

Differentiation of Market Samples of Chennai Nagakesara and Nagakesara by Chromatographic Techniques

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Subashini *et al.*: Differentiation of Chennai Nagakesara and Nagakesara

Efficacy of any herbal drugs depends on their chemical profile and their biological activity of ingredients. The anticipated efficacy of any drug could be achieved if authenticated ingredients that are collected as per good collection practices in the appropriate season. The general problem faced in herbal drug industry is correct identification of the source plant. The present study was aimed to develop chromatographic finger print profiles by using high performance thin layer chromatography and high pressure liquid chromatography of Nagakesara and Chennai Nagakesara/Cirunakappu to record the difference between them. Both samples were procured from Chennai market, extracted successively with n-hexane, chloroform, ethyl acetate and ethanol extracts using Soxhlet apparatus and subjected to high performance thin layer chromatography and high pressure liquid chromatography analysis. The thin-layer chromatography of hexane extract of Nagakesara showed more spots than Chennai Nagakesara/Cirunakappu and similarly with chloroform and ethanol extracts also. Maximum number of peaks was observed in all the extracts after derivatization with vanillin sulphuric acid reagent. In the high pressure liquid chromatography results of hexane extract maximum of 22 peaks were separated in Nagakesara whereas in Chennai Nagakesara/Cirunakappu only 14 peaks were separated; in contrast, in the ethanol extract of Chennai Nagakesara/Cirunakappu 17 peaks were separated while in Nagakesara it was five peaks only.

Key words: Madras Nagakesara, Cirunakappu, *Mesua ferrea*, *Mesua nagassarium*, *Cinnamomum wightii*

Siddha has encourages research efforts on scientific information about plants, plant extracts and various anatomical parts of plants as medicinal agents. Although herbal medicine exists since the dawn of time, our knowledge of how plants actually influence human physiology remains largely unexplored. This research is going on with a view to provide the phytochemical differentiation between the two plants with different analytical techniques like High-performance thin-layer chromatography (HPTLC), High-performance liquid chromatography (HPLC), Gas chromatography (GC), Gas chromatography–mass spectrometry (GC-MS), Liquid chromatography-tandem mass spectrometry (LC-MS/MS), Fourier-transform infrared spectroscopy (FTIR), Near-infrared (NIR), Raman spectra, etc.

In this communication, authors aim to differentiate the two drugs available in the crude drug market, in the name of Nagakesara. In Ayurveda, Nagakesara is equated to

Mesua ferrea (*M. ferrea*) Linn., whereas in classical Siddha literature, Madras Nagakesara/Cirunakappu is botanically equated as *Mesua nagassarium* (*M. nagassarium*) (Burm. f.) Kosterm^[1]. But in the crude drug market, immature fruits of *Cinnamomum wightii* (*C. wightii*) Meissn are sold as Madras Nagakesara/Cirunakappu in different places of Southern India^[2]. *Cinnamomum ovalifolium* (*C. ovalifolium*) Gardner ex Meisn is the synonym of *C. wightii*^[3]. Cirunakappu is a medicinal flower bud. It is carminative, astringent, acrid, purgative, diaphoretic and aromatic. *C. wightii* was used for treating wounds,

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fever, intestinal worms, headaches and menstrual problems and treat many diseases such as asthma, burning sensation in palm and soles, digestive impairment, gastric troubles, intercostal neuralgia, numbness of tongue, pleurodynia, spleen enlargement, leucorrhoea, hiccup, anemia, tuberculosis and kappa diseases. Essential oils from *Cinnamomum* species were isolated and screened for antimicrobial and anti-inflammatory activities in our laboratory. Future studies will focus on other types of bioassays, as this process is usually considered as the first step in the discovery of new drugs^[4,5].

Cirunakappu finds use in many Siddha formulation viz., Amukkarac curanam^[6-10] Civataic curanam, Elatic curanam, Ilaku Cantanatit Tailam, Incic curanam, Kantaka Racayanam, Karicalai Ilakam, Narattai Ilakam, Parankippattai Iracayanam, Talicaticcuranam, Talicati vatakam, Vivati Ilakam^[11], Ilankathi Curanam, Kadukkai Ilakam, Chandrakanthi Curanam, Naavalpattai Nei, Pooranathi Ilakam, Mahavilvathi Ilakam, Milaguth Tailam, Megathennai^[12] etc. It is known as Ceylon Iron wood in English, Nagakesara in Sanskrit, Telugu and Kanada, Nagkesar in Hindi, Nakappuvu in Malayalam. *Calophyllum nagassarium* Burm.f. and *M. nagassarium* (Burm. f.) Kosterm. are synonyms to *M. ferrea*^[13,14]. It is native to tropical Sri Lanka and a state tree of Tripura but it is disappearing from India. *M. ferrea* L. is locally known as Cobra's saffron (English), Nagakeshara (Hindi), Nagasampige (Kannada), Nageshwar (Assam), Sirunagam (Tamil). is found throughout Southeast Asia in tropical evergreen forests up to 1500 m elevation^[15].

Nagakesara (*M. ferrea*) is a medicinal stamen used in many Ayurvedic formulations viz., Bilvathi leha, Cyavanaprasha, Pugakha, Vyaghri haritaki, Eladi curna, Kalyaaka ghrta, Triphala ghrta^[16]; Amrtarista, Asvagandhadyarista, Babbularista, Dasamu larista, Draks arista, Jirakady arista, Kanak asava, Kadir arista, Pippalayady arista, Punarnavady arista, Madhusnuhi rasayana, Bhaskaralavana curna, Jatiphaladi curna^[17]; Haridra khanda, Narikela khanda, Arimedadi taila, Bala taila, Bhrngaraja taila, Kanaka taila^[18]. The main aim of this study is to develop a chromatographic methods for the differentiation of these two plants. As these drugs are commercially sold in the Indian market under the name Nagakesara/Cirunakappu, the purpose of our investigation is to aid its importance in the field of research and as well as its commercial gradient where it might be possibly adulterated with other marketed drug

like *M. ferrea* Linn.^[19,20].

MATERIALS AND METHODS

Plant material:

The plants in the name of Nagakesara/Madras, Nagakesara/Cirunakappu were purchased from Govindaraja Mudaliyar Sons Pvt. Ltd., the raw drug retail store in Chennai, Tamil Nadu and taken for the study.

Solvents and chemicals:

HPTLC pre-coated plates of silica gel 60 F₂₅₄ were procured from Merck India. All the solvents were of analytical reagent (AR) grade for HPTLC and HPLC analysis procured from Merck. Vanillin from Lobacheme and sulphuric acid from Merck was laboratory reagent (LR) Grade.

Preparation of extracts:

2 g of both plant samples were extracted using hexane, chloroform, ethyl acetate and ethanol successively using Soxhlet apparatus. Each extract obtained was filtered using whatman No. 1 filter paper, dried up to 5 ml using Rotovac and these solutions were used as test solution for HPTLC analysis. Then the extracts were dried and dissolved in methanol for HPLC analysis.

HPTLC instrument:

The CAMAG® HPTLC equipment composed of automatic Thin-layer chromatography (TLC) sampler 4 (ATS4), Visualizer and TLC scanner coupled with winCATS software, Chromatogram Immersion Device III for derivatization and TLC plate heater III.

HPLC instrument:

HPLC analysis was carried out in a modular Shimadzu LC-20 system which comprised of a LC-20AP pump, CTO-20AC column oven, a SPD-M 20A ultraviolet-diode array detector (UV-DAD) detector, CBM-10A interface and LC-20 workstation was utilized. A C18 column (250 mm length×4.6 mm inner diameter×5 µ particle size) from Shim-pack GSIT was employed at 30°. Separations were done in isocratic mode.

TLC/HPTLC procedure:

Sample application:

Solvent system: The selected mobile phases for hexane extract were hexane:ethyl acetate (6:1, v/v);

for chloroform, ethyl acetate and ethanol extracts was toluene:ethyl acetate (6:4, v/v).

Plate development: The samples loaded plate was kept in TLC twin trough developing chamber (after saturated with solvent vapour) with respective mobile phase and the plate was developed in the respective mobile phase up to 90 mm.

Photo-documentation: The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in photo-documentation chamber (CAMAG® Visualizer) and captured the images at white light, UV 254 nm and UV 366 nm.

Scanning: Before derivatization, the plate was fixed on the stage (CAMAG® SCANNER IV) and scanning was done at UV 254 nm and 366 nm. The

peak table, peak display and peak densitogram were noted.

Derivatization: The developed plate was sprayed with vanillin sulphuric acid as spray reagent and dried at 100° in hot air oven. The plate was photo-documented in white light and scanned at UV 510 nm for finger print development.

HPLC procedure:

After completion of HPTLC, all the extracts were dried and then redissolved in 5 ml of methanol, sonicated for 30 min and were filtered through 0.45 µ membrane prior to injection. The reverse phase column C18 was used for chromatographic separation. The eluting solvent chosen was 100 % acetonitrile with a flow rate of 1 ml/min. The volume injected was 20 µl. The

TABLE 1: HPTLC PEAK R_f AND AREA DETAILS OF SUCCESSIVE EXTRACTS OF CN AND NK

| | UV 254 nm | | | | UV 366 nm | | | | White light 510 nm | | | |
|--------------------|--------------------|--------|--------------------|--------|--------------------|--------|--------------------|--------|--------------------|--------|--------------------|--------|
| | CN | | NK | | CN | | NK | | CN | | NK | |
| | Max R _f | Area % | Max R _f | Area % | Max R _f | Area % | Max R _f | Area % | Max R _f | Area % | Max R _f | Area % |
| Hexane extract | 0.02 | 0.81 | 0.06 | 32.15 | 0.02 | 41.43 | 0.01 | 5.07 | 0.08 | 2.39 | 0.14 | 4.39 |
| | 0.13 | 6.67 | 0.14 | 11.94 | 0.07 | 10.35 | 0.05 | 11.37 | 0.12 | 40.94 | 0.22 | 11.28 |
| | 0.28 | 42.34 | 0.22 | 7.78 | 0.1 | 14.82 | 0.12 | 13.71 | 0.22 | 3.4 | 0.34 | 2.9 |
| | 0.38 | 9.68 | 0.31 | 7.03 | 0.18 | 8.68 | 0.14 | 39.28 | 0.27 | 6.76 | 0.43 | 1.63 |
| | 0.53 | 22.19 | 0.43 | 2.32 | 0.93 | 24.71 | 0.24 | 3.44 | 0.29 | 6.35 | 0.47 | 3.24 |
| | 0.74 | 4.55 | 0.49 | 9.29 | - | - | 0.28 | 3.21 | 0.46 | 16.96 | 0.62 | 26.55 |
| | 0.79 | 13.78 | 0.56 | 2.9 | - | - | 0.59 | 12.18 | 0.8 | 1.68 | 0.82 | 4.83 |
| | - | - | 0.61 | 13.04 | - | - | 0.82 | 11.72 | 0.82 | 1.96 | 0.85 | 8.82 |
| | - | - | 0.7 | 1.3 | - | - | - | - | 0.86 | 12.78 | 0.89 | 12.03 |
| | - | - | 0.87 | 12.25 | - | - | - | - | 0.91 | 4.19 | 0.92 | 10.37 |
| | - | - | - | - | - | - | - | - | 0.94 | 2.59 | 0.98 | 0.78 |
| | - | - | - | - | - | - | - | - | 0.99 | 13.17 | - | - |
| | 0.01 | 21.6 | 0.01 | 31.82 | 0.01 | 19.67 | 0.01 | 3 | 0.05 | 1.74 | 0.1 | 0.25 |
| | 0.08 | 44.49 | 0.12 | 0.65 | 0.04 | 8.2 | 0.03 | 12.66 | 0.1 | 3.89 | 0.16 | 0.27 |
| Chloroform extract | 0.2 | 19.1 | 0.18 | 28.25 | 0.79 | 72.13 | 0.09 | 8.76 | 0.2 | 25.62 | 0.19 | 16.3 |
| | 0.88 | 14.81 | 0.27 | 3.23 | - | - | 0.13 | 11.67 | 0.25 | 4.64 | 0.35 | 0.78 |
| | - | - | 0.41 | 2.11 | - | - | 0.19 | 13.26 | 0.33 | 3.09 | 0.42 | 2.62 |
| | - | - | 0.53 | 16.46 | - | - | 0.27 | 2.01 | 0.5 | 4.53 | 0.51 | 7.24 |
| | - | - | 0.63 | 6.32 | - | - | 0.3 | 1.05 | 0.58 | 28.21 | 0.59 | 10.37 |
| | - | - | 0.89 | 1.09 | - | - | 0.34 | 1.36 | 0.66 | 7.78 | 0.69 | 0.67 |
| | - | - | 0.93 | 10.06 | - | - | 0.43 | 0.58 | 0.73 | 3.73 | 0.85 | 0.65 |
| | - | - | - | - | - | - | 0.49 | 4.22 | 0.8 | 4.85 | 0.88 | 0.82 |
| | - | - | - | - | - | - | 0.58 | 6.93 | 0.87 | 3.09 | 0.9 | 1.15 |
| | - | - | - | - | - | - | 0.66 | 4.28 | 0.88 | 5.12 | 0.92 | 1.55 |
| | - | - | - | - | - | - | 0.74 | 1.2 | 0.92 | 1.24 | 0.96 | 1.51 |
| | - | - | - | - | - | - | 0.8 | 23.17 | 0.94 | 2.47 | - | - |
| | - | - | - | - | - | - | 0.88 | 2.73 | - | - | - | - |
| | - | - | - | - | - | - | 0.94 | 2.93 | - | - | - | - |

| | | | | | | | | | | | | |
|-----------------------|------|-------|------|-------|------|-------|------|-------|------|-------|------|-------|
| Ethyl acetate extract | 0.01 | 3.85 | 0.01 | 2.93 | 0.01 | 1.9 | 0.01 | 4.8 | 0.04 | 0.27 | 0.01 | 3.41 |
| | 0.06 | 4.56 | 0.07 | 1.5 | 0.04 | 2.9 | 0.05 | 10.53 | 0.08 | 0.24 | 0.06 | 0.72 |
| | 0.13 | 1.82 | 0.1 | 0.57 | 0.09 | 5.66 | 0.11 | 5.156 | 0.11 | 0.87 | 0.08 | 2.17 |
| | 0.15 | 6.79 | 0.14 | 2.24 | 0.13 | 6.75 | 0.15 | 10.27 | 0.15 | 15.8 | 0.17 | 15.81 |
| | 0.19 | 3.38 | 0.18 | 2.14 | 0.24 | 10.41 | 0.21 | 6.4 | 0.23 | 13.49 | 0.28 | 5.12 |
| | 0.23 | 2.6 | 0.2 | 12.62 | 0.82 | 72.37 | 0.25 | 5.24 | 0.32 | 3.95 | 0.34 | 1.55 |
| | 0.25 | 10.7 | 0.29 | 6.85 | - | - | 0.32 | 1.68 | 0.41 | 2.93 | 0.37 | 4.22 |
| | 0.28 | 9.54 | 0.36 | 8.45 | - | - | 0.41 | 2 | 0.52 | 39.51 | 0.43 | 3.53 |
| | 0.34 | 4.43 | 0.43 | 1.05 | - | - | 0.61 | 2.87 | 0.65 | 5.63 | 0.51 | 32.85 |
| | 0.44 | 1.19 | 0.46 | 1.25 | - | - | 0.72 | 42.2 | 0.69 | 7.14 | 0.65 | 17.98 |
| | 0.55 | 31.38 | 0.53 | 26.24 | - | - | 0.85 | 8.87 | 0.79 | 0.77 | 0.76 | 7.31 |
| | 0.79 | 0.81 | 0.66 | 1.93 | - | - | - | - | 0.86 | 0.63 | 0.84 | 0.58 |
| | 0.86 | 18.96 | 0.78 | 14.37 | - | - | - | - | 0.88 | 0.94 | 0.87 | 0.39 |
| | - | - | 0.89 | 15.87 | - | - | - | - | 0.92 | 3.47 | 0.89 | 4.34 |
| | - | - | - | - | - | - | - | - | 0.95 | 4.37 | - | - |
| Ethyl alcohol extract | 0.01 | 40.23 | 0.01 | 10.88 | 0.01 | 29.58 | 0.01 | 11.34 | 0.01 | 56.18 | 0.01 | 15.25 |
| | 0.1 | 1.44 | 0.08 | 0.33 | 0.29 | 9.8 | 0.1 | 11.74 | 0.21 | 9.75 | 0.08 | 0.46 |
| | 0.21 | 1.47 | 0.11 | 0.36 | 0.34 | 26.78 | 0.17 | 12.74 | 0.27 | 1.83 | 0.12 | 0.56 |
| | 0.26 | 8.49 | 0.14 | 1.65 | 0.52 | 18.39 | 0.28 | 9.09 | 0.4 | 0.66 | 0.17 | 0.33 |
| | 0.31 | 16.39 | 0.22 | 19.89 | 0.81 | 15.46 | 0.36 | 2.77 | 0.47 | 4.44 | 0.21 | 1.09 |
| | 0.47 | 10.61 | 0.31 | 12.28 | - | - | 0.39 | 4.16 | 0.51 | 4.74 | 0.26 | 1.18 |
| | 0.85 | 12.54 | 0.37 | 14.37 | - | - | 0.43 | 3.93 | 0.54 | 4.87 | 0.31 | 2.7 |
| | 0.94 | 8.84 | 0.42 | 5.81 | - | - | 0.51 | 12.71 | 0.68 | 8.02 | 0.37 | 5.32 |
| | - | - | 0.48 | 9.45 | - | - | 0.61 | 5.11 | 0.74 | 4.8 | 0.44 | 25.8 |
| | - | - | 0.56 | 4.27 | - | - | 0.72 | 7.9 | 0.89 | 1.77 | 0.65 | 11.58 |
| | - | - | 0.65 | 1.15 | - | - | 0.78 | 12.21 | 0.94 | 1.96 | 0.73 | 6.62 |
| | - | - | 0.75 | 9.15 | - | - | 0.87 | 6.29 | 0.98 | 1 | 0.78 | 14.09 |
| | - | - | 0.85 | 10.41 | - | - | - | - | - | - | 0.92 | 15.01 |

TABLE 2: HPLC PEAK R_t AND AREA DETAILS OF DIFFERENT EXTRACTS OF CN AND NK

| Peak | Hexane extract @ 230 nm | | | | Chloroform extract @ 200 nm | | | | Ethyl acetate extract @ 200 nm | | | | Ethanol extract @ 230 nm | | | |
|------|-------------------------|--------|--------|--------|-----------------------------|--------|-------|--------|--------------------------------|--------|--------|--------|--------------------------|--------|-------|--------|
| | CN | | NK | | CN | | NK | | CN | | NK | | CN | | NK | |
| | R_t | Area % | R_t | Area % | R_t | Area % | R_t | Area % | R_t | Area % | R_t | Area % | R_t | Area % | R_t | Area % |
| 1 | 1.8 | 6.994 | 1.697 | 10.285 | 1.611 | 30.18 | 1.796 | 21.965 | 1.605 | 21.842 | 1.744 | 45.98 | 1.489 | 64.767 | 1.395 | 9.672 |
| 2 | 2.052 | 13.042 | 1.954 | 8.517 | 1.859 | 6.81 | 2.044 | 28.634 | 1.699 | 42.469 | 2.316 | 16.513 | 1.758 | 14.337 | 1.562 | 59.321 |
| 3 | 2.163 | 9.72 | 2.145 | 6.947 | 1.97 | 4.71 | 2.609 | 12.523 | 2.316 | 16.035 | 2.471 | 18.733 | 1.957 | 6.78 | 2.149 | 9.135 |
| 4 | 2.325 | 6.783 | 2.321 | 6.664 | 2.321 | 3.23 | 3.282 | 2.25 | 4.904 | 0.929 | 4.88 | 4.955 | 2.165 | 3.16 | 2.291 | 16.766 |
| 5 | 2.471 | 8.34 | 2.589 | 13.889 | 2.941 | 3.7 | 3.381 | 1.271 | 16.067 | 18.724 | 15.991 | 13.06 | 2.316 | 3.033 | 4.894 | 5.107 |
| 6 | 2.634 | 4.983 | 4.894 | 15.951 | 3.526 | 18.17 | 3.55 | 3.851 | - | - | 17.059 | 0.758 | 2.452 | 2.998 | - | - |
| 7 | 2.741 | 6.395 | 6.27 | 3.844 | 4.497 | 2.65 | 3.811 | 5.676 | - | - | - | - | 2.636 | 2.869 | - | - |
| 8 | 3.352 | 6.299 | 7.415 | 1.857 | 5.788 | 10.259 | 3.936 | 23.397 | - | - | - | - | 3.9 | 1.326 | - | - |
| 9 | 3.523 | 6.375 | 8.733 | 2.721 | 7.997 | 1.41 | 4.305 | 0.052 | - | - | - | - | 4.896 | 0.487 | - | - |
| 10 | 3.781 | 7.975 | 10.241 | 0.619 | 10.739 | 0.3 | 4.415 | 0.223 | - | - | - | - | 7.436 | 0.018 | - | - |
| 11 | 3.908 | 16.1 | 11.132 | 0.671 | 11.44 | 0.236 | 5.903 | 0.157 | - | - | - | - | 7.783 | 0.016 | - | - |
| 12 | 4.895 | 4.66 | 11.384 | 0.764 | 14.355 | 10.946 | - | - | - | - | - | - | 8.291 | 0.012 | - | - |
| 13 | 5.27 | 1.622 | 11.915 | 1.105 | 15.416 | 7.23 | - | - | - | - | - | - | 8.759 | 0.039 | - | - |
| 14 | 15.982 | 0.712 | 13.581 | 0.318 | - | - | - | - | - | - | - | - | 10.268 | 0.014 | - | - |
| 15 | - | - | 14.631 | 2.034 | - | - | - | - | - | - | - | - | 16.057 | 0.052 | - | - |
| 16 | - | - | 15.242 | 2.643 | - | - | - | - | - | - | - | - | 17.255 | 0.024 | - | - |
| 17 | - | - | 16.02 | 14.627 | - | - | - | - | - | - | - | - | 19.966 | 0.067 | - | - |
| 18 | - | - | 17.097 | 2.301 | - | - | - | - | - | - | - | - | - | - | - | - |
| 19 | - | - | 19.915 | 1.942 | - | - | - | - | - | - | - | - | - | - | - | - |
| 20 | - | - | 21.565 | 0.629 | - | - | - | - | - | - | - | - | - | - | - | - |
| 21 | - | - | 22.504 | 0.818 | - | - | - | - | - | - | - | - | - | - | - | - |
| 22 | - | - | 23.324 | 0.855 | - | - | - | - | - | - | - | - | - | - | - | - |
| - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

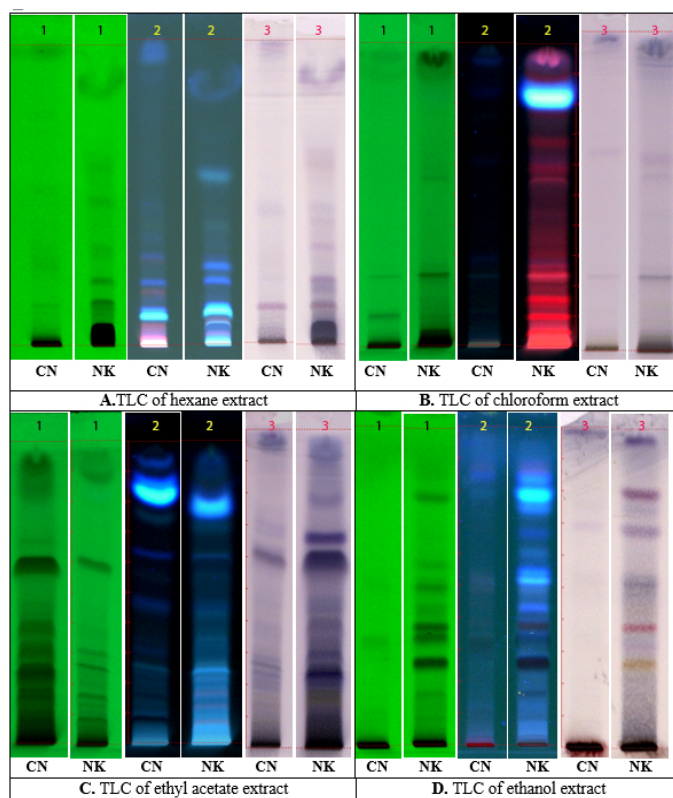


Fig. 1: TLC pattern of different extracts at 254 nm, 366 nm, white light after derivatization with VSR

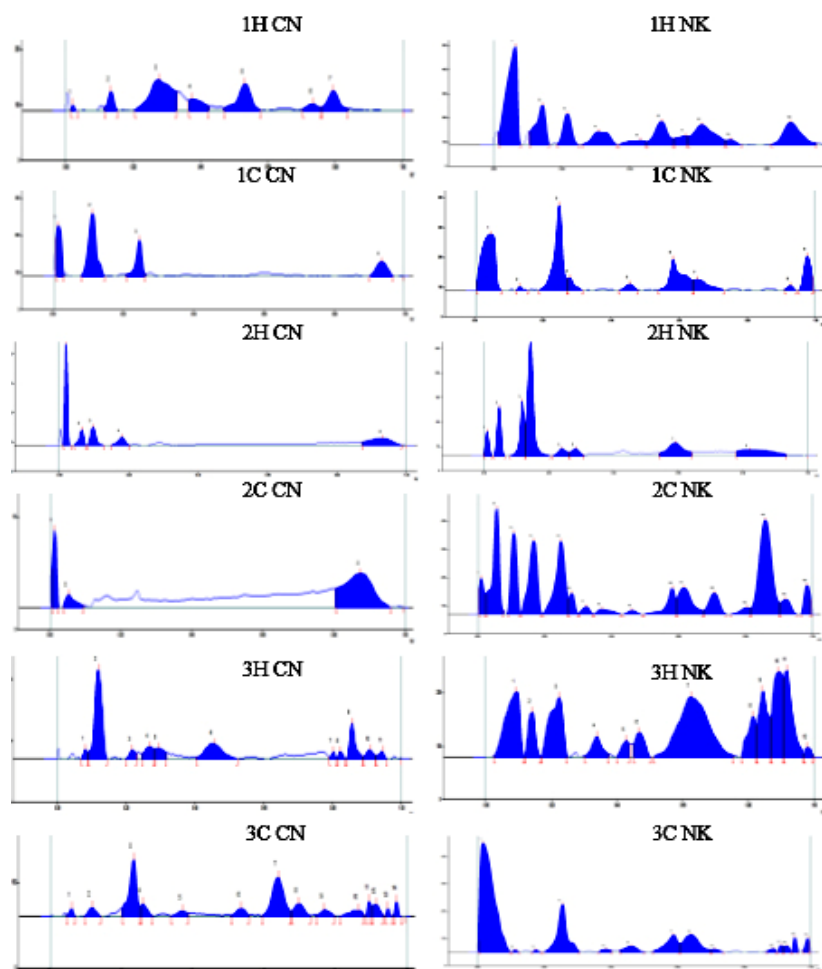


Fig. 2: Finger prints of hexane (H) and chloroform (C) extracts (1-λ254; 2-λ366; 3-λ520)

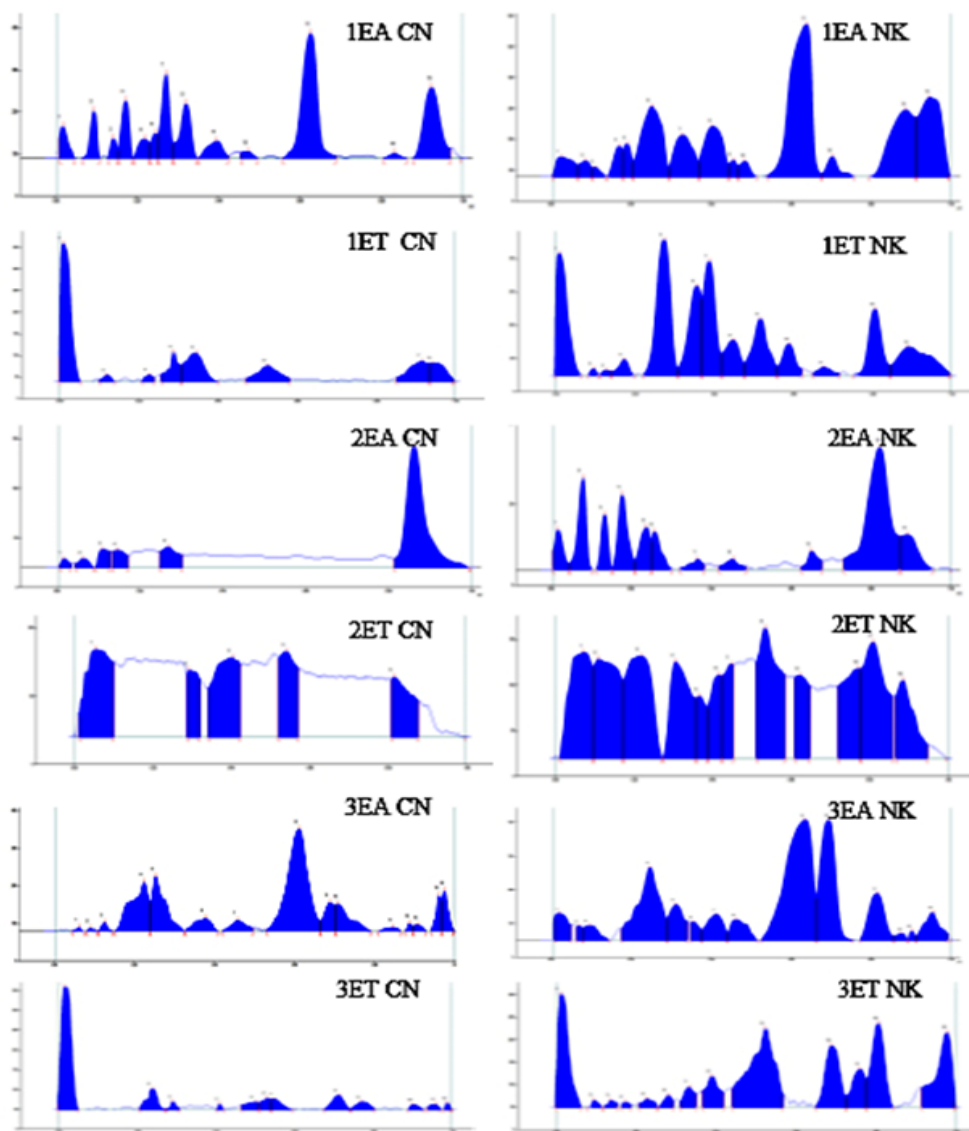


Fig. 3: Finger prints of Ethyl acetate (EA) and Ethanol (ET) extracts (1- λ 254; 2- λ 366; 3- λ 520)

separated peaks of hexane extract and ethanol extracts were monitored at 230 nm; chloroform and ethyl acetate extracts were recorded at 200 nm (Table 1 and Table 2).

RESULTS AND DISCUSSION

The TLC photo documentation of all the successive extracts of Chennai Nagakesara/Cirunakappu (CN) and Nagakesara (NK) are shown in fig. 1. TLC of hexane extract of CN showed spots at R_f 0.13, 0.27, 0.52 and 0.76 (all green) whereas NK showed 7 spots at R_f 0.14, 0.21, 0.29, 0.32, 0.47, 0.58 and 0.83 (all green) under UV 254 nm; CN showed 0.03, 0.06 (both pink), 0.09 (fluorescent blue), 0.12 (blue), 0.17 (pale pink), 0.21, 0.27 and 0.29 (all blue) while NK showed 9 spots at R_f 0.02, 0.04, 0.07, 0.11, 0.21, 0.26, 0.32, 0.38 and 0.55 (all blue) under UV 366 nm; after derivatization with VSR, CN showed 4 spots at R_f 0.04 (grey), 0.12, 0.21

and 0.44 (all purple) and NK showed spots at R_f 0.13, 0.18, 0.21, 0.32, 0.40, 0.45, 0.58, 0.63 and 0.87 (all purple).

Chloroform extract of CN showed two spots at R_f 0.11 and 0.23 (both green) while that of NK showed spots at R_f 0.23 and 0.55 (both green) under 254 nm; CN showed spots at R_f 0.12, 0.24, 0.29, 0.60, 0.76 and 0.84 (all pale blue) NK showed 14 spots at R_f 0.05, 0.11, 0.17, 0.24, 0.27, 0.32, 0.37, 0.45, 0.56, 0.60, 0.68, 0.77 (all pink), 0.83 (Fluorescent blue) and 0.89 (pink) under 366 nm; CN showed two spots at R_f 0.23 and 0.62 (both grey) while that of NK showed spots at R_f 0.23 (purple), 0.54 and 0.59 (both grey) after dipping in VSR as shown in fig. 2.

TLC of ethyl acetate extract of CN showed eight spots at R_f 0.09, 0.13, 0.16, 0.25, 0.31, 0.37, 0.59 and 0.88

(all green) under UV 254 nm; 0.08, 0.17, 0.26, 0.31, 0.47, 0.63, 0.75 (all blue), 0.83 (fluorescent blue), 0.86 and 0.93 (both blue) under UV 366 nm; 0.17, 0.20 (both grey), 0.23, 0.26, 0.28, 0.60, 0.68 and 0.70 (all purple); at the same time NK showed twelve spots at R_f 0.07, 0.10, 0.16, 0.18, 0.23, 0.32, 0.38, 0.43, 0.47, 0.59, 0.67 and 0.84 (all green) at UV 254 nm; ten spots at R_f 0.08, 0.13 (both pale pink), 0.17, 0.22, 0.25, 0.35, 0.44, 0.56, 0.63 (all pale blue) and 0.78 (fluorescent blue) UV 366 nm; nine spots at R_f 0.10, 0.18 (both grey) 0.24, 0.30, 0.39, 0.45, 0.59, 0.66, 0.78 (all purple) after dipping in VSR as shown in fig. 3.

TLC of ethanol extract of CN showed 4 spots at R_f 0.07, 0.11, 0.32 and 0.50 (all green) whereas NK showed

8 spots at R_f 0.09, 0.16, 0.25, 0.33, 0.43, 0.50, 0.56 and 0.78 (all green) under UV 254 nm; CN showed 5 spots at R_f 0.33 (brown), 0.49, 0.53, 0.85, 0.87 (all pink) while NK showed 12 spots at R_f 0.09, 0.17 (ash), 0.26 (black), 0.38 (brown), 0.43 (violet), 0.52, 0.54 (sky blue), 0.61 (greenish blue), 0.67, 0.75 (blue), 0.79 (sky blue) and 0.85 (blue) under UV 366 nm; after derivatization with VSR, CN showed 4 spots at R_f 0.04 (grey), 0.12, 0.21 and 0.44 (all purple) and NK showed spots at R_f 0.13, 0.18, 0.21, 0.32, 0.40, 0.45, 0.58, 0.63 and 0.87 (all purple).

In the HPTLC finger print profile of hexane extract under UV 254 nm of CN, the peaks at R_f 0.28 (42.34 %) and 0.53 (22.19 %) are the major peaks

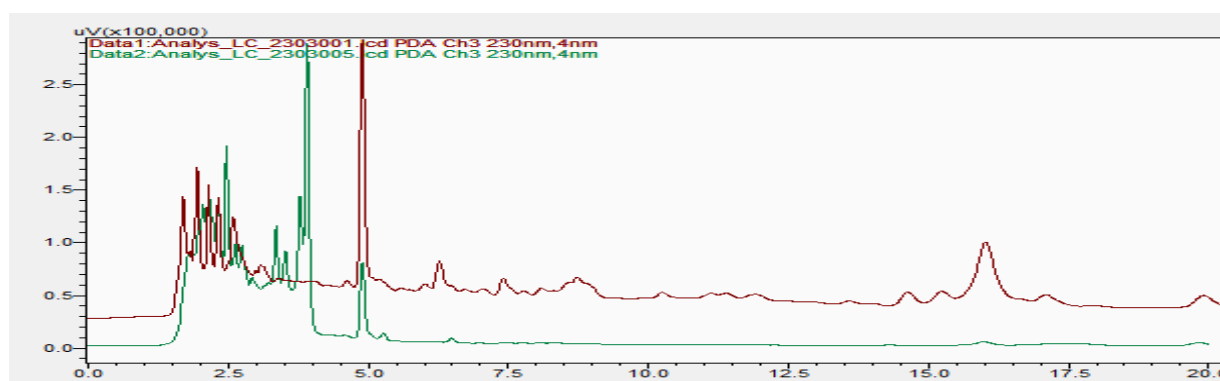


Fig. 4A: HPLC profile of hexane extract at 230 nm

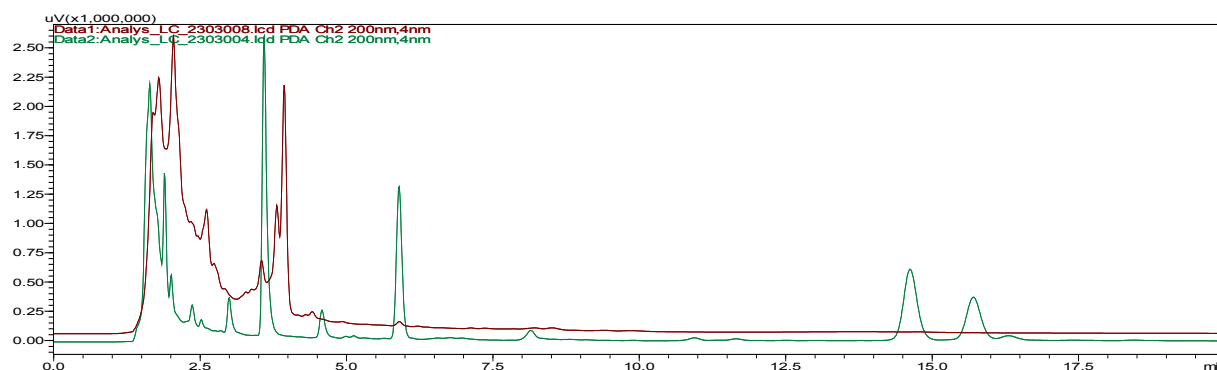


Fig. 4B: HPLC profile of chloroform extract at 200 nm

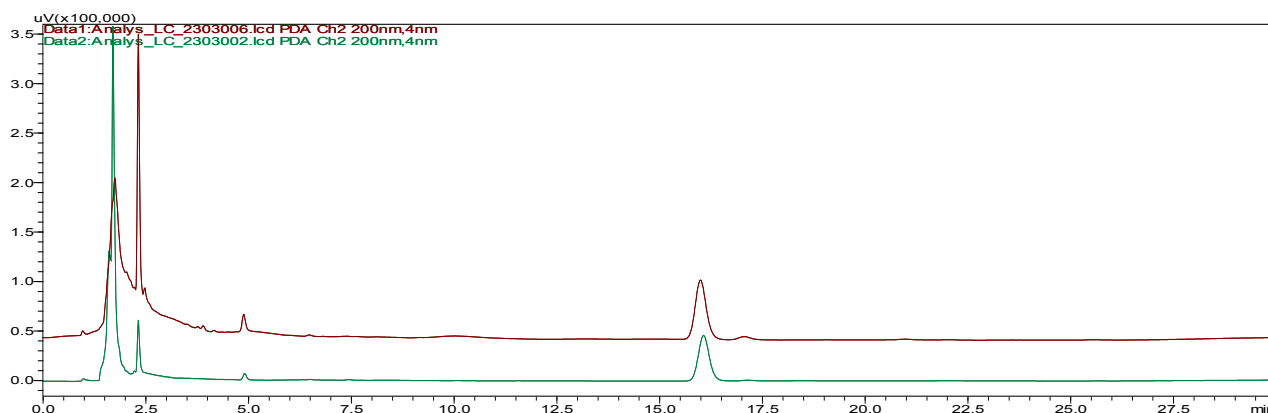


Fig. 4C: HPLC profile of ethyl acetate extract at 200 nm

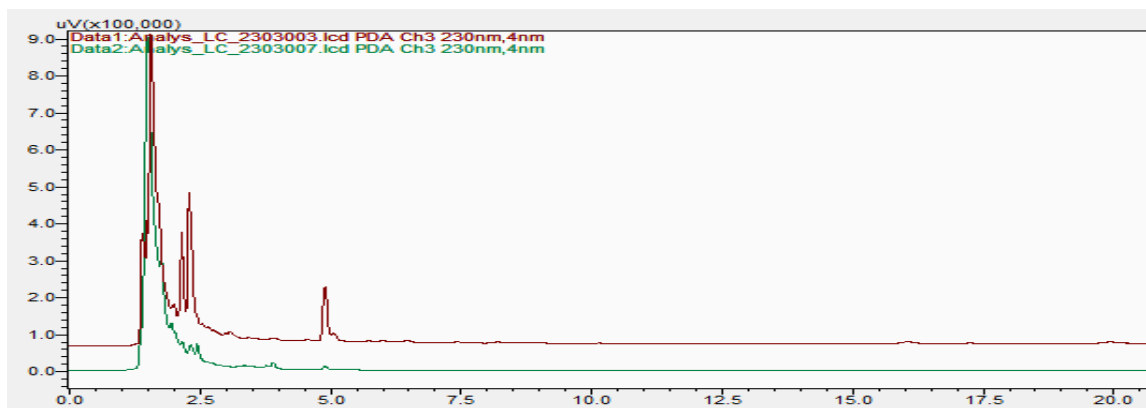


Fig. 4D: HPLC profile of ethanol extract at 230 nm

but in NK, the peaks at R_f 0.61 (13.04 %) and 0.87 (12.25 %) are the major peaks; under 366 nm the peaks at R_f 0.93 (24.71 %) and 0.02 (41.43 %) for CN and 0.14 (39.28 %), 0.12 (13.71 %) for NK; after derivatization three major peaks were separated at 0.12 (40.94 %), 0.46 (16.96 %), 0.86 (12.76 %) for CN and at 0.22 (11.28 %), 0.62 (26.55 %) and 0.89 (12.03 %) for NK.

In the HPTLC finger print profile of chloroform extract under UV 254 nm, three peaks separated at 0.08, 0.20 and 0.88 for CN, eight peaks separated for NK in which peaks at 0.18 (28.25 %), 0.53 (16.46 %) and 0.93 (10.06 %) are major; under 366 nm 2 peaks were separated in CN whereas in NK the peaks at 0.80 (23.17 %), 0.19 (13.26 %), 1.3 (11.67 %) were separated as major out of 15 peaks; after dipping in vanillin-sulphuric acid 14 peaks and 13 peaks were separated in CN and NK respectively.

In the HPTLC finger print profile of ethyl acetate extract under UV 254 nm, 12 peaks separated in which peaks at 0.55 (31.38 %), 0.86 (18.96 %), 0.25 (10.70 %) are major in CN, 13 peaks separated for NK in which peaks at 0.53 (26.24 %), 0.89 (15.87 %), 0.78 (14.37 %) and 0.20 (12.62 %) are major; under 366 nm 5 peaks were separated in CN whereas in NK the peaks at 0.72 (42.20 %), 0.05 (10.53 %), 0.15 (10.27 %) were separated as major out of 10 peaks; after dipping in vanillin-sulphuric acid 15 peaks in which 0.52 (39.51 %), 0.15 (15.80 %), 0.23 (13.49 %) and 14 peaks in which 0.51 (32.85 %), 0.65 (17.98 %), 0.17 (15.81 %) were separated as major in CN and NK respectively.

In the HPTLC finger print profile of ethyl alcohol extract under UV 254 nm, 7 peaks separated in which peaks at 0.31 (16.39 %), 0.85 (12.54 %), 0.47 (10.61 %) are major in CN, 12 peaks separated for NK in which peaks at 0.22 (19.89 %), 0.37 (14.37 %), 0.31 (12.28 %) and 0.85 (10.41 %) are major; under 366 nm

4 peaks were separated in CN whereas in NK the peaks at 0.17 (12.74 %), (12.71 %), 0.78 (12.21 %), 0.10 (11.74 %) were separated as major out of 11 peaks; after dipping in vanillin-sulphuric acid 11 peaks in which 0.21 (9.75 %) and 12 peaks in which 0.44 (25.80 %), 0.78 (14.09 %), 0.92 (15.01 %) were separated as major in CN and NK respectively.

The HPLC chromatograms of hexane, chloroform, ethyl acetate and ethanol extracts of CN and NK are shown in fig. 4A-fig. 4D. In the HPLC chromatogram of hexane extract at 230 nm, 14 peaks in CN and 22 peaks in NK emerged in which the peaks at R_t 2.052 (13.04 %), 3.908 (16.100 %) and 1.697 (10.285 %), 2.589 (13.889 %), 4.894 (15.951 %), 16.020 (14.627 %) were the major peaks; in the chloroform extract at 200 nm, 13 peaks in CN and 11 peaks in NK separated in which 1.611 (30.180 %), 3.526 (18.170 %), 5.788 (10.259 %), 14.355 (10.946 %) and 1.796 (21.965 %), 2.044 (28.634 %), 2.609 (12.523 %), 3.936 (23.397 %) respectively were the major peaks; in the ethyl acetate extract at 200 nm, 5 peaks at 1.605 (21.842 %), 1.699 (42.469 %), 2.316 (16.035 %), 4.904 (0.929 %) 16.067 (18.724 %) in CN and 6 peaks at 1.744 (45.980 %), 2.316 (16.513 %), 2.471 (18.733 %), 4.880 (4.955 %), 15.991 (13.060 %), 17.059 (0.758 %) in NK separated; in the ethyl alcohol extract, 17 peaks in which 1.489 (64.767 %), 1.758 (14.337 %) are major in CN and 5 peaks at 1.395 (9.672 %), 1.562 (59.321 %), 2.149 (9.135 %), 2.291 (16.766 %), 4.894 (5.107 %) in NK separated. All these finger print profiles are unique to every extract of CN and NK.

More than 300 medicinal plants have been listed as rare/red/threatened species and substitution for these herbs is the need of the hour^[21]. The most common criteria for adulteration is the morphological similarity between the species; secondly economic aspects. The rare/red/threatened species would be costlier and hence

adulterated with the cheaper one. Substitutions of herbs accomplish many goals though basic idea is to provide similar therapeutic effect as that of original drug. It provided a greater scope for the physician to utilize herbs that are easily available, cost effective and most appropriate for the clinical condition. It is not that all adulterations are intentional malpractice as stated in many literatures. With our experience it is noted that the herbal drugs are adulterated unintentionally also. Suppliers are not conversant and unaware about their spurious supply. Major reasons are confusion in name, non-availability and lack of knowledge about authentic plant. Even scientific community and traditional physicians are unaware of it. Dried floral buds of *M. ferrea* Linn, dried fruits of *Dillenia pentagyna* Roxb and dried fruiting inflorescence of *C. wightii* Meissn are used as Nagakesara in different regions of India^[23,24].

Cirunakappu, one of the study drug belongs to the genus *Cinnamomum*. Cirunakappu refers the flower as anatomical part of the plant but in market immature fruits are sold. If *Cinnamomum* species is to be used as Cirunakappu then the drug must originate from a flower not fruit. Otherwise, Cirunakappu might be the misnomer name of tender fruit of *C. wightii* like the fruit of *Cuminum cyminum* being called as cumin seed^[23,24].

Now a days, herbal drug industries follow high quality standards using modern techniques and instruments to maintain their quality. World Health Organization (WHO), in its publication on quality standards for medicinal plant materials, recommends rejecting any batch of raw material, which has more than 5 % of any other plant part of the same plant (e.g. stem in leaf drugs), nevertheless if they are derived from the authentic plant. Based on these standards, adulteration whether, intentional or unintentional, should be rejected. Also, suppliers and traders should be educated about the authentic sources.

The plant *M. ferrea* is used from the ancient time for its medicinal values. Most of the Ayurvedic and Siddha formulations prescribed for various diseases have Nagakeshara/Cirunakappu as one of the ingredient. But due to huge demand and unavailability of any medicinal plant, there is a common trend to use substitute herb for the morphological similarities or therapeutic value. In the name of Chennai Nagakeshara/Cirunakappu, *C. wightii* is sold out in the market. But in place of stamen of *M. ferrea* (Nagakeshara) similar looking flower but of *C. wightii* (Cirunakappu) might have been used as an adulterant.

Both the flower of *M. ferrea* (Nagakeshara) and the flower bud or the tender fruit of *C. wightii* (Chennai Nagakeshara/Cirunakappu) are entirely different in genus, morphology and chromatographic finger print profiling of chemical constituents. Hence the therapeutic value may also be different from each other. Pharmacological screening of both plants is necessary to scientifically prove the efficacy and use of latter species in place of former species.

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Conflict of interests:

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