	1	1	Light maroon
27.	0.38	0	0	1	1	Blue
28.	0.47	0	0	1	1	Blue
29.	0.56	0	0	0	1	Light band

Table 5: Comparative HPTLC analysis of methanol extract of *Nayopayam kwatha* and individual decoction at 254 nm, 366 nm and 550 nm

S. No.	Rf	Name of the extract				Band colour
		Sida	**Cumin**	**Ginger**	***Nayopayam***	
254 nm						
1.	0.02	0	1	0	0	Black
2.	0.03	1	0	1	0	Black
3.	0.05	1	0	0	0	Black
4.	0.06	0	1	0	1	Black
5.	0.09	1	1	0	1	Black
6.	0.1	1	0	0	0	Black
7.	0.12	0	0	0	1	Black
8.	0.2	1	1	0	1	Black
9.	0.27	1	1	0	1	Black
10.	0.41	0	1	0	1	Black
11.	0.53	0	1	0	0	Black
12.	0.72	1	1	1	1	Black
366 nm						
13.	0.01	0	1	0	0	Light red
14.	0.02	1	0	1	0	Light blue
15.	0.05	1	0	0	0	Light blue
16.	0.06	0	1	0	1	Light blue
17.	0.09	0	1	0	1	Light blue
18.	0.1	1	0	0	0	Ash
19.	0.12	0	0	0	1	Ash
20.	0.18	0	1	0	0	Light red
21.	0.23	0	1	0	1	Light blue
22.	0.27	1	0	0	0	Ash
23.	0.28	0	1	0	1	Red
24.	0.44	0	1	0	0	Ash
25.	0.5	1	0	0	0	Ash
26.	0.55	0	1	0	0	Ash
27.	0.68	0	1	0	0	Ash
28.	0.72	1	0	1	1	Ash
550 nm						
29.	0.03	0	1	1	0	Black -dark
30.	0.07	1	1	0	1	Black-dark
31.	0.08	0	0	1	0	Black - light
32.	0.18	1	0	0	0	Black-light
33.	0.23	0	1	0	1	Light green
34.	0.24	0	0	1	0	Black-light
35.	0.28	1	0	0	0	Black-dark
36.	0.51	0	0	0	1	Black-light
37.	0.57	1	1	1	1	Light violet
38.	0.72	1	1	1	1	Light violet

Table 6: Comparative HPTLC analysis of water extract of *Nayopayam kwatha* and individual decoction at 254 nm, 366 nm and 550 nm

S. No.	Rf	Name of the extract				Band colour
		Sida	**Cumin**	**Ginger**	***Nayopayam***	
254 nm						
1.	0.07	1	1	0	1	Black
2.	0.11	1	1	0	1	Black
3.	0.2	0	1	0	1	Black
4.	0.29	1	1	0	1	Black
5.	0.41	1	1	0	1	Black
6.	0.76	0	1	0	1	Black
366 nm						
7.	0.05	1	1	0	1	Blue
8.	0.09	0	1	0	1	Blue
9.	0.12	1	0	0	1	Blue
10.	0.16	1	0	0	0	Light yellow
11.	0.21	0	1	0	0	Light red
12.	0.25	0	1	0	0	Ash
13.	0.29	0	1	0	0	Red
14.	0.29	1	0	0	0	Ash
15.	0.46	1	0	0	1	Blue
16.	0.55	0	1	0	0	Light red
17.	0.73	0	1	0	1	Red

550 nm						
18.	0.04	0	0	0	1	Blue
19.	0.05	1	0	0	0	Dark blue
20.	0.05	0	1	0	0	Light grey
21.	0.09	0	0	0	1	Blue
22.	0.09	0	1	0	0	Light yellow
23.	0.12	1	0	0	1	Light Blue
24.	0.19	0	1	0	0	Yellow
25.	0.19	1	0	0	1	Blue
26.	0.28	1	0	0	1	Blue
27.	0.29	0	1	0	0	Yellow
28.	0.44	0	1	0	0	Light yellow
29.	0.46	1	0	0	1	Blue
30.	0.58	0	1	0	0	Yellow
31.	0.71	1	0	0	1	Light blue
32.	0.74	0	1	0	1	Yellow

Conclusions

The present study was an attempt to find out the chemical changes during the processing of polyherbal formuations by comparing it with the individual source plants. The present study also shows that HPTLC fingerprinting is a reliable method to assess these chemical changes during *Ayurvedic* processing. Quantitative and qualitative differences were also observed with the individual source plants and the polyherbal formulations.

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