Phytochemical Investigation of Nilavembu Kudineer Chooranam Ethyl Acetate Extract and Its Ability to Reduce Intracellular Antioxidant Levels in THP-I Cells

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Authors' contributions

This work was carried out in collaboration among all authors. Authors PK and MFV did the conception and design of the work. Author PK collected the samples and did the phytochemical screening, fluorescence analysis, pharmacognistic studies, pharmacognostic evaluation, TIC and HPTLC. Author PK wrote the manuscript. GCMS work was done by author SM. Antioxidant activity and intracellular anti-oxidant activity works were done by authors MFV and MRG proof read the whole manuscript and suggested the necessary changes and helps in designing manuscript. All authors read and approved the final manuscript.

Article Information

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ABSTRACT

Aims: Nilavembu kudineer chooranam is one of the best polyherbal formulations in Siddha tradition of medicinal practice. This formulation has nine herbs as ingredients. This research article explores the scientific analysis of the therapeutic and phytochemical potential of this herbal formulation.
Methodology: In this study, Nilavembu Kudineer Chooranam is extracted with hot ethyl acetate using a Soxhlet apparatus. This study focuses on the pharmacognosy, phytochemistry, physico-chemistry TLC analysis and antioxidant ability of Nilavembu Kudineer chooranam. High-

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performance thin-layer chromatography (HPTLC) is used to analyze the extract of Nilavembu Kudineer Chooranam to screen for active principles. Antioxidant activity of Nilavembu Kudineer Chooranam was determined using DPPH assay. Gas Chromatogram Mass Spectrometric method (GCMS) was carried out in the ethyl acetate extract of NKC for phytochemical analysis.

Results: The preliminary phytochemical analysis of the ethyl acetate extract of Nilavembu Kudineer Chooranam reveals the presence of phytochemicals. The quantitative phytochemical estimations show the presence of 0.082 μg of alkaloid, 5.81 μg of flavanoid, 4.32 μg of tannin and 0.736 μg of phenolics in ethyl acetate extract. The HPTLC analysis confirmed the presence of such phytochemicals that are well known for their medicinal properties. The percentage of moisture content, total Ash, acid-soluble ash, and a water-soluble ash content of NKC were found to be 3.7%, 9.07%,1.66%, and 0.75% respectively. The fluorescence analysis of NKC powder with different chemical reagents revealed the presence of several active principles in the extract. The antioxidant ability of NKC extract was also assessed by FACS analysis using DCFDA assay in THP-I (human monocyte) cells.

Conclusion: In this *in-vitro* assay NKC extract significantly reduced the intracellular antioxidant levels in THP-I cells. Thus the *in-vitro* assay further confirmed the antioxidant potential of NKC extract. It is apparent from the above results and discoveries that NKC makes a perfect health drink.

Keywords: Nilavembu kudineer chooranam; phytochemistry; pharmacognosy HPTLC; antioxidant; THP-I; GCMS; intracellular antioxidant.

1. INTRODUCTION

The plants that are having medicinal qualities have always been associated with the cultural behaviours and traditional knowledge of the people. Herbal medicine has fewer side effects and relatively low cost when compared with synthetic drugs [1]. It promotes natural healing and reinforces the immune system. It stabilizes hormones and metabolism. Most of the herbal medicines have curative properties to treat various diseases [2]. The major use of herbal medicine is for health promotion and therapy for chronic as opposed to life-threatening remedies increases when conventional medicine is ineffective in the treatment of diseases, such as in advanced cancer and the fever of new infectious diseases. Thus, they play a vital role in producing drugs.

Herbal drugs are an essential component of traditional medicine in several countries, including China and India. In India, Siddha, Ayurveda, and Unani are the three medicinal systems practised. Mostly the Siddha system of medicine trains in the Southern part of India [3]. The inherent use of the medicinal plants in Tamil culture remains empirical evidence to Siddha medicine. It is useful in curing all kinds of problems like urinary tract infections, diseases of the liver and the disorders in the gastrointestinal tract. Comprehension of the metabolism of medicinal plants throws light on the medicinal properties [4]. Based on biosynthetic origins,

natural plant products can be divided into major groups such as alkaloids, terpenoids and phenyl prostanoids and allied phenolic compounds. The synergy of the herbal formulation of Nilavembu Kudineer Chooranam (NKC) is attributed to the ingredients of nine plant materials. Nine components are Nilavempu (*Andrographis paniculata*), Vetiver (*Vetiveria zizanioides*), Vilamiccam ver (*plectranthus vettiveroides*), Santanam (*Santalum album*), Peyputtal (*Trichosanthes dioica*), Koraik kilanku (*Cyperus rotandus*), Cukku (*Zingeber officinale*), Milaku (*Piper nigrum*), Parpatakam (*Mollugo cerviana*) [5]. NKC in Siddha medicine is suggested for the prevention and management of all types of viral infections and fevers. It acts as immunostimulant and immunomodulator, which boosts immunity and modulates defence response in the body, which helps to product infections and their complications. It also plays a vital role in the human body against the dengue and chikungunya. The ingredients of the Kudineer possess anti-inflammatory, anti-microbial, analgesic, anti-oxidant, anti-viral, cytotoxic, hepatoprotective and antidiabetic activities [6]. The antioxidant is a compound that delays the oxidation of substrates even in very lower concentration than the oxidized substrate. The antioxidant can protect the body from damage caused by the free radical-induced by oxidative stress [7]. Thus the present studies deal with the pharmacognostic evaluation, preliminary phytochemical analysis, High-Performance Thin Layer Chromatography (HPTLC) and Antioxidant

activity properties of ethyl acetate extract of Nilavembu Kudineer Chooranam [8,9]. Antioxidants are the vital substances which possess the ability to protect the body from damage caused by the free radical-induced oxidative stress [10]. In this study, the Gas Chromatogram Mass Spectrometric method (GCMS) was carried out in the ethyl acetate extract of NKC for phytochemical analysis.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

Nilavembu Kudineer Chooranam has been selected for the present phytochemical study. The material was collected from Siddha hospital from Tirunelveli. Further, taxonomic identification was authenticated by Dr. G. Jeya Jothi, Taxonomist, Department of Plant Biology and Biotechnology, Loyola College, Chennai.

2.2 Preparation of Plant Extract

The materials were dried in shade for 5 days. The dried sample was ground to a fine powder. The whole powdered sample of Nilavembu Kudineer Chooranam was kept in an airtight container until extraction. 100 g of powdered materials of Nilavembu Chooranam were successively extracted with 500 ml of ethyl acetate by Soxhlet apparatus. For 24 hrs the extract was concentrated by using rotatory evaporator and subjected to freeze-drying till dry powder was obtained.

2.3 Physicochemical Parameters

The physicochemical parameters like moisture content; total ash value, water-soluble ash and acid insoluble ash were determined.

2.3.1 Determination of moisture content

2 g of the powdered sample was weighed in a weighed flat and thin porcelain dish, It was dried in the oven at 100ºC, It was cooled in a desiccator. The loss in weight was recorded as moisture [11].

2.3.2 Determination of total ash

Total ash was determined as formulated by the association of official analytical chemists, 2 gram of the sample was taken in a silica crucible which had been previously ignited and before weighing. The ignition was repeated until a constant weight was obtained [12].

2.3.3 Determination of Acid-soluble ash

1 g of ash was weighed and 10 ml of conc. H_2SO_4 was added to it. The insoluble matter was collected in a previously weighed sintered crucible, washed with hot water, dried to constant weight and weighed. The percentage of acid-insoluble ash was calculated [13].

2.3.4 Determination of water-soluble ash

1 g of ash was taken and 10 ml of distilled water was added to it. The mixture was shaken and filtered through Whatman filter paper. The ash that remained in the paper was kept in silica crucible and burnt again in a muffle furnace for 4 hours. The weight of ash was noted and the percentage of water-soluble ash was determined [14].

2.4 Qualitative Determination of Ash for Mineral Constituents

For detection of various inorganic elements in plant ash viz., Fe, Cl, P, S, etc., 1 g of ash material was dissolved in 25 ml of 50% HCl for 12 hours and then filtered through filter paper. The filtrate was treated with suitable reagents to identify the presence of elements qualitatively [15-17].

2.5 Phytochemical Screening and Estimation of Phytochemicals

The extract of Nilavembu Kudineer Chooranam was subjected to qualitative chemical examination for the presence of alkaloid, flavanoids, Glycoside tannin, Coumarin and phenolic compound. The phytochemical investigation of the extract of Nilavembu Kudineer Chooranam was carried out with standard protocol [18-21].

2.5.1 Estimation of alkaloids (by Singh and Archana Sahu method)

0.5, 1.0, 1.5, 2.0, and 2.5 ml (concentration varying from 5 to 25 µg) of the standard solution were pipetted out into a series of 25 ml standard flasks and 1.5 ml of the sample was pipetted out into a separate 25 ml standard flask. To all the flasks, including the blank, 1 ml of 0.01M SPI and 0.5 ml of 0.1 acetic acids were added. Then 10 ml of distilled water was added and kept in the boiling water bath for 10 minutes. After that, 2 ml of 0.01 M 3-Methylbenzthiazolinone-2(3H)-hydrazone (MBTH) was added into all the flasks

and that was boiled in a water bath for 2 minutes. All the flasks were cooled and made up to the mark with double distilled water. The blue colour formed was spectrophotometrically measured at λ630 nm. Then the graph was drawn by plotting the concentration of theophylline along with with the X-axis and the optical density reading along Y-axis. From the standard curve on the graph unknown sample concentration was calculated.

2.5.2 Estimation of flavonoids by aluminium chloride method (Zhishen, Mengcheng and Jianming)

0.5, 1.0, 1.5, 2.0 and 2.5 ml (concentration varying from 50 to 250 µg) of the standard solution were pipetted out into a series of test tubes. 0.1 ml of the sample was pipette out into a series of test tubes. To all the test tubes, including the blank, distilled water was added to make up to 2.5 ml. To all the test tubes, 75 µl of 5% $NaNO_2$ was added and incubated at room temperature for five minutes. Then 150 µl of 10% $AlCl_3$ was added at room temperature for six minutes. Then 0.5 ml of 1 M NaOH was added, mixed well and the pink coloured substance formed was spectrophotometrically measured at λ 510 nm. The graph was drawn by plotting the concentration of rutin along with the X-axis and the optical density reading along the Y-axis. The unknown concentration was calculated from the standard curve of the graph [22].

2.5.3 Estimation of tannin by the modified prussian blue method

0.1, 0.2, 0.3, 0.1, 0.4, and 0.5 ml (concentration varying from 10 to 50 µg) of the working standard solution was pipetted out into a series of test tubes. Then 0.1 ml of the sample was pipette out into a test tube. To all test tubes, including the blank, distilled water was added to make up to 7 ml. Then 1 ml potassium ferricyanide and 1 ml of $FeCl_3$ were added to all test tubes and mixed well. The absorbance was measured spectrophotometrically at λ700 nm. Then, the graph was drawn by plotting the concentration of tannin along the X-axis and the optical density reading along the Y-axis. From the standard curve of the graph. The concentration of the unknown sample was measured [23].

2.5.4 Estimation of phenolic compound

100 mg of the extract of the sample was weighed exactly and dissolved in 100 ml of triple distilled water (TDW). 1 ml of this solution was taken in a test tube, and then 0.5 ml 2N of the Folin-Ciocalteu reagent and 1.5 ml 20% of Na_2CO_3 solution was added to it. Now finally the volume was made up to 8 ml with TDW followed by strong shaking and lastly allowed to stand still for 2 hours. After which the absorbance was taken at 765 nm. These data were used to evaluate the total phenolic content using a standard calibration curve which was obtained from various diluted concentrations of Gallic acid [24].

2.6 Fluorescence Analysis

0.5 g of dried powder was taken into six clean and dried test tubes. To each test tube, 5 ml of dil. HCl, dil. HNO_3, aq. NaOH, $CHCl_3$, H_2SO_4 and distilled water were added separately. Then all the test tubes were shaken and allowed to stand for 20-25 minutes. The solutions obtained were observed under visible light for their characteristic colour reactions and were compared with a standard colour chart and colours were recorded [25].

2.7 TLC

Ethyl acetate solvent extract was subjected to thin-layer chromatography (TLC). The glass slides were evenly coated with a silica gel (for TLC) and dried. The TLC plate was cut into a size of 10 cm by 5 cm; a mark was made in pencil about 1.5 cm from the lower edge of the end of the plate which marked the origin. Glass capillaries were used to spot the sample on TLC plate. Each plate was kept in the beakers containing 3:4:3 ratio of Methanol, formic acid and toluene in ethyl acetate extract. After the run plates were dried and kept in the iodine chamber to detect the bands on the TLC plates. The procedure was repeated by using the different ratio and volume of solvent until a perfect separation was obtained [26].

2.8 HPTLC

2.8.1 High-performance thin layer chromatography

The known amounts of the ethanol extract of the plant (0.25 gm) were dissolved in 25 ml of methanol respectively and further sonicated in the ultrasonic water bath to dissolve and the volumes were made up to 25 ml with methanol and after filtration through Whatman filter paper (44). The resulting solution was used as the extract. Three spots of each extract of quantity 5 µl, 10 µl and 15 µl were applied in silica gel 60F254 TLC plate with the help of Linomat5

applicator. The plate was placed in the twin trough plate development chamber pre-saturated with the respective medium. The mobile phase was run up to about 80 mm. The developed chromo plate was dried by hot air. The plate was then photo-documented with the help of a digital camera (CANON P). The spots developed were scanned at a wavelength from 250 nm to 800 nm with the help of Scanner 111. The software Win cats interpreted the intensity and Rf. value of various peaks [27].

2.9 Antioxidant Activity

The free radical scavenging capacity of the extract was measured by using 1,1- diphenyl- 2- picrylhydrazyl (DPPH) assay. The extracts were added to the DPPH solution and incubated for 30 mins. Then the absorbance was measured at 517 nm using a UV-VIS spectrophotometer. Ascorbic Acid was used as a standard.

Percentage Inhibition (%) = (control -sample / control) × 100

Where control is the absorbance of the control and sample is the absorbance of the extract [28].

THP-I cells were grown to a confluence of 75% and seeded in 6-well plates at a concentration of about 1×10^6 cells/ml/well. The cells were treated with DCF-DA for 45 minutes at 37oC followed by NKC ethyl acetate extract and incubated for 4 hours. This treatment was followed by pelleting and washing the cells in PBS/FBS and analyzing them using BD FACS Calibur. The results were obtained as a histogram. Cell Quest Pro software was used for the analysis.

2.10 GCMS

The following chemicals Acetone (99.9%) – Cas No-67-64-1- Cat.No-11340-SRL, Chloroform (GC grade)-67-66-3-Cat.No-0103305-250 ml and Methanol- Product code-M0275 were purchased from Spectrochem®, SRL®, Acros Organics®, RANKEM®, Fisher Scientific®, and used as received without further purification. GCMS analysis of the ethyl acetate extract of NKC was recorded on Shimadzu QP2020 GC-MS spectrometer in EI mode. The oven temperature is maintained at 280ºC at a rate of 10ºC/min; the carrier gas with a flow rate of 1 ml/min. The split sampling technique was used to inject the sample in the ratio of 1:10. Retention indices (RI) of the compounds were determined by comparing the retention times of a series and identification of each component was confirmed by comparison of its retention index with data in the literature. Mass-Spectrum was carried out by using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components which was stored in the NIST library. The molecular weight, name, chemical structure and molecular formula of the components of the test materials were ascertained.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening

Preliminary Phytochemical screening was carried out and it was given in Table 1. The present study revealed that the ethyl acetate extracts of NKC contained alkaloids, flavonoids, phenols, tannins, and coumarin. This studies during the present investigations discovered that the NKC is mainly founded of many primary and secondary metabolites which can be measured for application in the therapeutic industry. The present study will prove useful to find the number of bioactive principles present in NKC. It may be concluded that NKC consists of many vital and novel Phytochemical. The Phytochemical screening of Ethyl acetate extract NKC is summarized

3.2 Pharmacognostic Studies

The present study revealed that the ethyl acetate extracts of NKC contained chlorine, sulphur, phosphorus and iron as shown in Table 2. The presence of mineral elements has the property to cure numerous health problems. It achieves a more biological function. For example, Iron is important for the carriage of oxygen to the tissue. Iron deficiency causes several diseases. This clearly shows that NKC has many therapeutic potentials. The results of fluorescence analysis of NKC powder with different chemical reagents are expressed in Table 3. Fluorescence study is an essential and required parameter for first-line standardization of crude drug. Characteristic fluorescence was observed after testing with different reagents like dil. HCl, $CHCl_3$, HNO_3, H_2SO_4, NaOH and H_2O. These studies help in proof of identity of the plant material further, it will use to detect adulterants and substituent and can help in upholding the quality. The moisture content of the crude drug and ash values

are shown in Table 4. The low moisture content of the crude drug reflects the availability of active principle in greater quantities. The minimum content of Moisture content of drugs showed clearly that it discouraged the growth of bacteria, yeast or fungi during storage.

3.3 Quantitative Estimation of Chemical Constituency

In the NKC extract phytoconstituents were dominated by Tannin (4.57 µg) and Flavonoids (4.37 µg) respectively and Alkaloids (0.09 µg) then followed by phenolic (0.736 µg) respectively

and it is given in Table 5 and Fig. 1. Flavonoids in NKC are antioxidants and free radical scavengers which prevent oxidative cell damage, have stronger anticancer activity and inhibit tumour growth.

3.4 TLC & HPTLC

TLC for the NKC extract in ethyl acetate 3:4:3 ratio Methanol, Formic acid and Toluene mobile phase was done. The spot got spread on the plate due to solvent. The result obtained under UV light at 360 nm reveals that six constituents are present in the NKC ethyl acetate extract is

Table 1. Preliminary phytochemical screening

Phytochemical		Ethyl acetate extract
Alkaloid	Mayer's test	+
	Dragendroff's test	+
	Hager's test	-
	Wagner's test	+
Flavonoid	Shinoda test	+
	Ammonia test	+
	Zn test	+
	Ehrlich's test	-
Coumarin	Modified Prussian blue test	+
Phenol	Ferric chloride test	+
	Gelatin test	-
	Lead Acetate test	+
Glycoside		+
Tannin		+

+ Present   - absent

Table 2. Detection of ash elements

S. No.	Experiments	Observation	Inference
1	Test solution +2-3 drops of dil.HNO_3 + 3-4ml distilled H_2O + few drops of $AgNO_3$.	White precipitate soluble more than ammonia indicates.	Presence of chlorine.
2	Test solution + 2-3 drops IIC1, warmed +10 drops $BaCl_2$.	The White precipitate of $BaSO_4$ indicates.	Presence of Sulphur.
3	Test solution + 3-4 drops HNO_3 soiled + 4-6 drops Ammonium Molybdate + 4-5 drops NH_4OH in water bath at 60^0C	Yellow precipitate ammonium phosphomolybdate soluble in NaOH indicates.	Presence of phosphorus.
4	Test solution + 3-4 drops potassium thiocyanate.	The reddish-brown colour of ferric thiocyanate indicates.	Presence of Iron.

Table 3. Fluorescence analysis

Reagents	Colour under visible light	Colour under UV light 365 nm
Powder + dil. HCl	Dark Brown	Green
Powder + HNO_3	Yellow	Green
Powder + NaOH	Brown	Green
Powder + $CHCl_3$	Dark Brown	Dark Green
Powder + H_2SO_4	Yellow	Green
Powder + H_2O	Light Green	Green

shown in Fig. 2. The HPTLC analysis of NKC extract verifies the presence of six different phytochemicals at 360nm. Tannin was concluded as the active constituent that gives peak with maximum % area (56.70%) in the ethyl acetate extract of NKC are shown in Fig. 3. The presence of tannin confirmed the anti-oxidant activity of NKC. HPTLC also provides information about the Rf values as shown in Table 6.

Table 4. Pharmacognostic evaluation

Properties	Percentage
Moisture content	3.7%
Total Ash	9.07%
Acid-soluble ash	1.66%
Water-soluble Ash	0.75%

3.5 Antioxidant Activity and Intracellular Anti-oxidant Activity of NKC Extract

The DPPH based antioxidant assay showed a direct correlation between the concentration of NKC extract and the free radical scavenging property of the extract, indicating the strong antioxidant ability for the NKC extract is shown in Fig. 4. Flow cytometric analysis of THP-1 cells treated with NKC extract (0.312 and 0.156 mg/ml) stained with 2,7 – dichlorodihydro-fluorescein diacetate (DCFH-DA), an indirect method for estimating the intracellular Reactive Oxygen species (ROS) level also matched with DPPH data. Flow cytometry analysis (FACS) revealed that the NKC extract pre-treated cells showed significantly reduced the intracellular ROS production in THP-1 (human monocyte) cell line compared to H_2O_2 + DCFH-DA (blue line) treated cells. The reduction in ROS as a concentration-dependent with a higher concentration of NKC extract (0.312 mg/ml-purple line) showing a lower intracellular ROS level when compared to the lower concentration of NKC extract (0.156 mg/ml-orange line) are shown Fig. 5. The FACS results in correlation with the DPPH assay results are in line with the phytochemical data which indicated a significant presence of antioxidants in the NKC extract. This indicates that NKC extract has strong potential to be exploited as an anti-inflammatory therapeutic agent.

Table 5. Amount of alkaloids, flavonoids, tannins and phenolic compounds

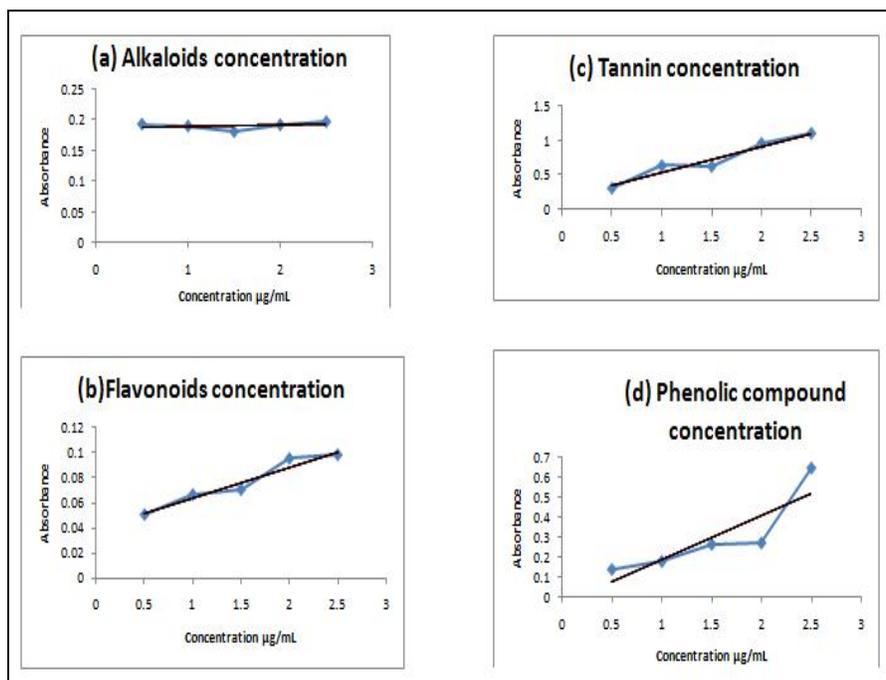| Type of extracts	Alkaloid	Flavanoid	Tannin	Phenolic
Ethyl Acetate Extract	0.09 μg	4.37 μg	4.57 μg	0.736 μg

Fig. 1. The concentration of ethyl acetate extract Nilavembu Kudineer Chooranam
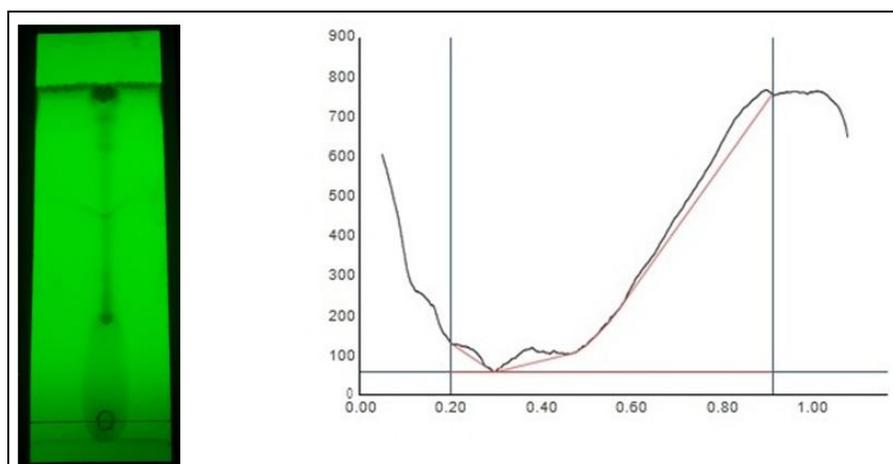
Fig. 2. TLC plate results in Fig. 3. HPTLC chromatogram
TLC plate result 3:4:3 ratio of Methanol,
Formic acid and Toluene in ethyl acetate extract.

Table 6. HPTLC Chromatogram of ethyl acetate extract of NKC

Peak	Start Rf	Start height	Max Rf	Max height	Max %	End Rf	End height	Area	Area %	Assigned substance
1	0.23	10.3	0.25	21.8	11.01	0.28	0.4	495.7	6.36	unknown *
2	0.32	14.3	0.38	38.4	19.35	0.40	22.6	1395.7	17.91	unknown *
3	0.42	13.0	0.43	17.7	8.90	0.46	1.4	223.0	2.86	unknown *
4	0.59	1.0	0.62	18.7	9.42	0.63	17.1	356.8	4.58	unknown *
5	0.66	17.3	0.70	32.4	16.36	0.72	29.9	903.7	11.60	unknown *
6	0.77	41.7	0.83	69.3	34.95	0.91	7.5	4418.6	56.70	unknown *
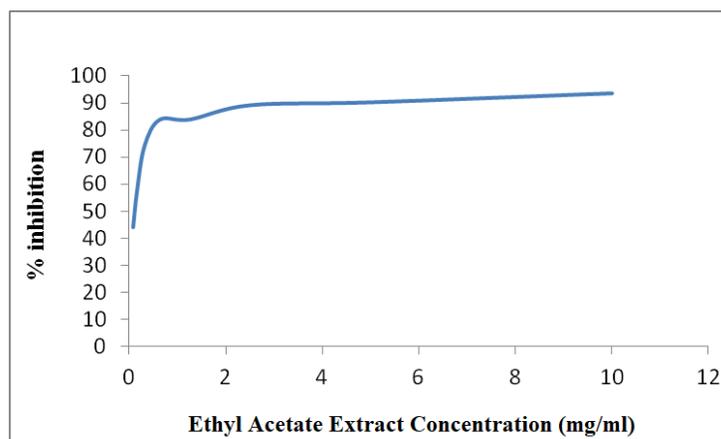
Fig. 4. DPPH antioxidant assay

3.6 GC-MS

GC-MS technique is used to find the constituents of hydrocarbons, alcohols, acids, esters, etc. Peak area, retention time and molecular formula were used for the confirmation of phytochemical compounds are shown in Fig. 6. The active principles with their Retention time (RT), Molecular formula, Molecular structure, Molecular weight (MW) and peak area in percentage are presented in Table 7. From the GC-MS analysis of ethyl acetate extract of NKC, the presence of fifteen compounds (phytochemical constituents) were revealed the

medicinal quality. Of the fifteen compounds identified, the most prevailing compound was Piperine compound (74.92%) followed by 1-(4-Hydroxy-3-methoxyphenyl)dec-4-en-3-one (5.01 %), n-Hexadecanoic acid (3.72%), 9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E) (2.05%) and remaining compounds are less than 2%. Compounds having anti-inflammatory, antibacterial, antifungal, skin conditioning properties have been identified and it will enhance the therapeutic potential of NKC.
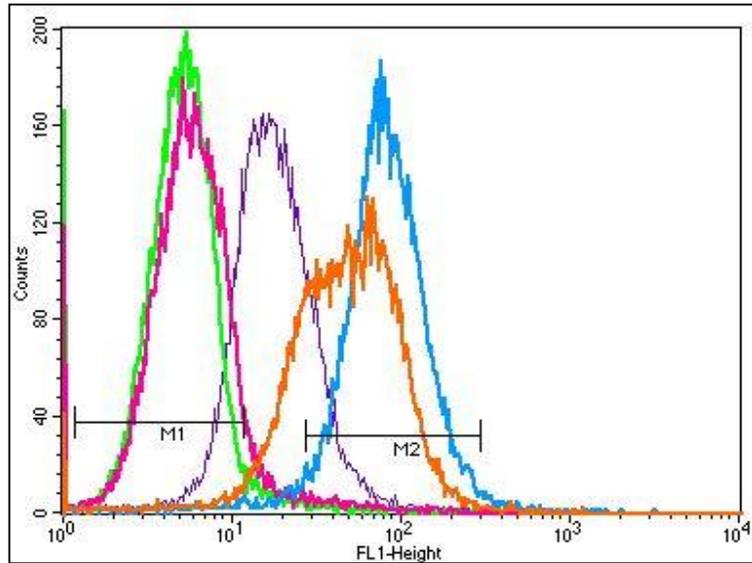

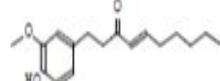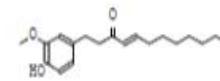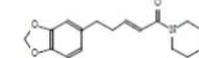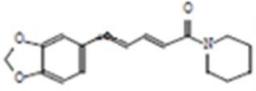**Fig. 5. Intracellular antioxidant activity of NKC extract**
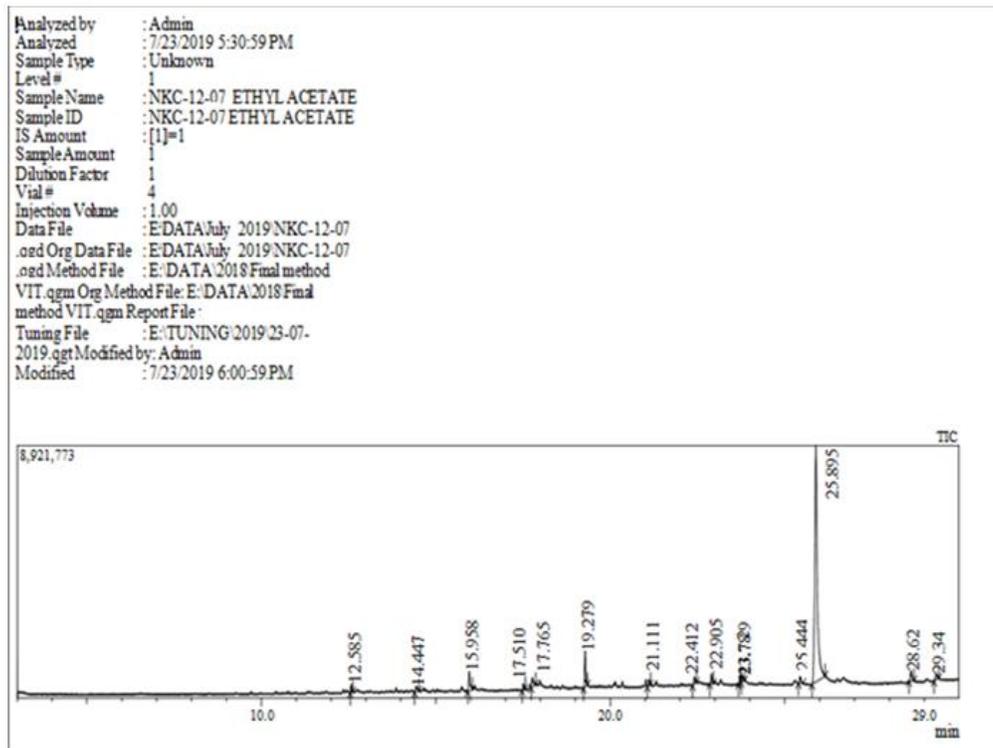
Fig. 6. Chromatogram of Ethyl Acetate Extract of NKC

Table 7. GCV Chromatogram of ethyl acetate extract of NKC

S. no.	Retention time	Peak Area %	Name of the compound	Molecular structure	Molecular weight & molecular formula
1	12.585	1.03	1-Naphthalenol, 1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyl-4-(1-methylethyl)-		$C_{15}H_{26}O$ & 222
2	14.447	1.13	(3S,3aR,6R,8aS)-7,7-Dimethyl-8-methyleneoctahydro-1H-3a,6-methanoazulene-3-carboxylic acid		$C_{15}H_{22}O_2$ & 234
3	15.958	3.72	n-Hexadecanoic acid		$C_{16}H_{32}O_2$ & 256
4	17.510	0.80	3,7,11,15-Tetramethylhexadec-2-en-1-yl acetate		$C_{22}H_{42}O_2$ & 338
5	17.765	2.05	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-		$C_{57}H_{104}O_6$ & 884
6	19.279	5.01	1-(4-Hydroxy-3-methoxyphenyl)dec-4-en-3-one		$C_{17}H_{24}O_3$ & 276
7	21.111	0.92	1-(4-Hydroxy-3-methoxyphenyl)dodec-4-en-3-one		$C_{19}H_{28}O_3$ & 304
8	22.412	1.19	(E)-5-(Benzo[d][1,3]dioxol-5-yl)-1-(piperidin-1-yl)pent-2-en-1-one		$C_{17}H_{21}NO_3$ & 287
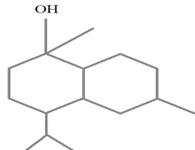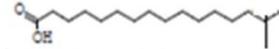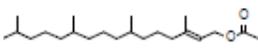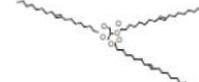
S. no.	Retention time	Peak Area %	Name of the compound	Molecular structure	Molecular weight & molecular formula
9	22.905	1.52	Dotriacontane		$C_{32}H_{66}$ & 450
10	23.720	1.35	(2E,4E,10E)-N-Isobutylhexadeca-2,4,10-trienamide		$C_{20}H_{35}NO$ & 305
11	23.789	1.37	(2E,4E)-N-Isobutyloctadeca-2,4-dienamid		$C_{22}H_{41}NO$ & 335
12	25.444	1.35	Tetrapentacontane		$C_{54}H_{110}$ & 758
13	25.895	74.92	Piperine		$C_{17}H_{19}NO_3$ & 285
14	28.623	2.24	Tetrapentacontane		$C_{54}H_{110}$ & 758
15	29.344	1.39	:(E)-9-(Benzo[d][1,3]dioxol-5-yl)-1-(piperidin-1-yl)non-8-en-1-one		$C_{21}H_{29}NO_3$ & 343

4. CONCLUSION

This preliminary work reveals that NKC extract contains many phytochemicals such as alkaloids, flavonoids, phenols, tannins, Glycoside and coumarin. The present study revealed that the ethyl acetate extracts of NKC contained chlorine, sulphur, phosphorus and iron. Many shreds of evidence gathered in earlier studies confirmed that the identified phytochemicals to be bioactive. The results of fluorescence analysis of NKC powder with different chemical reagents expressed the chemical nature of active principles. Ash values are less than ten percentage which shows that the purity of the sample. Low moisture content is always desirable for higher stability of drugs. The moisture content of the crude drug was found below 4%. The HPTLC methods have been developed for fingerprinting profile for NKC extract. Fingerprinting profiles were developed and pure spoils are required for the identification of various chemical components. Tannin was concluded as the active constituent that gives peak with maximum % area (56.70%) in the ethyl acetate extract of NKC. The DPPH based antioxidant assay showed a concentration-dependent decrease in the free radical scavenging property of the extract, indicating strong antioxidant ability. Anti-inflammatory activity indicates the therapeutic potential of NKC extract that can be exploited for therapeutic purpose. It is concluded from the data that extracts of NKC exhibited significant role in medicinal chemistry for the formulation of life-saving drugs. The analysis of GCMS on the ethyl acetate extract of NKC exposes the existence of vital medicinal bioactive components. The medicinal value of these components in ethyl acetate extract of NKC is similar to the components presents in the other plant extracts which have been already proved. So it is also proved that the extract taken is also equally effective. The work is in the process of evolution to determine its genetic activity and thereby to brighten its pharmacological profile in the field of traditional medicines. A plant having phytochemicals gains pharmaceutical reputation. The scope of the ongoing research is to isolate the phytochemicals and test their medicinal activities.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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