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Original Article

Powder Microscopic, Physicochemical, HPTLC and Antioxidant Studies on Noccik Kudinir Chooranam – A Polyherbal Siddha Formulation

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ABSTRACT

Objectives: To study pharmacognostical, physico-chemical, high performance thin layer chromatography and anti-oxidant activity potential of a Siddha drug "Noccik Kudinir Chooranam" (NKC). **Methods:** The raw drugs were collected, authenticated and the Kudinir Chooranam was prepared. Then the drug was subjected to powder microscopic diagnosis, physico-chemical parameters, Thin Layer Chromatographic photo documentation (TLC), High Performance Thin Layer Chromatographic (HPTLC) finger print profile of successive hexane, successive chloroform, successive ethanol, hydro alcohol (1:1) extract, ethyl acetate solubles of water extract, alkaloid fraction and direct ethanol along with ingredients. The methanol and aqueous extracts were evaluated for total phenol and flavonoid, DPPH radical scavenging activity and superoxide scavenging activities. **Results:** Different extracts of the drug showed distinct TLC and HPTLC finger print patterns which will be unique to this drug. The antioxidant activity showed significant results which are comparable to standards. **Conclusion:** The identified powder microscopic characters and the evolved physicochemical parameters would serve as reference tool for quality control and the anti-oxidant activity would find the much wider therapeutic importance of the drug.

Key words: Noccik Kudinir, *Vitex negundo, Allium sativum,* TLC, HPTLC, Antioxidant activity.

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INTRODUCTION

Siddha system of medicine has originated from Tamil Nadu, Southern state of India. Siddhars developed this medical system who possessed supernatural powers. Medicinal plants, also called medicinal herbs, have been identified and used in traditional medicine practices since prehistoric times in Siddha system of medicine. Plants synthesis humpty number of chemical compounds for functions including defence against insects, fungi, diseases and herbivorous mammals. It is estimated that in developed countries, herbals constitutes 25% of total drugs, while in developing countries, it is 80%. Medicinal plants are considered to be safe as there is nil or minimal side effects. In line with the increased demand, the herbal products require pharmacopoeial standards through botanical and chemical characterization, for the quality control. NKC is a poly herbal siddha drug used for the treatment of inflammation, swelling, fever, pain in joint and asthma.[1] Noccik Kudinir is a liquid form of Siddha medicine which could be prepared freshly and consumed for its therapeutic efficacy. The ingredients of the drugs are all well known; *Piper nigrum* and *Allium sativum* are available in all houses as kitchen culinary items and fresh leaves of *Piper betle* are available in market and only the *Vitex negundo* needs to be collected. In this communication, authors aimed to formulate NKC in the dried form with the composition of ingredients as per Siddha literature and subject to powder microscopy, physico-chemical parameters for the NKC (powder) and NK (liquid), TLC/HPTLC analysis, total phenol, total flavonoid, super oxide anion radical scavenging activity and DPPH radical scavenging activities.

The human system creates Reactive Oxygen Species (ROS), such as superoxide anion radical, hydroxyl radical and hydrogen peroxide by many enzymatic systems through oxygen consumption.[2] In small amounts, these ROS can be beneficial as signal transducers. However, during oxidative stress, large amounts of these ROS may favour some human disease conditions such as cancer, hepatic diseases, cardiovascular diseases, ageing and neurodegenerative diseases.[3]

MATERIALS AND METHODS

Preparation of NKC

Vitex negundo and *Piper betle* were collected from the nearby places. *Allium sativum* and *Piper nigrum* were procured from the market. All the plants were authenticated with reference to floras. The fresh leaves of *Vitex negundo* and *Piper betle* were shade dried, along with *Piper nigrum* and *Allium sativum* (fresh), powdered coarsely (NKC) and stored in an air tight container (Table 1).

Powder Microscopy

The powdered NKC was mounted in glycerine on clean microscopic slides after treating with 10% NaOH solution. Slides were observed under Nikon ECLIPSE E200 trinocular microscope and diagnostic characters were identified. Powder characters were magnified to 400X and photographed. The unique characters of individual herbals were identified.[4]

Table 1: List of ingredients and composition.

S.No	Tamil name of the plant	Botanical name of the plant	Anatomical part	Ratio as per Literature	Ration taken	Weight
	Nochi	*Vitex negundo* (Franch.) Rehder	Leaves	1 handful	13 handful	194 g
	Milagu	*Piper nigrum* L.	Fruit	8 g	104 g	104 g
	Poondu	*Allium sativum* (Sadler ex Rchb and Mill)	Bulb	4 g	52 g	52 g
	Vetrilai	*Piper betle* L.	Leaves	10 nos	130 nos	209 g

Preparation of Kudinir

Kudinir (water extract/decoction) was prepared by adding water (100 ml) to NKC (5 g), boiled till the water content is reduced to 1/4th of the water (25 ml) and then filtered.

Chemicals, solvents and materials

All the solvents used were AR grade (Merck). For visualizing purpose vanillin (1 g) sulphuric acid in ethanol (5%) solution (VSA) was used. Standards and reagents were AR grade and all other chemicals were LR (Sigma Aldrich).

Instrument

For HPTLC, aluminium plate precoated with Silicagel 60F_{254} (Merck) was used. Automatic sampler ATS4 for application on TLC plate, twin trough chamber (10 × 10 cm) for plate development, visualizer for photo documentation, Scanner 4 with win CATS software for finger prints, TLC plate heater for derivatization (CAMAG, Switzerland), Perkin Elmer's Lambda 25 UV-Visible spectrophotometer for absorbance measurement were used.

Physicochemical parameters

All the physiochemical parameters for NKC and NK were carried out as per standard methods.[5]

Preparation of extracts

NKC (1 g) was extracted successively with n-hexane, chloroform and ethanol using Soxhlet apparatus, filtered, concentrated and made up to 1 ml. NKC (100 mg) was sonicated with 1 ml 1:1 aqueous ethanol, filtered and used for TLC/HPTLC. The NK was extracted with ethyl acetate repeatedly, combined together, evaporated to 1 ml. NKC (5 g) was acidified with 5 % acetic acid solution and kept overnight. Then, filtered, basified with ammonia and brought the solution to basic pH. This solution was shaken with chloroform and chloroform layer was separated, dried and the yield was calculated. This residue was re-dissolved in 1 ml of chloroform for TLC of alkaloids.

Mobile Phases

The mobile phases for *n*-hexane extract, *toluene: ethyl acetate: formic acid* (8:0.8:0.5, *v/v/v/v)*; for successive chloroform, *toluene*: *ethyl acetate: diethyl amine* (7:3.5:1, *v/v/v*); for successive ethanol, *toluene: ethyl acetate: formic acid* (5:4:1, *v/v/v*); for hydro alcohol extract, *hexane: toluene: ethyl acetate: formic acid: acetic acid* (3:3:4:0.5:0.5, *v/v/v/v/v)*; for kudinir, *toluene: ethyl acetae: formic acid* (5:3:1,*v/v/v/v*); for alkaloid, *toluene: ethyl acetate: diethyl amine* (7:4:0.5, *v/v/v*), for ethanol extract with ingredients, *toluene: ethyl acetate: formic acid* (5:4:1, *v/v/v*) were finalised.

TLC/HPTLC

Extracts (15 µl), in 6 different plates (6x10 sq.cm) as 8 mm bands. The plates were developed in the respective mobile phases. The developed plates were air dried, viewed under UV 254 nm and 366 nm and the images were documented followed by multi wavelength scanning at these wavelengths using deuterium lamp in absorption/reflection mode. Then the plates were dipped in a dip tank containing VSA reagent and heated at 105°C till the appearance of coloured spots. Immediately the derivatized TLC plates were photo documented and scanned at a wavelength of 520 nm for finger prints.

Antioxidant activity

The total phenol content was determined by Folin-ciocalteau method.[6] The standard curve for total phenol using gallic acid and total flavonoid content[7] using quercetin is 2, 4, 6, 8, 10 µg/ml concentration. The sample concentration was 10 and 100 µg/ml respectively. For DPPH free radical[8] and superoxide anion radical scavenging activities[9] the extracts in the concentrations 1, 10, 15, 100 mg/ml, standard ascorbic acid in the concentration 25, 50, 75, 100, 200, 300, 400 and 500 µg/ml were taken.

RESULTS

The powdered compound formulation of NKC contains the characters pertaining to the bulb of *Allium sativum*, leaves of *Piper betel* and *Vitex negundo* and fruits of *Piper nigrum* (Figure 1). Elongated thin walled epidermis with a normocytic stomata and spiral vessel elements are the characteristic features of *A. sativum*; epidermal cells with glandular trichomes, stomata, prismatic crystals and spiral vessels are the distinctive features of *P. betel* leaves; Stone cells of hypodermis, bearer cells, perisperm cells and starch grains are the representative characters of

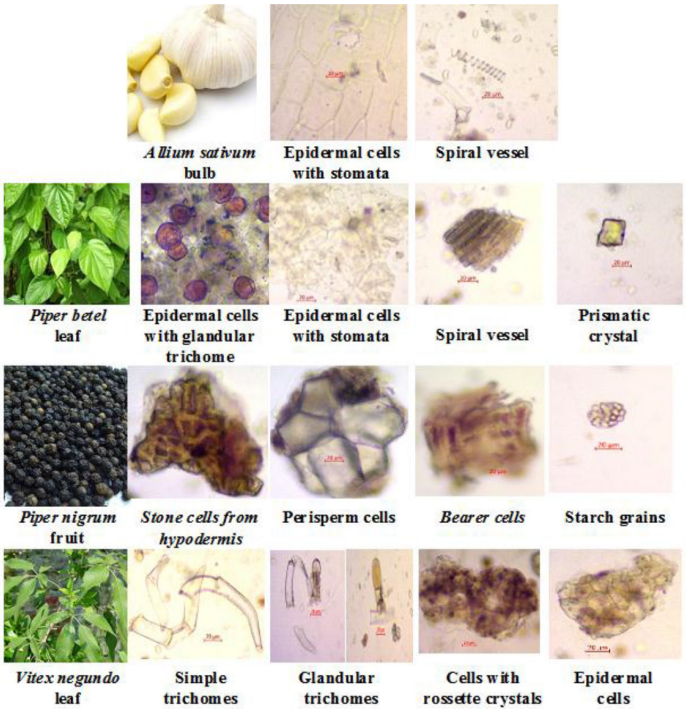

Figure 1: Ingredients and Powder microscopy of NKC.

P. nigrum fruit and simple unicellular to multicellular trichomes in addition to glandular trichomes, epidermal cells and parenchyma cells containing rosette crystals are specific characters of *V. negundo* leaves.

Physicochemical results of NKC and Kudinir are presented in the Table 2.

The TLC of *n*-hexane extract (Figure 2) showed seven spots at R*f* 0.25, 0.29, 0.34, 0.38, 0.39, 0.47, 0.52 (all green) under UV 254 nm; thirteen spots at R*f* 0.15 (pink), 0.24 (pale blue), 0.30 (pink), 0.33 (pale blue), 0.36 (yellow), 0.41 (pink), 0.42 (yellow), 0.47 (pink), 0.50 (bluish white), 0.55, 0.59 (both pink), 0.61 (blue) and 0.69 (pink) under UV 366 nm; eight spots at R*f* 0.03, 0.08, 0.18, 0.26 (all purple), 0.34 (yellowish brown), 0.48, 0.59, 0.97 (all violet). The TLC of successive chloroform (Figure 2) showed six spots at R*f* 0.14, 0.18, 0.58, 0.74, 0.85, 0.93 (all green) under UV 254 nm; fifteen spots at R*f* 0.04 (fluorescent blue), 0.06 (pink), 0.07 (brown), 0.09, 0.12 (both pink), 0.16 (brown), 0.18 (green), 0.32 (pink), 0.59 (violet), 0.64 (pink), 0.68 (violet), 0.74 (blue), 0.80, 0.85, 0.94 (all pink) under UV 366 nm; eight spots at R*f* 0.03 (Bluish Green), 0.08 (blue), 0.18 (pink), 0.26 (light pink), 0.39, 0.51 (both blue) o.57, 0.73 (both yellow) after dipping in VSR. The TLC of successive ethanol extract (Figure 2) showed 4 spots at R*f* 0.05, 0.49, 0.59 and 0.66 (all green) under UV 254 nm; fourteen spots at R*f* 0.03 (creamy white), 0.05 (pale pink), 0.36, 0.46, 0.51, 0.54, 0.59,0.63 (all pink), 0.67 (sky blue), 0.70, 0.77, 0.84, 0.90, 0.99 (all pink) under UV 366 nm; nine spots at R*f* 0.06, 0.35 (both violet), 0.49 (yellow), 0.51, 0.54, 0.58, o.67, 0.78, 0.98 (all purple) after derivatizing with VSR. HPTLC profile of hexane extract (Figure 2) under UV 254nm, major peaks (5,7,6 and 8) appeared at R*f* 0.34(area 34.79%), 0.48 (21.36%), 0.40(11.85%) and 0.52 (10.48%); under 366 nm, major peaks (3,4 and 2) at R*f* 0.35(50.69%), 0.41(15.66%) and 0.24(13.58%); after derivatization with VSR, at 520nm, showed major peaks (10,6,8,14 and 5) at R*f* 0.57(16.33%), 0.35(16.32%), 0.47(13.56%), 0.95(12.95%) and 0.27(10.21%) respectively. HPTLC profile of successive chloroform extract (Figure 2) under UV 254nm, showed major peaks (9,11,10 and 7) at R*f* 0.72(42.59%), 0.91(20.30%), 0.83(14.88 %) and 0.57(13.00%); under 366 nm, showed major peaks (12 and 13) at R*f* 0.93(33.69%), 0.96 (16.98%); after derivatization with VSR, at 520 nm, showed major peaks (10,11 and 13) at R*f* 0.74(32.42%), 0.90(19.09%) and 0.95(16.49%). HPTLC profile of successive ethanol extract (Figure 2) under UV 254 nm, showed major peaks (11, 10 and 8) at R*f* 0.68(19.93%), 0.60(14.89%), 0.49(12.31%); under 366 nm, showed major peaks (19, 20, 21 and 16) at R*f* 0.85(16.21%), 0.91(15.29 %), 0.96(14.84%), 0.63(10.41%); after derivatization with VSR, at 520 nm, showed major peaks (14,11 and 9) at R*f* 0.98(17.97%), 0.78(12.05%), 0.57(10.325%).

The TLC of hydro alcoholic extract (Figure 3) showed six spots at R*f* 0.54, 0.59, 0.68, 0.73, 0.80 and 0.84 (all green) under UV 254 nm; seven spots at R*f* 0.48(Blue), 0.55(Blue), 0.65(Blue), 0.68(Blue), 0.74, 0.66 (both Fluorescent Blue), 0.92(Blue) under 366 nm; five spots at R*f* 0.45, 0.53, 0.60, 0.70(all grey) and 0.74(brown) after derivatization with VSR reagent.

Table 2: Physicochemical results of NKC and NK.

S.No	Parameters	Mean (n=2)	S. No	Parameters	Mean (n=2)
	LOD at 105°C	19.87*		*Successive Extraction*	
	Total Ash	6.17*	7.	n-Hexane	4.00*
			8.	Chloroform	3.50*
			9.	Methanol	9.00*
	Water Soluble Ash	2.61*	10.	pH (10% solution)	6.49
	Acid Insoluble Ash	1.10*	11.	pH	6.57
	Water Soluble Extractives	41.40*	12.	Total Solids	2.75*
	Alcohol Soluble Extractive	27.60*	13.	Weight per ml (g/ml)	1.03

*means w/w %

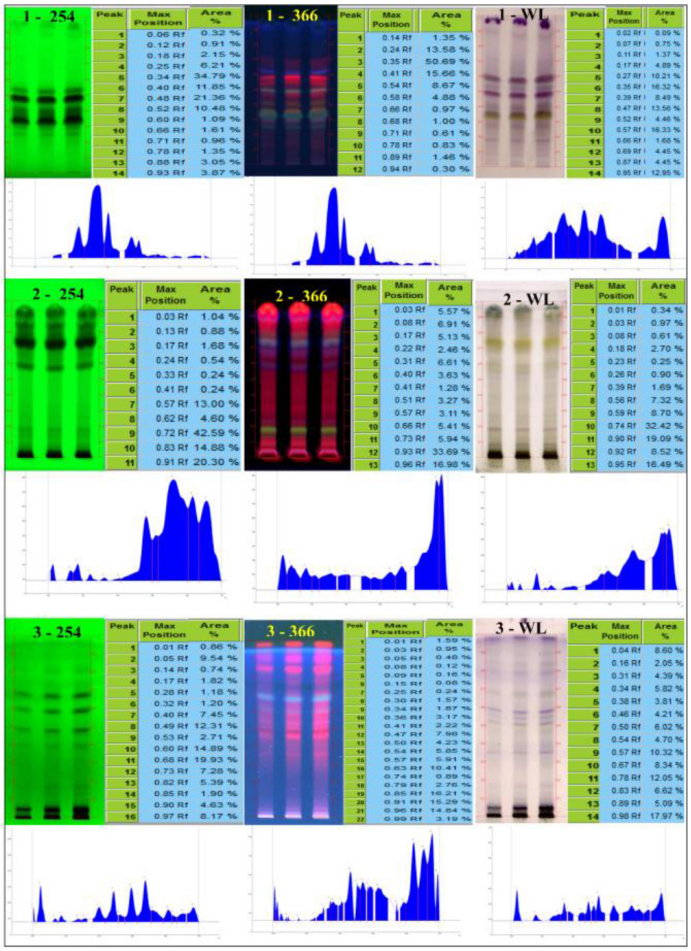

Figure 2: TLC/HPTLC of successive extracts of NKC 1 – n-Hexane; 2 – Chloroform; 3 – Ethanol.

The TLC of ethyl acetate solubles of Kudinir (Figure 3), therapeutic dosage form showed ten spots at R*f* 0.04, 0.05, 0.07, 0.09, 0.48, 0.59, 0.66, 0.80, 0.89 and 0.95 (all green) under UV 254 nm; twelve spots at R*f* 0.04 (Blue), 0.38 (Blue), 0.44 (Pale Blue), 0.47 (Pink), 0.53,0.59 (both Pale Blue), 0.64 (Fluorescent Blue), 0.69, 0.72, 0.79 (all Pale Yellow), 0.83 and 0.88 (both Pink) under UV 366 nm; ten spots at R*f* 0.06 (Violet), 0.38, 0.52, 0.54, 0.56 (all Purple), 0.66 (Yellow), o.69, 0.78, 0.84 and 0.93 (all Purple).

The TLC of alkaloid fraction of NKC (Figure 3) showed eight spots 0.12, 0.16, 0.45, 0.64, 0.73, 0.82, 0.92 and 0.98 (all green) under 254 nm; fourteen spots 0.03 (Fluorescent Blue), 0.14 (Blue), 0.19 (Greenish blue), 0.25, 0.31 (both Pink), 0.44 (Violet), 0.59 (Pink), 0.63 (Blue), 0.66, 0.70 (both Light Green), 0.86 (Pink), 0.90 (Light Green), 0.94 (Fluorescent Green) and 0.97 (Pink) under 366nm; two spots 0.63 and 0.97 (both orange) after spray with Dragendorff's reagent.

HPTLC finger print of hydroalcohol extract (Figure 3) under UV 254nm, showed major peaks (8 and 7) at R*f* 0.72(47.29%) and 0.69 (19.60%); under 366 nm, showed major peak (10 and 9) at R*f* 0.92(46.14%) and 0.71(19.83%); after derivatization with VSR, at 520 nm, showed major peaks (9 and 8) at R*f* 0.74(39.40%) and 0.70(19.42%).

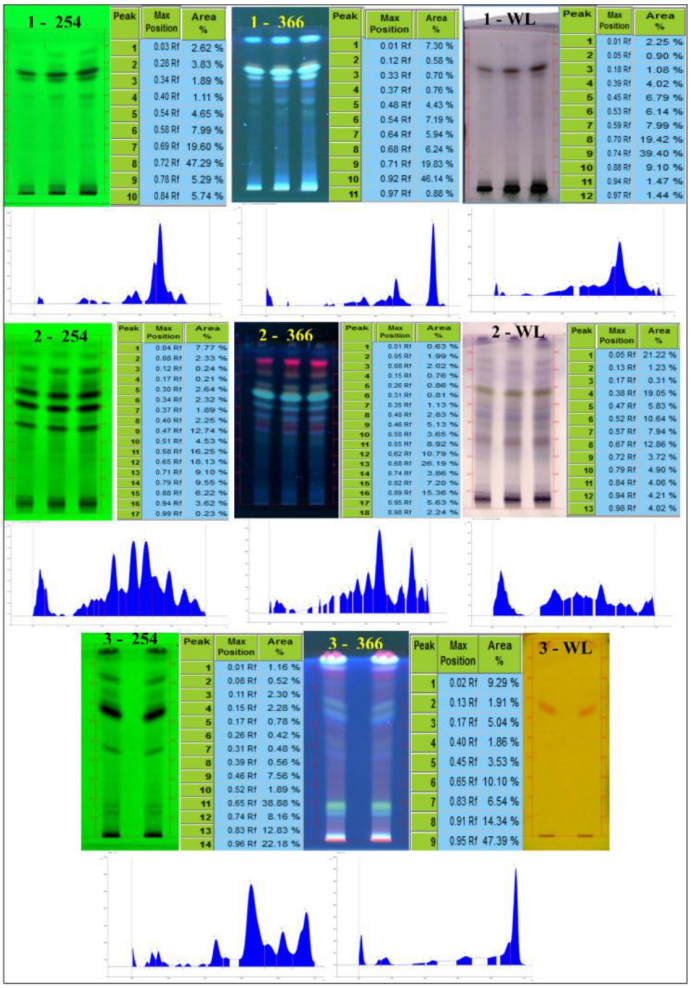

Figure 3: TLC/HPTLC of NKC 1-Hydroalcohol; 2–Ethyl acetate solubles of Kudinir; 3–Alkaloid fraction.

HPTLC finger print profile of ethyl acetate solubles of water extract of NKC (Figure 3), under 254 nm showed major peaks (12,11 and 9) at R_f 0.65(18.13%), 0.58(16.25%) and 0.47 (12.74%); under 366 nm, showed major peaks (13,16 and 12) at R*f* 0.68 (26.19%), 0.89 (15.36%) and 0.62(10.79%); after derivatization with VSR, at 520nm, showed major peaks (1,4,6 and 8) at R*f* 0.05(21.22%), 0.38(19.05%), 0.52(10.64%) and 0.67(12.87%).

HPTLC finger print of alkaloid fraction (Figure 3), at 254nm showed major peaks (11,14 and 13) at R*f* 0.65(38.88%), 0.96(22.18%) and 0.83(12.83%); under 366nm, showed major peaks (9,8 and 6) at R*f* 0.95(47.39%), 0.91(14.34%) and 0.65(10.10%). As the TLC plate showed two alkaloid spots at 0.63 and 0.97, finger print not documented after derivatization with Dragendorff's reagent. The TLC of alcohol extract with ingredients showed that spots of all ingredients in the NKC (Figure 4) and the 3D chromatogram also represented the ingredients peak in NKC.

Total phenol content was estimated as gallic acid equivalents (mg/g), 25.23±0.3mg/g for NKC aqueous extract and 32.32±0.406 for NKC methanol extract, the absorbance was measured at 750 nm. Total flavonoids estimated as quercetin equivalents (mg/g), 23.04±0.353mg/g for NKC aqueous extract and 23.26±0.829 for NKC methanol extract. The absorbance was measured at 435 nm. The results are represented pictorially in Figure 5.

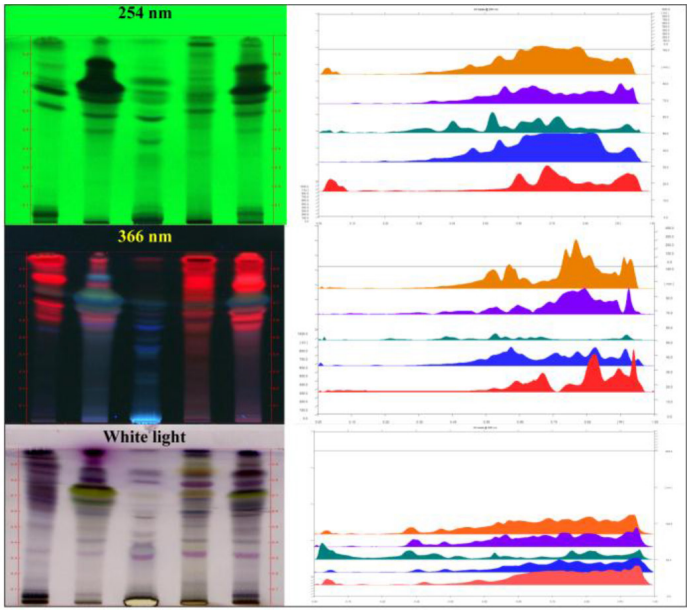

Figure 4: TLC and 3D chromatograms of ethanol extract of NKC with ingredients Track 1-5: *V. negundo*, *P. nigrum*, *A. sativum*, *P. betel* and NKC.

The DPPH radical scavenging activity of NKC extracts was detected and compared with standard ascorbic acid. The percentage of inhibition at various concentrations (Table 3) of NKC (aqueous-NKCAE and methanol-NKCME) as well as standard ascorbic acid (25-500µg/ml) were calculated and plotted in graph (Figure 5). The test drug shows higher inhibition of about 71.17% for NKCAE, 75.63 for NKCME and the standard ascorbic acid exhibits 77.66% of inhibition at 500 µg/ml sample concentration. In lower concentration 25µg/ml, the test drug shows inhibition 23.28% for NKCAE, 27.97% for NKCME and the standard ascorbic acid exhibits 38.98% of inhibition the absorbance was measured at 517nm using UV Spectrophotometer.

The superoxide radical scavenging of the extract of NKC were detected and compared with ascorbic acid standard (Table 3). The percentage of inhibition at various concentrations (25-500µg/ml) of drug as well as standard ascorbic acid (25-500µg/ml) were calculated and plotted in graph (Figure 5). The test drug shows higher inhibition of about 63.59% for NKCAE, 68.43% for NKCME and the standard ascorbic acid exhibits 72.49% of inhibition at 500µg/ml sample concentration. In lower concentration 25 µg/ml, the test drug shows inhibition 9.92% for NKCAE, 24.24% for NKCME and the standard ascorbic acid exhibits 18.26% of inhibition. The absorbance was measured at 560 nm using UV spectrophotometer.

DISCUSSION

The main reason behind the need for the evaluation of crude drug microscopically is to rule out any adulteration and substitutions if any. Microscopic examination of the powdered compounded formulation revealed the presence of elongated epidermal cells with anamocytic stomata and spiral vessels which is in accordance to the previous studies carried out in *A. sativum*;[10-13] thin epidermal cells with cyclocytic stomata, glandular trichomes with pearl glands and the prismatic crystals obtained during powder microscopy can be attributed to *P. betel* leaves and affirms the former studies;[14-17] broad and rectangular sclereids seen in abundance arranged laterally, bearer cells, starch grains granules both agglomerated and scattered and the vascular elements can serve as a diagnostic tool for identifying *P. nigrum* fruits and were consistent

Table 3: Free radical scavenging activity by NKC.

Concentration (µg/ml)	DPPH free radical Inhibition (%)			Superoxide anion radical inhibition (%)		
	Ascorbic Acid	CCAE	CCME	Ascorbic Acid	CCAE	CCME
25	38.98	23.28	27.97	18.26	9.92	24.24
50	42.58	32.03	39.92	29.88	20.97	33.26
75	47.97	51.48	53.05	33.03	29.88	41.26
100	56.95	61.02	68.36	47.46	33.26	51.18
200	62.42	61.56	73.52	55.13	58.62	61.10
300	66.48	66.09	74.92	66.85	60.20	62.80
400	70.63	67.27	75.23	70.91	62.12	63.92
500	77.66	71.17	75.63	72.49	63.59	68.43

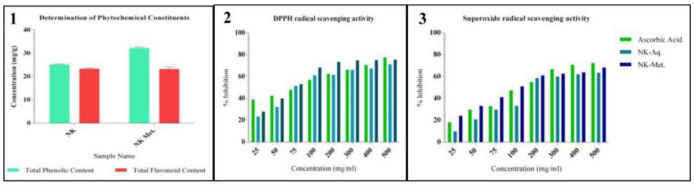

Figure 5: Antioxidant activity of NKC. 1 – Total phenol/flavonoid; 2 – DPPH radical scavenging; 3 – Super oxide anion radical scavenging.

with previous studies.[18-20] *V. negundo* leaf powder contains an immense amount of heteromorphic trichomes varying from simple to multicellular, short, conical, thick walled, warty and glandular trichomes and a large number of rosette crystals which were obtained in the current study and co relates to the previous studies.[21,22] The morphology of the various tissues and cells of the plants are species specific and diagnostic as a result the powder microscopic characterization can be effectively used as a diagnostic tool to identify the various components of compound formulations.

The physico-chemical parameters are the basic study carried out for herbal drugs. The loss drying gives the total content of moisture and volatile oil. All the ingredients of the study drug contain volatile to quantifiable amount and the moisture of fresh garlic also led to this high value of the loss on drying. However, the volatile oil present in the ingredient will prevent the drug from deterioration.[23] The total ash of the study drug, 6.17 % represents the inorganic salts which is a primary metabolite required for physiological functions of human body. Low intake of these nutrients may lead to loss of calcium and thereby leads to even death.[24,25] The water soluble ash of the study drug indicates that the 2.61% of ash are soluble in water and remaining is acid soluble ashes. The alcohol soluble and the water soluble extractives, 27.60% and 41.40% respectively are an indicative of the solubility of the secondary metabolite constituents in the high polar organic and inorganic solvents. The pH 6.49 shows that the drug is acidic despite the presence of alkaloid and would have longer shelf life. The kudinir (dosage form) also remains in acidic pH of 6.57. The specific gravity of the decoction is 1.03. The n-hexane soluble extract was calculated as 4% in which most of the non polar compounds would be extracted and the chloroform soluble extractive was evaluated as 3.50% which represents the medium polar compounds the methanol extractive indicates the presence of high polar compounds to the extent of 9%.

The non-polar compounds were separated and shown in the TLC of hexane extract, medium polar compounds in the TLC of chloroform extract, polar compounds in the TLC of ethyl acetate extract, high polar compounds in the TLC of ethanol, hydroalcohol and aqueous extracts.

The amount of total phenolic content was found to be 25.23±0.3 mg/g for NKCAE and 32.32±0.406mg/g for NKCME of gallic acid equivalents. The flavonoid content was found to be 23.04±0.353 mg/g for NKCAE and 23.26±0.829mg/g for NKCME of quercetin equivalents. The inhibitory activity of DPPH radical by NKCAE (IC_{50}=74.3±0.467µg/ml) and NKCME (IC_{50}=72.70±0.174µg/ml) was better when compared to the scavenging activity of ascorbic acid (IC_{50}=78.2±0.272µg/ml). The superoxide radical scavenging activity of ascorbic acid (IC_{50}=123.79±0.279 µg/ml) was better when compared to NKCAE (IC_{50}=179.24±0.345 µg/ml) and NKCME (IC_{50}=196.45±0.768µg/ml).

The phytochemical literature of the ingredients shows that the drug has variety of phytoconstitutes.[26-35] The ingredients were reported with many pharmacological activities.[36-51] Previous epidemiological studies report that these antioxidant compounds also possess anti inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial and antiviral activities to greater or lesser extent.[52-54] Hence the study drug would have anti-inflammatory, analgesic, anti-tumour, anti cancer, anti-arthritic and anti-rheumatic activities.

CONCLUSION

The identified microscopic characters would help to check the NKC drug for proper inclusion of individual ingredients. The physicochemical results, TLC/HPTLC of different extracts of NKC and Kudinir would be useful for the quality control investigation of the drug. As reported by earlier researchers, as the drug is exhibiting the DPPH free radical scavenging and superoxide anion radical scavenging activities, the drug leaves broad scope for the pharmacologist for further studies. For facilities and support.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

NaOH: Sodium hydroxide; **DPPH:** α, α – diphenyl-β-picrylhydrazyl.

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